ENCYCLOPEDIA OF MEAT SCIENCES

SECOND EDITION

EDITED BY

MICHAEL DIKEMAN
& CARRICK DEVINE

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MEAT SCIENCES

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VOLUME 1

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VOLUME 1

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

The material in the encyclopedia is not arranged by ordinary alphabetical order, but by alphabetical order according to 97 principal topic areas taken to allow all papers belonging to each principal topic to appear together in the same volume. Within each principal subject, article headings are also arranged alphabetically, except where logic dictates otherwise.

There are four features that help you find the topic in which you are interested:

1. The contents list.
2. Cross-references to other relevant articles within each article.
3. A full subject index.
4. Contributors list.

1 Alphabetical Contents List

The alphabetical contents list, which appears at the front of each volume, lists the entries in the order that they appear in the encyclopedia. It includes both the volume number and the page number of each entry.

2 Cross-References

All of the entries in the encyclopedia have been cross-referenced. The cross-references, which appear at the end of an entry as a See also list, serve four different functions:

i. To draw the reader's attention to related material in other entries.
ii. To indicate material that broadens and extends the scope of the article.
iii. To indicate material that covers a topic in more depth.
iv. To direct readers to other articles by the same author(s).

Example

The following list of cross-references appears at the end of the entry Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants.

> *See also*: Chemical Analysis: Sampling and Statistical Requirements; Standard Methods. Foodborne Zoonoses. Growth of Meat Animals: Metabolic Modifiers. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

3 Index

The index includes page numbers for quick reference to the information you are looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4 Contributors

At the start of each volume there is list of the authors who contributed to that volume.

PREFACE

The Encyclopedia of Meat Sciences, second edition, an extensive revision of the first edition published in 2004, covers all the essential meat topics, ranging from animal production, processing, analytical procedures, and food safety, to final consumption including health issues and nutritional aspects. There are more than 230 articles and these provide a greater breadth of coverage than any existing work on meat science. In addition to publication in print, the Encyclopedia is also available for licensing online that can allow regular updating. The articles are designed to bring a nonexpert up to a level of understanding the interactions among the various disciplines covered in the articles. Most articles are 3000–4000 words long and include a list of Further reading and Websites to expand the content beyond the immediate scope of this work. The Encyclopedia is, therefore, a valuable resource for several levels of education and experience.

The Editors gratefully acknowledge the contributions of the authors of the articles and the Editorial Advisory Board. The board not only proposed subjects to be covered, but also found contributors and then reviewed the articles. The work involved in an Encyclopedia such as this requires an extensive interactive cooperation among the Editors, the Editorial Advisory Board, the contributors, and the publishers, particularly the staff of the Major Reference Works division of Elsevier. The staff included Nancy Maragioglio, Donna de Weerd-Wilson, Anna Gebicka, Cari Owen, Will Bowden-Green, Sam Mahfoudh, Zoey Ayres, and Marise Willis.

The Editors are particularly grateful to Cari, Will, and Sam, who worked very closely with us and who diligently pursued all avenues to obtain contacts with contributors, maneuvered around obstacles, facilitated the day-to-day management, and linked everyone together to meet the deadlines.

Michael Dikeman and Carrick Devine
Editors, August 2014

INTRODUCTION

Meat consumption by hunter–gatherers predated the agricultural revolution. Consumption of meat and fish runs in parallel with human development that is still in process. Humans and animals have now coexisted for thousands of years for their mutual benefit, even though their relationship is changing. Meat does not come from a single, or even a few, animal species, but is derived from a wide variety of species ranging from poultry to pigs, cattle, sheep, goats, and wild game to thousands of species of fish. While many of these species are now intensively farmed, some still coexist with nomadic tribes, whereas, others are raised by families in small village communities, or are even hunted by remnants of hunter–gatherer communities. The second edition of the Encyclopedia of Meat Sciences discusses how the domesticated species evolved; the wide range of harvesting methods for animals, poultry and fish; the historical changes in production, processing and nutritional value, including the beneficial effects of optimum amounts of meat in a diet.

The meat industry is based on obtaining animals, poultry, and fish from pastures, feedlots and specialized intensive production systems, and from extractive industries such as fishing. It is understandable, therefore, that the genetics and management of animals and production systems are prominent in the Encyclopedia. However, the broad field of meat science is much more than harvesting animals and processing meat from them. It includes issues such as preslaughter stress and its effects on meat quality; religious issues; animal welfare; and humane slaughter techniques, all of which are extremely important to ensure that meat quality, cultural issues, and market requirements are harmonized.

Processing methods for the various species are different, but they have all historically developed to ensure, either by conscious design or by experience, that the underlying principles of physiology and biochemistry in the conversion of muscle to meat are optimized. Biochemistry and physiology are extremely important and fundamental disciplines, because they explain how unfortunate, undesirable processing defects such as PSE or cold shortening and toughening can occur and can be avoided. Progress in this area has also enabled significant changes in production and subsequent quality since the first edition of the Encyclopedia of Meat Sciences in 2004.

Understanding these changes requires an appreciation of the structure of carcass tissues, from gross carcass attributes to consideration and understanding of changes at the ultrastructural level. The form and function of muscle tissues, how they change through growth, how they impinge on meat quality, and the way that connective tissue and fat can be major contributors to the final product quality are all covered in these pages. Topics such as cold shortening that can cause meat toughening or inhibition of tenderisation are explained, as well as how procedures such as electrical stimulation evolved to prevent these problems. Assessment of meat quality from measurements such as muscle pH, tenderness prediction through spectral measurements on uncooked meat, color changes on display and storage, and reduction of microbial contamination are critical for many aspects of the meat industry and are also discussed.

There have been many and significant advances in meat animal production based on genetic, nutrition, growth biology, and metabolic modifier research. In regard to meat processing, advances in refrigeration and freezing technology, which is the foundation of perhaps the most important changes ever encountered for food is discussed. Even so, such advances also depend on the way in which microbiology and packaging are integrated to ensure wholesome products with a long shelf life, minimal spoilage, and desirable sensory attributes. However, there are many other ways to preserve food that are also important. Of ever-increasing importance is the topic of food safety, which must receive extensive attention because meat is a perishable product and is critical for a high quality of living and even for human survival. Meat marketing and pricing in all its forms, from wet markets to hotel, restaurant and institutional trade, and transportation are also important. Whole-tissue meat is usually cooked, so, many of the desirable attributes such as flavor development relate to the temperature interactions with various proteins and sugars during cooking. Other cuts are processed in various ways, from smoking to mincing to sausages and the technologies involved are covered.

Not all muscles or cuts of meat are suitable for the same cooking and preparation methods. Therefore, out of necessity, a vast range of highly desirable products has evolved with variations from one ethnic background to another. Other products are merchandized through fast-food restaurants. One can now consume a hamburger in China that is almost identical to that in Chile or in the United States owing to a consistency of product specifications that has become universal. Meat is not only a major source of quality protein and some vitamins and minerals; it often forms the central part of a meal, and is desirable to have the appropriate flavors, aromas, and appearance to conform to the expectations and the way meat is used in various cultures.

This second edition of the Encyclopedia of Meat Sciences also covers controversial health-related aspects of meat consumption and this aspect needs considerably more research. In recent years, the ready availability of meat and other foods has given rise to some health concerns. However, the issues are not always what they seem. The positive and potential negative health-related aspects of meat eating are addressed by experts in dietary and health aspects of meat consumption, but the effect of a single food item should not be considered in isolation.

The wide coverage of topics will ensure that this second edition of the Encyclopedia of Meat Sciences will be an important resource for students or professionals with an interest in meat science or those engaged in the livestock and meat industries. Most of the articles in the second edition are not only a revision of those in first edition but there are additional areas covered. The relatively short nature of the articles makes the Encyclopedia easy and interesting to read.

Michael Dikeman and Carrick Devine
Editors, August 2014

CONTENTS OF ALL VOLUMES

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ADDITIVES

Contents
Extenders
Functional

Extenders

E Mills, Penn State University, PA, USA

This article is a revision of the previous edition article by E Mills, volume 1, pp 6–11, © 2004, Elsevier Ltd.

Glossary

Casein The predominant protein in milk.
Collagen The predominant structural protein constituent of muscle connective tissues, the principle protein component of food grade gelatin.
Gluten A protein component separated during milling of grain, dietary concerns with respect to gluten focus on that from wheat, barley, rye, and oats.
Gums and hydrocolloids Long-chain polysaccharides derived from various plant sources or fermentation processes, give high viscosity in aqueous foodstuffs, for example, alginate, carrageenan, guar gum, locust bean gum, and xanthan gum.
Inulin and oligosaccharides Medium chain length polysaccharides comprised of glucose and fructose subunits.
Nonfat dried milk solids (NFDMS) Manufactured by drying skim milk, contains 52% lactose, 36% protein, and 8.4% mineral (predominantly potassium and calcium).
Retrogradation The viscosity decline in starch gels that occurs if the linear glucose chains reform into granules, accelerated by freezing and thawing.
Soy flour Milled soy grits derived from dehulled, defatted soy beans, contains 47% protein, 38% carbohydrate.
Starch Polysaccharide composed of glucose molecules arranged in linear and helical (amylose) and branched (amylopectin) structures.

Introduction

Extenders are added to meat products to reduce formulation costs or to contribute a variety of functions in the product. The effect of an extender on formulation cost may be significant especially when it facilitates increased product yield via addition of water. Nevertheless, nonmeat ingredients may be added to ground, comminuted, or whole muscle meat products for a variety of reasons including increased shelf-life, reduced fluid purge, increased slicing yield, improved flavor and juiciness, improved color, or cost reduction among others.

Ingredients described as extenders typically allow for reduced product cost while serving other important functions as well. A common functional property of most extenders is water-holding. Water-holding capacity is an especially important property because most cost reduction comes from the addition of water along with the extender. Ingredients are sometimes called binders when they are used primarily for increasing the water-holding capacity of a product or also if they improve fat-holding and emulsion stability. Thus, the terms extender and binder are often interchanged when referring to various ingredients. Extenders used in a particular meat product are

chosen based on their specific functional properties, compatibility with the product, and cost.

Functional Properties

A functional property is the ability of an ingredient to impart an economically important characteristic to the finished product in which the ingredient is used. Functional properties of extenders used in meat products include, for example, water-holding, texture modification, improvement of flavor or appearance, improvement of sliceability for luncheon meats, reduced cook loss or fluid purge, modification of heat-set or cold-set gelation, emulsification of fat, or simply improvement of flowability or mixability of a seasoning mix Table 1.

Many of the functional properties of extenders depend on the ability of the material to interact with water, protein, or fat. Water-holding by an extender material is largely dependent on available charged groups and void spaces within granules of the material. Interactions of extenders with meat protein often involve charged groups along with hydrophobic interactions. Interactions with fat are largely hydrophobic but, an extender may help emulsify fat if it includes both hydrophobic and hydrophilic regions. Protein-based extenders such as soy or milk proteins may interact with meat proteins to increase cohesiveness or with fat to increase emulsion stability. Starch-based extenders such as corn or potato starch increase water-holding but may interfere with protein–protein interactions, thus weakening the protein matrix within the product. By selecting the right extenders the manufacturer can increase or decrease specific product properties to achieve a desired functional result, often with a concurrent reduction in formulation cost.

Choosing the best extender for a particular application is a complex process. An ingredient such as starch may be chosen for its low cost and water-holding ability but it also imparts other properties such as pale color and a tendency to soften the product by interfering with protein–protein interactions. Another, more expensive, ingredient such as soy protein concentrate also holds water while increasing protein–protein interactions and emulsion stability.

Addition of extender ingredients changes the nutrient profile of the meat product. The change may be desirable with low use rates of protein ingredients such as soy or milk increasing the protein content and often changing the amino acid composition to improve biological value. At high use rates, approaching 50% extension, protein digestibility and biological value may be decreased. Milk ingredients also add calcium, which is often lacking in meat products. Even calcium-reduced milk ingredients have enough remaining calcium to measurably raise the calcium content of the meat product. Extenders usually reduce the fat content of the meat product by simple dilution. However, most extenders, except cellulose ingredients, are digestible and contribute to the total calories of the product.

Another significant consequence of extender addition is possible allergic responses. Soy, wheat, and milk proteins are common allergens but any protein ingredient may be allergenic in sensitive individuals. It is critical that extender materials be adequately described on the product label to let consumers know what they are buying.

Table 1 Functions of extenders in meat products

- Reduce formulation cost
- Increase water-binding
- Modify texture
- Improve flavor
- Modify appearance
- Modify cohesiveness
- Provide for heat-set or cold-set gelation
- Improve nutrient profile

Extender Addition to Meat Products

Extenders may be added to meat products by direct addition to comminuted meat or, for intact muscle, by injection along with water, or by surface application in a marinade and massage system.

Extenders are commonly used in certain sausage or sliced luncheon meat products where they are added along with seasonings and incorporated during mixing or chopping. In such applications the extender may be in a range of forms from finely milled powder to coarse grits or flakes. The dry extender may be rehydrated with water to form a slurry before adding it to the meat. Extender ingredients added as larger pieces such as grits or textured flakes may be visible on close examination in the finished product. Hamburger extended with soy grits and water has a different appearance than ground beef without an extender. However, the extender is usually not intended to be visible in the finished product.

For whole muscle products such as ham or chicken breast extender is added as a solution or suspension in water. In this process, the ingredients are commonly referred to as binders instead of extenders. The liquid containing the binder is injected into the muscle using the same techniques as in meat curing. Only finely milled or soluble materials are suitable for this application. Care must be taken to control rehydration, swelling, or gelation of the binder in the injection system as these may lead to fouling of the injector or loss of yield control. Continuous agitation is often needed to prevent the binder from settling out of the water as the liquid sets in the injector reservoir. Additionally, binders or extenders injected into muscle tend to remain in the channels made by the injector needles. When the product is sliced the bands of binder material may be visible within the muscle. This problem is minimized by increasing the number of injection needles and decreasing the amount of material delivered by each.

In some types of products the functional benefits of extenders can be realized by applying the material to the product surface as part of a marinade. Mechanical action in the form of tumbling, massaging, or mixing is required to promote incorporation of the extender material into the surface of the muscle. Penetration depth is minimal, only a few mm. Nevertheless, this approach can be used to modify surface properties of a product to improve color, texture, or reduce fluid loss.

Functional Components of Extender Ingredients

A variety of nonmeat ingredients may be used as extenders in meat products. Sources may include plant seeds, tubers, milk solids, or fermentation processes among others. But, in spite of their varied origins, the functional properties of most extenders are provided by their protein and carbohydrate components. Extender ingredients generally contain little or no fat. Water-holding capacity, flavor, texture, and visual effects of extenders in meat products depend on the types and amounts of carbohydrates and proteins present.

The proportions of carbohydrates and proteins vary greatly with the plant source used. Flour, commonly milled from wheat, contains significant amounts of both starch and protein (gluten). The variety of wheat and the milling process determine the proportions of starch and protein in the flour. More complex separation processes may be used to produce specific, concentrated components from the milled seeds. Wheat gluten, a by-product commonly removed during milling of wheat flour for baking purposes, is widely used as a meat product extender, especially in European countries.

Starches are commonly derived from corn, potato, rice, or wheat using specialized procedures that separate starch granules from protein and other carbohydrate components. The separation is achieved based on density or viscosity differences between starch and protein granules. Modified starches are manufactured using chemical or physical processes that impart unique functional properties to the starch and increase its cost.

High-protein ingredients such as soy protein, wheat gluten, or corn protein are also produced following additional processing steps. By combining these separated, purified components, a meat extender may be created with a unique set of properties that fit a specific product. Of course, the cost of such a specialized extender would be greater than that of a simple milled flour Table 2.

Ingredients Used as Meat Extenders

The list of ingredients that may be used as meat extenders is almost limitless. However, in practice only a few ingredients have found wide acceptance as extenders. Some of the more commonly used ingredients are described in the sections that follow. This is not intended to be a comprehensive listing. Instead, it is a collection of examples of commonly used ingredients or ingredients that demonstrate a unique principle.

Table 2 Types of meat product extenders

- Flour – milled whole grain or grain with seed coat or germ removed
- Proteins – protein fractions of grains, milk, or animal products
- Native starch – starch granules fractionated from grain or tubers
- Modified starch – starch granules pretreated to improve functionality
- Dextrin – from partial cleavage of starch
- Cellulose – long chain complex carbohydrate, dietary fiber
- Hydrocolloids – longer chain, charged side groups, high hydration

Soy Ingredients

Soy protein products represent a great model for discussing extenders in meat products. They are commonly used extenders in many countries. Soy products come in various types and physical forms and serve several different functions.

Soy flour is produced by pressing dehulled soybeans with a solvent to separate the soy oil. The resulting soy grits may be used directly or milled into soy flour. Soy grits or flour contain approximately 45–50% protein along with carbohydrate that imparts a distinctive 'beany' flavor to products. Soy flour may be heated and extruded to produce textured soy flour with a more meat-like texture compared to plain soy flour. Soy flour is an effective water-holder but in spite of its high protein content it has limited ability to participate in protein–protein interactions or fat emulsification. Soy flour may be partially purified by removal of much of the carbohydrate. The resulting soy protein concentrate (SPC) is approximately 70% protein, has much less beany flavor, and has improved protein functionality. SPC is often jet-cooked to further improve its protein functionality. Jet-cooked SPC exhibits excellent ability to interact with meat protein contributing to product firmness. It also has improved capability for fat emulsification. SPC may also be texturized by heating and extrusion to give a meat-like texture.

An extraction process is used to dissolve and then precipitate certain protein fractions from the soy flour to produce isolated soy protein (ISP). This material contains 90–95% protein and exhibits almost no 'beany' flavor. ISP has excellent protein functionality with capability for fat emulsification and increased fluid viscosity and gelation. Like soy flour and SPC, it may be texturized by thermal extrusion. It is also used to produce spun fibers, with improved meat-like texture, which perform nicely in production of meat analogs.

With each successive step from soy flour through isolated soy protein the cost of the material increases. In specific meat products or situations the improved functional properties and reduced flavor intensity of soy concentrate or isolated protein may justify the cost. However, soy flour or grits are most widely used as meat extenders. The beany flavor may become part of the product's flavor profile or seasonings may be used to mask the flavor. Dry soy flour is commonly rehydrated with 2.5–3.5 parts water for one part soy flour. The resulting slurry is added to the meat in the mixer or chopper. The added soy flour slurry may represent up to 50% of the finished meat product. Of course the amount used must be in compliance with applicable ingredient and labeling regulations. In the USA soy flour, SPC, and ISP are limited to 3.5%, 3.5%, and 2.0%, respectively, in cooked sausage products.

Milk Ingredients

Milk proteins are relatively expensive but are chosen by meat processors because of their unique ability to interact with meat protein to form heat-set gels and contribute to stable emulsions. Nonfat dried milk solids (NFDMS) are derived from fluid milk by fat separation and drying. It contains approximately 36% protein including casein and lactalbumin among others. NFDMS is used in meat products to increase water-holding and reduce cook loss. In finely comminuted products it contributes

to improved emulsion stability. Water-holding and emulsifying capabilities of NFDMS may be improved significantly by replacing much of its calcium with sodium. For this reason, the so-called calcium-reduced NFDMS is preferred for use in meat products even though it costs more. The high lactose content of NFDMS, approximately 52%, contributes to flavor and texture of the finished product and may be considered as undesirable in certain products. In the USA, NFDMS utilization is limited to 3.5% of finished product for comminuted meats.

Casein may be precipitated from milk and then resuspended as sodium caseinate. This process produces a concentrated protein (approximately 90% protein) and eliminates most of the calcium and lactose. Sodium caseinate has excellent water-holding and emulsifying properties, a mild flavor, and pale color. It tends to impart a smooth mouthfeel to fine ground sausages. In the USA, this ingredient is limited to 2.0% of finished product for comminuted meats and 1.5% of finished product for water-added ham.

The whey remaining after manufacture of cheese or precipitation of casein contains whey proteins, primarily lactalbumin. The whey may be dehydrated for use as a food ingredient but the dried whey contains up to 75% lactose and over 8% ash. Instead, whey protein concentrate is often produced using an ultrafiltration system to remove lactose and minerals. Dried whey protein concentrate is approximately 80% protein, 4.5% ash, and 6% lactose. It exhibits good water-holding capacity and forms an irreversible heat-set gel when heated above 70–75 °C.

Starch Ingredients

Various starches may be used as extenders in meat products. Starches are generally less expensive than protein ingredients and have low flavor and color intensity. Native starches represent stored carbohydrate in cereal grains and tubers. They are composed of two types of glucose chains, amylose (unbranched glucose) and amylopectin (branched glucose). The glucose chains are packed into starch granules within seeds (grains) or roots (tubers). The size and amylose/amylopectin makeup of the starch granule varies with plant source. Potato starch has larger granules whereas corn starch granules are small. Waxy corn starch contains only amylopectin although most starches include a mixture of amylose and amylopectin.

Starch granules are insoluble in cold water. On heating in water the granules suddenly swell and hydrate, eventually producing a viscous solution in a process called gelatinization. This process accounts for the high water-holding, increased viscosity, and gelling ability of starches. The gelatinization temperature varies for starches from different plant sources. In general, starches with larger granules swell and gelatinize at lower temperatures compared to those with smaller granules. Water-holding or viscosity of starch gels may be lost if the linear glucose chains reform into granules. This process called retrogradation leads to release of water and loss of product quality. When unmodified starch is used in meat products, retrogradation is likely during extended refrigerated storage. Starch retrogradation is accelerated by freezing and thawing.

Native starches are classified on the basis of their properties (viscous, watery, or stringy paste and strong or weak, clear or opaque gel on cooling) with cereal starches (corn, wheat, rice, and sorghum) setting to a strong opaque gel on cooling. Root and tuber starches (potato, cassava, and tapioca) are highly viscous and set to a clear, weak gel on cooling. Waxy starches (waxy corn, sorghum, rice) produce very high viscosity but do not form a rigid gel.

Native starches may be chemically modified to reduce gelatinization temperature or to alter viscosity or gel strength and to control retrogradation. Because most native starch granules do not gelatinize at typical meat cooking temperatures, modified starches are generally preferred for meat applications. Starch may be modified by acid or enzyme treatment, oxidation, or heating among others. Most modification processes involve partial cleavage of the starch to produce shorter glucose chains. This weakens the starch granule allowing for reduced gelatinization temperature. Viscosity and cooled gel strength are affected and the tendency for retrogradation is reduced.

Modified starches are used in meat products to increase cook yield, reduce fluid purge, increase product firmness, and improve sliceability. Overall firmness of the product may be increased due to reduction in free water. Certain starches may be used along with added water as fat replacers. Starches generally do not interact well with meat proteins so they can be used to reduce rubbery character in very low-fat products. The increased fluid viscosity produced by the starch is said to contribute an oily texture during chewing. This property is useful in fat replacement. Under some circumstances starches may cause reduced adhesion among meat pieces in restructured products. This is especially true if the starch is not injected but added in the blender or massager. The combination of starch source and modification process is critical in determining the performance of starch in a meat product.

Some practical issues arise when using starches in injection systems. Because the granules are not soluble, there is a tendency for rapid settling of the starch in the injector reservoir or other containers where the brine is allowed to set. The starch may settle out in the brine pump itself if it is stopped for a time. Continuous agitation is needed to keep the granules suspended during operations and the pump should be rinsed immediately after use. When using modified starches, care must be taken to assure that the temperature in the pumping system does not rise high enough to trigger gelatinization. The resulting change in viscosity can lead to loss of process control and possible equipment damage.

Inulin and Oligofructose

Some plants such as chicory, garlic, or onion do not produce much starch but instead store carbohydrate in the form of inulin and oligofructose. These are polysaccharides comprised of glucose and fructose subunits with varying chain length. Nutritionally, inulin and oligofructose are considered soluble dietary fibers. They are only minimally digested and do not elevate blood sugar levels. Inulin from some plants such as chicory root has a slightly sweet taste. When dissolved in water they impart a slippery, oily mouth-feel that may be quite beneficial in low-fat meat products. Thus, use of inulin in meat products is mostly as part of fat replacement system with

water. Inulin gel improves the texture of low-fat meat products. It also improves water-holding and has been shown to stabilize foams and emulsions in nonmeat food systems.

Gums and Hydrocolloids

Gum is a term that refers to a group of long-chain polysaccharides characterized by the ability to give highly viscous solutions at low concentrations. Ingredients in this group include exudate gums, seaweed gums, microbial gums, seed gums, and certain starch or cellulose derivatives. Ingredients in this category are used throughout the food industry but have found only limited use in processed meats. Unlike classical meat extenders, gums are commonly used at very low concentrations to improve yield, texture, and sliceability and to reduce fluid purge in products with high added water. The cost of gums is usually higher than for other extender ingredients but their exceptional performance may offset the cost.

The most commonly used gum or hydrocolloid in meat products is carrageenan. Carrageenan is a seaweed-derived gum extracted from red kelp. It is composed of linear galactose chains with varying amounts of sulfate side chains that contribute to its water-binding and gelling properties. There are three types of carrageenan, identified as kappa, iota, and lambda. On cooling, kappa carrageenan forms a rigid, brittle gel that tends to release fluid during storage. Iota carrageenan forms a weaker, more elastic gel that is reasonably stable through refrigerated storage, freezing, and thawing. Carrageenan gels may be melted and reformed repeatedly. Lambda carrageenan is used for thickening and viscosity but does not form a cold-set gel. In meat products, mixtures of kappa and iota carrageenan are combined in varied proportions for different applications. Benefits expected from use of carrageenan include improved cook yield, reduced fluid purge, improved sliceability and cohesiveness. Carrageenan has been used along with water in reduced fat products to improve juiciness and texture. Carrageenan is often used at a concentration of approximately 1% of meat weight. Highly refined carrageenan ingredients may give satisfactory results at concentrations of less than 0.5% of meat weight. USDA limits use of carrageenan to 1.5% of finished product for cured meat products.

Flavorings and Seasonings as Extenders

Proteins from plant or animal sources are commonly subjected to partial hydrolysis or other modifications to develop flavorings for food products. Such flavor ingredients appear on the product label and are regulated separate from similar proteins used as extenders. In most cases flavoring ingredients are considered to be self-limiting and thus are allowed at concentrations 'sufficient for purpose.' The hydrolysis process may be quite minimal and the flavor intensity low so that a considerable quantity is needed to alter product flavor. In this situation the meat processor may also consider water-holding and texture benefits in addition to the flavor contribution of the ingredient.

Seasoning ingredients may be an unconventional source of extender materials. An example is mustard seed. Ground mustard is a high protein (26–38%) ingredient with water-holding, gelling, and antibacterial properties. It is used in processed meats as a binder and extender and to enhance product flavor. Deheated mustard is a common ingredient in cooked sausages where it contributes to water-binding, fat emulsification, color, and flavor.

Animal-Derived Extender Ingredients

Consumer resistance to other-species ingredients has reduced the popularity of several animal-derived extender ingredients. Nevertheless, these ingredients are still available and in use for many meat products. Gelatin is probably the most widely used of these ingredients. Most gelatin protein is extracted from animal skins, bovine or porcine. Gelatin takes up water on heating and forms a reversible cold-set gel. It is typically added to processed meat products to reduce purge during refrigerated storage. Raw collagen in the form of powdered skin or muscle-connective tissue is also utilized. It has much lower cost than gelatin and reduced functional properties. This material takes up water on heating but is not soluble, so it is not easily used in injection systems. Blood serum proteins have excellent water-binding, foaming, and emulsifying capabilities. They may be particularly useful in hotdogs and luncheon meats where all these properties are desired.

See also: Additives: Functional. Chemical and Physical Characteristics of Meat: Protein Functionality; Water-Holding Capacity. Chemistry and Physics of Comminuted Products: Emulsions and Batters; Nonmeat Proteins; Other Ingredients. Processing Equipment: Brine Injectors

Further Reading

Berry, B.W., 1997. Sodium alginate plus modified tapioca starch improves properties of low fat beef patties. Journal of Food Science 62, 1245–1249.
Chavez, J., Henrickson, R.L., Rao, B.R., 1986. Collagen as a hamburger extender. Journal of Food Quality 8 (4), 265–272.
Correia, L.R., Mittal, G.S., 2000. Functional properties of some meat emulsion extenders. International Journal of Food Properties 3 (3), 353–361.
Eilert, S.J., Mandigo, R.W., 1997. Use of additives from plant and animal sources in production of low fat meat and poultry products. In: Pearson, A.M., Dutson., T.R. (Eds.), Production and Processing of Healthy Meat, Poultry and Fish Products, vol. 11. Glasgow, UK: Blackie Academic & Professional, pp. 210–225.
Janssen, F.W., Voortman, G., de Baaij, J.A., 1987. Detection of wheat gluten, whey protein, casein, ovalbumin, and soy protein in heated meat products by electrophoresis, blotting, and immunoperoxidase staining. Journal of Agricultural and Food Chemistry 35, 563–567.
McCormick, R.D., 1985. Numerous functional properties govern starch selection. Prepared Foods 154 (5), 173–174, 177.
Miles, C.W., Ziyad, J., Bodwell, C.E., Steele, P.D., 1984. True and apparent retention of nutrients in hamburger patties made from beef or beef extended with three different soy proteins. Journal of Food Science 49, 1167–1170.
Mittal, G.S., Usborne, W.R., 1985. Meat emulsion extenders. Food Technology 39, 121–130.
Reitmeier, C.A., Prusa, K.J., 1990. Addition of corn gluten meal and zein to ground pork of various fat percentages. Journal of Food Quality 13, 271–281.
Tarte, R., 2009. Ingredients in Meat Products: Properties, Functionality and Applications. New York, NY: Springer, 419 pages.
Verbeken, D., Neirinck, N., Van der Meeren, P., Dewettinck, K., 2005. Influence of k-carrageenan on the thermal gelation of salt soluble meat proteins. Meat Science 70, 161–166.

Zeidler, G., Pasin, G., Luh, B.S., Thompson, J.F., Rice, R.D., 1989. Reducing cooking time, yield losses and energy utilization of salisbury steaks as affected by various meat extenders and meat composition. Journal of Foodservice Systems 5 (3), 215–236.

Relevant Websites

http://www.fmcbiopolymer.com/Food/Ingredients/Carrageenan/Introduction.aspx
Carrageenan/Introduction, FMC BioPolymer.

http://www.foodadditives.org/food_gums
Food Gums, International Food Additives Council.

http://www.soyinfocenter.com/index.php
Soy Info Center.

http://en.wikipedia.org/wiki/Starch
Wikipedia entry on Starch.

Functional

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Glossary

Bacteriocin An antimicrobial protein produced by certain bacteria.
Comminuted meat The meat that has been ground or otherwise divided into very small particles.
Essential oil A volatile oil comprising aromatic components of spices that are responsible for distinctive aroma or flavor.
Maillard browning A chemical reaction between carbohydrates and proteins leading to a brown color with development of cooked flavor.
Monosodium glutamate A sodium salt of glutamic acid and a potent activator of umami taste sensor.
Myoglobin A red pigment found in meat, especially red meat.
Nitrosamine A compound resulting from reaction of nitric oxide with secondary amine, some of which are carcinogenic.
Nitrosylhemochrome The compound responsible for the familiar pink color of cured meat.

Regulations

Regulation of functional ingredients in meat products is quite variable around the world. No attempt will be made here to address specific regulatory issues for functional ingredients. Nevertheless, some general observations might be appropriate. Ingredients that impart obvious flavor or visual properties, such as salt and spices, are often considered to be self-limiting and are often unrestricted. Ingredients, such as sodium nitrite and sodium nitrate, can be quite toxic and thus are closely regulated in most parts of the world. Certain ingredients that might be improperly used to deceive an unwitting customer are also regulated in most countries. Examples in this category include sodium phosphates that contribute to increased water-holding ability or reducing agents that may stabilize color, even when the product is noticeably spoiled. In addition to these intuitively obvious regulations, the decisions to regulate specific ingredients are often a matter of tradition or circumstance. Thus, meat manufacturers often encounter a confusing patchwork of regulations when they market their products in other countries.

Adding Functional Ingredients to Meat Products

The addition of functional ingredients to meat products may be achieved using various methods, depending on the properties of the functional ingredients and the meat products. For comminuted or heavily macerated meats, all types of ingredients are easily dispersed by mixing or massaging. Mixing is a rapid process requiring only minutes to achieve uniform distribution of ingredients. Addition of ingredients to intact muscle is more complex. Low molecular weight, easily soluble ingredients such as salt or nitrite may be added by surface application of the dry ingredients, by injection of a water solution into the meat, or by immersion of the meat in a water solution. Injection is quite rapid whereas surface application or immersion requires days or weeks for ingredients to diffuse throughout the intact muscle. Larger molecular weight or insoluble ingredients might be added by injecting a suspension of the ingredients. Continuous agitation is needed to keep the ingredients in suspension during injection. Also, the suspended materials tend to remain in the path opened by the injection needle and become visible when the finished product is sliced.

Functional Ingredients

Salt

Salt (sodium chloride) is the most commonly used functional ingredient in meat product manufacture. It is used primarily for flavor with microbial inhibition, extension of shelf life, and increased protein hydration as secondary functions. Choices relating to the amount of salt to formulate in a product are usually based on taste preferences of customers. Finished product salt concentrations of 1.5–2.5% are common for processed, ready-to-eat meat products. Microbial inhibition and extended shelf life from salt addition are achieved by reducing water activity and, in some cases, by increasing the chloride ion content in the product. With very high salt content (6% or more), the product may be made shelf-stable due to reduced water activity. Such a product would have an intense salty flavor. Salt increases water-holding in meat protein systems and soluble protein in comminuted meat products. Much of the protein that is brought into solution with addition of salt is myosin. Soluble myosin has excellent emulsification and gelling properties. As a result, salt addition is critical for creating a stable and emulsion-like structure in finely comminuted products such as hot dogs.

Nitrite

Nitrite (sodium nitrite or potassium nitrite) is included in many processed meat products for the purpose of inhibiting

spore-forming microorganisms, especially *Clostridium botulinum*, and for stabilizing color and flavor. Meat products containing nitrite are commonly referred to as cured meat products. On addition to meat products, nitrite is reduced to nitric oxide, and this reactive intermediate accounts for most of the functions of nitrite. When nitric oxide from nitrite binds to the iron atom within myoglobin, its pigment properties are changed to a heat-stable form. This so-called pigment stabilization due to nitrite/nitric oxide leads to meat products that retain their pink color even after heating to well done. This heat-stable pink color, nitrosylhemochrome, is an identifying character of products cured with nitrite.

In addition to its effect on meat color, nitrite also influences meat flavor. Although nitrite or nitric oxide, at the low concentration used in meat curing, do not directly impart much flavor, it plays an important role in controlling lipid oxidation. When fresh meat products are cooked, a number of physical and chemical changes lead to accelerated oxidation of unsaturated fatty acids. This oxidation pathway is initiated, in part, by iron released from myoglobin and perpetuated by continuous formation of free radicals from the oxidized fatty acids. The resulting off-flavors are sometimes called warmed-over flavor. In cured meats, two mechanisms cooperate to limit lipid oxidation. First, nitric oxide stabilizes the heme iron in myoglobin and second, nitric oxide reacts with the free radicals to produce a nonreactive product and effectively stop the oxidation chain reaction. These important functions help cured meat products maintain their desirable flavor through extended storage.

In spite of its various benefits, nitrite is a toxic material with a lethal dose for humans of 22–23 mg per kg of body weight. The low amounts used in cured meat products, 200 mg per kg or less, virtually eliminate any risk of toxicity through consumption of cured meats. In addition to its direct toxicity, nitrite might react with certain amino acids to produce nitrosamines, some of which are carcinogenic. The nitrosamine-producing reaction is favored by high temperatures and acidic conditions. The high temperature developed when frying sliced bacon is known to lead to nitrosamine formation if the residual nitrite content is high. This occurrence is minimized by using a lower ingoing nitrite level for bacon and by including reducing agents that help deplete residual nitrite before frying.

Nitrate

There is currently little use of nitrate (sodium or potassium nitrate) in cured meat products. Nitrate is a precursor of nitrite in meat curing. Unlike nitrite, nitrate is comparatively stable in meat products. It reacts slowly, through reduction by microbial enzymes, to release nitrite over an extended period of time. Nitrate use is limited to products such as dry sausage, Prosciutto or Parma ham, and dry cured products that require long curing and aging times. Nitrate is much less toxic than nitrite and is found in many foods including fresh vegetables and drinking water.

Nitrate from Plant Sources

Consumer interest in 'Natural' labeled products in the USA has lead meat processors to manufacture products using nitrate found in certain plant-derived ingredients. Dehydrated celery juice powder is one commonly used ingredient. Such meat products, manufactured without the use of pure sodium nitrite or sodium nitrate, must be labeled as 'Uncured.' Nevertheless, the naturally occurring nitrate in celery juice powder may be converted to nitrite leading to typical cured meat properties. The shelf life, however, and especially cured color, of these 'Naturally Cured' products are considerably reduced compared with conventionally cured products.

Phosphate

Phosphates used in meat processing are usually alkaline polyphosphates. Sodium tripolyphosphate is a very commonly used linear polymer of three phosphate units. Other longer chain polymers can be used, but pH and buffering benefits of phosphate are reduced at longer chain lengths. On addition to meat products, the alkaline phosphate raises the pH away from the isoelectric point of meat proteins and thus increases the water holding. Over time, enzymes in the meat convert longer-chain polyphosphates into diphosphate (pyrophosphate). Pyrophosphate has the ability to break actomyosin cross-bridges that have not transformed into full rigor cross-bridges. Thus, pyrophosphate is the most effective form for increasing water holding and emulsifying ability of meat proteins. However, pyrophosphate has low solubility in water and tends to settle out of brine solutions as insoluble aggregates. Thus, meat curing brines are typically prepared using longer chain, more soluble polyphosphates. Even when using more soluble phosphates, great care must be taken to assure that the phosphate goes into solution before other ingredients, especially salt, are added. For sausage products, where phosphate solubility is not an important issue, it is common to utilize phosphate blends with a higher proportion of pyrophosphate. Thus, the maximum benefit of the phosphate may be realized immediately.

In addition to their influence on water binding and protein hydration, polyphosphates are also able to chelate metal ions that might otherwise catalyze lipid oxidation. Cooked meat products containing alkaline polyphosphates exhibit less lipid oxidation and flavor loss during storage than similar products without phosphate. Phosphates have also been shown to reduce microbial growth, especially that of Gram-positive bacteria. The effect is most pronounced for longer-chain polyphosphates but is also detectable for pyrophosphate.

Erythorbate

Erythorbate (sodium or potassium erythorbate) is a reducing agent used in cured meat products to facilitate the reduction of nitrite to nitric oxide. Sodium erythorbate and erythorbic acid are isomers of sodium ascorbate and ascorbic acid (vitamin C), respectively. Some manufacturers prefer to use the more expensive ascorbic acid in place of erythorbate because they want to list vitamin C on the ingredient statement. As reducing agents, erythorbate, erythorbic acid, ascorbate, and ascorbic acid are chemically equivalent.

Erythorbate is quite important in the curing reaction as it promotes the production of nitric oxide that binds to and stabilizes myoglobin. This is especially beneficial in products

such as frankfurters that might be manufactured and cooked in a very short time, less than 2 h. Use of erythorbate is also beneficial in products such as bacon where elevated residual nitrite might lead to formation of undesirable nitrosamines. In the USA, bacon is required to have 550 mg kg^{-1} of sodium erythorbate or equivalent in the formulation in order to reduce residual nitrite. Because nitrite is quickly converted into nitric oxide, its concentration has to be reduced before the high temperature of frying can lead to nitrosamine production.

Erythorbic acid or ascorbic acid may be used as reducing agents and oxygen scavengers to slow down light-induced fading of cured meat color. For this application, a solution is used to spray or dip the product before vacuum packaging. The application must not add appreciable weight to the product but can be quite helpful in extending the color shelf life of a cured meat product.

Sweeteners

Sucrose, dextrose, and corn syrup products, among others, are commonly used as sweeteners in manufactured meat products. These carbohydrate materials are usually included to impart a desired degree of sweetness. However, properties such as surface browning, water binding, mouthfeel, or smoothness, and the ability to be fermented by microorganisms are also important considerations in choosing the right sweetener. Nonnutritive sweeteners such as saccharin, cyclamate, aspartame, and sucralose have not been widely utilized in the meat industry due to high cost and limited functional benefits.

The sweetness of sugars is usually described in reference to sucrose (cane sugar). Considering sucrose to have a sweetness value of 100, the sweetness of some other sugars is as follows: fructose = 173, dextrose = 74, glucose = 74, and lactose = 16. Corn syrup and dried corn syrup products may be manufactured with a wide range of sweetness, depending on the degree of starch hydrolysis and dextrose isomerization to fructose. High fructose corn syrup with sweetness of approximately 130 is made by complete hydrolysis of corn starch to dextrose and maximal enzymatic conversion of dextrose into fructose. Low sweetness corn syrup products would be manufactured using only partial hydrolysis of corn starch to dextrose and no conversion of dextrose to fructose. Low sweetness corn syrup products are utilized more for their water-binding abilities than for sweetness.

Surface browning of meat products during cooking is a desirable process involving sugars in the product. Caramelization of sugars might lead to surface browning if very high temperatures greater than 190 °C are achieved as with radiant heat cooking. However, the Maillard browning reaction between protein and a reducing sugar is much more common in meat products. Dextrose, glucose, and corn syrup sweeteners all participate in the Maillard browning reaction. However, sucrose does not participate much in Maillard browning. The use of sucrose alone may lead to insufficient surface color during cooking and smoking of meat products.

Seasonings

Seasoning is the general term for ingredients used primarily to impart or modify flavor of food products. Many seasonings also contribute to product color. Seasonings include spices, herbs, and vegetables among others.

Spices are aromatic parts of plants. The particular part may be the fruit, seed, bud, flower, stem, leaf, or root of the plant. Herbs, a subclass of spices are dried aromatic leaves. The flavor or aroma intensity of natural spices varies with season, geographic source, and cultural conditions. Thus, the flavor and aroma of seasoned meat products may vary also. To improve product consistency, many processors have chosen to use essential oils or oleoresins of spices with standardized flavor and aroma intensities.

Essential oils include only the volatile, aromatic components extracted from the natural spices. They generally have little color and may not have a typical spice flavor because nonvolatile taste components are not included. Essential oils are usually extracted using steam distillation to recover the volatiles from the natural spice. Essential oils are widely used in the fragrance industry but are less common in the food industry.

Oleoresins are derived from natural spices by solvent extraction. They include both volatile and nonvolatile components of the spice. Thus, their flavor and aroma might closely match that of the natural spice. The solvents selected for extraction can greatly influence the content of the oleoresin. A low-polarity solvent, such as petroleum ether, will recover a different set of components than a higher-polarity solvent, such as acetone. Solvents are generally chosen so that the oleoresin includes the principal flavor, aroma, and color components of the natural spice.

Natural spices are harvested, dried, and ground with minimal heat treatment in order to protect the important volatiles present. The lack of a thermal kill step means that many bacteria, especially spore formers, survive on the ground spices. For many years, an ethylene oxide gas fumigation treatment was used to sterilize natural spices. Presently, irradiation is replacing ethylene oxide as the preferred sterilization technique. Both techniques are effective for destruction of bacteria. However, concerns with respect to dangers from small residual amounts of ethylene oxide are motivating many companies to switch to irradiation sterilization.

Flavorings

The term 'flavoring' is usually reserved for manufactured ingredients intended to impart or strengthen a specific flavor or flavor note. Most flavorings are made from high-protein materials such as soy protein, yeast extract, milk protein, or blood serum proteins. The protein is partially hydrolyzed to produce a mix of peptides that impart a particular flavor, such as beef or pork flavor.

The hydrolysis and chemical modification process used in preparation of flavorings is a highly protected art with much of the technology developed by trial and error. Flavor chemists share few of their secrets but some generalizations are well-established. Extensive hydrolysis to produce many short peptides tends to heighten the overall intensity, especially the bitter aspect of the flavoring. Complete hydrolysis of a protein with a high content of glutamic acid leads to production of monosodium glutamate (MSG). MSG is an effective flavor

potentiator and activates the umami receptor to give a savory note central to meat flavor. Very mild hydrolysis leads to a flavoring with low flavor intensity. This ingredient might be used when the processor wants to take advantage of other protein properties, i.e., water holding, in addition to the flavor.

Tenderizers

Tenderization of meat products can be achieved in several ways. Some of the ingredients used for tenderization include proteolytic enzymes, acids, salt, and phosphate, among others. See 'Tenderizing mechanisms: (c) Chemical/enzymatic' in this encyclopedia for a detailed discussion.

Antimicrobials

Many of the ingredients used in meat processing, for example, salt, nitrite, and phosphate, have antimicrobial properties. Nevertheless, only a few ingredients are used primarily for their antimicrobial capabilities. These are the focus of this section.

For many years, mold inhibitors were the principal class of antimicrobials used in the meat processing industry. Products in this category include potassium sorbate and propyl paraben. They are used in dry sausage manufacture to control surface mold growth. Casings for dry sausage may be dipped in a solution before stuffing, or the chubs or links may be dipped after stuffing.

Recent regulatory changes, including a zero tolerance for certain pathogens, especially *Listeria monocytogenes* and *E. coli* O157:H7, have led to a greatly increased interest in antimicrobials for fresh and ready-to-eat meat products. Many new ingredients or new applications of existing ingredients are in use or under investigation. Laboratory findings often fail to hold true in the field; so some or most of today's new antimicrobial ingredients might survive the test of time. Following is a description of some of the antimicrobials of interest.

Most of the recent work with antimicrobials has focused on surface sprays or dips that kill or limit growth of bacteria on fresh meat or ready-to-eat product surfaces. Organic acids, such as lactic, citric, and acetic, fall in this category along with sodium diacetate, acidified sodium chloride, acidified calcium sulfate, and cetylpyridinium chloride. Activated lactoferrin may also be used as a surface treatment to prevent bacteria from attaching to the meat surface, thus making them more susceptible to removal or destruction. Sodium or potassium lactate might also be used as antimicrobial ingredients in formulation of meat products, especially ready-to-eat items.

Bacteriocins, small proteins or peptides produced by certain bacteria, are another new type of antimicrobials of interest in the meat industry. Nisin, a bacteriocin produced by the lactic acid bacteria *Lactococcus lactis*, is used in the food industry as an inhibitor of Gram-positive organisms. When incorporated into the casing or packaging material of a processed meat product, nisin helps control bacterial growth on the product surface. Other bacteriocins also show promise for helping control pathogens or spoilage organisms in meat products.

Antioxidants

Lipid oxidation is a considerable problem for flavor of precooked meat items. On heating, physical and chemical changes take place in meat, making unsaturated fatty acids susceptible to rapid lipid oxidation. Oxidation also occurs slowly in fresh meat exposed to light and air. Initiation of oxidation may be controlled by limiting exposure to light and oxygen. The focus of this entry is control of lipid oxidation using antioxidant ingredients.

Lipid oxidation includes initiation and propagation events. Antioxidants work by preventing initiation or by stopping propagation. Initiation of oxidation in meat usually involves oxygen and a metal catalyst, such as iron or copper. The first step is the metal-catalyzed production of a free radical. Antioxidants that chelate metals prevent initiation by keeping the catalysts away from the substrate. Citric acid, polyphosphates, and EDTA (ethylene diamine tetraacetic acid) prevent oxidation by chelating metal ions that might otherwise catalyze initiation of lipid oxidation. Propagation of lipid oxidation over time occurs as free radicals formed during initiation react with oxygen and move on to attack another fatty acid double bond. This attack produces a new free radical that can repeat the process. This cascade process can be stopped by providing free radical acceptors that reduce the free radicals to a nonreactive form. BHA (butylated hydroxyanisol), BHT (butylated hydroxytoluene), TBHQ (tertiary butyl hydroquinone), PG (propylgallate), alpha tocopherol (vitamin E), nitric oxide from sodium nitrite, and the natural antioxidant in the spice rosemary, all act as free-radical scavengers donating electrons to reduce the free radicals to a stable form.

Lipid oxidation naturally occurs in the lipid, hydrophobic portion of meat products. Nevertheless, oxidation intermediates, such as free radicals, might be quite hydrophilic and move into the water portion of the product. Many antioxidants (BHA, BHT, PG, and tocopherols) segregate into the lipid portion whereas citric acid, polyphosphates, EDTA, and nitric oxide localize in the water portion. For best control of oxidation, it might be beneficial to include both a hydrophobic and hydrophilic antioxidant.

Control of lipid oxidation in cooked meat products often involves a combination of control measures. For example, smoked ham contains polyphosphates that control initiation and nitrite (nitric oxide) that acts as a free radical acceptor. In addition, ham usually contains ascorbate or erythorbate, an oxygen scavenger, and is vacuum packaged to limit oxygen exposure. When all these measures are in place to protect against oxidation, smoked ham might be stored under refrigeration for months without appreciable loss of flavor due to lipid oxidation. The same muscle cooked as a pork roast without any added antioxidant protection would exhibit detectable off-flavor within a few hours after cooking.

Acidifiers

Acidifiers are added to meat products to impart a tangy or tart flavor note, to extend shelf life, to tenderize fresh meat, or to promote protein denaturation and moisture release in dried snack products. Acidification is achieved by natural fermentation in traditional, long-process fermented sausages

(Hard Salami), and some whole-muscle products (Proscuitto Ham). However, direct addition of an acidifier allows the process to proceed more rapidly, achieving the desired pH reduction in a matter of minutes. Direct acidification allows the manufacture of many products that would not be practical using natural fermentation.

The most commonly used acidifiers in meat products are lactic acid and citric acid. Lactic acid is favored in products intended to compete with naturally fermented products because it has the same flavor profile. Citric acid is usually less expensive than lactic and is preferred in products such as dry sausage where its antioxidant properties are beneficial. Other acidifiers include acetic, adipic, fumaric, malic, phosphoric, and tartaric acids. Glucono-delta-lactone (GDL) is a unique acidifier sometimes used in the meat industry. It has little effect on pH until it is hydrolyzed to produce gluconic acid. This delayed acid release may be quite beneficial as discussed below.

Several important properties of meat, including color, water-binding ability, and protein functionality, are influenced by pH. Thus, addition of an acidifier to a meat product might have a variety of effects, both expected and unexpected. The normal pH of postmortem muscle is 5.6–5.8. This is somewhat above the isoelectric pH of muscle proteins. With addition of acid, the pH declines toward the isoelectric point where water binding is at its minimum and protein denaturation proceeds more rapidly. Below the isoelectric pH, protein denaturation continues with loss of fresh meat color and reduced protein solubility. Acid-denatured proteins are less able to bind water, emulsify fat, or form a heat-set gel that adheres meat pieces together.

The timing of acidification is often critically important in determining product properties. If acid is added at the start of the mixing process for a ground meat batter, the surface proteins on each meat particle will be denatured, causing bind failure. The finished product will be crumbly or mealy, and fat will separate during cooking. This problem may be managed by using GDL or an encapsulated acid and adding the acid very late in the mixing process. As mentioned above, GDL is acidic only after hydrolysis to gluconic acid. Encapsulated acid is similarly delayed from contact with the meat by virtue of a lipid coating around acid droplets. Encapsulated acid is not released until the cooking process when elevated temperature leads to melting of the lipid coating. At this point in the process, acid and heat denaturation of protein lead to a firm, cohesive texture.

See also: Additives: Extenders. Chemical Analysis for Specific Components: Curing Agents. Chemical and Physical Characteristics of Meat: Protein Functionality; Water-Holding Capacity. Chemistry and Physics of Comminuted Products: Nonmeat Proteins. Curing: Brine Curing of Meat; Natural and Organic Cured Meat Products in the United States; Production Procedures. Processing Equipment: Brine Injectors. Smoking: Liquid Smoke (Smoke Condensate) Application. Tenderizing Mechanisms: Chemical; Enzymatic

Further Reading

Alvarez, D., Castillo, M., Payne, F.A., *et al*., 2007. Prediction of meat emulsion stability using reflection photometry. Journal of Food Engineering 82, 310–315.

Ashie, I.N.A., Sorensen, T.L., Nielsen, P.M., 2002. Effects of papain and a microbial enzyme on meat proteins and beef tenderness. Journal of Food Science 67, 2138–2142.

Cassens, R.G., 1990. Nitrite-Cured Meat: A Food Safety Issue In Perspective. Trumbull, CT: Food & Nutrition Press Inc, 176pp.

Keeton, J.T., 1983. Effects of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. Journal of Food Science 48, 878–881.

McKeith, F.K., Merkel, R.A., 1991. Technology of developing low-fat meat products. Journal of Animal Science 69, 116–124.

Prabhu, G.A., Sebranek, J.G., 1997. Quality characteristics of ham formulated with modified corn starch and kappa-carrageenan. Journal of Food Science 62, 198–202.

Ruusunen, M., Puolanne, E., 2005. Reducing sodium intake from meat products. Meat Science 70, 531–541.

Sebranek, J.G., Bacus, J.N., 2007. Cured meat products without direct addition of nitrate or nitrite: What are the issues. Meat Science 77, 136–147.

Tarte, R., 2009. Ingredients in Meat Products: Properties, Functionality and Applications. New York: Springer

Relevant Websites

http://www.fao.org/docrep/010/ai407e/ai407e14.htm
Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific, CURED MEAT CUTS.

http://www.fao.org/docrep/010/ai407e/ai407e06.htm
Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific, NON-MEAT INGREDIENTS.

http://www.foodadditives.org/phosphates/q_and_a.html
International Food Additives Council, Phosphates Department.

ANIMAL BREEDING AND GENETICS

Contents

DNA Markers and Marker-Assisted Selection in the *Genomic* Era

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Glossary

Breeding value Sum of average effects of alleles, summed over the pair of alleles at each locus and over all loci affecting a trait. It is traditionally predicted based on the performance of the individual and relatives for the trait.

Genomic breeding value Prediction of the genetic merit of the animal based on information provided by high density panels of DNA markers (genomic information).

Genomic selection Selection is based on breeding values predicted from a very large number of estimated DNA marker effects across the whole genome. It is also a marker assisted selection because genomic data is combined with other sources of information to enhance accuracy of breeding values at an early age and facilitate the selection of new traits.

Marker-assisted selection (MAS) The process of using information provided by DNA-markers to predict the genetic merit, assisting the identification of the best animals to be used as parents of the next generation. The DNA marker information should contribute to improve the accuracy of selection and increase the rate of genetic progress by identifying animals carrying desirable genetic variants for a given trait at an earlier age.

Molecular marker Identified segment of the DNA in the genome with known sequence and location on a chromosome. Nowadays, single-nucleotide polymorphisms (SNPs) are commonly used markers in association studies and genomic selection.

Quantitative trait Phenotypes (characteristics) that vary in degree and can be attributed to polygenic effects and their environment.

Quantitative trait loci (QTL) Chromosome segments containing or linked to the genes that underlie quantitative traits.

Introduction

Most economically relevant traits in livestock production systems are under genetic control, which implies that they can be genetically improved by exploiting the genetic variability within and between breeds. Significant genetic improvement rates have been reported in many characteristics such as growth performance, wool production, and milk composition. Less emphasis has been given in selection schemes in livestock species to those attributes which are related to carcass composition, meat quality in particular. This is partially explained by the difficulties and high costs of measuring them.

However, more attention has lately been given by breeders and geneticists to carcass and meat quality traits due to the stronger influence that consumer satisfaction has had on the supply chain in the past few decades; consequently, increased efforts are being directed toward the genetic improvement of carcass traits and meat quality.

Advances in molecular genetics are leading to valuable applications in the meat industries, such as providing accurate paternity tests or certifying the origin of specific products. The limitations of identifying superior genotypes for meat quality, due to the difficulties of collecting phenotypic data on these traits, may be overcome by molecular genetics. The very recent developments in structural and functional genomics, as novel and promising techniques, will provide a more comprehensive understanding of the genetics and metabolic paths, with direct application on genetic improvement. This article presents main concepts on deoxyribonucleic acid (DNA) markers and marker-assisted selection with emphasis on carcass and meat quality. The potential of the novel genomic tools and their implications for the genetic improvement of these traits are discussed.

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Role of Molecular Markers on Genetic Improvement of Carcass and Meat Quality Traits

Most of the relevant carcass and meat quality characteristics are quantitative traits whose phenotypic expression is the result of the joint action of several genes and environment. In general, the evaluation of the genetic merit of individuals and breeds is based on the analysis of phenotypic records plus pedigree information.

Although phenotypes are not perfect predictors of breeding values, conventional animal breeding methodologies have been effective in the genetic improvement of traits under selection. The limitation on obtaining phenotypic information of carcass and meat quality is a significant restriction for their genetic improvement, especially for meat quality. Modern *in vivo* noninvasive techniques allow the inclusion of carcass composition in the selection schemes using measurements on breeding animals, but the assessment or prediction of meat quality traits still relies on the implementation of siblings or progeny tests, which lead to longer generation intervals and, consequently, slower genetic progress.

The inclusion of the genetic information provided by molecular markers can make a significant contribution to the genetic improvement of carcass and meat quality traits. Estimations of genetic merit can be available for breeding animals at younger ages with levels of accuracies that were not possible before. Higher selection accuracies and shorter generation intervals will lead to higher rates of genetic improvement. In addition, it would be possible to consider in breeding programs some very difficult and expensive traits to measure, such as fatty acid composition or flavor. Furthermore, it will be possible to investigate the differential expression of genes in different muscles and different *in vivo* and postmortem phases, by the application of transcriptomics and proteomics. Based on the information provided by the transcriptome and the proteome, these new 'omics' give insight into the genes being expressed and the proteins influencing metabolic pathways, thus complementing the understanding achieved through genomics.

Markers and Quantitative Trait Loci

Genetic variability at DNA level can be directly assessed by using genetic markers. They are segments of DNA with a known position in the genome, which can be identified by laboratory tests (genotyping). By analyzing DNA samples that are very easy to extract from tissue samples, such as hair, blood, or meat, the variant (allele) of each genetic marker which an animal carries can be detected. Markers are of different types. In the recent past, markers called microsatellites were the ones of preference because they were very polymorphic (many alleles) and, therefore, highly informative. However, the genotyping of this type of marker is relatively expensive and difficult to standardize. Nowadays, single nucleotide polymorphisms (SNP) are extensively used. They are abundant and the genotyping shows low rate of errors at a low cost with current technologies.

In some cases, the marker is the gene of interest, or it is a fragment of DNA within the gene. If this is the case, knowledge of the marker variant directly indicates which the gene's variant (direct marker) is (**Figure 1**). However, in most cases, genetic markers are nonfunctional or neutral genes, which are linked to the gene of interest (indirect marker). Despite being nonfunctional, indirect genetic markers can provide valuable information not only for the identification of the target gene but also for selection on the trait of interest. Direct and indirect genetic markers can be used in genetic improvement schemes, although they require different strategies and lead to different responses to marker-assisted breeding, which will be discussed in the Section Identification of QTL and Genes.

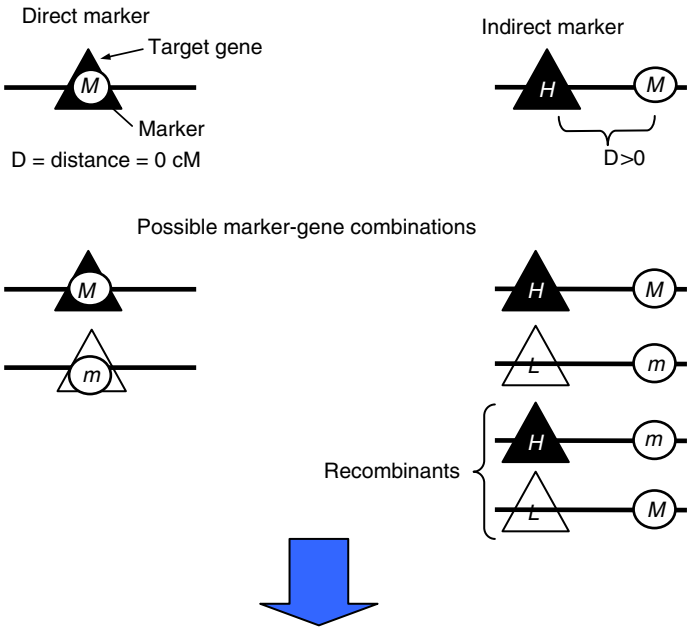
Genetic markers have become strategic tools for the identification of loci underlying the expression of quantitative traits, known as quantitative trait loci (QTL). Methodologies used in genetic evaluations are based on the assumption that quantitative characteristics are controlled by an infinite number of genes, each with infinitesimal (very small) effect. Nevertheless, genes or QTL with moderate effects have been identified. In general, important progress has been made in the identification and location of QTL affecting traits that are economically relevant for livestock production systems, including carcass and meat quality attributes. Information on QTL identified in cattle, sheep, pigs, and chickens are available on online databases with free access (AnimalQTLdatabases www.animalgenome.org/QTLdb). Other very useful public databases on genes and markers are also available (i.e., GenBank, www.ncbi.nlm.nih.gov/genbank; FunctSNP, www.csiro.au/science/FunctSNP).

QTL and Genes Affecting Carcass and Meat Quality

Quality is a complex concept that is a function of several traits that vary according to the species and target markets. **Table 1** presents some carcass and meat characteristics for which significant QTL have been identified, whereas **Table 2** summarizes significant findings for muscularity and tenderness, which are major carcass and meat quality traits, respectively.

Tenderness has been defined as one of the most important eating quality traits for consumer. Important efforts have been dedicated to unravel the genetic background of this attribute. The genes responsible for μ-calpain (CAPN1) and calpastatin (CAST) with effects on meat tenderness have been identified and SNP markers associated with them have been reported. The role of the calpain/calpastatin system on tenderness is described in other article. Although independent validations have confirmed the effect of these markers, their effects need to be validated in different commercial populations, because of differences in genetic frequencies in *Bos indicus* and *Bos taurus* breeds and the likely interaction between these genes.

Muscle development and shape in the carcass are relevant to define value in terms of actual meat yield or conformation and muscularity. The Myostatin (*GDF8*) gene has been associated with double-muscling phenotypes in some European breeds of cattle, such as Belgian Blue, Charolais, and Piedmontese, and with improved muscularity in sheep. The larger muscle development is due to an increased protein synthesis translated into a higher number of muscle fibers (hyperplasia). In the case of this gene, in most of the studies, neutral or favorable effects on meat tenderness were reported.



Marker genotype	Inferences on genotype with	
	Direct marker	Indirect marker (D=10 cM)
MM	HH ✓	HH ✓ (20.7%) HL × (4.1%) LL × (0.2%)
Mm	HL ✓	HH × (4.1%) HL ✓ (41.3%) LH × (0.4%) LL × (4.1%)
mm	LL ✓	HH × (0.2%) HL × (4.1%) LL ✓ (20.7%)
Total ✓	100%	83%

Figure 1 Direct and indirect genetic markers. When a direct marker is available for the gene of interest, the marker variants indicate precisely the allele of the target gene. The genotypes for the genes of interest can be inferred directly from the marker genotypes. In this example, the marker alleles M and m are associated with the high (H) and low (L) performance alleles, respectively. Therefore, an MM genotype in a direct marker indicates accurately that the animal is homozygous for the favorable allele (HH), whereas Mn and mm imply that the genotypes for the gene of interest are HL and LL, respectively. An indirect marker is located close to the gene, but it is not the gene itself. The distance D between marker and gene will determine the magnitude of the linkage. It is considered that M and H and m and L are linked. However, recombination events can lead to new combinations of marker and gene alleles (M–L and m–H). Owing to these possible combinations between marker and target alleles, it cannot be surely told that which marker allele is associated with the gene variants, and thus the confidence in the inference from marker genotypes is lower. Considering D = 10 cM, 83% of our inferences are correct, but in 17% cases, marker genotypes indicate the wrong allele is the gene of interest. Obtaining markers closer to the target genes will reduce this uncertainty because recombination rates are lower.

In sheep, the Callipyge gene (*CLPG*) has a pronounced effect on hindquarter muscularity. Characterization of Callipyge lambs indicates greater dressing percentages and heavier and leaner carcasses than in normal lambs. However, the gene also has a severe detrimental effect on tenderness of high-value muscles. The increased toughness of Callipyge meat has been explained by a reduced rate and extent of postmortem proteolysis that results from increased levels of calpastatin. Carwell is another gene in sheep that increases muscle development, but its effect is limited to the longissimus muscle. Although it may have a mild unfavorable effect on tenderness, this is not commercially relevant and can be removed by postmortem treatment.

Identification of QTL and Genes

The traditional approach used to map QTL in livestock species is termed as genome scan. In previous approaches, markers were selected to cover the whole genome with an average distance of 20 cM between them. An extensive list of major DNA-marker trials in farm livestock developed in the 1990s is presented in Table 3. The identification of QTL is based on the combined analysis of molecular and phenotypic information by searching for significant associations under specific experimental designs. One common design in farm animals was to map QTL segregating in crosses based on parental populations that are highly divergent for the traits of interest. Figure 2 shows the general concept that underlies the identification of QTL linked to genetic markers.

Although QTL experiments provided very valuable information, only few QTL with moderate or larger effect were

Table 1 Carcass and meat quality characteristics for which significant QTL have been found

	Beef cattle	*Pigs*
Carcass quality	Carcass weight	Carcass length
	Dressing percentage	Dressing percentage
	Predicted saleable beef yield	Proportion of lean
	Eye muscle area	Fatness
	Rump (P8) fat depth	Backfat
Meat quality	Marbling score	Ultimate pH
		Color
	Tenderness	Water-holding capacity
		Intramuscular fat

Table 2 Some major genes affecting carcass and meat quality

Trait	*Name*	*Specie*	*Chromosome*	*Locus*
Muscularity	Callipyge	Sheep	18	–
	Carwell	Sheep	18	–
	Myostatin	Cattle	2	GDF8
		Sheep		
Tenderness	μ-Calpain	Cattle	29	CAPN1
	Calpastatina	Cattle	7	CAST

Table 3 Genome scans searching for QTL affecting meat and/or carcass attributes

Species	*Population*	*Research group*	*Country*
Pigs[a]	Large White × Pietrain	Liège University	Belgium
	Meishan × Large white	INRA	France
	Pietrain × (Meishan or wild boar)	Hohenheim University	Germany
	Meishan × Large white	Agricultural University of Norway	Norway
	Landrace × Iberian breed	IRTA–INIA	Spain
	Wild boar × Large white	University of Uppsala	Sweden
	Meishan × Large white	Roslin Institute	United Kingdom
	Chinese breeds × Yorkshire	Iowa State University	USA
	Berkshire × Yorkshire	Iowa State University	USA
	Meishan × Large white	University of Minnesota	USA
	Meishan × Synthetic line	USDA	USA
Beef cattle[b]	Charolais × Brahman	Cooperative Research Centre	Australia
	Limousin × Jersey	AgResearch – Adelaide University	New Zealand – Australia
	Angus × Brahman	Texas A&M	USA
	(Brahman × Angus) × MARC III	US Meat Animal Research Centre	USA
	(Brahman × Herford) × MARC III	US Meat Animal Research Centre	USA
	(Piedmontese × Angus) × MARC III	US Meat Animal Research Centre	USA
	Belgian blue × Marc III	US Meat Animal Research Centre	USA
Sheep[c]	Texel × Coopworth	AgResearch, Sydney University, Adelaide University	Australia – New Zealand
	Awassi × Merino	Sydney University	Australia
	INRA401	INRA	France
	Fat and lean selection lines	AgResearch	New Zealand
	Texel, Suffolk, and Charollais commercial sire reference animals	Roslin Institute, Univeristy of Edinburgh, and Scottish Agicultural College	United Kingdom
	Scottish Blackface lean and fat selection lines	Roslin Institute	United Kingdom
	Rambouillet × Romanov	USDA Clay Centre	USA
	Suffolk × Romanov	USDA Clay Centre	USA

[a]Bidanel, J.P., Rothschild, M., 2002. Current status of quantitative trait locus mapping in pigs. Pig News and Information 23, 39N–53N.
[b]Burrow, H.M., Moore, S.S., Johnston, D.J., Barendse, W., Bindon, B.M., 2001. Quantitative and molecular genetic influences on properties of beef: A review. Australian Journal of Experimental Agriculture 41, 893–919.
[c]Crawford, A.M., 2001. A review of QTL experiments in sheep. Proceedings of the 14th Conference of the Association for the Advancement of Animal Breeding and Genetics, pp. 33–38. Queenstown: Association for the Advancement of Animal Breeding and Genetics.
Abbreviations: INRA, Institut National de la Recherche Agronomique; IRTA–INIA, Institut de Recerca i Tecnologia Agroalimentàries–Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria; USDA, United States Department of Agriculture.

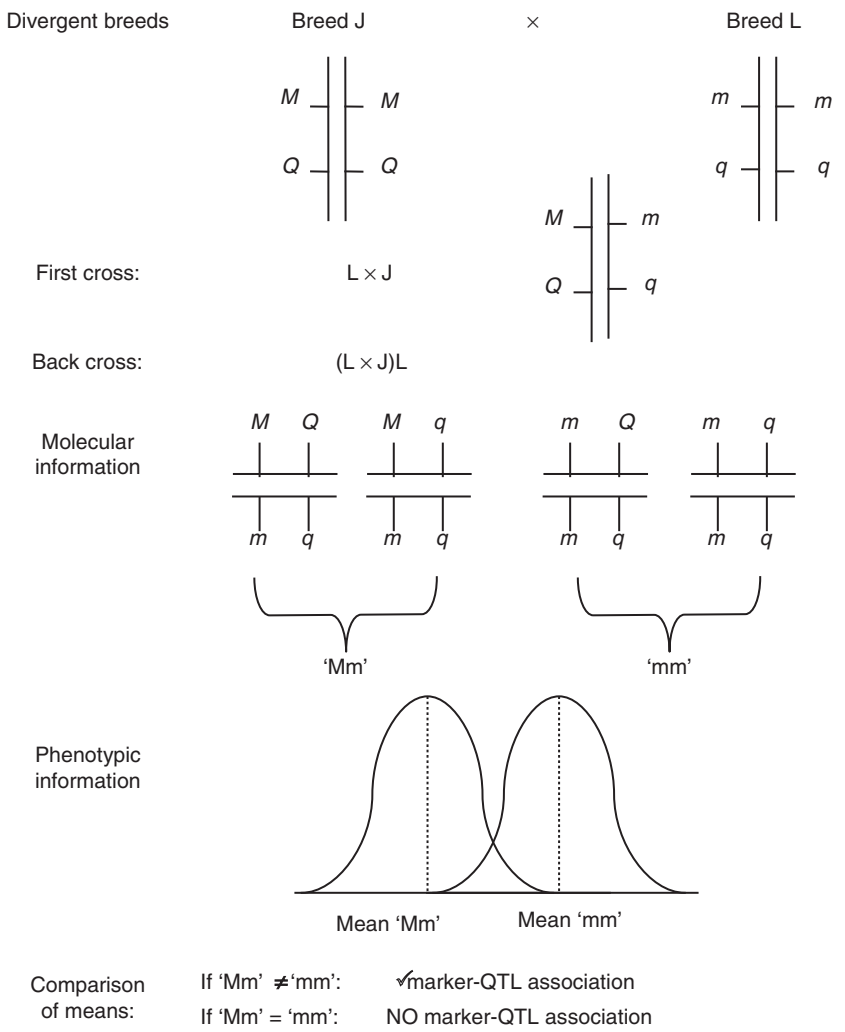


Figure 2 Searching for QTL–genetic marker associations in a backcross design between two breeds. Breeds L and J are two divergent breeds, which are assumed as homozygous for different alleles of both QTL (Q, q) and genetic marker (M, m). F1 animals are heterozygous for the marker and linked QTL. Backcrossed animals are obtained by mating F1 crosses to L individuals. There are four classes of gametes formed by the F1: the parental gametes MQ and mq and the recombinant gametes Mq and mQ. Because parental breeds are homozygous, L gametes are all mq. The resulting segregation in the backcross may allow the identification of the QTL. Genotyping provides the information of marker genotypes and phenotypic recording supplies data on the trait of interest. If the average of the Mm individuals is significantly different from the average of those with mm genotype, it is then concluded that the QTL affecting the trait of interest is linked to the genetic marker.

detected and mapped, given the power of the QTL experiments. In addition, further studies were needed before incorporating QTL into breeding programs to confirm that QTL mapped in crosses between divergent breeds or in a different breed were relevant to the genetic variation within the target breeding population. The size of the effects of an identified QTL is needed to be reestimated in commercial populations because they may differ between genetic backgrounds, environments, or production systems.

Fine mapping of QTL was used to reduce the broad chromosomal region derived from genome scans. Additional markers in the flanking region were tested in QTL linkage studies, thus improving the precision of QTL location. Furthermore, the fine-mapping approach has the potential of narrowing down the number of candidate genes and eventually identifying the causative polymorphism. A candidate gene is a known gene in another species that is related to the physiology underlying the trait of interest. After the candidate gene is chosen, the association between polymorphism/alleles for that gene and the phenotypic expression of the trait is investigated. It is important to mention that the assistance provided by genetic markers in terms of genetic improvement does not necessarily require the identification of the causative genes. Nevertheless, the detection of the gene and the development of direct marker tests were expected to lead to a more effective utilization of DNA technology in genetic improvement.

Only a few commercial DNA tests are available for economically relevant traits, although many of them are related to carcass or meat quality traits. Examples for beef tenderness are the DNA tests Pfizer GeneSTAR® and Ingenity TenderGENE®, which include the CAPN1 and CAST genes. In sheep,

LoinMAX® and MyoMAX® provide information for carcass quality based on the Carwell and Myostatin gene, respectively. In addition to these tests, there are new tools which can take into account simultaneously the information captured by hundreds or thousands of markers after their calibration using training populations. These genomic tools are explained later in this article.

Despite considerable efforts in QTL mapping and application of marker-assisted selection, the incorporation of DNA markers into the breeding programs has been low and the general impact poor. Under the QTL mapping approach previously described, marker densities were insufficient to find markers that were linked with QTL at the population level (population-wide linkage disequilibrium). Owing to the low marker densities, QTL of minor effect were not detected or their effects were overestimated, leading to inconsistent results across QTL mapping studies and reinforcing the relevance of carrying out independent validations studies before utilization in breeding programs.

Validation studies of commercial DNA tests for economically relevant traits showed that effects were not necessarily similar in all populations. On the basis of phenotypic data and DNA samples from reference cattle populations, a very interesting initiative was put in place by the US National Beef Cattle Evaluation Consortium (NBCEC) to independently verify the associations claimed by commercial genotyping companies. The results are in the public domain and can be found in the NBCEC web page.

Table 4 SNP Beadchips available for cattle, pigs, and sheep

Specie	*Product name*	*Number of SNP*	*Company*
Cattle	BovineSNP50K	54 001	Illumina
	Bovine3K	2 900	Illumina
	BovineLD	6 090	Illumina
	BovineHD	777 962	Illumina
	Axiom Genome–Wide BOS 1 Array	648 855	Affymetrix
Pigs	PorcineSNP60	62 163	Illumina
Sheep	OvineSNP50	54 241	Illumina

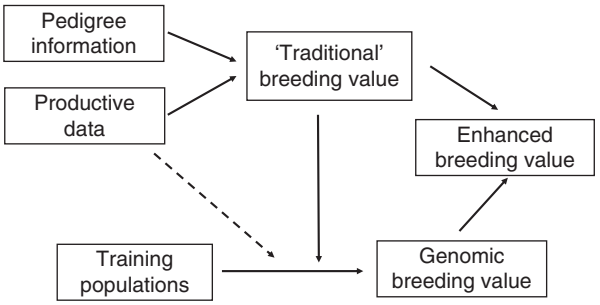


Figure 3 Combining genomic information in breeding programs. Breeding values are predicted on the basis of phenotypic (performance records) and pedigree data. The enhanced breeding values result from adding the genomic breeding values. The estimation of genomic breeding values relies on the genomic information of the animals and the SNP effects. The analysis of the genomic and phenotypic data of training populations provides the magnitude of the SNP effects.

Genomic Selection

Recent developments in DNA technology and genome sequencing have led to the detection of thousands of SNPs, making possible a very dense coverage of the genome, at affordable genotyping costs. Dense arrays of SNPs (SNP chip) have been developed for many livestock species that are commercially available (Table 4).

The availability of genome-wide dense markers has allowed the implementation of genomic selection in which the estimation of the genetic merit is assisted by the information provided by thousands of markers. Based on this methodology, and because of the high density of markers used, every QTL would be in population-wide linkage disequilibrium and all QTL affecting any trait would be considered simultaneously, independently of the magnitude of QTL effects.

The implementation of genomic selection can be defined in two steps. In the first step, the SNP effects are estimated in a training population that comprises animals with genomic data as well as with information on the relevant traits. The second step is to use the estimated effects of the SNP to predict the genetic merit of the breeding animals. Using genomic information, it is possible to estimate genetic merit just with the information obtained from a DNA sample, for very young animals. This information allows making earlier selection decisions that will reduce the generation interval. It may also increase the number of animals with information of their merit, which will have a positive effect on selection intensity.

In practice, genomic information is integrated to the genetic evaluation engines in addition to pedigree and phenotypic data, which are the 'traditional' sources of information to predict the genetic merits. The flow of information is illustrated in Figure 3. The higher accuracies in this case due to more precise relationships will also contribute to obtain higher rates of genetic progress.

Genetic evaluations including genomic information are already in place in many dairy cattle, pig, and poultry breeding programs. Regarding beef cattle breeds, the American Angus Association, using SNP data, is publishing estimations of genetic merit (www.angus.org/Nce/WeeklyEvalGenomicData.aspx), whereas other breeds such as Hereford are developing their training populations in the context of their international genetic evaluation program, which involves USA, Canada, Uruguay and Argentina Hereford data simultaneously (www.hereford.org/static/files/0711Genomics.pdf). Genomic breeding values are also now available in the sheep genetic programs run by Sheep Improvement Limited in New Zealand (www.sil.co.nz/News/Sheep50k-breeding-values-available.aspx).

In contrast with marker-assisted selection, genomic selection does not require a QTL detection step, as all markers, either significant or not, are considered. In fact, a one-step methodology to estimate enhanced breeding values (traditional plus genomic breeding values) is being implemented in some dairy cattle and in pig breeding programs. Nevertheless, the improvement of accuracy due to the genomic information can be enhanced by ensuring that markers linked to relevant QTL are present in the SNP chip being used.

Genome-Wide Association Studies and Functional Genomics

The information provided by the high density SNP chips enables not only the implementation of genomic selection but also genome-wide association studies that provide very valuable information for the identification of specific polymorphism with favorable effects on carcass and meat quality. Fine mapping, however, may not be sufficient to achieve this objective. A more comprehensive understanding is possible by combining the information provided by functional genomic tools to high-density genotyping or DNA genome sequencing data. Gene expression/transcriptomic profiling can give new insights to the actual SNP with influence on the traits of interest. The contribution of proteomics to meat quality is discussed in other article.

For both genomic selection and genome-wide association studies, the size of the training population and the quality of the data being recorded are of relevance. The volume of carcass and meat quality information with genomic data have a direct association with the accuracy of genomic breeding values and, therefore, on the additional genetic progress to be achieved. Similarly, the power of the genome-wide association studies will be stronger, and more accurate findings will be obtained. In this sense, the availability of accurate, less expensive, and time consuming methods to assess carcass and meat quality will make a significant contribution. New methods such as near-infrared spectroscopy and video-image analysis for the prediction of carcass and meat quality, respectively, will be useful sources of data to be collected directly in abattoirs in large number of animals. A comprehensive characterization of meat and carcass quality attributes using more expensive and laborious evaluation methods will be always very valuable, particularly as part of more detailed studies exploiting the potential of all new genomic tools.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Chemical and Physical Characteristics of Meat: Palatability. Classification of Carcasses: Beef Carcass Classification and Grading. Conversion of Muscle to Meat: Aging. Meat Marketing: Market Requirements and Specifications. Proteomic Technologies and Their Applications in the Meat Industry

Further Reading

Aguilar, I., Misztal, I., Johnson, D.L., *et al*., 2010. A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. Journal of Dairy Science 93, 743–752.

Barendse, W., 2009. Genetic-based diagnostic tools for predicting meat quality. In: Kerry, J.P., Ledward, D.A. (Eds.), Improving the Sensory and Nutritional Quality of Fresh Meat, first ed. Cambridge: CRC Press, pp. 292–317.

Barendse, W., 2011. Haplotype analysis improved evidence for candidate genes for intramuscular fat percentage from a genome wide association study of cattle. PLoS ONE 6, e29601. doi:10.1371/journal.pone.0029601.

Bishop, S.C., Karamichou, E., 2009. Genetic and genomic approaches to improving sheep meat quality. In: Kerry, J.P., Ledward, D.A. (Eds.), Improving the Sensory and Nutritional Quality of Fresh Meat, first ed. Cambridge: CRC Press, pp. 249–263.

Dekkers, J.C.M., 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. Journal of Animal Science 82 (E-supplement), E313–E328.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, fourth ed. Harlow, UK: Longman.

Hayes, B.J., 2007. QTL Mapping, MAS, and Genomic Selection. A Short-Course Organized by Animal Breeding & Genetics Department of Animal Science. Iowa State University. 4–8 June 2007. Available at: http://www.ans.iastate.edu/section/abg/shortcourse/notes.pdf (accessed 06.10.13).

Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819–1829.

Mullen, A.M., Pannier, L., Hamill, R., 2009. New insights into the biology of meat quality from genomic and proteomic perspectives, with particular emphasis on beef. In: Kerry, J.P., Ledward, D.A. (Eds.), Improving the Sensory and Nutritional Quality of Fresh Meat, first ed. Cambridge: CRC Press, pp. 199–224.

Rothschild, M.F., Hu, Z.L., Jiang, Z., 2007. Advances in QTL mapping in pigs. International Journal of Biological Science 3, 192–197.

Schefers, J.M., Weigel, K.A., 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. Animal Frontiers 2, 4–9. doi:10.2527/af.2011-0032.

Van Eenennaam, A.L., Li, J., Thallman, R.M., *et al*., 2007. Validation of commercial DNA tests for quantitative beef quality traits. Journal of Animal Science 85, 891–900.

Traditional Animal Breeding

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Glossary

Best linear unbiased prediction A statistical procedure for predicting the breeding values of animals.
Estimated breeding value (EBV) An estimate of an individual's true breeding value (genetic merit) for a trait, based on the performance of the individual and its relatives for the trait, and for genetically correlated traits.
Expected progeny difference It is EBV divided by two. The difference in expected performance of future progeny of an individual, compared with expected performance of future progeny of an individual of average genetic merit.
Heterosis Advantage of crossbred progeny over the average performance of the individual parent breeds.
Intermuscular fat It is defined as the fat deposited between muscles.
Intramuscular fat It is the fat deposited within muscles.
Subcutaneous fat It is defined as the fat deposited under the skin.

Introduction

Meat production can be influenced to a large extent by animal breeding and genetics. Several breeding strategies that can affect carcass composition and meat quality will be reviewed in this article, that include (1) selection between breeds within species; (2) crossbreeding to combine desirable characteristics from more than one breed or strain, or to exploit heterosis in crossbred progeny; and (3) genetic selection of superior breeding stock within a breed. Genetic influences on carcass and meat quality and selection programs designed to improve these traits will also be discussed.

Differences among Breeds

When selecting among breeds within a species, it is important to choose a breed that is able to perform well within the relevant environment as well as meet the appropriate market demands. Differences among breeds are only relevant in the environments in which they have been measured, because of possible genotype by environmental interactions. Most breed comparisons of carcass composition and meat quality have been performed in temperate climates, using animals on a high level of nutrition. Studies performed in more extreme environments (e.g., tropical or subtropical), or when food is less abundant, have been fewer in number and have shown less convincing evidence of breed differences. It is also of note that any breed comparison is a snapshot in time, as breeds evolve as a result of selection, so results from breed comparisons may change over time.

Carcass Composition

Large between-breed differences exist within all farm animal species for growth and carcass composition traits. As an animal matures, it undergoes an increase in the ratio of muscle to bone, followed by a decrease in muscle growth rate and an increase in the ratio of fat to muscle. However, breeds vary in their rate of maturation and average mature weight. Therefore, standardizing measurements of body composition (proportions of muscle, fat, and bone) to the same stage of maturity of body weight (ratio of actual weight to expected mature weight) results in much less variation in carcass composition than standardizing to the same age or weight. One exception to this rule is the Texel breed of sheep, which shows less total body fat than expected for its mature size (Table 1).

In beef cattle, late-maturing breeds, such as the Continental European breeds, are often preferred under conditions of good nutrition, producing heavier carcasses with little fat. Early-maturing beef breeds, such as the traditional British breeds (e.g., Angus, Hereford, and Shorthorn), can be harvested at lighter weights and may be preferred for grass-based systems, when food supply is limited or for certain markets such as those rewarding higher intramuscular fat. Similarly, in lamb production systems, the use of early-maturing breeds (e.g., Southdown) will allow faster finishing of small lambs with good carcass composition. However, the use of larger breeds that mature later (e.g., modern Suffolk strains) will result in heavier lambs with less fat. Traditionally, early-maturing pig breeds (e.g., Middle White) were used for pork production and late-maturing breeds (e.g., Large White) for bacon production. Strains and hybrids of improved pig breeds that are now used in pork and bacon production (e.g., Piétrain, Landrace,

Table 1 Heritability ranges for carcass composition traits across species

Trait	*Heritability*[a]
Ultrasound muscle depth/area	Moderate–high
Ultrasound fat depth	Moderate–high
Carcass weight	Moderate–high
Carcass length	High
Dressing percentage	Low–moderate
Lean yield	Moderate–high
Lean:bone ratio	Moderate–high

[a]Low = 0–0.25; moderate = 0.25–0.5; and high = 0.5–1.

Hampshire, and Large White) have better carcass composition than that of traditional British pig breeds (e.g., Tamworth, Gloucester Old Spot, and Saddleback), owing to reduced fat levels and increased muscle percentage.

Breeds may partition fat and muscle differently between body depots. Dairy breeds of sheep and cattle have a higher proportion of body fat in internal depots than meat breeds, which have higher proportions of subcutaneous fat. In general, maternal sheep breeds that have higher reproductive rates and higher levels of milk production also have increased proportions of noncarcass fat. During growth and development, intermuscular fat is deposited before subcutaneous fat, which is deposited before intramuscular fat. Therefore, relative to subcutaneous fat, large late-maturing cattle breeds have a higher proportion of intermuscular fat than small early-maturing breeds, which have increased levels of intramuscular fat (e.g., British beef breeds vs. Continental European breeds). Breed comparisons in pigs have found that the Duroc, Meishan, and Berkshire breeds have a high proportion of intramuscular fat compared with other improved breeds, and for some markets, the level of intramuscular fat in pure Duroc and Berkshire pigs is too high for consumer acceptability.

Meat Quality

In addition to yield and carcass composition, meat quality is determined by traits such as color and composition of muscle and fat, level of intramuscular fat, juiciness, tenderness and texture, and flavor and aroma. Objective laboratory-based techniques that are discussed in other articles have been developed to quantify many of these traits. These measurements are often referred to as technological traits and some examples are listed in Table 2. The most widely used of these techniques is 'shear force,' which measures the force required to cut through samples of cooked meat. Trained sensory panel analysis is still considered the most relevant measure for many meat quality traits and Table 2 also gives examples of some of these sensory traits. There is substantial evidence of between-breed variation in technological and sensory meat quality traits. Examples include the following:

- Paler, more watery muscle, with more exudation of fluids during storage, in 'improved' pig breeds compared with traditional British pig breeds (e.g., Pietrain vs. Bekshire).
- Yellower fat in Channel Island cattle breeds compared with other cattle breeds.
- More tender, fine-grained meat in smaller breeds of cattle, owing to smaller muscle bundles.
- More tender meat in 'double-muscled' Piedmontese cattle compared with some other breeds.
- More tender meat in *Bos taurus* cattle breeds than in *Bos indicus* (humped cattle) breeds. *Bos indicus* cattle show increased calpastatin activity in the muscle, which is known to inhibit postmortem tenderization.
- More tender meat in Duroc pigs compared with most other breeds, owing to an increased amount of red muscle fibres and increased intramuscular fat.
- Increased flavor, juiciness, and tenderness in pigs with an increased percentage of Duroc, Berkshire, or Meishan genes.

Table 2 Heritability ranges for meat quality traits across species

Trait	*Heritability*[a]	
	Technological (objective)	*Sensory (subjective)*
Color		
Lean color		Low–moderate
Fat color		Low
Lean color reflectance	Low–moderate	
Myoglobin content	Moderate–high	
Juiciness		Low
Water-holding capacity	Low	
Drip loss	Low–moderate	
Tenderness		Low–moderate
Lean texture/firmness		Low–moderate
Shear force	Moderate–high	
Calpastatin activity	Moderate–high	
Myofibrillar fragmentation index	Moderate–high	
Intramuscular fat content		
Marbling score	Moderate–high	
Chemical intramuscular fat %	Moderate–high	
Ultimate pH	Low–moderate	
Flavor		Low
Overall acceptability		Low

[a]Low = 0–0.25; moderate = 0.25–0.5; and high = 0.5–1.

Breeds can also react differently to on-farm management, transport, preslaughter, slaughter, and processing methods. For example, leaner and lighter animals are more likely to suffer from cold shortening in the carcass post-mortem.

Crossbreeding

Crossbreeding can be used for several reasons that can be exploited simultaneously. One reason may be to combine desirable characteristics from more than one breed or strain. This is termed 'complementarity.' In the pig and poultry industries, different breeds or specialized lines, selected for different characteristics, are commonly crossed to produce commercial hybrids. Sire lines are often selected to be heavier and faster growing, whereas female lines may be selected for reproductive traits, low maintenance requirements, or other economically important factors. An example of such a system for pigs is given in Figure 1. The breeds or strains chosen for crossing depend on their suitability for specific environments as well as market requirements. Crosses between different breeds of sheep and cattle are also used to combine desired characteristics. In the United Kingdom, hill ewes (e.g., Scottish Blackface and Swaledale) are often crossed to rams from upland breeds (e.g., Blue-faced Leicester and Border Leicester) with high maternal performance. The crossbred female progeny are then mated to terminal sire breeds (e.g., Texel, Suffolk, and Down breeds) that have been selected for improved carcass and growth traits, to produce a high number of better-quality lambs.

Another reason for crossbreeding is to exploit heterosis, or hybrid vigor (advantage over the average performance of the

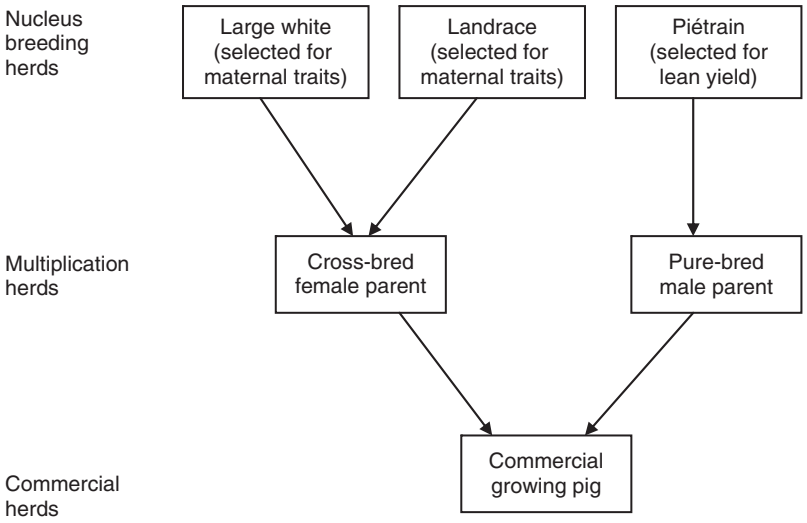

Figure 1 Example of a pig breeding program.

individual parent breeds). Heterosis is widely exploited in the pig, beef cattle, and poultry industries. The 'combining ability' of different strains is tested to increase production. Some strains combine well only when used as the male or the female parent. The pure-bred lines that are used for crossing can be either selected to improve specific traits within line (e.g., meat yield and fertility) or selected on the performance of their crossbred progeny (reciprocal recurrent selection). Heterosis is usually greater for traits with low heritability (see Section Genetic Parameters for Carcass Composition and Meat Quality Traits), such as those affecting overall fitness, reproduction, or survival, and less for production or carcass traits. Some evidence suggests that cross-bred cattle mature more quickly and are thus heavier at a given age, with more marbling fat, total fat, and muscle. However, after adjusting for weight, heterosis for carcass composition traits tends to be low. Effects of heterosis on most technological and sensory meat quality traits have not been studied widely, but available estimates tend to be low for juiciness, tenderness, flavor, cooked color, and overall desirability. Results for the effect of heterosis on shear force in beef are more widely reported and range from slightly unfavorable to moderately favorable.

Differences within Breeds

Substantial genetic improvement can be made within breeds for desired characteristics. This method relies on the fact that the traits to be improved are heritable, and that more animals are produced than need to be kept for replacement stock, to allow selection among progeny for preferred breeding animals.

Genetic Parameters for Carcass Composition and Meat Quality Traits

Heritability is the proportion of the total phenotypic (observed) variation in a trait that is explained by genetic variation. It is, therefore, a measure of how much a trait is controlled by genes (or, more precisely, genes that act additively), as opposed to environmental influences. Heritability is expressed on a scale of 0 to 1, where a value of 1 suggests that the trait is completely controlled by an animal's genes, and management, feeding, and other environmental factors play no part in determining the expression of the trait. Traits with higher heritability allow a higher rate of genetic improvement than traits with low heritability. Estimates of heritabilities for the main carcass and meat quality traits vary between studies but tend to fall in the ranges identified in Tables 1 and 2. There is a good agreement across species for heritability estimates of these traits. Relatively few studies have been conducted on genetic parameters of eating quality traits compared with carcass composition. However, in general, the heritability of most carcass composition traits is moderately high, and objective technological measures of meat quality are more heritable than sensory traits determined by sensory panel analysis. Selection responses in sensory meat quality traits are, therefore, expected to be low because they are less heritable and more difficult to evaluate.

It is important to note that the magnitude of the environmental variation and, therefore, the heritability depends very much on the ability to standardize measurements. The better the meat quality traits are measured, the higher their heritability and the higher the selection success. A low heritability does not necessarily mean that there is too little genetic variance for selection; rather, it may reflect the difficulties in measuring the traits in a standardized, repeatable, and accurate manner. Genetic parameters for meat quality may also differ according to the type of muscle tested within an animal and owing to differences in preslaughter conditions (e.g., stress during transport or at the abattoir) or processing methods (e.g., electrical stimulation, conditioning, and hanging method). For example, shear force has been reported to be more variable in carcasses that have not undergone electrical stimulation. In particular, genetic parameters for meat tenderness vary, probably because this is a very complex trait, depending on many factors (pH and temperature changes postmortem, glycolysis, and processing) and the interactions among them.

Selection on one trait will often lead to correlated responses in other traits, which are not always monitored but could have very important economic consequences. A selection program designed to improve carcass composition should also be concerned with the effects of these changes on sensory meat quality traits and should monitor effects on reproductive traits (e.g., fertility and dystocia) and functional fitness. There are concerns that selection for reduced fat levels may be associated with reduced fertility of females and it may delay puberty, as observed, for example, in pigs and cattle. Genetic improvements in pig and poultry production have also been related to a decrease in meat quality in terms of flavor and texture. In general, intramuscular fat percentage has a negative genetic relationship with meat yield and a positive genetic relationship with total fat. Intramuscular fat in pigs was ignored in early studies, as its importance for eating quality was underestimated. As pigs were selected for reduced back fat, the level of intramuscular fat was also reduced, which was later linked to a reported decline in the eating quality of pig meat. Attempts are now under way in some countries to increase intramuscular fat in pig meat without increasing total fat. Studies in some sheep and cattle populations suggest that this may be possible, as low to moderate genetic correlations have been found between levels of fat in different depots. There is also evidence in pigs that different fat depots are at least partially under different genetic control. This may allow selection in these species to reduce one fat depot (e.g., subcutaneous), whereas maintaining moderate levels of fat in other depots, to reduce or avoid the unintended consequences mentioned, or to maintain marbling and meat quality (intramuscular fat).

Correlations among carcass composition and technological and sensory meat quality traits differ among studies, as a result of which few strong trends have emerged. In general, the literature suggests that selecting for leanness might have slight negative effects on eating quality traits (e.g., water-holding capacity, tenderness, juiciness, pH, and drip loss). Sensory quality traits such as tenderness, flavor intensity, and juiciness tend to be positively correlated to one another, and genetic correlations between these traits and shear force tend to be negative (lower shear force equals more tender). Shear force also has a low to moderate negative genetic correlation with intramuscular fat. Tenderness is widely thought to be the most important determinant of meat quality to consumers. However, genetic parameters for this trait differ, depending on the muscle tested and the method of measurement (myofibrillar fragmentation index, calpastatin activity at 24 h, shear force, or sensory panel assessment of tenderness). The majority of results suggest that calpastatin activity is highly genetically correlated with shear force (higher calpastatin activity = higher shear force), but the phenotypic correlation between the two measurements is only moderate. Correlations of tenderness among different muscles are moderately low and the correlation between shear force and sensory panel evaluation of tenderness also varies between muscles. As a result, selection for tenderness may be difficult and further work is needed to determine the relationships between technological and sensory measurements. (At least in beef, and the research at K-State, the correlation between trained sensory panel tenderness and Warner-Bratzler shear force (WBSF) is high and negative.)

(There are animals that defy the antagonisms between carcass composition and meat quality. For example, the expected progeny differences (EPDs) of an American Simmental bull at ABS global (a company specialising in bovine genetics and reproduction), which has a global rank in the top 5% for marbling, in the top 5% for longissimus muscle area, and in the top 5% for WBSF. In other words, using this bull can simultaneously increase muscling, increase marbling, and decrease WBSF in progeny.)

Selection Programs

In genetic improvement programs, animals are selected on their own performance, on the performance of their relatives, or on a combination of both. First, the breeding goal or goals – i.e., the traits to be improved – must be decided and subsequently the selection criteria determined. The latter are the measurements that will be taken and then selected on in order to improve the breeding goal. In some cases, the breeding goals and the selection criteria are the same, but often they are not, especially with carcass and meat quality traits. Traits to be used as selection criteria must be highly repeatable and practical to measure on-farm or online during animal processing. Selection criteria should be heritable and should show sufficient variation within the population. The design of the breeding program should define the number of male and female animals that are to be selected each year, the age at mating, the generation interval, and other such factors.

Genetic selection programs differ in complexity. A relatively simple approach is to select breeding animals on the basis of their own phenotypic performance (e.g., ultrasound data obtained from selection candidates). However, the impossibility of collecting actual phenotypic carcass and meat quality data on selection candidates has restricted the use of this method of selection for many of these traits. 'Independent culling levels' in one or more traits are often used to improve the genetic merit of the flock or herd. This method involves choosing animals for breeding only if they reach a certain threshold in each trait of interest (e.g., over a certain weaning weight and/or below a certain ultrasound fat depth).

Using a selection index is a more complicated, but effective, method of selecting animals on more than one trait, based on the performance of the individual and its relatives. This method allows selection of traits that can be measured directly or indirectly (using predictor traits) on the selection candidates themselves and also traits that can only be measured on relatives (e.g., slaughter and meat quality traits). Accurate recording of performance data and pedigree structure is vital in these programs. The amount of emphasis or weighting on each trait in a multitrait index can be altered and is usually determined by economic importance. Estimated breeding values (EBVs) for each trait for each animal are produced, and then are combined into an overall 'index score.' Selection decisions are based on these index scores. Response to selection in any individual trait per generation using a multitrait index is smaller than what could be achieved by selecting for that trait alone. However, index selection will lead to the highest rate of change in overall economic merit.

EBVs are calculated, in most selection programs, using a statistical procedure known as best linear unbiased prediction (BLUP). This procedure predicts the genetic effects for each trait separately from management and environmental influences. The EBV is determined by the genetic merit of the animal itself, plus that of its relatives, for the trait of interest and reflects the genetic or breeding merit of that animal compared with the population mean. Because, on average, each breeding animal passes half of its genes to its offspring, the breeding value for each trait is often expressed as the expected progeny difference (EPD), which is half the EBV of the breeding animal.

If the breeding goal of a selection program is to improve carcass composition, the selection criteria will often include predictors of composition taken on the live animal. As live weight is a composite trait (meat, fat, and bone), it is usually not a sufficient predictor of carcass composition. However, carcass composition can be estimated *in vivo* using techniques such as mechanical and optical probes, ultrasound scanning, or computed tomography (CT) scanning. Ultrasound is commonly used in selection programs for sheep, cattle, and pigs to measure depths and areas of subcutaneous fat and muscle (Figure 2) and greatly improves the predictions of body composition above those estimated from live weight alone. In cattle and pigs, intramuscular fat has also been estimated using ultrasound measurements taken on live animals. CT scanning increases the accuracy of predictions of total carcass fat, muscle, and bone compared with ultrasound and allows the measurement of tissues in different body depots and regions (Figure 3). Measurements of average muscle density resulting from CT scanning can also provide moderate predictions of intramuscular fat levels in live sheep and pigs. Two-stage selection can be carried out in sheep and pig populations, where ultrasound scanning is used on-farm to screen large numbers of selection candidates, and then a small number of top-ranking animals are CT scanned to make final selection decisions based on conformation or composition of breeding stock.

Because there are few live-animal predictors of technological and sensory meat quality traits, breeding programs designed to improve meat quality use mainly measurements taken on slaughtered relatives of selection candidates to calculate EPDs (or EBVs depending on the scale used) for these traits. For example, most cattle breed associations in the United States now produce EPDs for marbling and a few have published EPDs for tenderness measured by shear force. Similar traits are included in some cattle breeding programs in the UK and Australia.

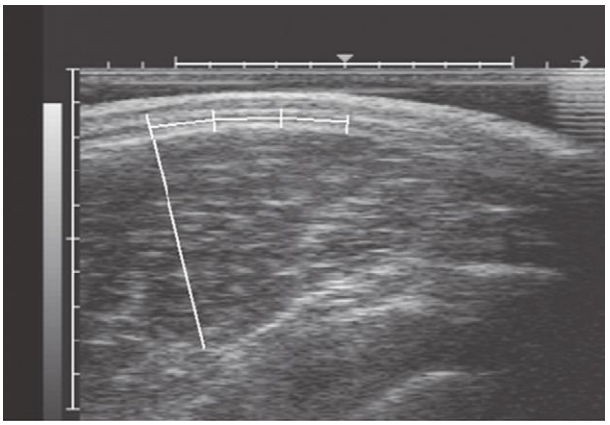

Figure 2 Example of an ultrasound scan taken in beef cattle.

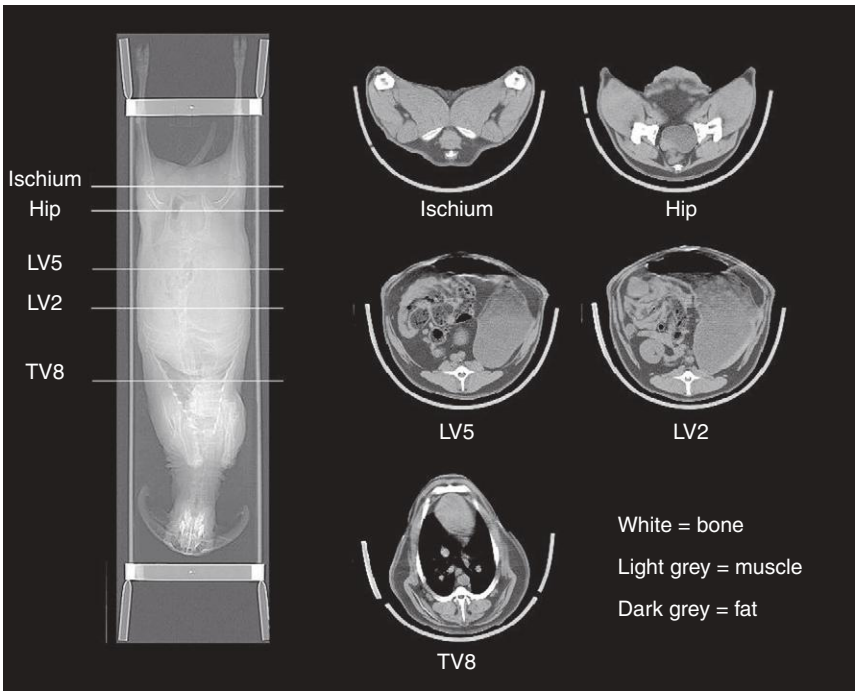

Figure 3 Example of CT scan images from a sheep. LV, lumba vertebra; TV, thoracic vertebra.

In many countries, a relatively low proportion of beef cattle and sheep are performance recorded and, therefore, are included in genetic improvement programs. In these industries, there are many small-scale breeders and although abattoirs usually provide some financial incentives to improve conformation and reduce fat levels, few incentives are given to improve other aspects of eating quality. However, a few countries are now trying to implement grading systems that also reward for improved meat quality traits such as marbling levels, pH, and color (e.g., Meat Standards Australia). There has been considerable genetic progress in lamb and beef carcass composition due to selection programs. In several countries (e.g., United States, Canada, and Europe), 'central testing' has been used to identify sheep or cattle of superior genetic merit, where high-ranking individuals from different farms are tested together at a central station to reduce environmental variation and allow the measurement of 'difficult to measure' traits. There is some concern over the effectiveness of this approach, especially if animals are submitted at later ages, and the use of this method seems now to have decreased. Its importance could, however, increase if genome-wide selection methods are employed. 'Progeny testing' can also identify superior breeding animals by recording data on progeny of high-ranking animals, either at a central testing station or on-farm. This method allows carcass and meat quality traits to be measured directly on progeny of breeding stock. However, central and progeny testing are time consuming and expensive and are only likely to be used to select sires for use in widespread artificial insemination programs. Group breeding and sire reference schemes are now being used by sheep and beef breeders in several countries. Group breeding schemes usually involve a nucleus breeding flock or herd of elite animals taken

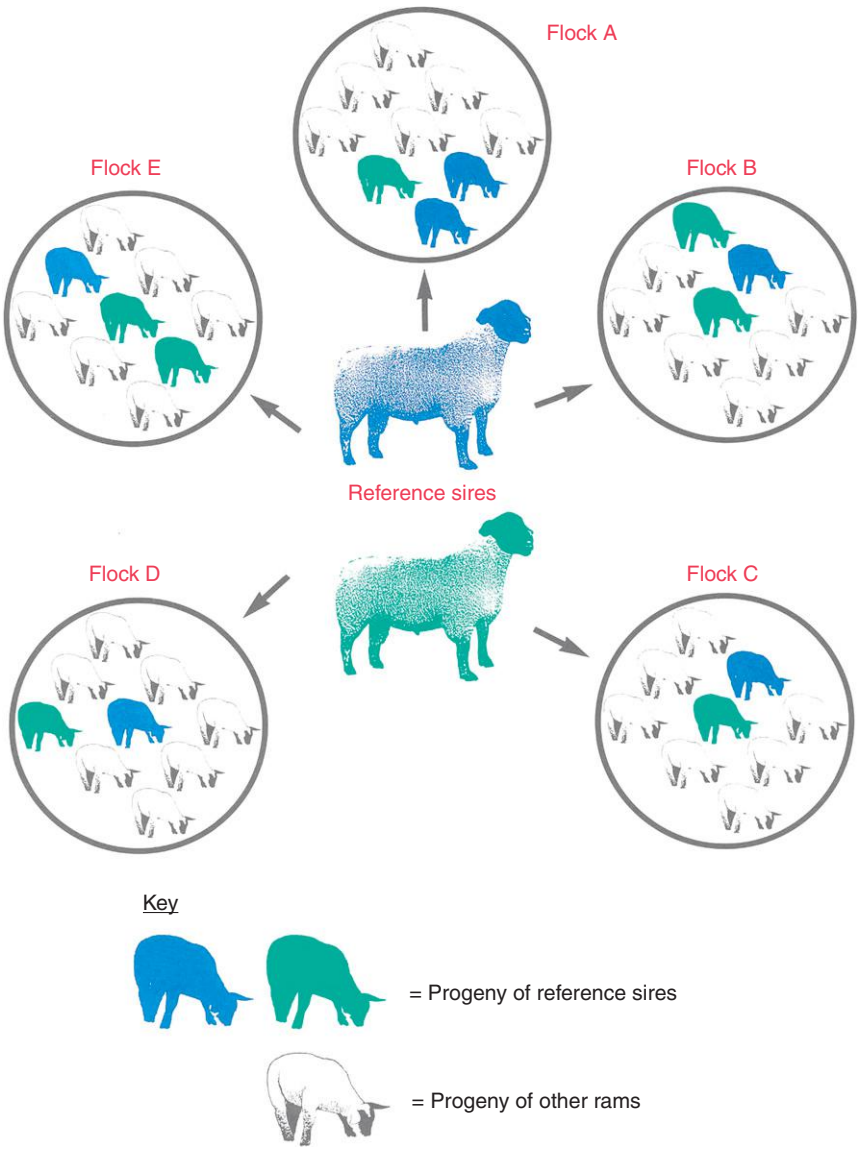

Figure 4 Schematic diagram of a sire referencing scheme (see plate 1). Reproduced from Simm, G., Wray, N.R., 1991. Sheep Sire Referencing Schemes – New Opportunities for Pedigree Breeders and Lamb Producers. SAC Technical Note T264. Edinburgh: SAC.

from different group member farms. This nucleus undergoes intensive recording and selection in order to produce breeding animals (usually males) of high genetic merit so that they can be used on breeders' farms. More popular now are sire reference schemes, in which all flocks or herds are linked by the use of common sires on a proportion of females on each farm (Figure 4). These schemes use BLUP on data from all farms to produce EPDs (or EBVs) that are comparable across all member flocks or herds.

Breeding populations in pig and poultry production are controlled mainly by relatively few large national or international breeding companies. In these industries, 'production pyramids' exist, where intensive selection takes place in the elite breeding herds or flocks. The resulting animals, of superior genetic merit, are multiplied in number and usually crossed to produce commercial animals for meat production (Figure 1). All tiers of the industry are therefore influenced by improved genetics in the top breeding herds or flocks. High selection intensities, short generation intervals, and reduced environmental influences on production in these species maximize the output of high-quality product. As a result of this structure, there have been industry-wide improvements in growth rate, uniformity, muscle yield, feed conversion efficiency, and fat levels in both pigs and poultry.

Major Genes

Most production traits are continuous in their distribution and are controlled by the action of many genes, each having a small effect. These are termed polygenic or quantitative traits. However, some traits are substantially influenced by a single major gene or quantitative trait locus. Some major genes are known to have large effects on carcass composition and meat quality traits in the populations in which they are found. Examples are given in Table 3.

Phenotypic records from relatives can be monitored to detect the presence of major genes and identify individuals and families with the desired genotypes. The use of molecular techniques to identify animals with different genotypes will allow much greater exploitation of these major genes or other genes with smaller, but important, effects. More advanced molecular and reproductive techniques, such as cloning and genetic modification of livestock species, may also play important roles in the meat industry in future.

Future Considerations

Traditionally, the aim of selection was to increase production efficiency and lean yield in farm animals raised for meat production. However, recent consumer preferences for healthy and convenient meat products produced in welfare-friendly systems call for different breeding goals and selection traits. Future selection objectives are likely to incorporate more meat quality issues. Genetic variation has been blamed for an inconsistent product. However, genetic variation provides the opportunity to increase meat quality within livestock populations. The potential to improve meat quality by traditional breeding methods would be greatly increased by the

Table 3 Examples of major genes affecting carcass composition and meat quality

Major gene/Quantitative trait locus (QTL)	*Species: Breed*	*Effects on carcass and meat quality*
Dwarfism	Poultry	*Reduces*: growth rate; mature weight
Myostatin gene ('double muscling')	Cattle: Belgian Blue, Piedmontese, and other breeds Sheep: Texel and other breeds	*Reduces*: subcutaneous fat; marbling; collagen content; lean color intensity; and flavor. *Increases*: muscularity; muscle:bone ratio; muscle:fat ratio; eye muscle area; dressing percentage; and water content
Diacylglycerol acyltransferase 1 (*DGAT1*)	Cattle Sheep	Affects intramuscular fat; marbling; and tenderness (sheep)
Calpain gene (*CAPN1*)	Cattle	Affects tenderness
Calpastatin gene (*CAST*)	Cattle	Affects tenderness
Callipyge	Sheep: Poll Dorset originally	*Reduces*: carcass fat; marbling; and tenderness. *Increases*: muscularity; lean yield; dressing percentage; and connective tissue content
Carwell (Loin-Max)	Sheep: Poll Dorset originally	*Increases*: lean yield; eye muscle depth. *Reduces*: tenderness slightly, but this effect can be eliminated by enhanced processing
Texel Muscling QTL (TM-QTL)	Sheep: Texel	*Increases*: loin muscling. *Reduces*: tenderness slightly, but this effect can be eliminated by enhanced processing
Ryanodine receptor 1, 'halothane gene' (RYR1)	Pigs	*Reduces*: ultimate pH; water-holding capacity. *Increases*: pale, soft, exudative (PSE) meat; meat quality variation
PRKAG3 (new alleles of the 'RN'gene)	Pigs: Hampshire	*Reduces*: processed meat yield; ultimate pH; lean color intensity. *Increases*: drip loss; variation in meat quality
Fatty acid-binding protein (*FABP*) genes	Pigs: Meishan originally Poultry	Affects intramuscular fat and fat deposition
IGF2 gene	Pigs Cattle	Affects weight of primal cuts, lean meat yield, backfat thickness, and eye muscle area

development of tools to measure or predict meat quality *in vivo*. The incorporation of such measures into large-scale, organized breeding programs would allow direct selection for meat quality traits.

See also: Chemical and Physical Characteristics of Meat: Adipose Tissue; Color and Pigment; Palatability; pH Measurement; Water-Holding Capacity. Species of Meat Animals: Meat Animals, Origin and Domestication

Further Reading

Burrow, H.M., Moore, S.S., Johnston, D.J., Barendse, W., Bindon, B.M., 2001. Quantitative and molecular genetic influences on properties of beef: A review. Australian Journal of Experimental Agriculture 41, 893–919.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, fourth ed. Harlow: Longman.

Lawrie, R.A., 1998. Lawrie's Meat Science, sixth ed. Cambridge: Woodhead Publishing.

Macfarlane, J.M., Simm, G., 2008. The contribution of genetic improvement for lamb meat production. Proceedings of the 3rd International Symposium about Goat and Sheep Meat Type – 3rd Sincorte, Tecnol. & Ciên. Agropec., João Pessoa, v.2, n.3, pp. 7–14.

Marshall, D.M., 1999. Genetics of meat quality. In: Fries, R., Ruvinsky, A. (Eds.), The Genetics of Cattle. Wallingford: CAB International, pp. 605–636.

Sellier, P., 1998. Genetics of meat and carcass traits. In: Rothschild, M.F., Ruvinsky, A. (Eds.), The Genetics of the Pig. Wallingford: CAB International, pp. 464–495.

Simm, G., 1998. Genetic Improvement of Cattle and Sheep. Tonbridge: Farming Press.

Simm, G., Wray, N.R., 1991. Sheep Sire Referencing Schemes – New Opportunities for Pedigree Breeders and Lamb Producers. SAC technical note T264. Edinburgh: SAC.

Thompson, J.M., Ball, A.J., 1997. Genetics of meat quality. In: Piper, L., Ruvinsky, A. (Eds.), The Genetics of Sheep. Wallingford: CAB International, pp. 523–538.

de Vries, A.G., Faucitano, L., Sosnicki, A., Plastow, G.S., 2000. Influence of genetics on pork quality. In: Wenk C., Fernández J.A., Dupuis M. (Eds.) Quality of Meat and Fat in Pigs as Affected by Genetics and Nutrition, pp. 27–35. Proceedings of the joint session of the EAAP commissions on pig production, animal genetics and animal nutrition, Zurich, August 1999. The Netherlands: Wageningen Academic publishers.

Wilson, D.E., 1992. Application of ultrasound for genetic improvement. Journal of Animal Science 70, 973–983.

ANIMAL HEALTH RISK ANALYSIS

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Glossary

Hazard identification A process of identifying pathogenic agents that might be associated with the commodity considered in a risk analysis.
Risk A function of the likelihood of an event occurring and the likely magnitude of the biological and economic consequences of that event.
Risk analysis A science-based process composed of hazard identification, risk assessment, risk management, and risk communication.
Risk assessment A process to evaluate the likelihood and biological and economic consequences due to the entry, establishment, and spread of a pathogen in an importing country.
Risk communication The exchange of information, opinions, and results with potentially affected and interested parties during a risk analysis.
Risk management A process to identify, select, and implement measures to reduce identified risks.
Sanitary measures Measures applied to imported goods that protect animal or human health or life in the importing country from risks associated with the entry, establishment, or spread of a pathogen.
Uncertainty A lack of knowledge about a parameter being assessed. Uncertainty can often be reduced by further studies or surveys.
Variability The effect of natural chance on a parameter. Unlike uncertainty, variability cannot be reduced by further studies or surveys.

Introduction

The international trade in animals and animal products presents a degree of risk to the importing country because of the possible presence in the commodity of pathogens that might threaten the resources (human, animal, or environmental) of the importing country, although this risk should not be used as an unjustifiable barrier to trade.

Animal health import risk analysis is a tool that provides an objective and defensible method of identifying and managing disease risks associated with the importation of animals, animal products, animal genetic material, feedstuffs, biological products, and pathological material.

Risk is defined as a function of the likelihood of a disease entering a country and the likely consequences of the disease affecting other animals. Import risk analysis provides a systematic approach to the identification of hazards (pathogens) that might be associated with an imported commodity, assessment of the likelihood and consequences of introducing diseases, formulating sanitary measures to manage this risk, and communicating the findings to others.

Transparency

Although import risk analysis is based on science, the process must accommodate knowledge gaps and uncertainty. Because of this, transparency (the comprehensive documentation of all data, assumptions, methods, results, discussion, and conclusions) is essential. Without transparency, the distinction between facts and the analyst's value judgments might blur.

Uncertainty and Variability

Incomplete knowledge or a lack of understanding of a pathogen will result in uncertainty, which is likely to be present in any risk analysis. This must be distinguished from the natural heterogeneity inherent in any biological system that will result in variability in a risk pathway.

If significant uncertainty is encountered when conducting a risk analysis, a precautionary approach to managing risk may be considered. However, sanitary measures selected must be based on a risk analysis that takes into account available scientific information and any precautionary measures should be reviewed as soon as additional information becomes available. A transparent rationale should always be presented to support selected sanitary measures.

Qualitative and Quantitative Methods

Qualitative risk assessments express likelihood estimates in nonnumerical terms such as high, medium, low, or negligible. This approach is suitable for the majority of risk assessments, and is routinely used for decision making. In some situations, a

quantitative approach might be considered desirable as an adjunct to a qualitative assessment. Quantitative assessments express their inputs and outputs (results) numerically, but are not in themselves any more objective or precise than a qualitative approach. Quantitative models also present significant challenges in interpreting and communicating their results. Regardless of which approach is chosen, it is essential that the analysis is transparently documented and subjected to peer review.

International Obligations

Under the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), members can employ sanitary measures to imported commodities but these measures must not be applied arbitrarily, or result in discrimination between members where similar conditions prevail, or constitute a disguised restriction on trade.

The SPS Agreement requires WTO members to base their sanitary measures on international standards, guidelines, and recommendations, where they exist. The SPS Agreement recognizes the World Organisation for Animal Health (OIE) as the international organization responsible for the development and promotion of international standards, guidelines, and recommendations for animal health and zoonoses. However, members may choose to adopt measures that result in a higher level of protection than that provided by international standards, although these must be based on the outcome of an import risk analysis.

The OIE's Terrestrial Animal Health Code provides recommendations and principles for conducting transparent, objective, and defensible risk analyses for international trade. The four components of risk analysis described by the OIE are hazard identification, risk assessment, risk management, and risk communication.

Hazard Identification

To effectively manage the risks associated with imported commodities, any organisms that could potentially cause harm and could be introduced into the importing country must be identified. The potential hazards identified are those associated with the species being imported, or from which the commodity is derived, and which might be present in the exporting country. It is then necessary to identify whether each potential hazard is already present in the importing country, and whether it is subject to control or eradication in the importing country and to ensure that import measures are not more trade restrictive than those applied within the importing country. Hazard identification also needs to consider whether strains of a potential hazard found in the importing country are likely to be less virulent than those reported internationally or in the exporting country, or if the proposed import will increase the exposure to a potential hazard in the importing country.

Depending on the nature of the commodity or the degree of processing, some categories of pathogenic agents may be excluded from consideration. For example, arboviruses such as West Nile virus, which replicate in bloodsucking arthropods and are transmitted by bite to a vertebrate host, need not be considered in a risk analysis for fresh or frozen meat or meat products.

The methods of production, manufacturing, or processing might also exclude certain categories of pathogenic agents. Provided details of these production methods and a verifiable quality control program, which includes testing, are included as part of a commodity description, these pathogenic agents do not need to be considered individually in a risk analysis. Where categories of pathogenic agents are excluded, a description of the category and the justification for their exclusion should be included as part of the hazard identification process. For example, provided meat and meat products have been derived from animals that have been subject to antemortem and postmortem inspection in slaughter and processing plants, which operate effective Good Manufacturing Process (GMP) and Hazard Analysis and Critical Control Point (HACCP) programs, then parasites restricted to the intestinal tract do not need to be considered as potential hazards in these commodities.

If hazard identification fails to identify any potential hazards associated with the imported commodity, then the risk analysis can be concluded at this point. If the importing country adopts international standards recommended in the OIE Terrestrial Animal Health Code, then there is also no need to continue a risk analysis beyond this point.

Risk Assessment

Risk assessment is the evaluation of the likelihood and consequences of an exotic pathogen being introduced into the importing country. A risk assessment consists of four interrelated steps:

- Entry assessment: How likely is the imported commodity to be contaminated with the hazard, leading to its introduction into the importing country?
- Exposure assessment: What risk pathways exist that could lead to exposure of animals and humans in the importing country to the hazard, and how likely are they to occur?
- Consequence assessment: What would be the consequences of exposure to the hazard?
- Risk estimation: A combination of the results from the entry, exposure, and consequence assessment to summarize if the identified hazard presents a risk.

It is important to note that all of the above steps might not be necessary in all risk assessments. If the likelihood of entry is negligible for a potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises where the likelihood of entry is nonnegligible but the exposure assessment concludes that the likelihood of exposure to susceptible species in the importing country is negligible, or where both entry and exposure are nonnegligible but the consequences of introduction are concluded to be negligible.

Entry Assessment

Entry assessment consists of describing the biological pathway(s) necessary for an importation activity to introduce

pathogenic agents into a particular environment, and estimating the probability of that complete process occurring. The entry assessment describes the probability of the 'entry' of each of the potential hazards under each specified set of conditions with respect to amounts and timing, and how these might change as a result of various actions, events, or measures.

For meat and meat products to act as a vehicle for the introduction of pathogens, the agent must be able to:

- Infect the species from which the commodity is derived from
- Disseminate to those tissues likely to be present in the traded commodity
- Persist in those tissues during the processing and handling conditions to which the commodity is likely to be subject.

The following are examples of factors that might need to be considered in an entry assessment for the importation of meat and meat products:

Biological factors

- The influence of age, breed, and sex of animals on the susceptibility to the potential hazard. For example, only Muscovy ducks and their hybrids are susceptible to Derzsy's disease so the likelihood of entry for this disease is negligible for meat commodities derived from other duck species.
- Means of transmission (horizontal or vertical) of the potential hazard. For example, provided OIE guidelines on breeding flock hygiene are followed, of the OIE-listed avian diseases, only highly pathogenic avian influenza (HPAI), Newcastle disease, and avian mycoplasmosis have a non-negligible likelihood of entry in poultry hatching eggs.
- Infectivity, virulence, and stability of the potential hazard. *Chlamydia psittaci* is an obligate intracellular organism that depends on living host cells for high-energy metabolites, so will not be viable in meat.
- Routes of infection (oral, respiratory, etc). Inoculation of ducks with *Riemerella anatipestifer* via the intravenous or subcutaneous routes results in significant mortality at all challenge doses, whereas very high doses are needed to cause harm by oral challenge.
- Agent predilection sites. Studies have shown that low pathogenicity avian influenza (LPAI) cannot be transmitted to susceptible birds by feeding meat derived from an infected bird because virus replication is largely limited to the respiratory tract. LPAI has a negligible likelihood of entry in imported poultry meat. In contrast, HPAI replicates in a much wider range of tissues and feeding meat from an infected bird is known to transmit virus to a susceptible bird.
- Impact of vaccination, testing, treatment, and quarantine. Vaccination against some diseases, such as leptospirosis, might reduce the excretion rate of the organism and, therefore, the contamination of the environment. Vaccination of poultry has been shown to prevent HPAI virus replication in skeletal muscles.

Country factors

- Incidence and prevalence. The incidence rate of a disease agent affects the probability of a harvested animal being infected. For such information to have value, it should be derived from statistically based surveys reporting the disease prevalence, not just the clinical disease.
- Evaluation of veterinary services, surveillance and control programs, and zoning systems of the exporting country. The quality of information relating to the disease status of a country reflects the standard of veterinary services and the programs they manage.
- Existence of disease-free zones and compartments. If surveillance and disease management procedures allow recognition of such areas, then importation from these reduces the risk associated with that organism.
- Farming and husbandry practices. Traceability is recognized as an important food safety issue. Ruminants reared totally on pasture are exposed to risk factors different from those of ruminants spending part of their time housed or confined and fed concentrates. The likelihood of animals in the United Kingdom in the 1980s being exposed to the bovine spongiform encephalopathy agent depended on whether they had been fed meat and bone meal.

Commodity factors

- Risk increases with increasing volume of trade.
- Likelihood of contamination. Only healthy animals should be presented for harvest and the distance traveled before harvest should be minimized. Quality control techniques such as GMP and HACCP will help minimize contamination at harvest. The recovery of *Salmonella* Gallinarum-Pullorum from poultry meat has only been reported in environments with poor hygiene practices.
- Effect of processing. The pH of meat drops with rigor mortis. If the pH of meat falls below 6.0, then foot-and-mouth disease virus (FMDV) is likely to be inactivated. However, the pH might not fall below 6.0 if an individual is stressed prior to harvest and, as a result, FMDV may persist in the carcass. However, even in healthy animals, the pH will not fall below 6.0 in lymph nodes, blood clots, viscera, and bone marrow, so these tissues pose a risk for the introduction of FMDV.
- Effect of storage and transport. Freezing is recognized to inactivate a number of pathogens such as Aujeszky's disease virus, leptospires, and hydatids. Heat treatment can also be relied on to inactivate a number of pathogens although a few, such as infectious bursal disease virus, are recognized as being able to withstand domestic cooking temperatures.

Several of these factors might be influential in determining the likelihood of a potential hazard entering a country through an imported commodity. Figure 1 illustrates how these factors might impact the likelihood of introducing porcine reproductive and respiratory syndrome virus (PRRSv) in imported pig meat.

Exposure Assessment

Exposure assessment consists of describing the biological pathway(s) necessary for exposure of animals and humans in the importing country to the hazards (in this case the

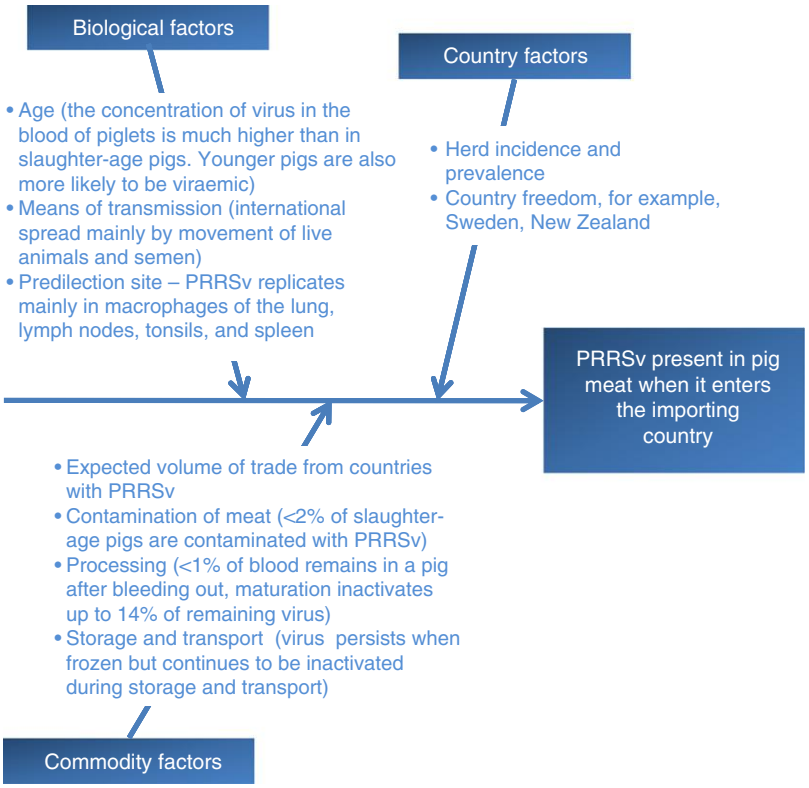

Figure 1 Example of the factors that influence the entry assessment for PRRSv in imported pig meat.

pathogenic agents) associated with the imported commodity, and estimating the probability of the exposure(s) occurring.

The exposure assessment is often the area where there is greatest uncertainty in a risk assessment. For contaminated meat products to serve as vehicles for the introduction of animal disease, the following criteria must be met:

- The pathogen in the meat must be present at an infectious dose.
- Scraps of meat must be fed to a susceptible animal of the appropriate species in the importing country.
- The pathogen must be able to establish infection when given via the oral route.
- If an infection is established in the importing country, local conditions must be such that the disease could spread.

With the exception of pathogens that are able to survive the processes involved in meat and bone meal manufacture, for imported meat to introduce a disease the contaminating pathogen must be able to infect a carnivorous or omnivorous animal when eaten. Ruminants, being herbivores, are extremely unlikely to be infected directly by pathogens carried in meat. The greatest risk of exposure, therefore, occurs in countries with significant pig populations, although imports of poultry meat could expose local poultry to certain disease risks. However, domestic legislation controlling the feeding of waste food to pigs and poultry might have some impact on this risk.

The concentration of the pathogen in meat and the dose of the pathogen required to establish infection via the oral route need to be considered. Continuing the example described in Figure 1 of PRRSv in imported pig meat:

- Meat containing an infectious dose of PRRSv at harvest is likely to come from pigs that are at the peak of viraemia. The mean concentration of PRRSv in the blood of pigs at the peak of viraemia can be estimated from published studies to be approximately $10^{3.18}$ $TCID_{50}$ ml^{-1} blood.
- The residual blood content of lean meat is 2–9 ml kg^{-1} muscle, so the viral titer of meat after bleeding out will be no more than 0.9% of the peak viraemic titer.
- Maturation and the delay before the product arrives at the point of retail result in a further reduction of the amount of viable virus that can also be quantified using published studies.
- The results of experimental studies published in 2005 have established the dose–response relationship between the oral dose of PRRSv and the probability of infection.

Knowing the likely concentration of virus in imported pork at the point of retail (taking into account the effect of bleeding out, maturation, and refrigeration or freezing), it is possible to predict the probability of a known weight of pork taken from a viraemic individual to contain an infectious dose of virus if fed to a susceptible recipient. Furthermore, as the available evidence suggests that only 1.2% of meat samples taken from pigs at harvest are likely to harbor an infectious dose of PRRSv, one can also estimate the probability of a known weight of pork imported from a country where PRRSv is present to contain an infectious dose of the virus. Such calculations have shown that

1 kg of pig meat imported from a country with PRRSv has a probability of 1.8×10^{-3} of containing an infectious dose of virus. However, caution is needed when extrapolating outside experimental results when using purely mathematical models fitted to experimental data. Because of this, the results reported here should be considered highly conservative (i.e., overestimating the probability of infection). For low-dose calculations, a mechanistic model (e.g., β-Binomial, β-Poisson, or Weibull-Gamma) is more plausible. Further discussion of this is beyond the scope of this article.

Consequence Assessment

Consequence assessment describes the relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process should exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socioeconomic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. Examples of consequences that should be considered include:

Direct consequences:

- Animal infection, disease, and production losses
- Public health consequences.

Indirect consequences:

- Surveillance and control costs
- Compensation costs
- Potential trade losses
- Adverse consequences to the environment.

Risk Estimation

Risk estimation consists of integrating the results from the entry assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset. Risk estimation takes into account the whole of the risk pathway from hazard identification to unwanted outcome.

For a quantitative assessment, the final outputs might include:

- estimated numbers of herds, flocks, animals, or people likely to experience health impacts of various degrees of severity over time;
- probability distributions, confidence intervals, and other means for expressing the uncertainties in these estimates;
- portrayal of the variance of all model inputs;
- a sensitivity analysis to rank the inputs as to their contribution to the variance of the risk estimation output; and
- analysis of the dependence and correlation between model inputs.

Risk Management

Risk management is the process of selecting and implementing measures to reduce the risk to an acceptable level, whilst ensuring that negative effects on trade are minimized. The objective is to manage risk appropriately to ensure that a balance is achieved between a country's desire to minimize the likelihood or frequency of disease incursions and their consequences, and its desire to import commodities and fulfill obligations under international trade agreements.

Because risk is a function of probability and consequences, risk management may either seek to reduce the probability of an event occurring or the consequences of it occurring. Continuing the example of the risk of PRRSv in imported pig meat, options that could be considered to manage this risk may include:

- Removal of high-risk tissues. PRRSv has a high affinity for lymphoid tissues, although replication does not appear to be significant in bone marrow. Therefore, the removal of major carcass lymphoid tissues, especially those of the head and neck, and also the major regional lymph nodes, can be considered to significantly reduce the risk in meat.
- Stabilized herds. Pigs that are naturally exposed to PRRSv after having recovered from a previous infection with the same virus strain are known to have a shorter duration of viraemia than naïve animals exposed for the first time, providing the basis for the concept of the 'stabilised' herd. Thus, in situations where infection is known to have occurred several months prior to harvest, even if the animals have been recently reinfected, the likelihood of viraemia in harvest age pigs can be considered to be significantly lower than in situations where a herd of naïve animals has been infected just a few weeks prior to harvest.
- Treatment of pig meat to inactivate PRRSv. PRRSv is known to be relatively sensitive to pH; outside the range of pH 6.0–7.5, the virus is rapidly inactivated. On this basis, a wide range of salamis can be considered to pose negligible risk of PRRS. Similarly, holding meat chilled for a week has been shown to reduce the level of infectivity present by 90%, so curing for 12 months such as in the production of Parma ham is considered to result in insignificant levels of infectivity. PRRSv has been shown to be inactivated following exposure to 56 °C for an hour, so pig meat that is cooked at this level or greater is considered to pose negligible risk.
- Measures that reduce the likelihood of exposure. Any form of meat that minimizes trimming or cutting during its preparation prior to cooking can be expected to pose a lower risk than whole carcasses because of the lower likelihood that scraps will be generated prior to cooking that might be fed to pigs in the importing country. For example, quantitative modeling in New Zealand has shown that if imports of pig meat are restricted to consumer-ready cuts weighing less than 3 kg, then the probability of a PRRSv incursion would be equivalent to a mean of once every 1227 years.

Risk Communication

Risk communication is the exchange of information and opinions with potentially affected and interested parties during a risk analysis, and the communication of the results of a risk assessment and proposed risk management measures with

the decision-makers and interested parties in the importing and exporting countries.

A risk communication strategy should be put in place at the start of each risk analysis and this iterative process should continue throughout. The communication of the risk should be an open, interactive, iterative, and transparent exchange of information that may continue after the decision on importation. The assumptions and uncertainty in the model, model inputs, and the risk estimates of the risk assessment should be communicated.

Peer review is a crucial component of risk communication in order to obtain scientific critique and to ensure that the data, information, methods, and assumptions used in a risk analysis are the best available.

See also: Hazard Analysis Critical Control Point and Self-Regulation. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbiological Safety of Meat: Emerging Pathogens; Prions; Viruses. Modeling in Meat Science: Meat Quality; Microbiology; Refrigeration. Parasites Present in Meat and Viscera of Land Farmed Animals. Risk Analysis and Quantitative Risk Management

Further Reading

Cobb, S.P., 2010. Import Risk Analysis: Turkey Meat Wellington: Ministry for Primary Industries. Available at: http://www.biosecurity.govt.nz/files/biosec/consult/draft-ra-turkey-meat.pdf (accessed 29.10.13).

Cobb, S.P., 2011. The spread of pathogens through trade in poultry meat: Overview and recent developments. Revue Scientifique et Technique de l'Office International des Epizooties 30, 149–164. Available at: http://web.oie.int/boutique/extrait/12cobb1149164.pdf (accessed 29.10.13).

Haas, C.N., Bose, J.B., Gerba, C.P., 1999. Quantitative Microbial Risk Assessment. New York, NY: John Wiley & Sons, Inc.

MacDiarmid, S.C., 1991. The Importation into New Zealand of Meat and Meat Products. A Review of the Risks to Animal Health Wellington: Ministry of Agriculture and Fisheries. Available at: http://www.biosecurity.govt.nz/files/regs/imports/risk/meat-meat-products-ra.pdf (accessed 29.10.13).

MacDiarmid, S.C., Thompson, E.J., 1997. The potential risks to animal health from imported sheep and goat meat. Revue Scientifique et Technique de l'Office International des Epizooties 16, 45–56. Available at: http://www.oie.int/doc/ged/D9115.pdf (accessed 29.10.13).

Murray, N., Pharo, H., 1996. Import Risk Analysis: Porcine Reproductive and Respiratory Syndrome (PRRS) Virus in Pig Meat Wellington: Ministry for Primary Industries. Available at: http://www.biosecurity.govt.nz/files/regs/imports/risk/prrs-risk-analysis.pdf (accessed 29.10.13).

Pharo, H., Cobb, S.P., 2011. The spread of pathogens through trade in pig meat: Overview and recent developments. Revue Scientifique et Technique de l'Office International des Epizooties 30, 139–148. Available at: http://www.oie.int/doc/ged/D10756.pdf (accessed 29.10.13).

Vose, D., 2008. Risk Analysis: A Quantitative Guide, third ed. Chichester: John Wiley & Sons, Ltd.

World Organisation for Animal Health, 2010. Handbook on Import Risk Analysis for Animals and Animal Products: Volume 1 Introduction and Qualitative Risk Analysis, second ed. Paris: World Organisation for Animal Health.

World Organisation for Animal Health, 2012. Terrestrial Animal Health Code Paris: World Organisation for Animal Health. Available at: http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/ (accessed 29.10.13).

AUTOMATION IN THE MEAT INDUSTRY

Contents

Cutting and Boning

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Glossary

Christmas tree A vertical stainless-steel rack containing a large number of hooks arranged concentrically around the vertical shaft and used to store and transport a large number of pork hams within a meat processing plant. The Christmas tree is suspended from the top on an overhead rail or chain. It gains its name due to its resemblance with a Christmas tree when fully loaded with cuts.

Feather bones The bone that protrudes vertically from each thoracic vertebra in beef, lamb, and pork carcasses. These are often referred to as baby back ribs, riblets, or button ribs.

Forequarter pistola cut A particular cut of beef incorporating the forequarter, forequarter ribs, and flank after removal of the hindquarter pistol cut from a beef side.

Frenched racks A rack of lamb is a cut that contains a split backbone and 7 or 8 ribs. The rack is often frenched, whereby the rib bones are exposed by removing the fat, meat, and connective tissue from the end of the ribs to a fixed distance from the main muscle (eye muscle or Longissimus dorsi). Rack frenching is the process of removing the intercostal muscles from between the ribs on a 7- or 8-rib rack).

Hindquarter pistola cut A particular cut of beef incorporating the hindquarter, vertebra, and up to 10 ribs, with the flank removed. So-named because of its pistol-like profile.

Line boning The removal of boning involving a number of boning tables arranged adjacent to a central conveyor. Primal meat cuts are placed on the infeed of the conveyor and at each boning table, one or more boners select the appropriate cut, perform a specific operation on the cut, and then return the finished cut to the conveyor for further processing by a subsequent boner further down the conveyor.

Middles The primal cut produced from the middles section of a carcass containing the central vertebral column and the associated ribs.

Pace boning Pace boning is a new boning concept that replaces traditional table boning. Pace boning occupies less floor space and is claimed to result in smoother production and to be more efficient. Individual boning tasks are performed by boners whereas the product remains on a central conveyor, with each boner performing a specialized task, resulting in increased productivity. The decreased throughput time per product on pace boning lines are also claimed to contribute to greater food safety, as the deboned products can quickly be returned to the cold store.

PLC-control A programmable logic controller (PLC) is a digital computer used for automation of industrial machinery and automation.

Primal cuts The basic sections that a carcass is normally cut into before being further subdivided into steaks and other table-ready cuts are produced. Although different countries and cultures differ in how they divide carcasses of different species into primals, the carcass is generally divided into three primal regions – the hindquarter, middle, and the forequarter. In some cases carcasses may also be split vertically down the backbone, separating each primal region into two individual cuts.

Rail boning The practice of removing primal and whole muscle cuts from a beef side where the side is suspended from an overhead rail. Removed cuts are dropped onto an adjacent table or conveyor for subsequent further processing or trimming.

Introduction

The issues of industrialization with respect to organization, work specialization, and use of production lines, such as for slaughter line operation, apply particularly to cutting and boning technology. Indeed, such technologies are probably the area that are mostly used by the robot-assisted and computer-controlled manufacturing systems.

This article begins with a general analysis of the opportunities and challenges associated with the development of automation solutions for cutting and boning followed by a section for each of the three species covered:

- Porcine (pork) cutting and boning
- Bovine (beef) carcass cutting and boning
- Ovine (sheep and lamb) cutting and boning

The three sections related to the species include an overview of currently (2012) available automation technologies and some reviews on the historical development of the technology.

Opportunities and Challenges

The strongest incentives for the meat industry to adopt automation technology is related to labor – the drive for improved productivity through reduced labor also creates a more rewarding and safer working environment.

Cutting and in particular boning operations are very labor intensive and require skilled, hard, and hazardous work. Automation offers the potential to remove arduous work and as a result introduces more rewarding jobs in terms of planning, supervision, and control of the new technology.

Automation can also improve hygiene and product yield. In some cases, dedicated automation can further increase the production flexibility and facilitate compliance with a wide range of customer specifications without losing cost efficiency. The latter aspect is of particular benefit to export intensive companies serving a wide variety of markets.

Despite these drivers, cutting and boning operations throughout the world are still very labor intensive. Automation in the pig slaughter industry has mainly been adopted in regions with high labor costs such as Northern Europe. In New Zealand, the lamb industry has been progressive in developing and using automation technology to increase processing efficiency and overcome the challenges in recruiting, training, and retaining skilled labor for demanding and manually intensive tasks. Automation in the beef industry is limited because of the complexity associated with handling of the size and biological variation of the carcasses and cuts.

Barriers for automation include the high cost and complexity associated with the development of automation technology, combined with the limited market size, with most of the growth in production occurring in meat-producing regions that have ready access to low-cost labor.

The development of automation for cutting and boning imposes engineering challenges due to the complex structures of hard bone and softer meat and fat and the inherently variable nature of carcass geometry due to genetic variability. This requires flexible and robust machinery and tools combined with advanced measuring systems and control software.

The breakdown of carcasses for all species generally follows the same process of

- separating the carcass into primal – representing the fore – middle, and rear regions of the carcass;
- further cutting the carcass into subprimals and bone-in cuts;
- boning to produce table-ready cuts, either part-boned or bone free; and
- trimming cuts to meet customer specifications regarding shape, weight, fat cover, and presentation.

However, the inherent differences in each species have resulted in critical differences in the process for each species, by which carcasses in each species are processed and disassembled into meat cuts and related coproducts. The automation of boning and cutting processes is, therefore, addressed separately for each species in the following sections.

Pork Cutting and Boning

Concept Design for Automated Cutting

Figure 1 shows an example of a concept design for automated cutting of pig carcasses. The concept has been developed by the Danish pig meat industry, The Danish Meat Research Institute (DMRI), Denmark, and different suppliers of machinery for the meat industry.

Automatic Primal Cutting

Automatic machines for primal cutting have been available for several years, for example, from the two Danish companies, Attec and KJ Industries. The machines separate half carcasses (whole carcasses are split down the backbone as part of the slaughter process) into the three primal cuts: fore-end, middle, and hind leg by sawing after precise measurements and positioning. The two machines operate with different measuring technologies. Attec's machine applies servo-controlled mechanical sensors, whereas the machine from KJ Industries makes use of computer vision analysis.

Automatic Cutting of Pork Middles

Figure 2 shows a machine for automatic cutting of middles supplied by Attec, Denmark.

Attec also supplies a machine for automatic cutting of middles. Cuts from the middle-cutting machine are shown in Figures 3 and 4. In its basic version, the machine splits the pork middle into loin and belly and cuts off the rib-top. In an extended version, the machine further performs precutting of the ribs, without cutting in the meat. The extended version may also include removal of the feather bones. The rib precut and splitting is done based on individual recipes specifying quality parameters. Feather bone removal is also performed at this stage. Each of the different cuts are delivered on separate belts for further processing and handling.

The benefits of the middle-cutting machine are both reduced labor costs and improved yield. The yield increase is

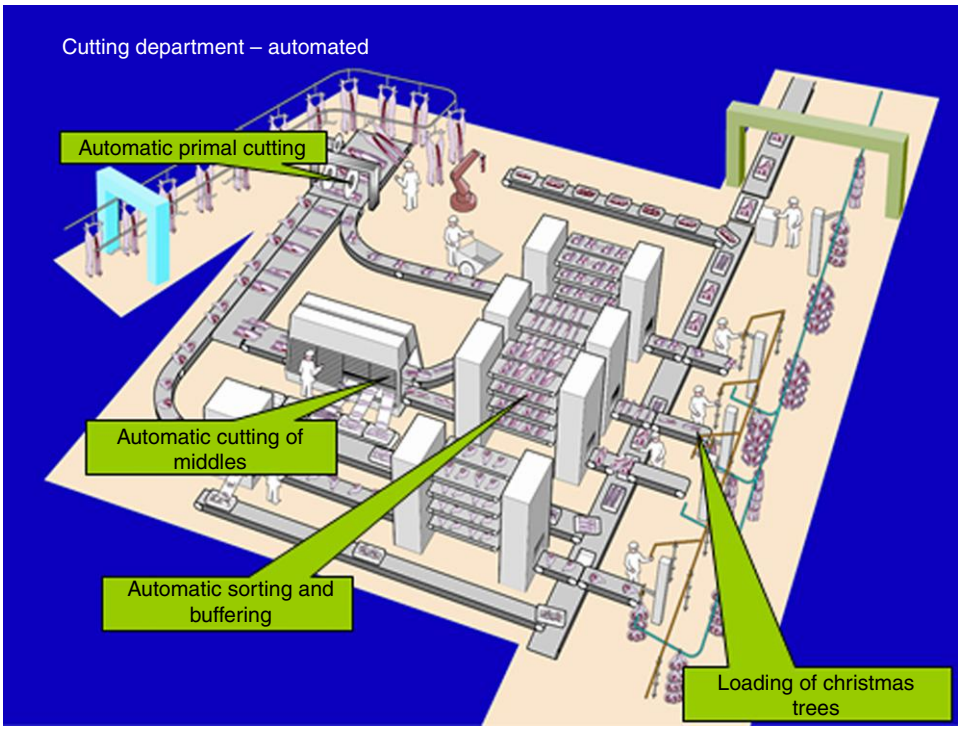

Figure 1 Concept design for automated cutting of pig carcasses. Courtesy of DMRI.

Figure 2 Automatic cutter for pork middles. Photo courtesy of Attec.

achieved because sawing of the rib-tops can be more accurate and the division of the loin and the belly can be optimized according to product specifications. Because the production recipes can be changed instantly, the machine can produce in accordance with current information of customer demands.

Automatic Sorting and Buffering

A system for automatic sorting with buffering capacity is supplied by the Danish company, KJ Industries. In the buffer storage, the different cuts are sorted according to customer specifications such as weight, meat percentage, fat thickness, pH values, and breed.

An automatic S-curved conveyor and a buffer system ensure a continuous product flow and also ensure that cuts are forwarded according to the correct sorting class. From the buffer, the cuts are automatically transported to further processing, packaging, or storage.

Semiautomatic Loading of Cuts on 'Christmas Trees'

When internal transport takes place by use of 'Christmas trees,' the semiautomatic system for loading of cuts can be applied to avoid manual heavy lifts. The cuts from the buffer storage, now arranged in quality classes, will be moved automatically to a number of loading stations and loaded on the 'Christmas trees' by the use of semiautomatic devices. A system for unloading the cuts at the destination for subsequent boning is also available.

Automation of Boning Processes

Boning is difficult to automate because of the subtleties of the process itself and the characteristics of the items to be processed. Cuts from animal carcasses are very complex, irregular, and unpredictable in terms of shape, texture, and internal structures consisting of a geometrically irregular mix of hard and soft tissue. Therefore, only a few automation solutions have been developed for boning till date.

Skinning/Derinding

One process, however, was automated – or rather mechanized – very early, namely the skinning or derinding of cuts. Ray Townsend (USA) invented the first pork skinner in 1946, and the

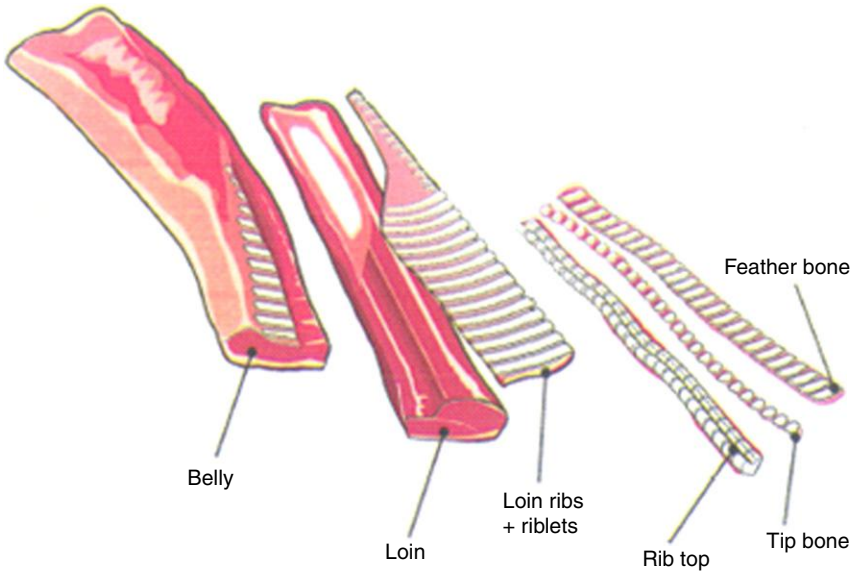

Figure 3 Processed cuts from the automatic cutter for pork middles. Image courtesy of DMRI.

Figure 4 View of splitting the middle into the loin and belly in the automatic cutter for pork middles. Photo courtesy of Attec.

Figure 5 The Attec pork ALDT. Photo courtesy of Attec.

company Townsend (now part of Marel, based in Iceland) has since been one of the leading suppliers of skinning equipment.

Today, a number of manufacturers offer this type of machine. One of the technical challenges with this process is to ensure that the skin is removed while leaving a fat layer of a specified thickness at the product. This is not easy, if possible at all, to ensure by means of conventional mechanical devices. At International Frozen Food Association 2013 in Frankfurt, however, a new development from Attec and DMRI was exhibited. The Attec pork automatic loin 3D trimmer (ALDT) is an automatic equipment using a number of individually actuated knifes controlled by a measuring unit combining ultrasound and vision measurements allows much higher precision in trimming and higher yields (Figure 5).

Industrial Robot Solutions

Although the equipment described is custom designed, dedicated machines designed as machinery equipped with PLC-control systems based on industrial robots also exist.

The German companies, Banns and e+v Technology GmbH (e+v), are examples of suppliers of such solutions based on industrial robots combined with automatic vision measurement systems (e.g., Figure 6). At present (2012), applications for primal cutting, cutting shoulders, and middles are available.

Beef Carcass Cutting and Boning

Primal Cutting and Quartering

Traditionally, most beef carcasses are fully chilled and almost equilibrated at temperature generally below 5–10 °C before further partition of the carcass sides. However, some plants in Australasia and Scandinavia conduct hot boning immediately after slaughter, or semihot boning with core muscle

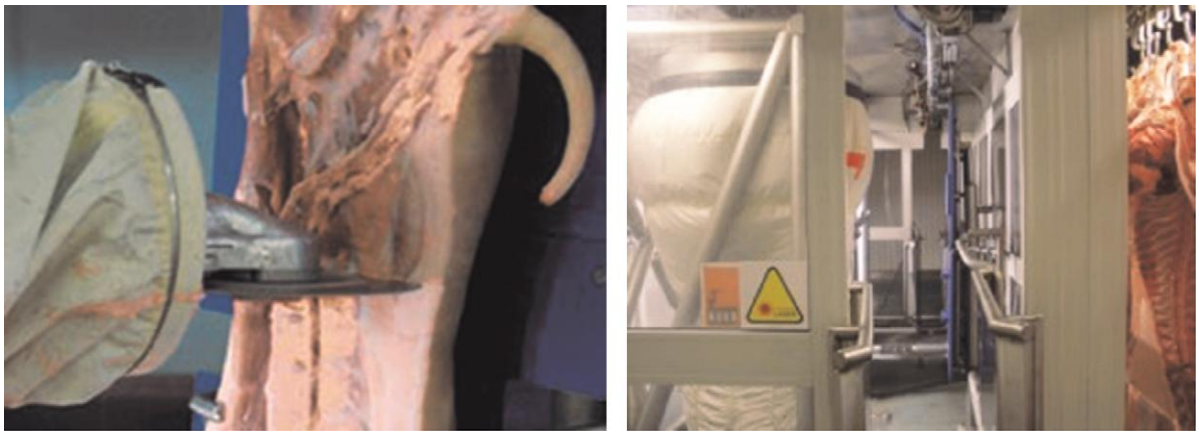

Figure 6 Robot for conventional precutting (primal cutting) with four individual cuts. Robot system from Banns and e+v technology, using a Kuka robot. Photos courtesy of e+v Ltd.

temperatures of 10–20 °C. With hot boning, certain cuts may be wrapped in plastic to prevent muscle contraction, resulting in lower tenderness.

The predominant quartering process used includes cutting and sawing sides to halves or pistol/hindquarter. It is a manually operated process using powered sawing and cutting tools. Several initiatives to automate this 'scribing' by the use of standard robots mounted with saws and controlled by vision systems have been taken, for example, a joint Machinery and Robotics Pty. Ltd. (MAR, Australia), Meat and Livestock Australia (MLA)/Australian Meat Processor Corporation (AMPC) project, and a German e+v project where similar commercial systems are in place for pork. In a French-led project, robotic Z cutting into pistola and forequarter has been researched. The first commercial scribing system came in operation in 2009. Uptake of the technology so far has been limited.

Cutting and Trimming

Beef quarters are progressively broken down into individual cuts with the quarters either manually placed at tables (table-boned) or hung from the hook (rail-boned). This process requires operator skill in order to follow muscle seams and delivering presentable cuts. The work load on operators is considerable and introduction of simple force rams to aid in the pulling and separation process has been used for several decades. The systems assist the operator by pulling bone from meat or meat from bone. Likewise, systems that position the quarter in more favorable position for cutting by tilting the quarter during removal are in use. There are several similar systems are available, for example, Carne Liberator system.

More recently, a more adaptive controlled pulling device called Hook assist has been developed in an MLA/AMPC and Scott Technology Ltd (Scott, New Zealand) effort (Figure 7). The machine now being introduced in Australasia is a manual assist device developed to make boning of aitchbones and knuckles less physically demanding. The device is a mechanical arm that acts to provide the pulling force an operator requires for rail boning. Force feedback technology is used to give a boner a meat hook that can pull with four times the force of a human. The force is finely controlled by the operator using a joystick principle.

Strain on operators arises from uneasy work positions and required force for maneuvering muscles and knife trajectory. In modern plants, adjustable platforms to compensate difference in operator heights have become standard, and for rail boning, height-adjustable rams are used. Traditional layout boning room includes individual deboning table where an operator bones out quarters moved to his position.

Separating the boning process on consecutive operators allows specialization and easier design of a more ergonomically correct work position, for example, pace boning and line boning.

Automatic Loin Boning

The biological variation in beef carcasses compared with, for example, pork and poultry with respect to animal age, weight, and skeletal differences is a challenge for the development of automatic boning solutions. Flexible tools and sensors may be required to successfully deal with the biological variation. The company BLM Engineering in New Zealand developed together with MLA the Beeftech loin boning machine, where the loin cuts fixed on a moving conveyor passes a solid curved knife separating muscle from meat. This machine has a throughput of up to six carcasses per minute. The machine has been developed to a commercial version but has yet to see a widespread industry uptake.

Another recent approach for loin boning using several consecutive tools was inspired by the pork middle deboning equipment developed by Attec and DMRI. DMRI with MLA started exploring the feasibility of a loin boning concept using consecutive moving tools like sawing, a rigid knife, and several flexible knives working on a fixated strip loin piece.

Yield Management and Traceability

The increased demand for traceability, yield recording, and monitoring of operator performances have led to introduction of IT solutions with information monitors and scales at many operator positions in the boning room. An advanced example

Figure 7 An adaptive controlled pulling device called Hook assist developed in an MLA/AMPC and Scott effort. Photo courtesy of Scott Technology Australia P/L.

of this is the Marel stream line system, where cutting instructions and performance information is given on monitors. The operators are assigned cuts with specific customer specification and by weighing after trimming it is possible for management and the operator to monitor the performance of the individual operator. A built-in traceability mechanism at all levels of product handling ties the animal origin and carcass information with the individual cuts, ensuring that all product information may be used for labeling and for product recall. Although the cost of these advanced systems is significantly higher, the potential is increased yield recovery and a data-based continuous feedback to both the operator and boning room management. This enables worker pay rates to also be linked to worker performance according to their individual performance and throughput data recorded in the system.

Ovine Cutting and Boning

New Zealand and Australia are the world's largest exporters of lamb and mutton, with over 93% of all lamb and 91% of mutton slaughtered being available for export. Historically, the vast majority of this trade was in the form of frozen carcasses, primarily supplying the UK with frozen lamb in whole carcass form. Today, the vast majority of cross-border lamb exports are in a bone-in or boneless form, much of this in table-ready form and exported chilled rather than frozen. For example, in the year ended September 2010, less than 5% of New Zealand lamb and mutton was exported in whole carcass form, 20% going as boneless product and 75% going as cuts.

The trend toward producing a wide range of further processed and table-ready cuts has resulted in dramatic increase in the range and complexity of cut specifications. The typical New Zealand lamb processor will have many hundreds of individual cut specifications on file and be required to produce these regularly. Although the differences between specifications will often involve subtle differences in packaging and presentation of final trimming, this plethora of specifications will result in a typical lamb cutting and boning room being required to implement changes in processing operation to accommodate the need to produce different cuts during a single shift.

The typical lamb carcass breakdown is shown in Figure 8 and includes the most common cuts produced from each of the three primals (forequarter, middle, and hindquarter).

Lamb, unlike beef and pork, carcasses are generally not split down the backbone but instead cut into these three primals. Each of these primals is then further broken down into further processed and table-ready cuts before being packaged, boxed, and then sent to refrigerated storage before load out.

Such a wide range of complex cutting specifications offers significant opportunity for automation; however, with many variations in the cuts required, the technical complexity has represented a major hurdle to the development of automation. The constant change in the cutting operations in response to changing cut specifications has also focused automation developments on the production of higher value cuts or on operations that are common to a wide range of cuts.

Drivers for Automation

Traditionally, lamb carcasses are cut into their three primal cuts manually using band saws. This is a highly dangerous operation, with significant risk of serious hand injuries and amputations of fingers. This high risk operation has led to the development of Bladestop™, a joint development by MAR and MLA. Bladestop™ comprises an operator arm sensor attachment with a unique blade brake mechanism, all under microprocessor control. Bladestop™ mechanically stops the band saw blade when the sensor detects that a person has come into contact with the blade, stopping the blade within 10 ms and therefore significantly reducing the chance of serious injury.

Band saws also produce a significant amount of wastage, with the average band saw generating significant meat saw dust as a part of the cutting operation. There is also the risk of contamination from bone and meat dust, particularly when a

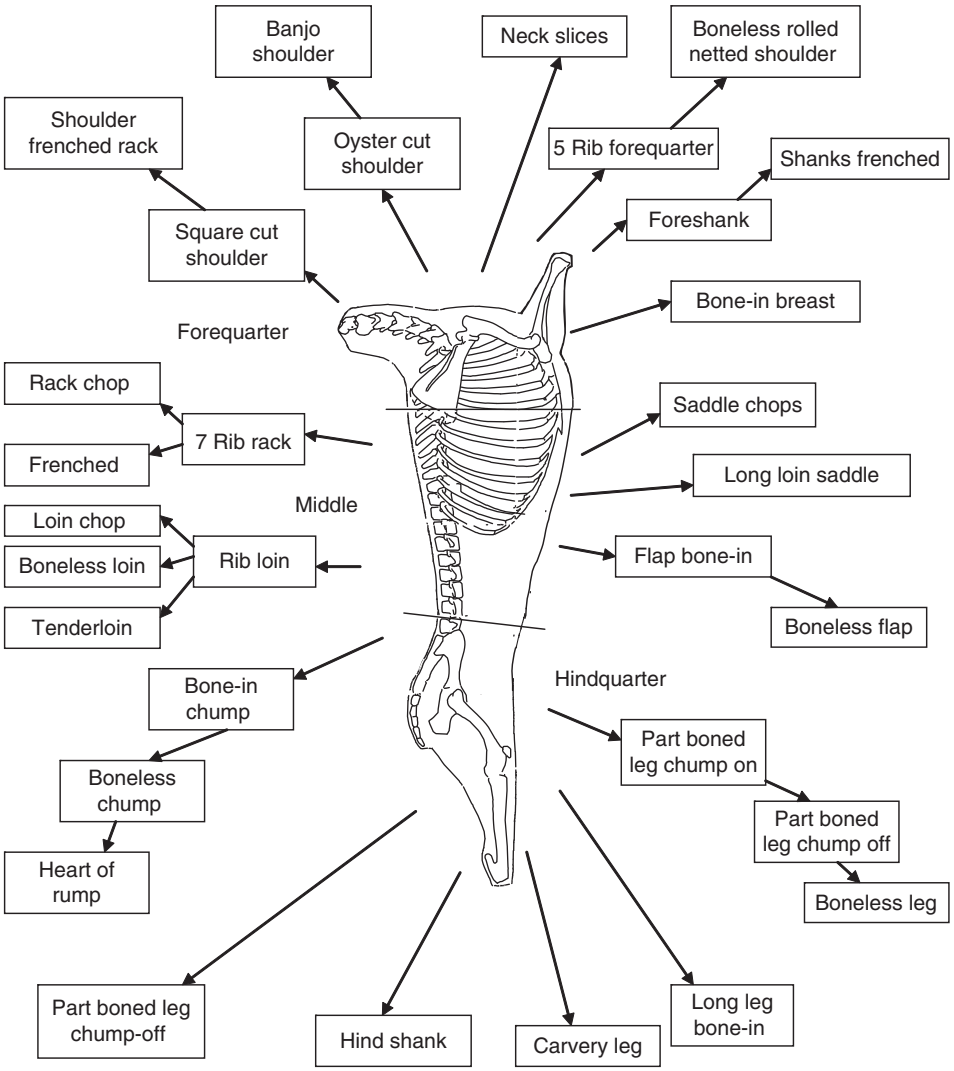

Figure 8 Typical lamb carcass break-out.

cut is made through the spinal column. Alternatives to band saws include circular cutting disks, such as those manufactured and marketed by Freund, Germany. These disks generate almost no dust, are capable of cutting through meat, fat, and bone, and offer significant advantages in terms of safety and hygiene over conventional toothed blades and band saws.

Much of the subsequent processing of primal is undertaken manually using sharp boning knives, representing a further safety risk as well as being time consuming and thus labor intensive. Automated key cutting tasks offer the potential to reduce this risk, also offering the benefits of increased hygiene through reduced cross-contamination as well as increased yield and more consistent presentation of the cut surface.

Mechanized Task Replacement

In the 1980s and 1990s, the Meat Industry Research Institute of New Zealand Inc. (MIRINZ) developed a number of mechanical systems for automating key aspects of lamb cut production. Two of these that have met with significant commercial success are the loin/saddle removal machine and the Chine bone removal machine, now manufactured and marketed by BLM Engineering in New Zealand (BLM, Figure 9). These machines perform the same repeatable task on manually loaded cuts, generating yield improvements of 10% and 8%, respectively, at throughput rates of up to 9 cuts per minute, using only a single operator. There are more than 100 of these machines currently in service within the lamb industry worldwide.

MIRINZ also developed mechanical prototypes for automating the primalling of carcasses using large, automated band saws, fleecing shoulders of the forequarter and mechanically removing the intercostals from rack saddles to produce frenched racks, but none of these developments proceeded to a commercial stage.

MACPRO abt, New Zealand, in conjunction with the MLA, also developed a number of mutton boning systems, including leg and trunk boning machines. There has been little commercial uptake of these machines by industry. The same developers, under the brand name EXOS, have recently launched

Figure 9 The loin/saddle removal and chine bone removal machines. Photos courtesy of BLM Engineering.

a new lamb loin/saddle boning machine that operates in a similar manner to the BLM machine.

Attec has developed a number of machines for lamb cutting. These include a semiautomated lamb primal cutting system that uses one or more circular disk knives to provide a cut that produces no meat or bone dust. The carcass is laid flat on a table and either aligned with the knife manually or the knives aligned by the operator positioning an optical indicator on the carcass that aligns the knives automatically. A number of installations are currently in commercial use around the world.

Attec has recently announced an automated middle cutting machine called the LMPC-200. This machine is able to perform flap trimming and chine bone removal on lamb saddles in a single operation. A lamb shoulder square cutting machine is also in development. Both of these machines require manual operators to load and unload product.

Rack frenching (the process of removing the intercostals muscles from between the ribs on a 7-rib rack) is a skilled and manually intensive task, requiring between three and six workers at typical lamb-processing rates of three to six carcasses per minute. Frenched racks are also one of the most highly valued, table-ready cuts produced from lamb carcasses, and customer specifications dictate that the intercostal tissue must be trimmed to a set distance from the eye muscle and that appearance and finish to the product be of a uniform high standard. Although a mechanical cutting solution to this manual task is yet to be commercially developed, a number of systems are currently in use applying high pressure water jets to remove the intercostal tissue from between the ribs to a consistent distance from the eye muscle. These machines require only a single operator and thus offer significant benefits in terms of labor savings and reduction in the risk of knife-based injuries. However, the disadvantages of this approach include:

- High use of water.
- Requirement to process the resulting slurry.
- Loss of the intercostals as an edible product and a loss in yield.
- The application of water limiting the shelf life of the product when stored chilled.

McLaren Engineering (New Zealand) has recently developed iFRENCH, a continuous processing French rack machine that uses water to process racks at a sustained rate of 24 racks per minute.

Robotic Automation

Before 2000, boning and cutting automation involved the mechanization of a fixed operation, with minimal, if any, modification of action to accommodate variations in carcass geometry or anatomy. More recently, a number of developments that incorporate robotics with sensor feedback have been developed, aimed at addressing the more challenging boning and cutting operations.

One such development is the Robotic Ovine Cutter (ROC), which is commercially available through MAR. This joint initiative between Midfield Meats in Australia, BMC (UK), and e+v combined an off-the-shelf robot and e+v's image vision system to automatically primal lamb carcasses. The carcass is first scanned by the vision system while still on the rail and the primal cut positions calculated. Using a custom gripper, the robot then removes it from the rail and passes it through circular disk blade before returning the hindquarter to the rail. The original system was capable of processing 200 carcasses per hour. Subsequent developments have increased the throughput to 400 carcasses per hour through the mounting of the circular disk blade on a second robot to speed up the cycle time. Higher throughput systems proposed by MAR involve additional robots to gain further reduction in cycle time. Reported results indicate that more than 90% of square shoulder cuts are made within plus or minus half a rib.

More recently, Robotic Technologies Limited (RTL), a joint venture between New Zealand's largest meat processing company Silver Fern Farms Ltd. and Scott, has revealed its vision for a fully automated lamb boning room (**Figure 10**). This development is aimed at producing an automated room for producing bone-in products at throughputs of up to 10 carcasses per minute.

The system comprises a real-time X-ray system that images each carcass from two aspects to generate the necessary information of external and internal anatomy, and in particular

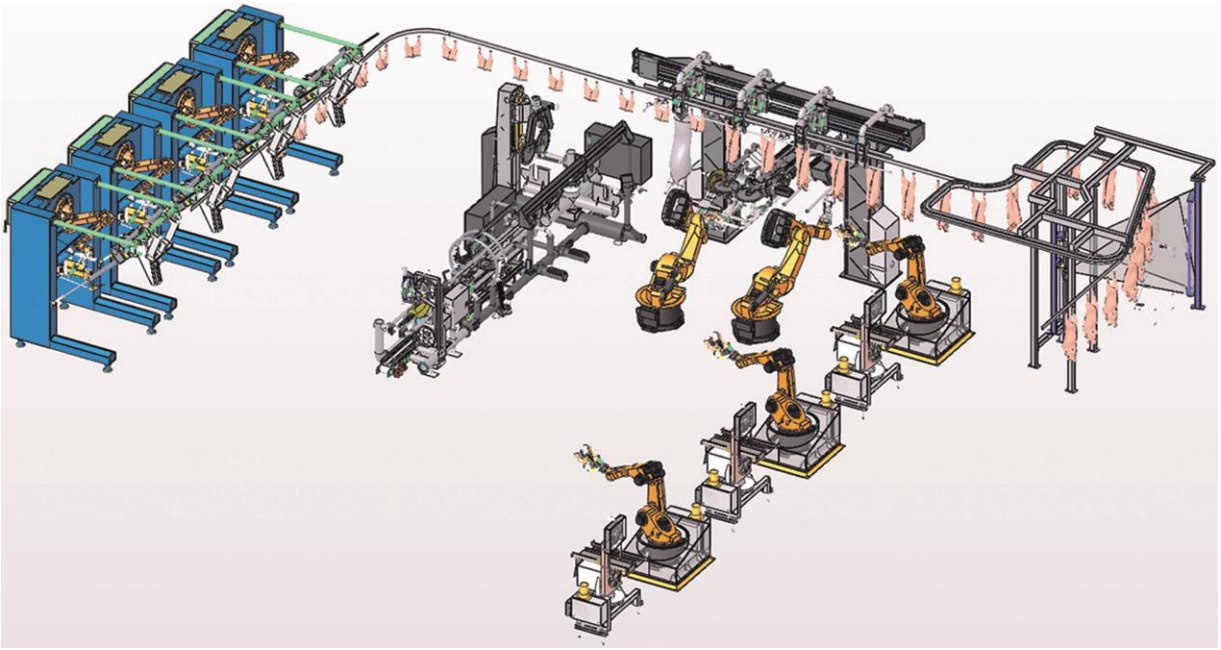

Figure 10 RTL's full automated boning room. Image courtesy of RTL Ltd.

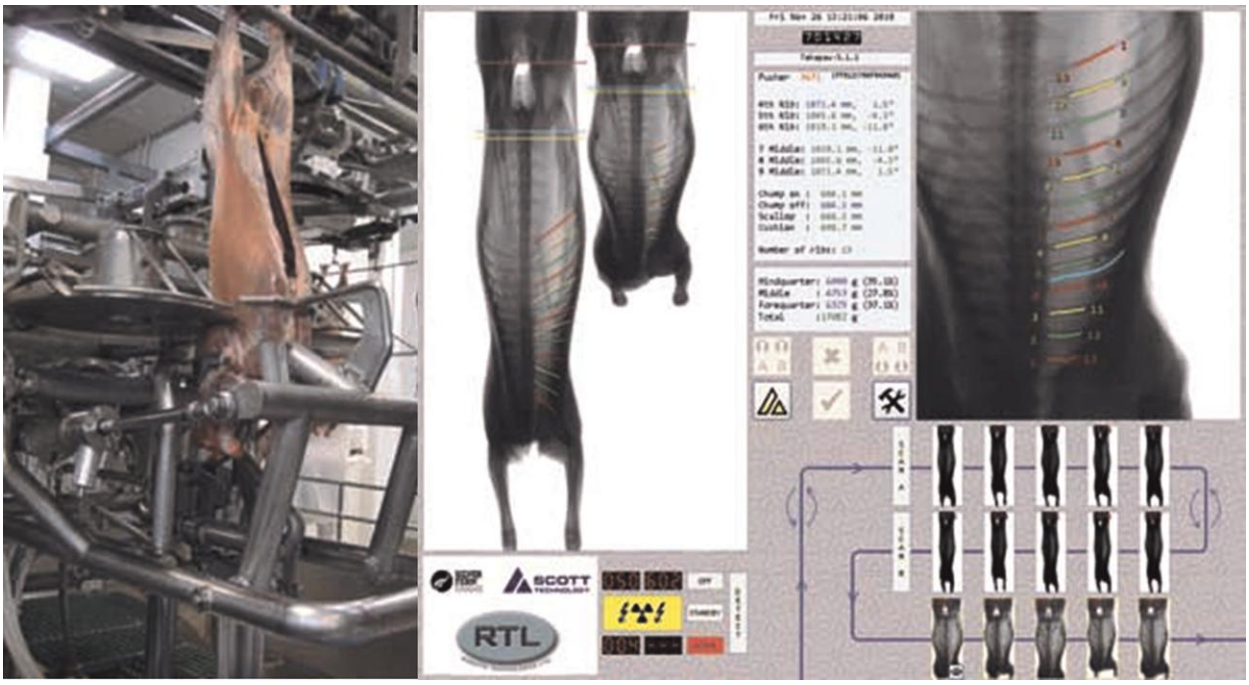

Figure 11 RTL's primal robot and the output of RTL's X-ray imaging system. Image courtesy of RTL Ltd.

the internal location of skeletal structure. It is this information that enables much of the subsequent automation.

Carcass primaling is then performed by two robotic cutting stations. The first uses circular disk knives to separate the forequarter from the carcass, with the robot being able to angle the cut in order to optimize meat yield in the higher value primal while accurately placing the cut with respect to the ribs and vertebrae. The second robot uses a blade knife to separate the hindquarter from the middle primal using a variety of cut positions (chump on, chump off) while also angling the cut to clear the pelvic bone and again optimize the meat yield in the more valuable loin area. These two robots are operational in meat plants in New Zealand and Australia.

The three primals are then handed off the three separate robotic stations. The hindquarter station uses a conventional robot, equipped with a conventional knife blade, to cut and

separate the two legs from the pelvic bone. RTL are also working on an automated knuckle tipping machine for the legs after removal form the pelvic bone.

The middle robotic station incorporates automatic spinal column material removal, saddle crosscut, flap and brisket trimming, and chine and feather bone removal. These cuts are all optional dependent on the required cut specification. This station is at the in-plant testing stage.

The forequarter robotic station uses a robot to grip and cut the forequarter using a conventional band saw. This system is able to implement cuts to the brisket, knuckle tips, and neck as well as splitting the forequarter, depending on the required cut specification. This station is installed in at least one New Zealand lamb processing plant.

Each robotic station is developed as a standalone module. In this way, they can be implemented separately or together, integrated with a carcass X-ray imaging system or independently integrated with other sensing systems installed adjacent to the robotic stations (**Figure 11**). Producing significant savings in labor and hygiene, the ability to optimize cut placement according to cut specification results in an increase in yield of higher value cuts of approximately 1%, contributing significantly to the payback of this technology, particularly in higher throughput installations.

See also: Automation in the Meat Industry: Slaughter Line Operation. Carcass Chilling and Boning. Cutting and Boning: Hot Boning of Meat. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals

Further Reading

Anon, 2001. Slaughter-line for pigs is completed. An automated evisceration process is integrated into the slaughter-line at Danish Crown. Fleischwirtschaft 81 (5), 111–115.

Anon, 2011. Compendium of New Zealand Farm Facts, 35th ed. Wellington: Beef + Lamb New Zealand.

Folkmann, P., Christensen, F., 2003. Innovation and advanced technology behind a new machine for automatic cutting of pork middles. Fleischwirtschaft 83 (3), 89–91.

Guire, G., Sabourin, L., Gregory, G., 2010. Robotic cell for beef carcass primal cutting and pork ham boning in the meat industry. Industrial Robot: An International Journal 37 (6), 532–541.

Hansen, F., 2004. Robot ready for 47 million fore-ends. Fleischwirtschaft 84 (1), 31–32.

Hardin, W., 2011. X-ray imaging delivers a better cut. Vision Systems Design 16 (7), 17.

Hinrichsen, L., 2010. Manufacturing technology in the Danish pig slaughter industry. Meat Science 84 (2), 271–275.

Madsen, K.B., Nielsen, J.U., 2002. Automated meat processing. In: Kerry, J., Ledward, D. (Eds.), Meat Processing – Improving Quality. Abington: Woodhead Publishing, pp. 283–296.

Madsen, K.B., Nielsen, J.U., 2004. Encyclopaedia of Meat Sciences I. Amsterdam: Elsevier, pp. 43–49.

Madsen, N.T., Nielsen, J.U., Mønsted, J.K., 2006. Automation – The meat factory of the future. Proceedings of ICoMST. The Netherlands: Wageningen Academic Publishers. ISBN-10: 90-8686-010-9, ISBN-13:978-90-8686-010-4.

Nielsen, J.U., 1999. Automatic evisceration of pigs. Meat Automation – Proceedings on Special User's Meeting on New Automation Technologies for Meat Production, pp. 78–81. Brussels, Belgium: Woodhead Publishing.

Purnell, G., Loeffen, M., 2006. Automation for the modern slaughterhouse. In: Nollet, L.M.L., Toldrá, F. (Eds.), Advanced Technologies for Meat Processing, third ed. USA: CRC Press, pp. 43–71.

Ulrich, J., Mønsted, N.K., Madsen, K., 2005. Automation in pig slaughtering, cutting and boning. Food Science & Technology 19 (1), 17–19.

Zink, J., 1995. Application of automation and robotics to pig slaughtering. Proceedings of the 41st Congress of Meat Science and Technology, pp. 420–424. San Antonio TX: Wageningen Academic Publishers.

Relevant Websites

http://www.argusrealcold.com
Argus Realcold.

http://www.attec.dk
Attec Food Technology, Denmark.

http://www.banss.de
BANSS, Germany Gmbh.

www.beefboning.com
Beef Tech.

http://www.danishcrown.com/page22686.aspx
Danish Crowns Facility in Horsens, Denmark.

http://www.eplusv.de/index.html
e+v Technology GmbH.

http://www.exos.co.nz
Exos.

http://www.freunduk.com
Freund.

http://www.marel.com/
Icelandic Marel.

http://www.frenchrackmachine.co.nz
John Knight Ltd.

http://www.kjindustries.com:
KJ Industries, Denmark.

http://www.macpro.co.nz
MACPRO*abt*.

http://www.mclarenstainless.co.nz
Mclaren Stainless.

http://www.scott.co.nz/
Scott Technology Limited.

http://wassco.co.nz
Wassco.

Slaughter Line Operation

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Glossary

Bung Term used to describe an animal's rectum.
Cod Region of a beef animal in the region of the udder or scrotum.
EUROP Grid method of carcass classification implemented in 1981 by the European Economic Community to ensure the uniform classification of adult bovine animals. The EUROP grid consists of a five-point scale in which each conformation and fat class is subdivided into low, medium, and high classes resulting in 15 classes. Conformation is assigned according to the EUROP system where E is excellent, U is very good, R is good, O is fair, and P is poor. Fat class is a numerical scale where 1 is low, 2 is slight, 3 is average, 4 is high, and 5 is very high.
Flare fat Visceral fat deposit surrounding the kidneys and inside the loin of pig carcasses. It is the source of the highest grade of lard, known as leaf lard.
Paternoster lift An elevator that consists of a chain of open compartments that move slowly and vertically in a continuous loop without stopping.

Introduction

For many years slaughtering has been industrialized with respect to organization, work specialization, and use of production lines. It was from seeing the conveyor systems used by the beef slaughter industry in Chicago in the beginning of the twentieth century that Henry Ford I had the idea of implementing assembly lines that revolutionized the car industry. At present the meat industry is getting inspiration from the robot-assisted and computer-controlled manufacturing systems applied in the car industry.

This article is organized in a section with a general analysis of the opportunities and challenges associated with the development of automation solutions for slaughter houses followed by a section each for the three species covered:

- Porcine (pig) slaughter automation.
- Bovine (beef) slaughter automation.
- Ovine (sheep and lamb) slaughter automation.

The three sections related to the species include an overview of currently (2012) available automation technologies and a review of the historical development of the technology.

Opportunities and Challenges

The strongest incentive for the slaughter industry to adopt automation technology relate to labor – the drive for improved productivity through reduced labor, while also creating a more rewarding and safer working environment. Slaughtering operations are labor-intensive and require hard and repetitive work. This situation can be improved by automation, which not only removes arduous work but in addition introduces more rewarding jobs in terms of planning, supervision, and control of new technology. Automation also improves the hygiene by less manual product handling and less cross contamination between carcasses because the tools in the machines can be cleaned more effectively between each carcass. Slaughter quality and yield can also be improved by automation through more accurate, consistent, and repeatable execution of key slaughter tasks.

Despite these drivers, slaughter houses throughout the world are still very labor intensive. Automation in the pig slaughter industry has mainly been adopted in regions with high labor costs such as Northern Europe. In New Zealand, the lamb slaughter industry has been progressive in developing and using automation technology to increase processing efficiency and overcome the challenges in recruiting, training, and retaining skilled labor for demanding and manually intensive tasks. Automation in beef slaughter is limited because of the complexity associated with the handling of the biological variations of beef carcasses.

Barriers for slaughter line automation include the high cost and complexity associated with the development of slaughter automation technology, combined with the limited market size, with most of the growth in production occurring in meat producing regions that have ready access to low-cost labor.

The development of automation for slaughter houses imposes engineering challenges because the machines are to perform transformation of a material composed of complex structures of soft and hard material. Further, the machines must be able to adapt to considerable and unpredictable variations of the input material. This requires flexible but still robust machinery and tools combined with advanced measuring systems and control software.

Automation of Pig Slaughter Lines

The most common approach to developing slaughter line automation is task replacement: specialized machines dedicated to performing a specific slaughter task. More recently, specialized machines have been complemented by standard

Table 1 Overview of the pig slaughtering processes and automation

	Process	*Automation*
Unclean part		
Live animal handling	Transport to slaughterhouse	
	Unloading of pigs	Vision monitoring of animal welfare
	Veterinary antemortem inspection	
	Lairage	Semiautomatic
	Transfer to stunning	Semiautomatic
	Stunning	Semiautomatic
Sticking and bleeding	Shackling	
	Sticking and bleeding	Vision control
Surface treatment	Scalding	Mechanical
	Deshackling	
	Dehairing	Mechanical
	Gambrelling	
	Singeing	Mechanical
	Rind scraping/polishing	Mechanical
Clean part		
Evisceration and trimming	Carcass opening	Automatic machines
	Removal of secondary organs	Automatic machine
	Removal of stomach and intestines	
	Removal of plucks	
	Separation of pluck sets	Automatic machines
	Carcass splitting	Automatic machine
	Neck cleaning	Automatic machine
	Removal of flare fat residues	Automatic machine
Inspection and quality measurements	Veterinary inspection of carcasses and organs	Electronic data collection
	Carcass weighing	Automatic
	Carcass classification	Automatic
Downstream processes	Carcass chilling	Computer-controlled transportation systems
	Sorting into quality groups	
	Storage and temperature equilibration	

industrial robots equipped with special tools and designed specifically to survive the harsh environmental slaughter lines.

An overview of the handling of pigs for slaughter from transport of the live animals through the slaughter processes until the equilibration chilling room are shown in Table 1. The current automation possibilities are indicated.

Automation of Unclean Part of a Pig Slaughter Line

Figure 1 illustrates the unclean part of a pig slaughter line configured with available (2012) automation and mechanization technology (yellow text-boxes).

The set up shown in Figure 1 is based on gentle and animal-friendly handling of the pigs and in groups from delivery at the farm, during transportation, and until CO_2 stunning at the slaughter house. At the slaughter house, the pigs are kept in their familiar groups at the lairage and further during moving to the CO_2 stunning system by means of automatically driven gates. The pigs are stunned in groups of 5–7. The groups are led into a paternoster lift, which is lowered into the CO_2 where the pigs lose consciousness before sticking. The benefits achieved from this concept are high animal welfare, low mortality during transport, reduced drip loss, and improved meat quality.

For most of the remaining processes except shackling, sticking, gambrelling, and reading of farm identification numbers, automated mechanical solutions are available. For higher line speeds, a separate equipment for each process is used. For lower line speeds, combination systems are available, for example, systems combining scalding, dehairing, and singeing.

Automation of Clean Part of a Pig Slaughter Line

Figure 2 illustrates an automation concept for the clean part of a pig slaughter line. Automatic machines are indicated by the yellow text boxes.

In Figure 2, the shown concept has been developed in partnership between the Danish pig meat industry and a division in the Danish Technological Institute (DMRI), Denmark. The individual machines were developed in cooperation with machine suppliers, for example, Danish supplier of meat processing equipment (SFK) Systems, Denmark, that delivers a number of the shown automatic machines. The automation concept may reduce the required manning by 30–40% compared to manual operation.

Below are shown photos of selected machines for the slaughter of pigs together with a short description of the operation (Figures 3–5).

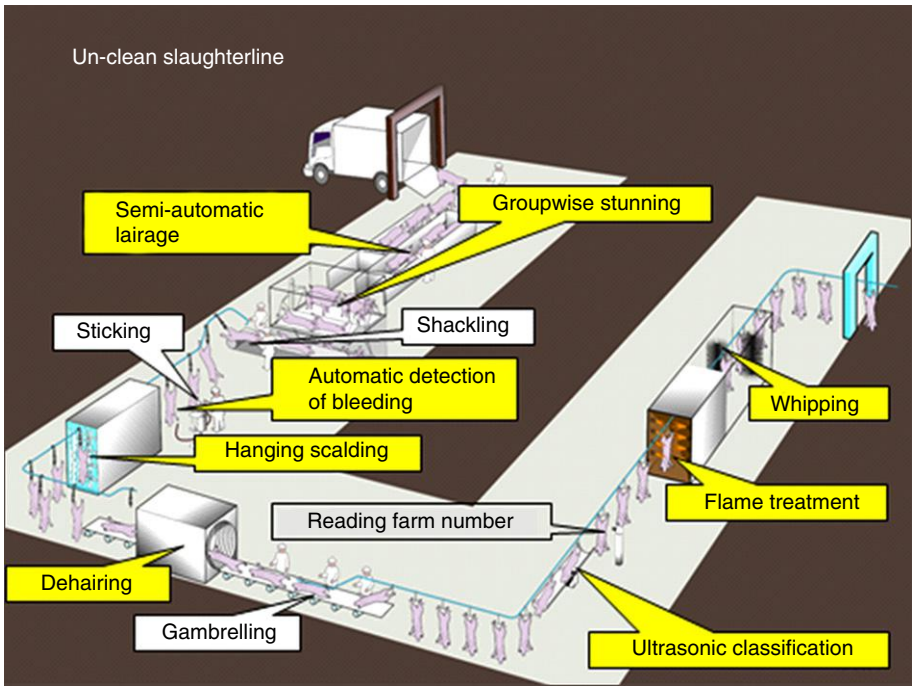

Figure 1 Unclean pig slaughter line configured with up-to-date technology.

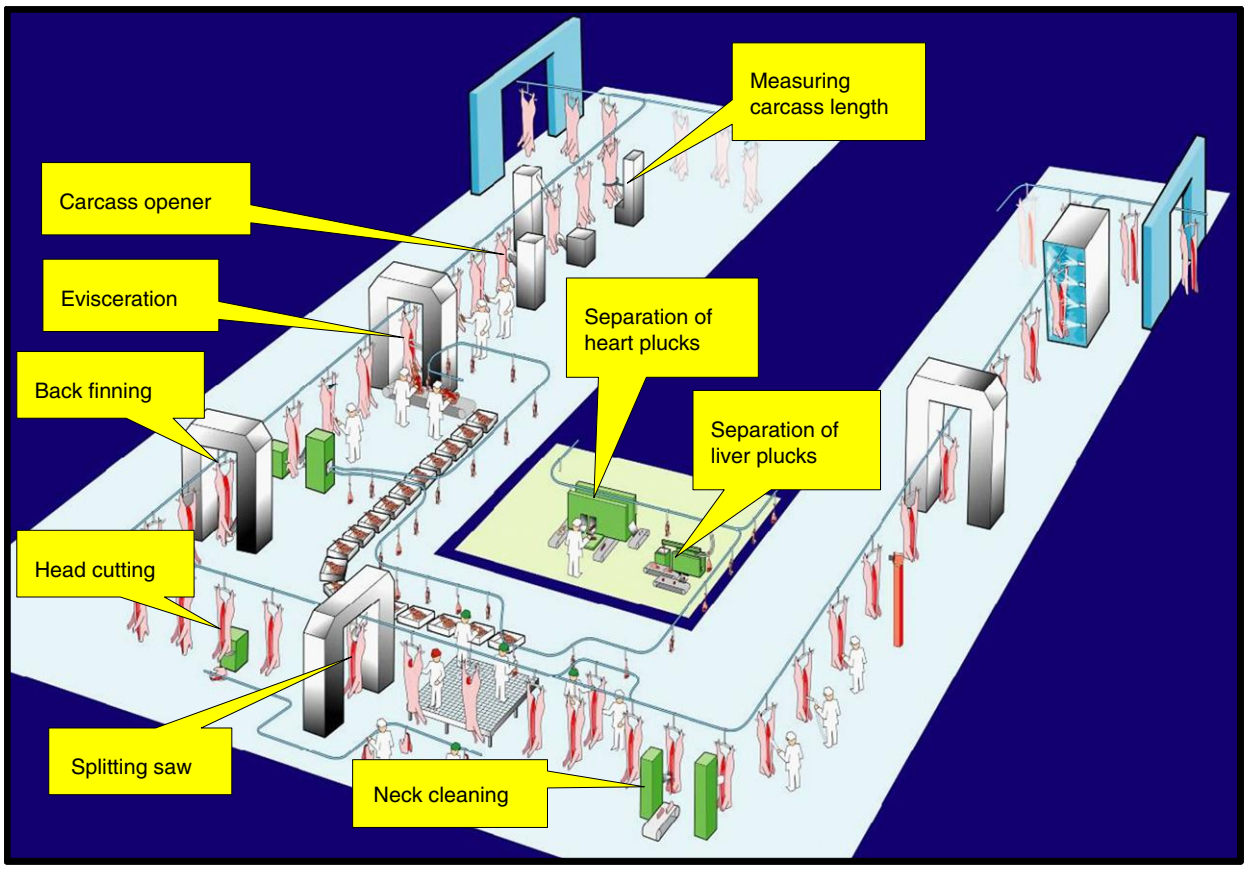

Figure 2 Clean pig slaughter line configured with state-of-the-art automation technology, 2011.

Figure 3 Automatic evisceration. The automatic evisceration machine cuts the diaphragm, tears off the flare fat, and loosens the intestinal tract such that the entire set of intestines and organs is removed from the carcass before separation (Anon, 2001a,b). Because the separation takes place outside the carcass, it can be performed consistently and reliably with lower risk of fecal contamination.

An alternative range of machines (*F*-line) for slaughter line automation is available from the Dutch company Meat processing systems (MPS). The *F*-line includes automated precutting, belly opening, rectum drilling, neck cutting, leaf lard removal, splitting, and marking.

Automation solutions based on the use of industrial robots are offered by the Germany slaughter technology company. The technology base is six-axle industrial robots in hygienic configuration, combined with high resolution visual recording systems and evaluation software as well as tools that have been designed especially for robot application. Robots are currently available for the following processes: fore-paw cutter, bung dropper, w-bone cutter, belly-and-breast opener.

Beef Slaughter Automation

Slaughter procedures for cattle are still very manual with little automation and limited technological development and application. Most of the development has been in the area of manually operated, power-assisted tools that have been improved to ease the physical work for operators or in the area of tools developed for improving the hygienic quality of slaughter.

The majority of cattle in the world are, however, still slaughtered at low line speeds. Most of the animals are slaughtered at meat plants run in single shifts at line speeds from approximately 30–75 h^{-1} and the plants are seldom specialized but are required to slaughter all types of cattle as they are delivered. This implies that new technology must be flexible and match the large biological variation in dimensions.

In the USA, larger meat plants have higher line speed up to approximately 400 carcasses per hour achieved by increasing

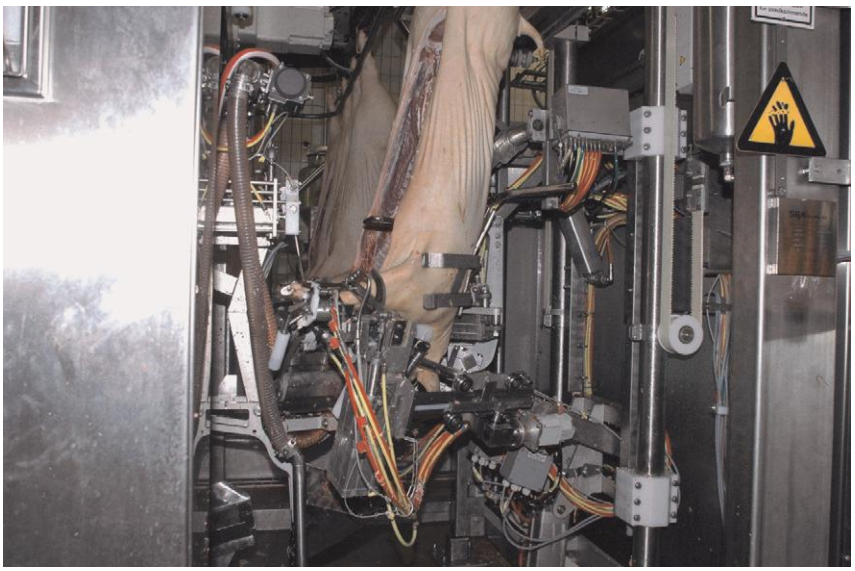

Figure 4 Automatic neck cleaning and cutting of forelegs. The machine automates some of the critical cleaning of the carcasses after carcass splitting. The machine automatically trims the neck region for glands and blood residues and from the breast part it removes the heart fat. Further, the machine cuts off the forelegs. This cut is done more precisely than by manual operation.

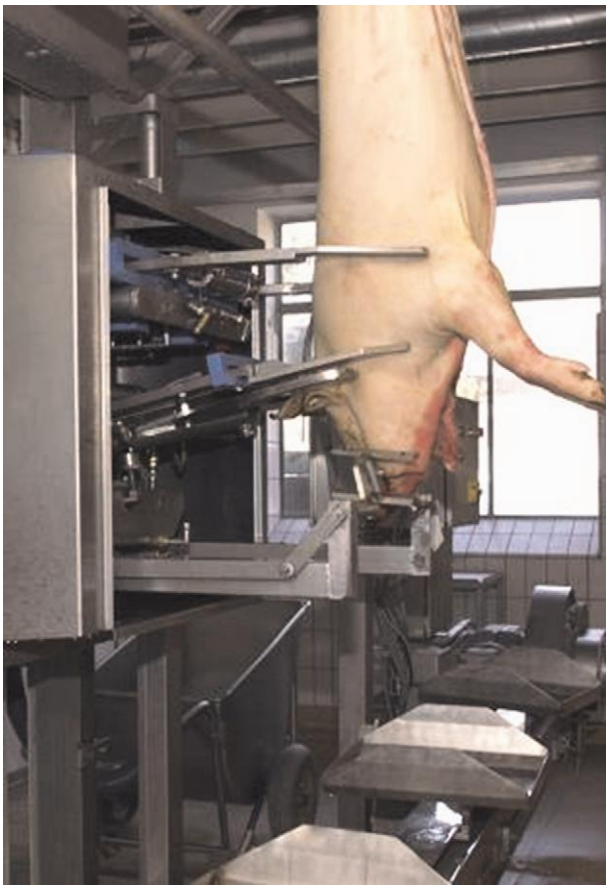

Figure 5 Head-cutting machine. The machine operates in two modes. It can either cut off the head entirely or leave it on a separate conveyor system or alternatively the machine can cut the neck only and leave the head hanging at the carcass by the jowls. Cutting off the head before carcass-splitting reduces the risk of contamination from the mouth and throat regions. The machine also provides better yield by leaving more neck meat on the carcass.

manning levels, sharing tasks across more operators, and by ensuring that the animals slaughtered are relatively homogenous in size, with slaughter lines tailored for steers and heifer. Often, equipment is duplicated and some tasks are progressively and sequentially performed by several machines, for example, hide pulling.

Lairage and Stunning

Moving cattle into a stunning box without electric prodders can be difficult. DMRI developed a stunning box with associated automatic drive gate, which gently pushes the animal into the stunning box; the system is used in different versions in several Scandinavian plants with a capacity of up to 60–70 animals per hour. Research has been carried out with automatic placement of the captive bolt stunner using vision and a standard industry robot, however, without any commercial uptake. In Australia, Meat and Livestock Australia (MLA) has undertaken research into automating the tracking of the head using machine vision to automate the stunning task (in conjunction with research into very high frequency stunning and alternative stunning technologies), but this has not progressed to commercial implementation. Automatic electric stunning boxes with head fixation are widely used in New Zealand and are in the market from Jarvis, New Zealand and BANSS, Germany. The box is controlled by an operator who activates the stun box for fixating the head, engages electrodes, and monitors the process. Although the electric stun with no penetration of the skull limits risk of special risk material (SRM) from the brain spreading, there are some reports of more incidences of blood splash in the muscles compared to captive bolt stunning. In the automatic electric stun box the operator has no interaction with the animal, which has been the case with captive bolt stunning without head fixation of the animal, a process that is required from 2013 in EU. Shackling is one of the most dangerous processes with reflex kicks injuring operators occasionally when putting on the chain. MLA has initiated research to automate this process by developing a prototype shackling tool, which will put on the chain by a vision-controlled industrial robot.

Hock Cutting

The first standard robot to be introduced on the beef slaughter lines is used for cutting hocks of forelegs. This solution is available from Jarvis, USA, and also trialed in Australia by MLA and Machinery and Robotics (MAR) including clipping of horns.

Dehiding

The dehiding process is one of the most mechanically assisted steps with many different versions of mechanic hide pullers in place. Guidance from an operator is crucial to achieve high hygienic quality. In the US, where line speeds are high, the process is performed in three consecutive steps. The ideal fully automatic process for this has yet to be developed. Nearly all hide-pulling equipment requires a human operator to attach the equipment to the hide, and has minimal sensing and no automated feedback to modify its action.

Bunging and Sealing

During the 1990s, a semiautomated beef bung bagging machine was developed in Australia by Australian Meat Technology Pty Ltd. (AMT) and MLA. This machine is a mechanical aid that provides a high degree of reliability in carrying out the bunging operation and delivers a significant improvement in hygiene through the reduction in the risk of contamination, see Figure 6. This machine is commercially marketed by SFK Systems A/S.

Carcass Opening

In the 1990s, Industrial Research Limited (IRL), New Zealand, developed an automated beef belly rip robot. This used a proprietary robot to perform the opening cut on beef animals. This was evaluated in North American beef plants but was unable to deliver the required performance at the throughput required, or cater for the variability in stock and stock

Figure 6 The AMT beef bunging machine. Photo courtesy of Meat Processing Systems (http://www.mps.org.au).

presentation, largely due to the limitation of the technology available at the time. With technology advancements, there is renewed interest in revisiting this and other automation challenges along the slaughter board.

Evisceration

Taking out the organs is arduous work and critical for slaughter hygiene. So far no viable automation or aid for evisceration is available to assist the operator. Some relief for the operator is gained from still more adaptive moving lift platforms that allow better positioning of the operator both in vertical and horizontal direction. Modern platforms are also being equipped with automatic cleaning of areas that may touch the carcass.

Carcass Splitting

Band saws operated manually is the predominant tool for splitting beef carcasses even at the highest line speed but with duplication of operators and saws. Circular saws mounted in a dedicated machine were used for automatic splitting in Sweden for many years. Automatic circular splitting saws are, for example, provided by MPS Meat Processing Systems, The Netherlands (MPS). However, it has been reported that the saws that are mechanically controlled require monitoring and adjustment for delivering a sufficient precise result similar to what can be achieved be a skilled operator with the ability to correct for carcass differences. MLA and MAR in Australia have researched using band saws mounted on standard industrial robot controlled by different sensing principles, for example, ultrasound and X-ray. The current technical limitation of this technology is in the sensing, which is yet to reach the level of performance and robustness required to justify commercial implementation and industry uptake. Correct placement and positioning of the carcass properly at the start of the process also adds to total automation cost, placing the technology out of reach of lower throughput plants. DMRI proposed to combine splitting and spinal cord removal in one machine to make it more cost-effective. The feasibility of this combination remains to be shown.

Cleaning of the Carcass

Slaughter hygiene is essential and steam vacuuming of contaminations, bone dust, and fragments are processes tying up several operators. Likewise, SRM removal from the carcass requires operators and equipment. Presently these operations are manual but trials with standard robots operating the steam vacuuming tools have been carried out by MLA and MAR. In the USA, carcass decontamination also involves cabinets from companies such as Birko (USA) for hide washing after sticking and different cabinets for washing, steaming, or mild acid spraying during the slaughter process.

Classification and Grading

Traditionally carcass classification was an entirely manual process. Vision systems became commercially available in the mid-1990s but commercial uptake was slow, but with the advent of more robust technology and ongoing development to improve the performance, vision-based classification and grading systems are now a mature technology. Vision systems for classifying carcasses according to EUROP conformation and fat classes, as well as rib eye cameras for automatically predicting United States Department of Agriculture (USDA) quality grades, are now in common use. In the 2000s, uptake increased as EU regulations for approval of machine grading were enforced, and likewise USDA approved rib eye camera grading. There are several suppliers of technology, some supplying local markets only, whereas some are marketing globally, for example, Cedar Creek Company, Australia, Carometec, Denmark, e+V, Germany, NORMACLASS, France, and Research Management Systems (RMS), USA.

Ovine Slaughter Automation

New Zealand and Australia are the world's largest exporters of lamb and mutton, with more than 90% of all lamb and mutton slaughtered being available for export. Over the past few decades, the New Zealand meat industry has invested heavily in the development of mechanical and automated slaughter and dressing technology for sheep and lambs, resulting in the vast majority of the annual kill being slaughtered in modern, up-to-date processing facilities. Although the main driver for automation has been to reduce the cost of labor, there has been a strong focus on maintaining stringent hygiene (critical when the vast majority of product is exported chilled to distant markets by sea) and to improve the quality of hides and related coproducts so as to extract the maximum value from each and every carcass.

Inverted Dressing

Mechanization and automation on the slaughter board had its genesis in the development of inverted dressing in the 1970s. Inverted dressing enables the hygienic processing of lamb

carcasses where the depelting procedure starts from opening cuts in the (cleaner) forequarter region, and the pelt is removed downwards toward and off the (more contaminated) hind legs. Traditionally, this process involves both manual (e.g., tunnel punching) and mechanical (e.g., shoulder and hide puller) operations that are labor- and time-intensive and can result in downgrading of the fleece if not conducted consistently.

The inverted dressing system has many benefits, including a significant reduction in manpower. However, the most significant benefit is that, with the carcass suspended simultaneously by the fore- and hind legs (called the hammock position), it is now possible to introduce automation to key tasks.

The majority of mechanization and automation has been applied to the pelting and evisceration stages, justified by the combined benefits of increased hygiene, reduced contamination and labor costs, and increased worker safety. In countries where major export customers include Muslim markets, religious requirements have impacted somewhat on the degree to which the stunning and bleeding tasks can be automated.

Mechanization for Task Replacement

Initial developments were purely focussed on creating direct mechanical alternatives to specific tasks on the slaughter chain. Mechanization was seen as a logical replacement for manual workers where the task was considered highly repetitive and routine or required significant force. Mechanical equipment was developed to replicate the key action required but still required human interaction to grip or position part of each carcass appropriately, or to perform the correct prework, to ensure correct operation. Many of these used minimal or no sensing or measurement, and were programmed simply to perform the same task repeatedly with no modification. For example, the shoulder and final pelt puller machines, originally developed by the Meat Industry Research Institute of New Zealand (MIRINZ) and commercialized by Millers Mechanical Ltd. (now Milmeq Ltd, New Zealand), once synchronized with the chain, performed their tasks without any input or feedback on the variation in size, shape, or presentation of each carcass. Additionally, the shoulder puller required an operator to clamp the foreleg pelt regions before activating the machine's operational cycle. Although the final puller automatically gripped the pelt as it hung below the carcass, additional manual workup was sometimes required to ensure satisfactory pelt removal for all variations in carcass age, size, breed, and condition. The design of these machines was in part determined by the relative immaturity of commercially available sensing and automation systems available at the time.

Where full task replacement was not deemed cost-effective, semiautomated or task-assistance machines have been developed and are now effectively deployed on the chain. These machines require manual guidance and, while making a task easier, faster, and safer, often contribute to a reduction in labor units but do not eliminate labor from the chain. Examples of these include the brisket roller machine and flanking tool, which both require manual operation and hands-on guidance but significantly reduce the effort required to do their respective tasks and thus reduce both the effort required and overall manning levels required, particularly at higher chain speeds.

A further implication of this task-replacement approach to automation has been that consideration to subsequent tasks and in particular the controlled positioning of the carcass for subsequent tasks has not been incorporated in equipment design. For example, rear hock removal is often undertaken once the pelt has been fully pulled down over the rear legs as a sock. However, pelt removal often results in overall carcass length increasing by some 10% or more due to the downward forces imparted on the carcass, therefore potentially rendering any previous carcass measurement invalid as an input to subsequent automation tasks. Although it is technically possible from an automation perspective to cut the hocks as part of the pelt pulling operation, existing hock cutting equipment has been developed as a separate piece of equipment (mostly due to commercial drivers).

One of the enablers of subsequent developments in automation has been the technological advancement in sensing, automation systems, and industrial robots. Many of the mechanical open-loop machines developed initially have been updated with more advanced sensing technology and augmented by more modern robotics to enable them to accommodate carcass-to-carcass variation as appropriate.

Table 2 gives an overview of the key tasks for a typical ovine slaughter board, along with task-replacement automation currently available.

In addition to these commercially available systems, a number of developers are working on automated solutions on a number of other tasks, including but not limited to:

- Front and hind hock tipping (clean cut).
- Tail cutting.
- Neck sanitizing.
- Bung cutting.
- Fully automated sock ringing.
- Automated gas depleting.
- Bung sealing.
- Foreleg rolling and fully automated brisket rolling.
- Sock splitting.

Robotic Automation

More recently, developments in Australia and New Zealand have seen the next generation of automation that does incorporate sensing feedback to customize each robot's operation according to specific carcass measurements. These developments have combined robots with custom end effectors and sensing technology to automate key tasks on the slaughter chain that cannot be automated by simple open-loop mechanical systems due to the complexity and degree of variation that exists between carcasses.

One of the first developments of this kind is the Y-cut robot, which automatically makes the opening cut in the pelt down the inside of each foreleg and the neck, see Figure 7. This development was a collaborative initiative by IRL, Motion Design Ltd., New Zealand, and New Zealand Meat Research and Development Council (now Beef and Lamb New Zealand). The original prototype used a proprietary robot developed specifically for this task but current commercial

Table 2 Overview of the lamb slaughtering processes and automation

Subprocess	*Tasks*	*Manual/automated*
Stun and bleed	Stun	Mechanical
	Throat cut and stick (Halal)	Manual
	Bleed	
	Hindleg hang	Manual
Pelt opening	Anus removal	Manual
	Y-cut and forequarter workup	Robotic
	Hang forelegs	Manual
	Brisket clear	Manual/mechanical assist
	Sock cut	Manual
	Cod removal	Manual
	Head removal	Mechanical
	Invert carcass	Manual
	Carcass decontamination – forequarter and hindquarter	Robotic
Pelt removal	Workup	Manual/mechanical assist
	Shoulder puller	Manual/mechanical assist or robotic
	Final puller	Mechanical
	Rotary puller	Mechanical
	Hock removal	Mechanical
	Invert carcass	Manual
Evisceration	Brisket cut	Robotic
	Carcass invert	Manual
	Kidney fat removal (Australia)	Robotic
	Evisceration	Robotic
Grading	Trim	Manual
	Inspection and detain	Manual
	Grading	Manual/vision

versions now use third-party commercial industrial robots, with custom sensing and end effectors to measure the carcass and make the opening cuts, respectively. Systems are currently in operation on both Australian and New Zealand meat processing plants at throughput rates of up to 8 carcasses per minute, and have demonstrated significant improvements in carcass hygiene compared to manual operation.

Another example where sensing has further enhanced automation is the variable independent path (VIP) Shoulder Puller, developed by Milmeq in conjunction with Alliance Group Limited, one of New Zealand's largest ovine processors and exporters. This machine, shown in Figure 8, based on the original MIRINZ shoulder puller, uses variable force feedback and enhanced grip design to autoload, eliminating the labor unit previously required to load the machine and delivering a more uniform pull, resulting in less pelt damage.

At present there are several autoloading pelt removal machines, including the Pelt-O-Matic, developed in Australia and now marketed by Food Equipment Australia as well as SFK Systems in Europe. The Pelt-O-Matic is based on the original four-pronged puller developed by MIRINZ in the 1990s. It is an automated rotary system for pelt removal that comes

Figure 7 IRL's Y-cut robot opens the pelt inside each leg. Photo courtesy of Callaghan Innovation, formerly IRL, and Ovine Automation Limited.

in a number of configurations covering 2–10 carcasses per minute.

The combination of custom-sensing technology and standard industrial robots has also been applied to individual human task replacement on the slaughter board. In conjunction with MLA, MAR has developed fully robotic machines that can directly replace manual operators for the following tasks:

- Kidney fat removal.
- Vacuum sanitization of the neck, forelegs, brisket, and hind legs.
- Brisket cutting using a Jarvis brisket cutter (carcass inverted).
- Neck and tail tipping cut.
- Hock tipping and cutting.

Because each of these machines is designed to operate autonomously and automates only a single task, each machine is fully self-contained and incorporates the necessary sensing equipment, robotic arm, and end effector. Further, each sensing system is proprietary and has been developed specifically for the specific task. This has resulted in quite extensive development costs which, when combined with the sensing and robotic hardware costs result in a significant capital cost for each system. With the ovine processing industry looking for a payback period normally lower than 24 months (two seasons), the cost–benefit of these systems will depend significantly on the number of carcasses processed each year, throughput (carcasses per hour), number of shifts per day, and slaughter board configuration (order of tasks). Often, in smaller plants, automation does not fully replace a complete person due to the fact that, as well as the task being automated, the person is also performing other related tasks due to the chain configuration and speed. This has limited the industry uptake of these developments to date.

This limitation of focussing on automation from a direct task-replacement perspective is currently being addressed through a more integrated approach to slaughter board automation. An example of the task-integration approach is the

Figure 8 Milmeq's VIP shoulder puller.

(a) (b)

Figure 9 Brisket-cutting robot (a) and evisceration robot (b). Photos courtesy of Milmeq Ltd., New Zealand, and Ovine Automation Ltd., New Zealand.

recent development of robotic brisket cutting and evisceration equipment by Ovine Automation Limited (OAL). OAL is a consortium of nine major New Zealand ovine processing companies, with support from New Zealand Ministry for Science and Innovation and MIRINZ Inc. (OAL), see Figure 9. These two items of automation have their genesis in the mechanical brisket cutter and evisceration machines originally developed by MIRINZ in the 1990s and are currently in commercial operation in one New Zealand lamb processing plant. Although these two machines automate tasks previously undertaken manually as stand-alone machines, OAL has also undertaken further development that has extended the impact of these machines beyond simply direct labor replacement. The brisket cutter is designed to open the brisket in a manner that is directly compatible with the evisceration robot. Further, the evisceration robot directly places the gut set and viscera into the gut trays and has facilitated a new approach to viscera inspection and sorting. As a result, rather than just replacing between one and two labor units, a brisket cutter robot and evisceration robot have the potential to eliminate up to five labor units on a typical 8-min lamb chain per shift. OAL is also currently pursuing an integrated approach to automating

the opening up of the pelt down the forelegs and belly of the carcass.

The benefit of this approach is that automation is able to address a suite of operations systematically rather than in isolation, with consistency and integration of process control and operating components leading to cost and development savings. More importantly, this approach enables the overall process and order of tasks from a systems perspective, enabling compound rather than incremental improvements to be achieved for a wide range of throughput operations.

Classification and Grading

As with beef and pork, carcass classification was traditionally a manual process, based on hot carcass weight, fat depth at the GR site (fat depth at the 12th rib 11 cm from the vertebrae), and sometimes combined with a subjective conformation (carcass shape) assessment, with the majority of plants still using manual grading. Although fat depth probes are widely used in the pork industry (e.g., Hennesey GP-2, Hennesey Grading systems, New Zealand, and Ultra-FOM, Carometec, Denmark), they have not seen widespread uptake for sheep. Vision systems for grading (based on one or more 2-D color images of the carcass taken at the grading station) are supplied from several companies such as Cedar Creek Company, Australia; e+V, Germany; and RMS, USA, being the most common of automated vision systems in use for ovine.

See also: Automation in the Meat Industry: Cutting and Boning. Carcass Chilling and Boning. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Nutrition of Meat Animals: Pigs. Species of Meat Animals: Cattle

Further Reading

Anon, 2001a. Slaughter-line for pigs is completed. An automated evisceration process is integrated into the slaughter-line at Danish Crown. Fleischwirtschaft 81 (5), 111–115.

Anon, 2001b. Sheep and lamb dressing. Meat Technology Update 01/6, MLA.

Folkmann, P., Christensen, F., 2003. Innovation and advanced technology behind a new machine for automatic cutting of pork middles. Fleischwirtschaft 83 (3), 89–91.

Hansen, F., 2004. Robot ready for 47 million fore-ends. Fleischwirtschaft 84 (1), 31–32.

Madsen, K.B., Nielsen, J.U., 2002. Automated meat processing. In: Kerry, J., Ledward, D. (Eds.), Meat Processing – Improving Quality. Abington: Woodhead Publishing, pp. 283–296.

Madsen, K.B., Nielsen, J.U., 2004. Encyclopaedia of Meat Sciences I. Amsterdam: Elsevier, pp. 43–49.

Madsen, N.T., Nielsen, J.U., Mønsted, J.K., 2006. Automation – The meat factory of the future. Proc. ICoMST.

Nielsen, J.U., 1999. Automatic evisceration of pigs. Meat Automation – Proceedings on Special User's Meeting on New Automation Technologies for Meat Production. Belgium: Brussels, pp. 78–81.

Purnell, G., Loeffen, M., 2006. Automation for the modern slaughterhouse. In: Nollet, L.M.L., Toldrá, F. (Eds.), Advanced Technologies for Meat Processing, third ed. US: CRC Press, pp. 43–71.

Zink, J., 1995. Application of automation and robotics to pig slaughtering. In: Proceedings of the 41st Congress of Meat Science and Technology, San Antonio, TX, USA. pp. 420–424.

Relevant Websites

http://www.jarvisengineering.com
Automatic meat processing machinery supplier.

http://www.banss.de
BANSS, Germany GmbH, supplier of robotic solutions for pig slaughter lines.

http://www.butina.eu/products/backloader/
Butina, Denmark delivers systems for CO_2 stunning of pigs in groups.

http://www.fea.net.au
Food Equipment Australia offers design, manufacture and installation of sheep, cattle and goat processing equipment and slaughter systems.

http://www.kjindustries.com
KJ Industries, Denmark delivers combination equipment for combining scalding, dehairing and singeing.

http://www.redmeatinnovation.com.au
MLA web site containing an overview of MLA-supported automation developments in the red meat sector.

http://www.mps-group.nl
MPS meat processing systems, Holland supplies the F-line range of automated machines for pig slaughter.

http://www.milmeq.com
Ovine slaughter board automation.

http://www.machineryautomation.com.au/
Robotic automation for beef and small stock slaughter and boning operations.

http://www.sfk.com/
SFK Systems, Denmark delivers a range of automatic pig slaughter line machines.

http://www.danishcrown.com/page22686.aspx
Virtual tour of the world's most modern pig slaughterhouse, Danish Crowns facility in Horsens, Denmark.

B

BACON PRODUCTION

Contents
Bacon
Wiltshire Sides

Bacon

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Introduction

There are a variety of products that fit under the bacon name, including: belly or streaky bacon, pancetta (round bacon), Wiltshire bacon, Canadian-style bacon, jowl bacon, shoulder bacon, cottage bacon, beef bacon, etc. The article on Wiltshire bacon covers basic processing and this article will focus on the production of pork belly bacon that is popular in the United States.

Belly Bacon

Belly bacon has significantly increased in popularity over the past 20 years or so. As recently as 1978, an American Can Company survey of 2000 US female heads of households found that 38% of households were consuming less bacon than previously, primarily because people were either eating breakfast less frequently, found bacon to be too expensive, were eating fewer eggs, or were on diets that restricted their bacon consumption (1978). As late as 1989, it was stated that "bacon consumption is evidently in a long-term eroding trend…".

In the early to mid-1990s, bacon shifted from being primarily a breakfast item to being added as a condiment to many quick-serve hamburgers. By this time, McDonalds had started adding round bacon to their hamburgers. Also, as a result of many quick-serve restaurants increasing the doneness of hamburgers to 'well-done,' bacon was added to improve the palatability of these hamburgers. At about the same time, precooked bacon became available, which provided a less messy, more convenient bacon for both retail and food service customers. As a result of the increased convenience, consumers started using precooked bacon on 'bacon lettuce and tomato sandwiches' (BLT's) and other sandwiches at meals other than breakfast. Belly bacon consumption has increased around the world, partially due to the introduction of precooked bacon slices and use of bacon bits on salads and other entrees, bacon as a pizza topping, and due to the introduction of Western breakfasts in international hotels. The form that bacon is sold in has expanded, to include diced, strips, and ground, in addition to the more traditional shingled, layout, bits, and stack pack.

In recent years, bacon has had nearly a cult following, with new products containing bacon or bacon flavor, such as confectionary products (doughnuts, chocolate, etc.), alcoholic beverages, sausage products, ice cream, etc. The most recent addition to this list may be bacon jerky.

History of Bacon

It is generally believed that bacon originated in the British Isles, however, there are other claims that the first bacon was

made in China. The word bacon appears to have originated from the Germanic word bakkon, which meant the 'back of the animal.' It became 'bacon' in French, and the English started using the word in the twelfth century to mean cured back bacon. Bacon in the United States refers to belly bacon. It is not clear, but belly bacon may have originated in the United States.

Precooked bacon may also have been developed in the United States. It has become popular as it is convenient for the end user, and is shelf stable, not requiring refrigeration. Rendered bacon fat resulting from this process is considered of higher quality than traditional bacon grease, as it is generated at lower cooking temperatures. This rendered bacon fat is used in other food products as a flavoring ingredient. Disadvantages of precooked bacon are reduced aroma and flavor, and a chewier texture, compared to traditional pan-fried bacon. The flavor problem has been remedied by increasing concentrations of ingredients that contribute flavor, such as salt, sweeteners, liquid smoke, etc.

The pork belly, from which belly bacon is made, has the notoriety of being the only meat cut to be traded on the Chicago Mercantile Exchange. Beginning in 1961, pork bellies were traded for 50 years in the futures market as a hedge against fluctuating pork prices.

On the other hand, pork bellies have been largely ignored in swine genetics research, as traditionally hams and loins were more valuable to processors than the bellies. As hogs have been bred to produce leaner carcasses, bellies have become leaner which results in less desirable bacon. Because of their monogastric stomachs, the diets of pigs will be reflected in the quality of the fat. Current use of distillers grains, a by-product of the ethanol industry, as a feed source, results in softer bellies, which is also not good for producing high-quality bacon.

The Raw Material Used for Bacon Production

Belly bacon is produced from pork bellies. The bellies are skinned or derined and the ribs and sternum are cut off as a sheet (spare ribs). The flank muscle is typically removed from the belly (called facing or robbing the lean) to reduce the chances of developing pickle pockets. The teat line is removed from the ventral edge of the belly to eliminate the presence of mammary glands in the final product. The complete specification for trimming pork bellies in the United States, according to the National Association of Meat Processors (NAMP, 2010) is as follows:

> The belly is prepared from the side after removal of the leg, shoulder, loin, fat back and spareribs, bones and cartilages, and practically all leaf fat, shall be excluded. The fat back shall also be excluded by a straight cut not more than 1.5 in. (3.8 cm) from the outermost dorsal curvature of scribe line. The anterior (shoulder) and posterior (leg) ends of the belly shall be reasonably straight and parallel. No side of the belly shall be more than 2.0 in. (5.0 cm) longer than its opposing side. The width of the flank muscle (rectus abdominis) shall be at least 25 percent of the width of the belly on the leg (sirloin) end. The fat on the ventral side of the belly and adjacent to the flank shall be trimmed to within 0.75 in. (19 mm) from the lean. The area ventral to the scribe line shall be free of scores and "snowballs" (exposed areas of fat) that measure 3.0 square inches (19.4 sq. cm.) or more. The belly shall be free of enlarge, soft, porous, dark, or seedy mammary tissue. The scribe line is not considered a score but shall not be more than 0.25 in. (6 mm) in depth at any point.

The weight of pork bellies after skinning and trimming is 65–85% of the skin-on weight, depending on how closely the bellies are trimmed. The amount of lean or fat that is removed during squaring up the ham end, or how far the back fat edge is trimmed from the scribe line, will determine the trimming yield (Figures 1 and 2). The amount of trimming that is done to bellies will determine the quality and cost of the final bacon.

Lighter-weight bellies are more commonly used for precooked bacon, which is sold by the slice. However, heavier bellies are more commonly used for making retail bacon, which is sold by the pound. Bellies are sorted or graded by weight, in 2 lb increments. Within weight ranges, bellies may also be sorted by thickness (Figure 3).

Belly bacon is primarily made in the United States from fresh (never frozen) bellies, however, using frozen bellies may be necessary to survive seasonal fluctuations in belly prices and availability. Obviously, frozen bellies present additional problems to processors, particularly the need to defrost the

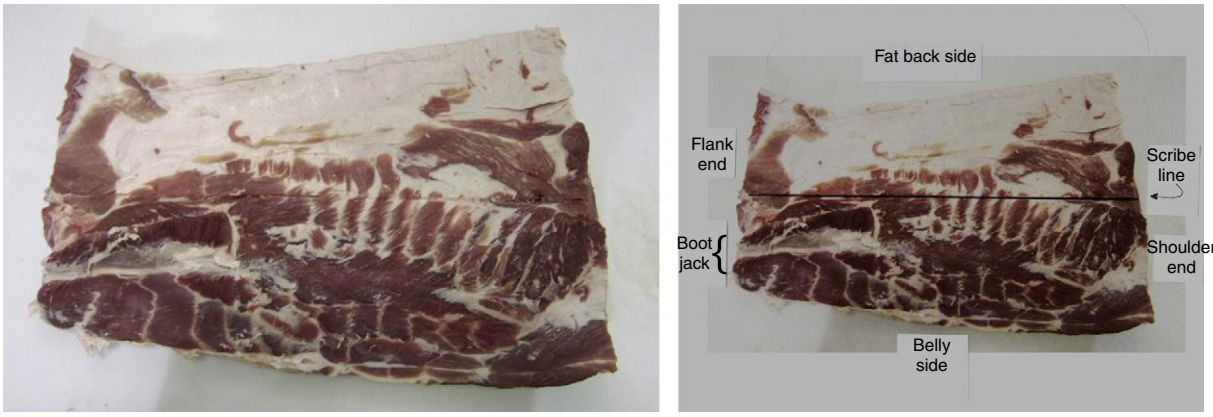

Figure 1

frozen bellies before curing. However, if done properly, using frozen bellies can be profitable for a processor. Defrosting was traditionally done by submerging the frozen bellies in water, but is currently done primarily by air tempering.

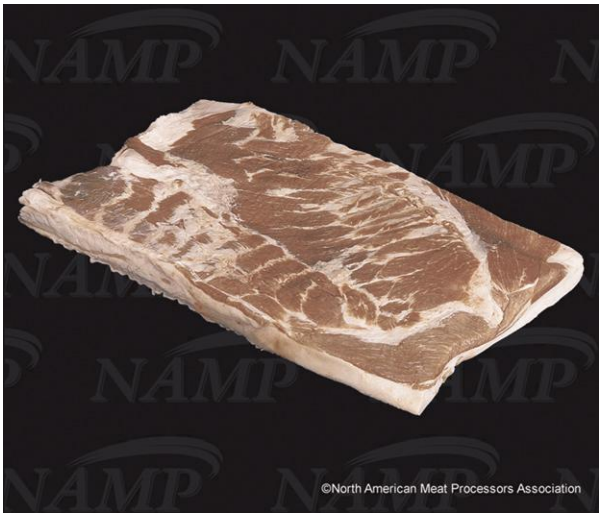

Figure 2

Fat is the most variable component of meat, and likewise, pork bellies are the most variable cut made from a pork carcass in terms of composition. Sorting bellies by thickness results in more uniform bacon, than sorting by weight.

The increase in leanness of pigs, as well as some of the ingredients that some pigs are currently fed, has resulted in a relatively higher content of unsaturated fat in the pork. Higher percentages of unsaturated fatty acids results in softer belly fat, which makes high-speed slicing difficult. Also, since fat is a major contributor to the flavor of bacon, leaner bellies may not be as desirable for making bacon as fatter bellies.

Curing Methods

Curing is the process of adding nitrite, as well as salt or sugar to meat to preserve, and enhance the color and flavor of the meat. To make bacon, pork bellies are cured with one or a combination of the following methods.

Injection

Currently, the most common method of curing is done by injecting a curing solution into the bellies, which is referred to

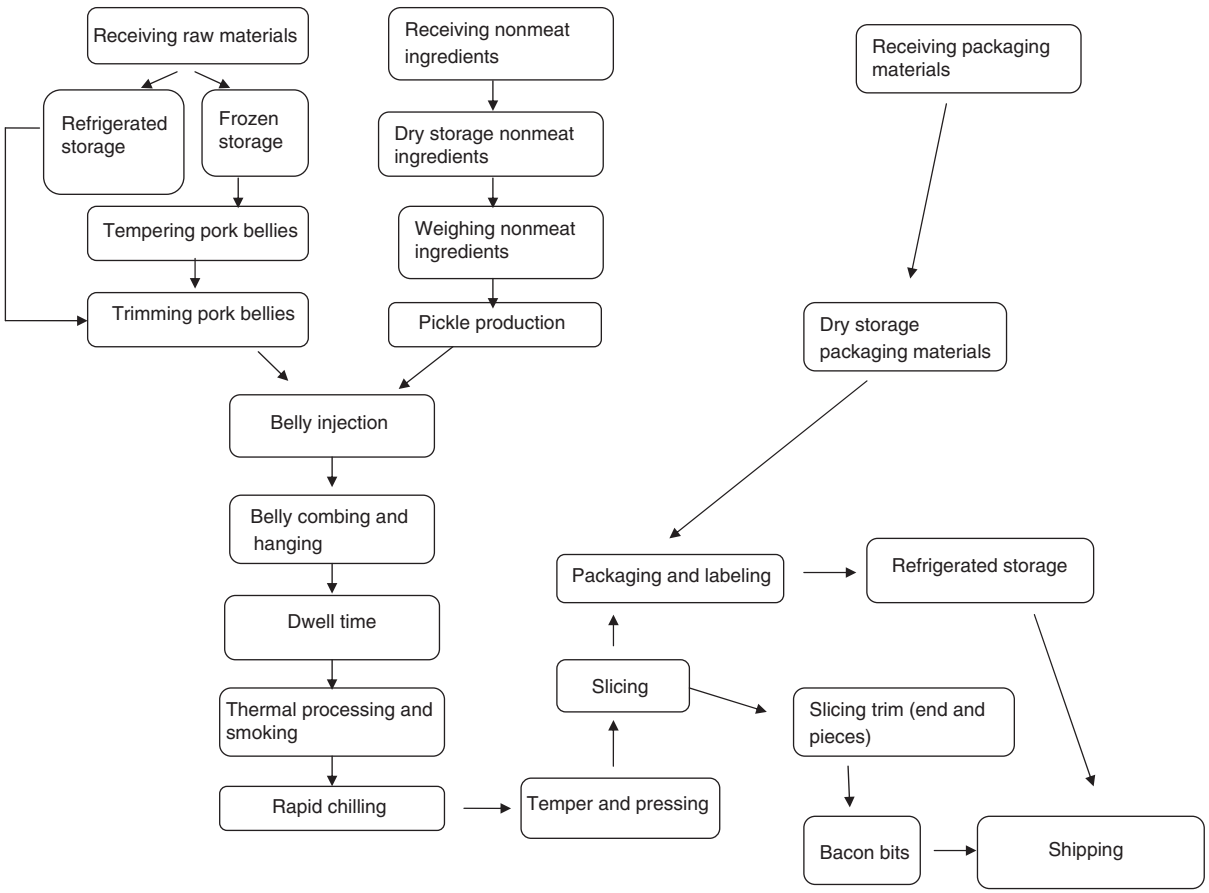

Figure 3

as stitch pumping or pickle curing. Initially, this process involved the use of a hand pump, but after World War II, more automated injectors became available.

The basic ingredients used in bacon curing solutions and their functions, include:

- Sodium chloride – flavor and preservation
- Sodium nitrite – cured color development and stability, contributes to cured meat flavor, and reduces oxidation (development of rancidity) of fat
- Ascorbate or erythorbate – curing aids that accelerate color formation and stabilize the color
- Phosphates – added to improve water-holding capacity and to reduce oxidation (development of rancidity) of fat and lean
- Sugars – for example, dextrose and glucose, counteracts salt flavor
- Spices – flavor contributors.

The order of addition of the above ingredients to water is very important. If phosphates are to be added, they need to be added to the water as the first ingredient. It is important that the phosphates are well dissolved before adding additional ingredients, particularly salt, otherwise ingredients added subsequently will precipitate out and accumulate as a white sludge on the bottom of the mixing tank.

Mixing or agitation of the curing solution is helpful in getting phosphates and other ingredients dissolved in water, however, caution should be taken to avoid over-mixing, which could whip air into the solution. Injecting foamy brine into bellies should be avoided.

In the United States, regulations require that bellies shrink back to their green (or preinjected) weight, during the smoking process, so typically bellies would be injected at an uptake level of 11–12% of the green belly weight. To calculate this level of solution uptake, a 'drip time' is typically assigned between the injection process and the final weighing process. This drip time should be kept consistent to make comparisons between batches of product. Other countries may not have the weight gain restriction, so bellies might be injected to higher percentages in those countries.

Even and consistent distribution of the curing solution is important in making bacon, and meeting injection targets for solution uptake is important to producing a consistent product. Whereas pump pressure, conveyor speed, and needle design are important in consistently meeting injection targets, consistency of belly temperatures during the injection process may influence solution uptake more than any other factor. A larger number of needles combined with warmer belly temperature will result in being able to reduce injection pressure, which will ultimately reduce incidence of pickle pockets.

After the curing process, comb hooks are placed in the flank end of the bellies, entering from the lean side, and the bellies are hung on smoke racks. It is important to catch the cutaneous trunci muscle, with the comb, to hold it in place during the smoking process. Improper combing can reduce the yield of good bacon slices by more than 8%. A drip period before smoking helps to prevent streak marks from the smoke on the smoked slabs. Some recommend a holding time of up to an hour after the last bellies are injected before the smoking process begins for improved uniformity in cured color across an entire batch of product.

Dry-Curing

Before the availability of injection systems, bellies could have been cured by either a dry-cured process, an immersion process, or a combination of the two. Dry-curing involves surface rubbing of bellies with the curing ingredients and holding the rubbed bellies for 7 days per inch of belly thickness before smoking to obtain even cure distribution. With dry-curing, sodium nitrate was used in addition to nitrite because of the additional curing time. Dry-curing is sometimes used in modern processing operations to make food service bacon, as this bacon shrinks and curls less than injected bacon during the final cooking process.

Immersion Curing

Immersion curing involved submerging the meat into a curing solution, which resulted in uniform distribution of curing ingredients throughout the meat more quickly than the dry-curing method.

Either the injected or dry-rubbed bellies may be immersed in a solution, or cover pickle, until they are smoked. Injected bellies are typically held for only 2 or 3 days in the cover pickle.

Cooking and Smoking

Traditionally, belly bacon was not fully cooked, but is only cooked sufficiently to apply a good surface smoke color and to firm up the slab to allow for efficient slicing. Typically, the endpoint temperature for bellies during smoking ranges between 52 and 53 °C (126 and 128 °F). Therefore, in the industry this process is often called smoking, rather than cooking.

Fully cooked bacon strips are cooked using conveyorized, microwave ovens, using a conveyor belt system that holds slices flat to minimize curling during the cooking process. This product is considered shelf stable, not requiring refrigeration after packaging.

Bacon bits are typically deep fried.

Post-Cook Chilling

After cooking, bellies should be rapidly chilled to 5 °C (44.6 °F), then more slowly chilled from 5 to −6.5 °C (20.3 °F) to allow the fat to set up properly. Chilling too rapidly can result in the formation of ice crystals in the cooked bacon.

Tempering

Before pressing, bellies are tempered to temperatures of −5.5 to −3.3 °C (22–26 °F) for best results. As salt content is reduced in bacon, forming temperatures can be increased to get the same sliceability. The ideal slab temperature for slicing will

vary, depending on the percent salt and injection levels of the bacon and the type of slicing equipment used. Lower salt bacon would require lower temperatures for good sliceability.

Pressing/Forming

Cooked and chilled bellies are pressed hydraulically into a rectangular shape that will result in more uniformly shaped bacon slices. Slab widths typically vary from 9.5 to 11 in., but the slab lengths may vary depending on the level of trimming done to the belly.

Slicing and Packaging of Bacon

The majority of bacon is still retailed as sliced bacon, and slicing is, therefore, a central operation in the production of many bacon products. This is the step in the process in which a processor determines how much number one product they have to sell to their customers.

Bacon slice thickness is referenced in the meat industry by the slices per pound or per kilogram. These designations include:

Thick sliced bacon	11–16 slices per pound (7–8 per kg)
Regular sliced bacon	16–22 slices per pound (8–10 per kg)
Thin sliced bacon	22 and up slices per pound (>10 per kg)

Shingle-sliced bacon is typically vacuum-packaged in 1 lb or part kilogram quantities for retail sales, using a paper L-board for the label and to support the product.

Layout bacon involves slices being placed edge-to-edge on paper for convenient loading of bacon onto oven sheets or griddles for quantity preparation of bacon. Since this product is intended for food service, it is sold by slice numbers and is packaged in a modified atmosphere to reduce the incidence of slices sticking to the paper.

Precooked, or fully cooked, bacon is typically sliced at 22–24 slices per pound (10–11 per kg), although it is sold in food service on a per slice count, rather than by weight. Precooked bacon thickness is more commonly described as slices per inch, and the most common count is 13–15 slices per inch (range: 8–16 slices per inch or 3–5 slices per kilogram).

Precooked bacon is packaged in a modified atmosphere package. Bacon jerky is an adaptation of precooked bacon made by fully cooking thick slices of bacon using the microwave process.

Canned bacon was more common in the past, and was used for US military rations, but can once again be found, particularly in camping supply stores.

Microbiology, Color, and Flavor Development

The initial microflora on bacon is that originating from the carcass, but the bacon can be contaminated via brine, especially when recirculated. Vacuum packaging results in a succession of microflora changes. While micrococci initially dominate, lactobacilli will eventually dominate. The salt content prevents putrification, but salt-tolerant bacteria (e.g., lactobacilli) will grow and result in a souring and reduction of shelf life.

The characteristic red color of bacon is formed during the curing process when the muscle pigment myoglobin forms nitrosylmyoglobin, involving reduction of nitrite. The cured flavor is a characteristic of nitrite-cured meat products and is found to be one of the most important quality attributes.

See also: Bacon Production: Wiltshire Sides. Chemical Analysis for Specific Components: Curing Agents. Curing: Brine Curing of Meat. Production Procedures. Packaging: Modified and Controlled Atmosphere; Overwrapping; Vacuum. Smoking: Liquid Smoke (Smoke Condensate) Application. Traditional

Further Reading

Anonymous, 1978. What's Happening to Bacon? Meat Industry, pp. 64–65.
Anonymous, 2010. Meat Buyers Guide, sixth ed. Reston, VA.: National Association of Meat Processors. p. 162.
Kramlich, W.E., Pearson, A.M., Tauber, F.W., 1973. Processed Meats. Westport, CT: The AVI Publishing Company.
Roger, M., 2010. Bacon processing. Meat Processing Technology Series. Champaign, IL: American Meat Association.
Moody, R.L., 1997. Overview of the Bacon industry. In: Proceedings of the Reciprocal Meat Conference. University of Connecticut.
Oppedal, A., 1998. Marketing Belly Burden. Hog Farm Management, pp. 18–20.
Young, B., White L., 2008. Bacon technology 101. 2008 Tech Journal Series. The National Provisioner.

Relevant Websites

http://en.wikipedia.org/wiki/Bacon
http://en.wikipedia.org/wiki/Pork_belly
http://en.wikipedia.org/wiki/Pancetta

Wiltshire Sides

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Glossary

'Live' brine An immersion brine used in the production of Wiltshire cured bacon that was continually replenished with curing salts and reused, having a characteristic salt-tolerant microflora.

Wiltshire curing A traditional method of producing bacon that originally involved the curing of whole sides of pork and included an extended period of maturation after several days of immersion in a 'live' brine.

Introduction

According to The Columbia Encyclopedia, bacon is "flesh of hogs – especially from the sides, belly or back – that has been preserved by being salted or pickled and then dried with or without wood smoke." Curing of meat is one of the most common preservation techniques that can be traced back to the ancient Greeks. Records show that Great Britain's traditional breakfast of bacon and eggs was sold as far back as 1560 by a London cheese monger. Local farmers processed bacon by rubbing the sides in salt, and the contamination of the salt with saltpeter plus bacterial action resulted in the typical red color of nitrite-cured meat.

In the middle of the eighteenth century, bacon production was simultaneously organized in the southern part of Great Britain and in Ireland. In England, pigs were transported from counties in the South West of the country to be marketed in London. On route they were rested in a town called Calne in the county of Wiltshire. Any pigs that were not fit enough to travel onwards were purchased by the Harris family for slaughter and processing into Wiltshire cured bacon. A royal warrant to supply Wiltshire cured bacon to the royal household was awarded to Thomas Harris in 1864. During the nineteenth century, bacon production went through technological developments and in 1847 the successful importation of bacon to Great Britain from Denmark was begun.

In several countries, codes of practice were established for the production of Wiltshire-type bacon to ensure the required high standards of skill and quality control in its production. Thus, from 1906 to 1989, Danish bacon was produced according to the rules given in The Danish Regulation for Bacon Production, which was administered by the Danish Ministry of Agriculture. A code of practice for the production of tank-cured Wiltshire bacon was produced in 1958 by the British Bacon Curer's Federation (which later became the British Meat Manufacturers Association and then the British Meat Processors Association). Despite the introduction of such quality schemes, bacon in most of the bacon-producing countries is now produced according to specific customer requirements.

Bacon Pigs

Bacon is one of the few processed pork products that has had a direct influence on the anatomy of modern pigs. These are referred to as 'baconers.' Particularly in Denmark, the breeding of pigs for bacon to fulfill the requirements of the British market changed the anatomy of Danish Landrace pigs from 1920 to 1970. The selection of the longest pigs for breeding resulted in an increase in length of approximately 8 cm and the introduction of two extra pairs of ribs (from 13 to 15 pairs). Simultaneously, the fore-ends of the pigs were reduced. The average thickness of backfat in Danish pigs has also reduced drastically (by more than 50%) during the past 70 years.

Feeding is known to be a critical factor for the quality of bacon. Feed with a high content of polyunsaturated fatty acids (e.g., from fish meal) is known to result in problems during processing (soft fat and off-flavor) and storage (development of rancidity). Consequently, feeding programs and optimum slaughter weight are the vital parameters for obtaining optimum bacon quality. The increase in lean meat content during the recent decades has automatically increased the ratio between polyunsaturated, monounsaturated, and saturated fats, and this has to be considered in the processing of bacon.

Wiltshire Bacon Processing

After chilling and splitting of the carcass into sides, the hind feet are sawn off (the length of the leg varies between nationalities), the tenderloin, aitch bone, cervical vertebrae, part of the sternum, and the front feet are removed; the belly is trimmed; the blade bone is removed; and the chine bones are trimmed, ending up with a traditional side as illustrated in Figure 1.

In the early days of bacon production, dry curing was the normal process. This was a slow process taking 6–8 weeks. This method has been completely replaced by systems using tank curing. The flowsheet in Figure 2 gives a schematic representation of the unit operations in the production of Wiltshire bacon.

Initially, the sides are injected with brine, often corresponding to 8–10% of the weight of the side; however, only 6–8% will be retained. In the past, this injection was done manually into the large muscles, but later multineedle injections became the normal practice, first in manually loaded machines (Figure 3) and later in continuous machines. The curing brine is typically 17°Be, which is equivalent to a sodium chloride content of 17.2% (w/v), 0.15% (w/v) potassium nitrate, and 0.08% (w/v) sodium nitrite. Subsequently, the sides are stacked in brine tanks (traditionally in large, white-tiled

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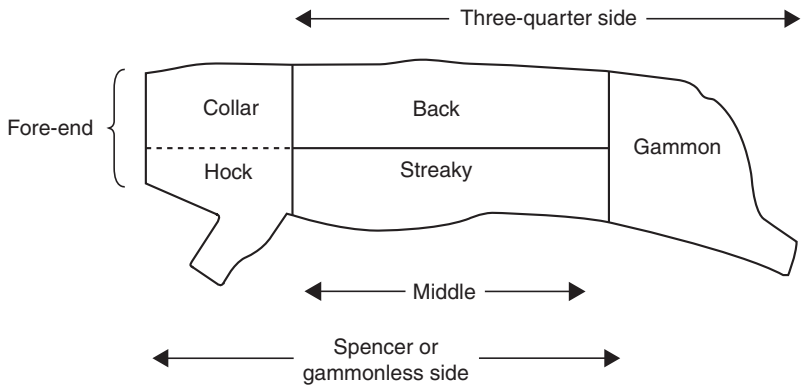

Figure 1 Bacon side with names of the typical cuts.

brick tanks; more recently in stainless-steel tanks), up to 10 sides high and with the lean face upwards and sprinkled with 300 g of salt per side to ensure curing of the areas that touch the side above or below. The cover brine is then added. The sides are kept below the surface with battens (these were originally made of timber, but have been replaced with plastic or stainless-steel battens). The cover brine is unique in traditional tank curing production, as it is based on reuse of brine from the preceding bacon production batches (some tank brines have been known to reach an age of more than 30 years), which results in the development of a specific microflora in the brine – the procedure was called 'blackslopping.' The brine itself was known as 'live brine' due to its characteristic microflora. The tank brine is typically made up to 23°Be by adding a dry salt mixture after each use. This is equivalent to a sodium chloride content of 24% (w/v), 0.20% (w/v) potassium nitrate, and 0.10% (w/v) sodium nitrite. The sides remain in the tanks for not less than 96 h and up to 120 h at a brine temperature of 5 °C. During the curing process, the sides lose weight ($\sim$2.7% of the weight before curing) and the final salt content in the meat depends on the fat content, the time in the tank, and the part of the side.

After curing, the sides are taken out of the tanks and placed in maturation rooms, where the sides are stacked on the floor or on pallets stacked up to eight high, with the rind upward at a temperature of 3–4 °C. During the drainage period and a maturation period of at least 5 days, the salt distribution equalizes in the meat. The maturation period is claimed to be essential for the production of high-quality bacon. The product is known as green sides or green bacon.

Care of the Immersion Brine

The continual reuse of the same immersion brine is a major characteristic of Wiltshire curing. Maintaining a stable brine is crucial and requires the following steps:

- Immersion brine temperature must not exceed 5 °C,
- salt, nitrate, nitrite, and pH must be measured after each use and the brine restrengthened accordingly,
- insoluble solid debris must be removed from the brine by filtration after each curing cycle,
- the pH should be between 6.3 and 6.5 and stable,
- if the brine pH is $>$6.8 it must be discarded and replaced with fresh brine,
- the total viable microbial count (on 4% salt agar at 22 °C for 5 days) should not exceed 500 000 cfu ml^{-1},
- brine should not remain unused for a lengthy period and must be aerated frequently if not in regular use, and
- a salt-tolerant microflora should prevail, which results in a predictable and controlled conversion of nitrate to nitrite.

Processing of Other Traditional Bacon Sides

In addition to the most common 'Wiltshire sides,' alternative processing methods for bacon sides also exist. Of these, Ayrshire cure is the most noteworthy. The sides have the rind removed and bones taken out before they are injected with pickle and placed in brine tanks. In the production of traditional products, such as Scottish Ayrshire rolls and Ulster rolls from Ayrshire-cured sides, the middles are defatted and tightly rolled and tied.

Brine Ingredients and Their Function in the Manufacture of Bacon Sides

The basis for curing is the brine (pickle) ingredients:

- Common salt (sodium chloride)
- Saltpeter (sodium nitrate or potassium nitrate)
- Sodium nitrite.

Common Salt

Sodium chloride is used because of its preservative quality, its physical (structural changes) and chemical interactions, and its organoleptic characteristics.

Saltpeter

Nitrate is a nitrite reservoir, as it is reduced to nitrite in the presence of nitrate-reducing bacteria. Moreover, nitrate stimulates the activity of nitrate-reducing bacteria, which can contribute to flavor development in meat.

Sodium Nitrite

Nitrite has many functions; it contributes to color development and color stability, influences the cured meat flavor

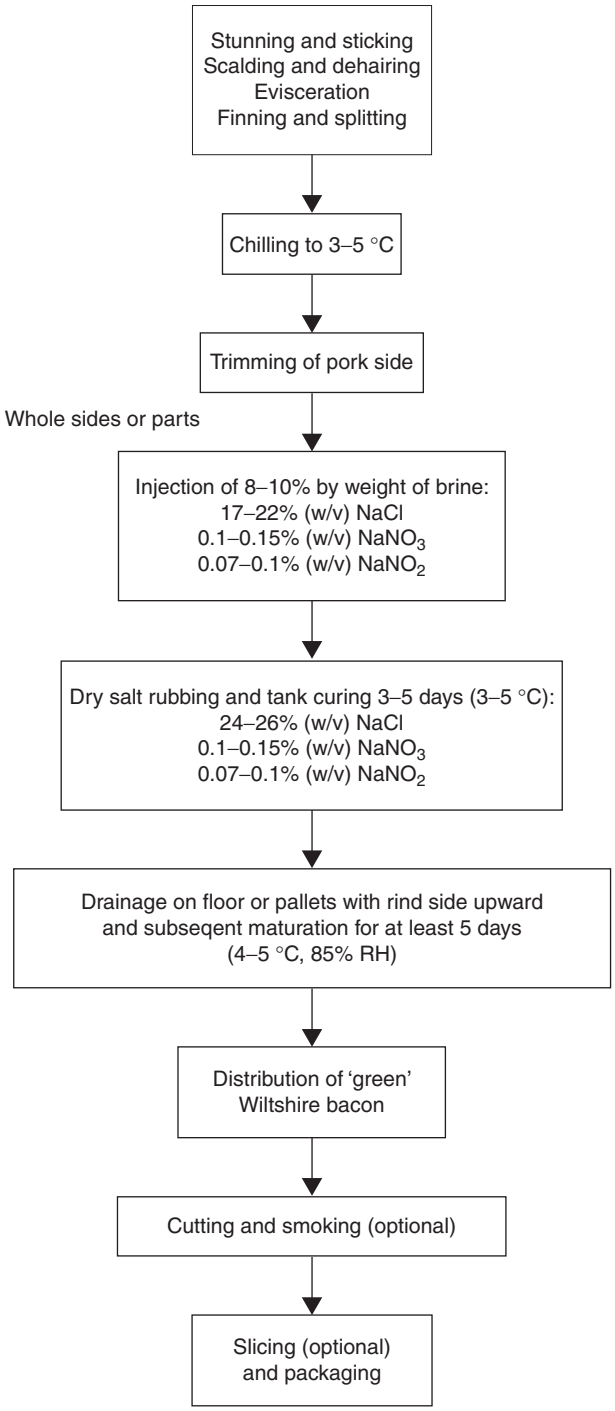

Figure 2 Flowsheet representing the general unit operations during the production of Wiltshire bacon.

(possibly owing to its antioxidant properties), and is a preservative.

Microbiology of Wiltshire Bacon

The initial microflora is that of the carcass. Salt (curing salt) used in the processing might contain spore-forming bacteria. The curing brines also contribute considerably to the microflora of bacon sides.

- Injection brines: The total colony count (in colony-forming units, CFU) of freshly prepared injection brine is normally $<10^3$ CFU ml^{-1}, but might vary considerably. Brines used in multineedle injection machines often contain a higher total colony count than registered in hand-injection brines.

This is due to the common use of recirculated surplus brine, which unavoidably transfers microorganisms to the fresh brine despite filtration. Injection brines have shown a predominance of *Micrococcus, Acinetobacter, Flavobacterium*, and coryneform bacteria, all of which are common contaminants of pork carcasses. Moreover, lactic acid bacteria, enterococci, and *Vibrio* spp. are found in recirculated brines. *Vibrio* spp. are known to cause spoilage of bacon (see below), and their presence in multineedle injection brine is due either to inadequate cleaning of the machine or to contamination with cover brine. See Table 1 for established advisory hygienic standards of injection brine.

- Cover (tank) brine: Immediately after preparation, these brines contain the same flora as injection brines. However, conventional cover brines were most often based on the 'backslopping' technique and were used repeatedly (brines could be 5–10 years old, some have even been reported to be up to 30 years old) and they, therefore, contain both microorganisms originating from the meat and some surviving in the tank brines. This results in a heterogeneous flora and a high number of bacteria (10^6–10^7 CFU ml^{-1}). In general, most saprophytic bacteria are able to survive in curing brines for varying lengths of time, and it might thus be concluded that any species found on pork to be cured can be found in brine. However, none of them can grow in brine. Organisms belonging to the genus *Vibrio* are a natural part of the flora in cover brines. The total count and the composition of the bacterial flora of cover brine is decisive for the quality of the brine, and an unfavorable count and composition gives rise to an 'unstable' cover brine resulting in unacceptable shelf life of the final product (Table 2). In contrast, cover brines with a moderate presence of *Vibrio* spp. are reported to enhance the flavor characteristics of the final product (see below).

Drainage and maturation of tank-cured bacon changes the microbial environment on the surface of the product, making growth of salt-tolerant microorganisms possible, especially if the curing brine has been unstable (Table 2). Drying of the product surface during maturation can give rise to unwanted growth of molds. Moreover, high room humidity dilutes the salt content on the surface of the product, enhancing growth conditions for salt-tolerant spoilers. Consequently, a controlled relative humidity (~85%) is most often used in the maturation/storage rooms.

When cuts or slices of Wiltshire-type bacon are placed in impermeable packages (vacuum/modified atmosphere packaging), a succession of changes in the microflora follows. Micrococci continue to dominate among the rising bacterial population, especially in the presence of nitrate. However, ultimately lactobacilli will dominate, possibly via a transitional phase in which enterococci dominate.

Figure 3 From 1970 to 1986 Danish bacon sides for Britain were injected using a special machine in which 109 needles injected the brine evenly from both the meat and rind sides into the muscles. Nowadays, it is common practice to use continuous multineedle injectors for the production of bacon cuts.

Microbial Spoilage of Wiltshire Bacon

The salt content in bacon prevents putrefactive microorganisms (e.g., pseudomonads) from multiplying. However, other salt-tolerant bacteria, such as lactobacilli, continue to grow (especially when the products are vacuum packed or packaged in a modified atmosphere), even under refrigeration, and may reach millions per gram, which results in souring and thus a reduction in the shelf life of the product. Moreover, heavy contamination with proteolytic *Vibrio* spp. may further reduce the shelf life of the product.

During processing of Wiltshire bacon products, changes in the natural meat microflora proceed toward a salt-tolerant flora. In general, a higher total colony count appears on the rind compared with the meat surface. Consequently, the microflora on sides of Wiltshire bacon varies in both number and species as a result of the heterogeneous nature of the bacon side. Surface spoilage (e.g., slime formation or discoloration) of refrigerated

Table 1 Advisory standards for injection brines

Category: UK	*CFU (NA)*[a] *(10^3 ml^{-1})*	*Category: Canada*	*CFU (PCA)*[b] *(10^3 ml^{-1})*	Escherichia coli *per 5 ml*
Good	<0.5	Excellent	<0.3	Absent
Fair	0.5–1.0	Good	0.3–1.0	Absent
Poor	1.1–5.0	Fair	1.0–2.0	Absent
Very poor	>5.1	Poor	>2.0	>1

[a]Colony-forming units on nutrient agar including 4% (w/v) NaCl, 22 °C.
[b]Colony-forming units on plate count agar including 4% (w/v) NaCl, 25 °C.
Source: Data from Gardner, G.A., 1973. Routine microbiological examination of Wiltshire bacon curing brines. In: Board, R.G., Lovelock, D. (Eds.), Sampling – Microbiological Monitoring of Environments. London: Academic Press, pp. 21–27 and Dempster, J.F., 1981. Microbiological guidelines for bacon curing brines. Farm Food Research 12, 190–192.

Table 2 Advisory guidelines for Wiltshire cover brines

Category: UK	*CFU (NA)*[a] *(10^3 ml^{-1})*	*Escherichia coli (ml^{-1})*	*Category: Canada*	*CFU (PCA)*[b] *(10^3 ml^{-1})*	*Escherichia coli (ml^{-1})*
Good	<50	<1	Excellent	<100	Absent
Fair	50–100	1–10	Good	100–500	1–20
Poor	101–500	11–100	Fair	500–1000	1–20
Very poor	>500	>100	Poor	>1000	>20

[a]Colony-forming units on nutrient agar including 4% (w/v) NaCl, 22 °C.
[b]Colony-forming units on plate count agar including 4% (w/v) NaCl, 25 °C.
Source: Data from Gardner, G.A., 1973. Routine microbiological examination of Wiltshire bacon curing brines. In: Board, R.G., Lovelock, D. (Eds.), Sampling – Microbiological Monitoring of Environments. London: Academic Press, pp. 21–27 and Dempster, J.F., 1981. Microbiological guidelines for bacon curing brines. Farm Food Research 12, 190–192.

bacon has been associated with growth of several species, but *Micrococcus, Vibrio, Acinetobacter,* and yeasts are the most common spoilers. Molds (e.g., *Aspergillus, Alternaria, Fusarium, Mucor, Rhizopus, Botrytis,* and *Penicillium*) can also be common in Wiltshire-type bacon spoilage if the humidity during maturation and storage is not properly controlled.

Color and Color Problems in Bacon

The characteristic red color of 'green bacon' is formed during the curing process, when the muscle pigment myoglobin binds nitric oxide and forms the bright red heme pigment nitrosylmyoglobin. Nitric oxide is formed in a series of complex reactions involving reduction of nitrite added directly or produced during bacterial breakdown of added nitrate.

Raw bacon color is quite stable if not exposed to light and oxygen simultaneously. If bacon is exposed to oxygen and light, the cured meat pigment, nitrosylmyoglobin, oxidizes rapidly to the brown metmyoglobin, giving the bacon a brown-grayish appearance. This process is accelerated if the meat surface is drying out, as seen under certain retail conditions. Oxidation of the cured meat pigment is a reversible reaction, and bacon color can, therefore, be regained if exposure of fresh discolored bacon to oxygen is prevented, for example, through vacuum or modified atmosphere packaging.

Flavor Development in Wiltshire Bacon

'Cured flavor' is a characteristic of nitrite-cured meat products and is found to be one of the most important quality parameters of these products. The nature of the 'cured flavor' is not fully understood. The high number of unit operations in the production of Wiltshire bacon causes multiple reactions that might contribute to the final flavor development. Salt in itself contributes a salty flavor of cured products. Moreover, salt is known to accelerate lipid oxidation in meat products, resulting in the formation of rancid off-flavors. Consequently, one of the most important factors in the formation of cured meat flavor is the addition of nitrite. At the levels added for curing, nitrite itself does not contribute any flavor. However, owing to its antioxidant activity it hinders the formation of rancid off-flavor. Consequently, the characteristic 'cured flavor' is probably merely the result of limitation of the formation of compounds known to give rise to rancid off-flavor and is thus due to improved human perception of the flavor characteristics of the salted meat, which would otherwise be drowned by a rancid off-flavor had the addition of nitrite not limited its formation.

Wiltshire bacon is found to have specific flavor characteristics not found in other bacon types. These flavor characteristics have been attributed to the use of the 'backslopping' technique of immersion brines, which gives rise to a characteristic microflora. This microflora has a proteolytic activity, leading to the formation of peptides and amino acids that are subsequently catabolized by the microorganisms, resulting in flavor-active compounds characteristic of Wiltshire bacon.

Packaging and Transport of Bacon Sides

In the original trade practice, bacon sides were placed in bales and wrapped for transport after drainage and maturation. At first, bales of bacon contained four green sides, with each pair of sides placed together, meat to meat, to preserve color and minimize contamination. However, trading practice has moved completely to containerization after the disappearance of small exporting factories. The introduction of refrigerated containers revolutionized bacon export because it extended the shelf life of the bacon.

Cutting of Sides and Smoking

In the early days of the bacon trade, sides were exported and further cutting and processing (boning, smoking, slicing, cooking, etc.) took place at the wholesaler's cutting plant. Subsequently, prepackaging became popular, and most bacon sides are now cut into the three traditional cuts (middle, forend, and hind leg) for easier handling. The complete side is often cut into the five cuts shown in Figure 1 and vacuum packed before retail distribution.

Smoking is a process that has always been closely associated with bacon, particularly in Scotland and Southern England. The use of smoking improves the shelf life of the bacon (it reduces both the microbial spoilage and the development of rancidity). Traditional smoking of bacon resulted in a weight loss of approximately 5%, but the introduction of alternative smoking methods (e.g., the use of liquid smoke) has minimized such losses. In addition to the preserving effect,

smoking also contributes significantly to the flavor of the bacon.

Conclusion

Curing of whole sides – traditional Wiltshire bacon – is a disappearing method of production as the costs are too high in relation to the price of bacon. The designations Wiltshire and Ayrshire bacon are still used in the branding of high-quality bacon, but these products are now made directly from cuts, as is the normal procedure in modern bacon production, and the time in immersion brine and the subsequent maturation period are drastically reduced to make the production more economical. Nowadays, immersion brines are fresh brines, and 'live' brines are limited to a few specialist producers of Wiltshire cured bacon.

See also: Packaging: Modified and Controlled Atmosphere; Vacuum

Further Reading

Andersen, H.J., Hansen, T.B., 1996. Cured meats and poultry, including cooked cured meats. In: Milner, J. (Ed.), LFRA Microbiology Handbook. 2. Meat Products. Leatherhead: Food Research Association, pp. CU1–CU29.

Dempster, J.F., 1981. Microbiological guidelines for bacon curing brines. Farm Food Research 12, 190–192.

Gardner, G.A., 1973. Routine microbiological examination of Wiltshire bacon curing brines. In: Board, R.G., Lovelock, D. (Eds.), Sampling – Microbiological Monitoring of Environments. London: Academic Press, pp. 21–27.

Gardner, G.A., 1982. Microbiology of processing: Bacon and ham. In: Brown, M.H. (Ed.), Food Microbiology. London: Applied Science, pp. 129–178.

Gardner, G.A., 1983. Microbial spoilage of cured meats. In: Roberts, T.A., Skinner, E.A. (Eds.), Food Microbiology. Advances and Prospects, Society for Applied Bacteriology Symposium Series no. 11. London: Academic Press, pp. 179–202.

Gray, J.I., Pearson, A.M., 1984. Cured meat flavour. Advances in Food Research 29, 1–86.

Hogan, W.J., 1978. The Complete Book of Bacon. London: Northwood Publications.

Kramlich, W.E., Pearson, A.M., Tauber, F.W., 1973. Processed Meats. Westport, CT: AVI.

Sheard, P.R., 2010. Bacon. In: Toldra, F. (Ed.), Handbook of Meat Processing. Iowa: Wiley-Blackwell, pp. 327–336.

Taylor, A.A., Shaw, B.G., Jolley, P.D., 1980. A modern dry-salting process for Wiltshire bacon. Journal of Food Technology 15, 302–310.

Taylor, A.A., Shaw, B.G., Jolley, P.D., Nute, G.R., 1982. Hot curing Wiltshire bacon. Journal of Food Technology 17, 339–348.

BIOFILM FORMATION

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Glossary

Brownian movement of bacteria A random movement of cells suspended in a fluid resulting from their collision as opposed to motility mediated by cellular appendages.

Hydrophobic Lacking an affinity for water; insoluble in water; or repelling water.

Planktonic cells Individual free-floating cells.

Quorum sensing A method of communication among bacterial cells by the release and sensing of small diffusible signal molecules.

Spore-forming bacteria Bacteria that have the ability to develop spores, a resting stage of live bacteria encased in a 'shell' that is capable of protecting the cell under adverse conditions. Under appropriate growth conditions, the spores will germinate and cells will become active.

Introduction

The concept of microorganisms attaching to surfaces to form biofilms is not new. There are several different definitions of biofilms, but common to all is that bacteria form communities that are attached to solid surfaces. There is a general tendency for microorganisms to attach to wet surfaces upon which they multiply. They produce exopolymeric substances (EPS) in which they become embedded. EPS assist bacteria to adhere to surfaces and to each other, and protect the bacteria from adverse conditions. When these processes proceed without interruption, a biofilm is formed. A biofilm consists of a biologically active matrix of cells that is embedded in EPS produced by the cells and associated with or attached to a solid surface. The formation of biofilms by prokaryotic cells is a biologically unique developmental process in which the activities of the cells are coordinated to obtain effects of mutual benefit, such as the maintenance of open water channels. Such coordination of cellular activities requires there to be inter- and intraspecies communication. Various systems of cell-to-cell signaling have been identified and are currently being investigated.

Many *in vitro* studies of biofilms have been undertaken using one or more bacterial species; however, the study of biofilms *in situ* remains challenging despite the availability of sophisticated molecular, electronic, and microscopic techniques. The application of *in vitro* findings for understanding of bacterial behavior in food processing environments is often not valid, so more study of natural biofilms is needed. Since bacteria living within biofilms can be very resistant to environmental stresses, such as drying and industrial sanitation processes, prevention of their development is an ongoing challenge for the meat industry. Of particular concern are nooks and crannies in commercial meat processing equipment that are hard to access and difficult to clean.

Formation of Biofilms

Stages of the Process

The formation and development of biofilms involves several stages. In the literature, there are different approaches to how these stages are defined and described (Table 1). Essentially, cells of microorganisms attach, first reversibly and then irreversibly, to a substratum that has been preconditioned with molecules in the environment around them. Once attached, and very early in biofilm development, the bacteria, and the microenvironment they create, become resistant to adverse or disruptive environmental conditions. If not disturbed, a mature biofilm will be formed. The formation of a mature biofilm can be achieved within a few hours but might instead take several weeks. Figures 1 and 2 are diagrammatic representations of the processes governing biofilm formation.

Surface Conditioning

When a solid material is placed in a liquid, solutes from the liquid will concentrate on the surface of the solid material and form a conditioning film. The physicochemical properties of the surface, such as surface free energy, hydrophobicity, and electrostatic charges can change. In a food processing environment, the properties of the work surfaces might change depending on the type of conditioning film. For example, if surfaces in the facility are hydrophobic, they could show a high affinity for fat and the conditioning film on all surfaces could be high in fat content. Meat juice has been shown to reduce the negative charge on stainless steel, which would be expected to change the overall properties of the stainless steel.

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Table 1 Stages of biofilms formation as described by various authors

Stage 1	*Stage 2*	*Stage 3*	*Stage 4*	*Stage 5*	*Stage 6*	*Stage 7*	*Stage 8*	*Stage 9*	*Reference*
Conditioning of a surface	Adhesion of cells	Formation of microcolony	Biofilm formation	Detachment and dispersal of biofilms					Kumar and Anand (1998)
Initial attachment of cells to the surface	Production of EPS resulting in more firmly adhered 'irreversible' attachment	Early development of biofilm architecture	Maturation of biofilm architecture	Dispersion of single cells from the biofilm					Stoodley *et al.* (2002)
Preconditioning of the adhesion surface	Transport of planktonic cells from the bulk liquid to the surface	Adsorption of cells at the surface	Desorption of reversibly attached adsorbed cells	Irreversible adsorption of bacterial cells at the surface	Production of cell–cell signaling molecules	Transport of substrates to and within the biofilm	Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm	Biofilm removal by detachment or sloughing	Breyers and Ratner (2004)
Deposition of organic molecules (conditioning of a surface)	Biologically active molecules attracted to surface	Some microbial cells remain after cleaning and sanitizing, and initiate growth	Larger biofilms formed with the help of expression of genes and quorum sensing						Shi and Zhu (2009)

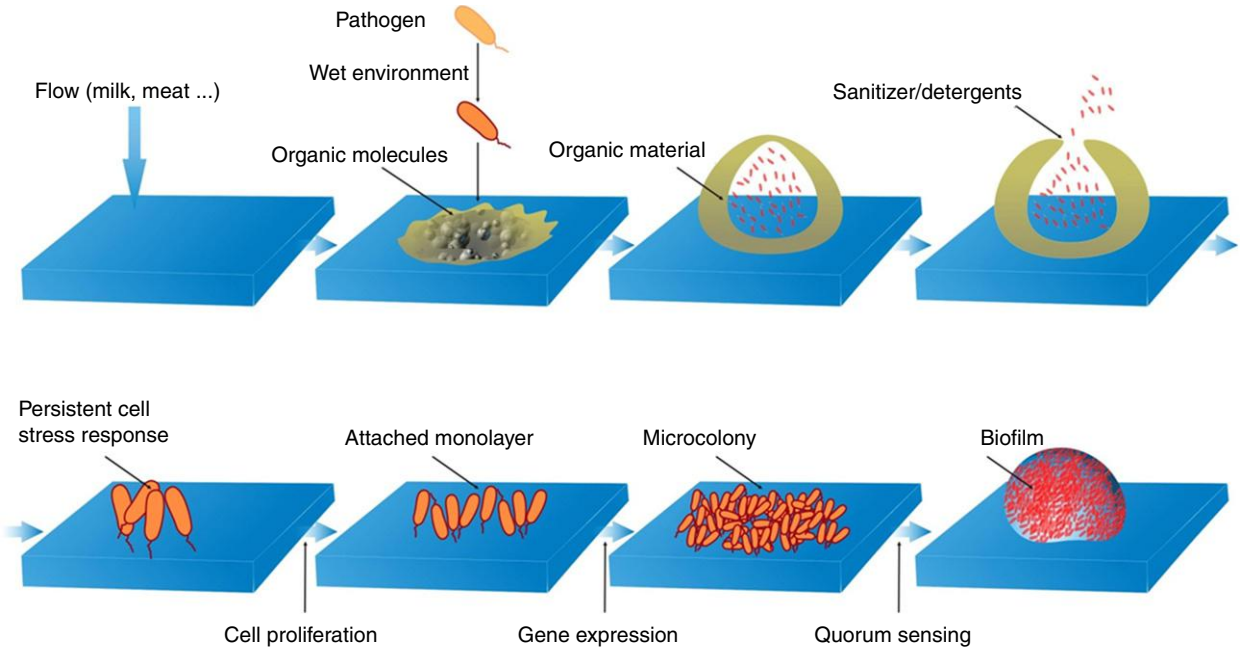

Figure 1 Sequence of events in biofilm formation on food contact surfaces. Firstly, organic molecules from food are deposited on the surface of equipment and form a conditioning film. Secondly, biologically active microorganisms are attracted to the organic molecules. Thirdly, persistent microbial cells remain after cleaning and sanitizing and initiate growth. Lastly, the biofilm forms with the expression cellular genes and quorum sensing. Reprinted from Shi, X., Zhu, X., 2009. Biofilm formation and food safety in food industries. Trends in Food Science and Technology 20, 407–413.

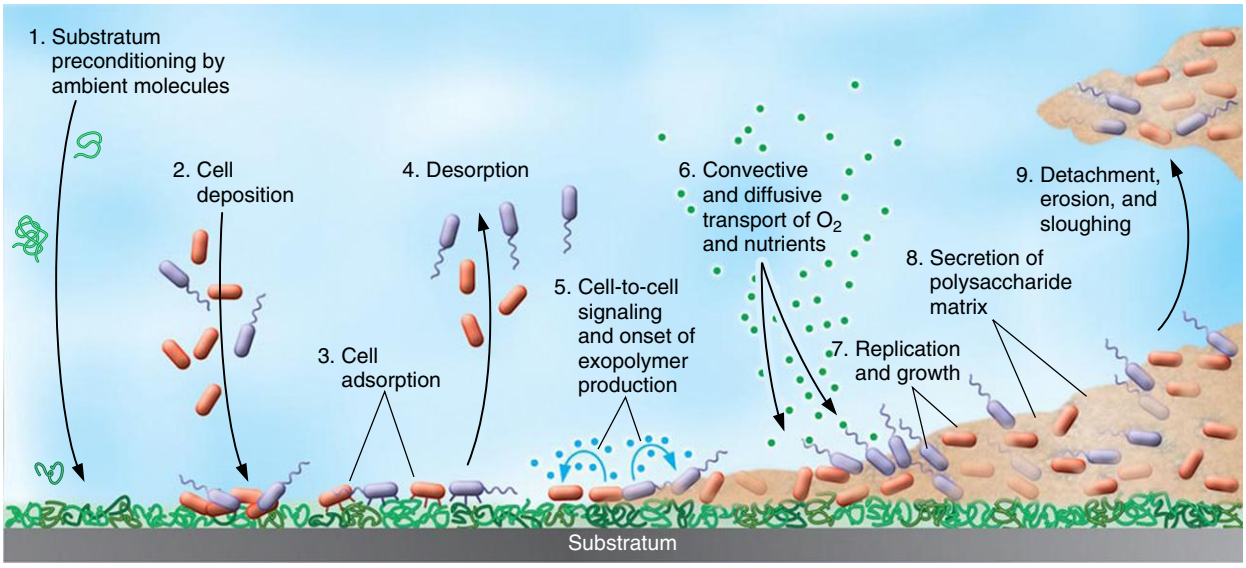

Figure 2 Distinct processes govern biofilm formation. Reprinted from Bryers, J.D., Ratner, J.P., 2004. Bioinspired implant material befuddle bacteria. American Society of Microbiology News 70, 232–237, with permission from ASM.

Transport of Planktonic Cells to Surfaces and Adhesion

Factors that cause cells to undergo the transition from planktonic mode to biofilm mode or to become attached to or detached from surfaces are associated with a variety of environmental and physiological triggers that include quorum sensing, nutrient and oxygen availability, shear stress, microbial metabolic activity, microbial gene expression, and cellular stress. Planktonic cells can move toward a conditioned surface actively, by means of flagella, or passively in response to forces such as gravity, diffusion, or fluid dynamics. Once cells are close to the surface, adhesion will be affected by factors like nutrient availability in the fluid, growth stage of the cells, and the microtopography of the contact surface. The first stage of adhesion is reversible and is the result of long-range interaction forces: electron acceptor–electron donor

interactions and hydrophobic interactions that keep cells close to the conditioned surface. At this stage, the bacteria are not firmly attached and will continue to show Brownian movement. They can still be easily removed, if the microtopography of the surface does not keep cells trapped, by processes that produce shear forces, such as rinsing, or changes in charge and hydrophobicity. Until the bacterial surface appendages become involved, the bacteria usually cannot make direct contact with the surface due to repulsive forces between the bacterial cells and the surface. Bacterial cells produce surface appendages, including flagella, fimbriae, pili, and EPS fibrils that reduce the surface area that contacts the inert surface and by which they can overcome repulsive forces such as negative charges on both cell and surface to become attached.

Flagella, composed of fine threads of flagellin protein, permit motility, which is hypothesized to overcome repulsive forces between cells and the surface so that a monolayer of cells can form on the surface. Flagella can also enable bacteria to move along the surface and allow growth and spread of the developing biofilm or form adhesive bonds between bacterial cells and surfaces.

Fine, filamentous, hair-like proteinaceous appendages, including pili, and fimbriae present in both Gram-negative and Gram-positive bacteria are involved in various processes including conjugation, adherence, and twitching motility. All of these can affect biofilm formation and the characteristics of the bacteria in the biofilm. Although bacteria with fimbriae can adhere strongly to other bacterial cells and inorganic particles, and can probably overcome the initial electrostatic repulsion barrier between the cells and the surface, fimbriae are not always involved in the biofilm attachment process. They have, however, been shown to have a critical role in the initial stable cell-to-surface attachment for *Salmonella* spp. and numerous pathogenic and nonpathogenic *Escherichia coli*. A bacterial cell might be covered in fimbriae, giving the cell a hairy appearance.

There are different types of pili, and cells can change the characteristics of the pili they produce to correspond to their environment. Cells may have one or several pili. The ends of pili are sticky and make cells adhesive so that bacteria with pili adhere strongly to other bacterial cells and inorganic particles. Pili are able to retract to bring the bacterial cell closer to the surface and Type IV pili enable cellular locomotion.

Enterobacteriaceae produce proteinaceous fibers termed curli that are implicated in cell adhesion, cell aggregation, biofilm formation, and pathogenesis. Research into these fibers is relatively limited, but there is evidence that co-expression of curli and cellulose leads to the formation of networks of tightly packed cells that are aligned in parallel and that are highly hydrophobic. EPS also has a role in stabilizing the attachment of bacteria to surfaces.

Production of Exopolymeric Substances, Irreversible Attachment, and Development of Biofilm Architecture

The production of EPS, irreversible attachment, and development of biofilm architecture are linked. An important characteristic of biofilms is that the cells are embedded in an EPS matrix. This matrix has a variety of functions, including cohesion and adhesion of cells and particulate matter; protection from biocides and other chemicals; entrapment of molecules and nutrients; binding of cations, toxic metallic ions and other substances; and resistance to dessication. The polysaccharides and proteins form the structural elements of biofilms and determine their mechanical stability. The roles of the other components are not well established. At some time after attachment of cells to a surface, this attachment becomes irreversible. The cells grow and divide to form a microcolony. The microcolony enlarges, produces EPS, and coalesces so that it becomes anchored to the surface and stabilized. Microcolonies become protected from environmental stresses, and cells can show increased resistance to antimicrobial agents within a few hours of adhesion, even before they become embedded in the EPS matrix. Over time, cellular activity continues and the biofilm matures. Biofilms can be composed of different organisms or they can contain only one species. The organization of the cells ranges from a single layer to 3-D structures with the bacteria living within a large, complex, and organized ecosystem. There might be mechanisms whereby some species are encouraged to populate a biofilm, whereas attachment of other species is inhibited. *In vitro* studies show that multispecies biofilms are thicker and more stable than single species biofilms. The distribution of bacteria within biofilms is not even. Bacteria grow within the microcolonies that are surrounded by the EPS matrix and water channels are formed among them. These channels facilitate the transport of substrates, including oxygen, metabolites, and nutrients to the biofilm and within the biofilm; and also transport products, including waste products, out of the biofilm.

The physiological state of biofilm bacteria appears to be different than that of planktonic bacteria. The combination of physiological modifications of biofilm-associated cells, including reduced growth rates and production of enzymes that can degrade antimicrobial substances, and physical protection provided by the biofilm matrix itself might be responsible for the extreme resistance to antimicrobials of biofilm bacteria.

Part of the 'life cycle' of a biofilm is that damaged individuals are eliminated from the population. Programmed cell death and lysis can be a function of spatial orientation within the biofilm. Since deoxyribonucleic acid (DNA) is a component of the EPS and is thought to be related to the stability of the biofilm, the expectation is that cell lysis has a role in maintaining the stability of the biofilm structure.

Biofilms are known to spread, but factors that control release of cells and detachment of sections of the biofilms are not well understood. They can release 'pioneer' cells or daughter cells individually, or there might be a sloughing or detachment of a relatively large part of the biofilm. When single cells or small clusters of cells detach, the effect on the biofilm is limited to the surface. When a large portion of a biofilm is sloughed off, the whole biofilm is impacted and may be lost.

Cellular Control of Biofilms – The Role of Cell-to-Cell Signaling Molecules and the Molecular Basis of Biofilm Formation

The defining characteristic of biofilms is the formation of an integrated bacterial community. This requires self-organization

and cooperation among cells, rather than the classical 'competitive' natural selection of individual microorganisms. Bacteria, which are colonial by nature, must be able to sense environmental changes and respond to them. They must also communicate with each other. Bacteria modulate gene expression and have systems of intercellular interactions and communications that are currently the focus of much study.

The molecular changes that occur in a bacterium when it changes from its planktonic form to become sessile (permanently attached to the substrate) can be very complex and are only beginning to be understood. For example, in *Listeria monocytogenes*, expression of many proteins has been shown to be upregulated, including some related to stress response, protein synthesis, carbon metabolism, and regulation. These diverse molecular shifts show that the central metabolism of *L. monocytogenes* is affected during biofilm development.

A major area of interest is the role of quorum sensing in development and maintenance of biofilms. Cell-to-cell signaling is mediated by small, diffusible molecules called auto-inducers that are produced and secreted during bacterial growth. As the bacterial population in the biofilm increases, the concentration of these small molecules increases too. When they reach a threshold or quorum level, the cells respond to them, with a variety of phenotypic responses being displayed as a result of the regulation of target genes that are quorum-sensing dependent. Quorum sensing seems to be involved in all stages of biofilm formation and maintenance. Some of the biofilm-related processes that can be controlled by quorum sensing are the establishment of bacteria in a mixed biofilm community, survival in food processing environments that are hostile to bacteria, the production of surface appendages, and motility. Since the discovery that bacterial cells communicate is relatively recent, much information is still required to fully explain the role of quorum sensing in the activities of biofilm bacteria. There is particular interest in the possibility of controlling the development of biofilms through disruption of the quorum sensing system.

Studying Biofilms

The study of biofilms *in situ* is extremely difficult for many reasons. As a result, most knowledge about biofilms has been obtained from *in vitro* studies. Such results might not be relevant to circumstances in the food industry. Many disinfection processes are designed using bacteria that are planktonic, which are more sensitive to antimicrobials than bacteria embedded in biofilms. Enumerating or determining the types of bacteria residing within biofilms can be difficult. It has been estimated that for each planktonic bacterial cell detected, another 1000 cells could be present within a biofilm. In food processing environments, surfaces are usually treated with antimicrobial chemicals during cleaning, might not provide a lot of nutrients, can be hot or cold and dry, and might be otherwise hostile environments for bacteria causing them to be stressed and/or injured and nonculturable. The types of bacteria within a biofilm and the environment in which they develop will determine its characteristics. Researchers cannot as yet wholly duplicate naturally occurring biofilms.

A variety of microscopic techniques including scanning electron microscopy, transmission electron microscopy, and laser scanning microscopy are the tools used to observe biofilms and to monitor their development under controlled conditions. One instrument being studied as a way to monitor biofilm formation online is the mechatronic surface sensor. This type of sensor is based on the analysis of the vibration response of the surface and can detect biological and chemical characteristics of a variety of surfaces such as stainless steel and polyvinyl chloride.

Biofilms in the Food Industry

The major focus of research on biofilms has been in relation to medical matters, but they are also very important in many industries, including the food industry. The bacteria in biofilms can cause food spoilage, compromise food safety and affect performance, and longevity of industrial equipment. Several pathogenic and spoilage bacteria including *L. monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *E. coli*, *Pseudomonas* spp., and lactic acid bacteria have been associated with biofilm formation. In the meat and dairy industries, bacteria, including species of *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Flavobacterium*, yeasts, and *Kluyvera* were recovered from biofilms associated with clean floor materials. *L. monocytogenes* will form biofilms on floor drain surfaces, in storage tanks, on hand trucks, on conveyor belts, and on other food contact surfaces; and individual strains have been shown to persist in food plants for several years. Bacteria in biofilms can catalyze chemical and biological reactions to cause corrosion in pipelines and tanks, and if the biofilms are allowed to become thick, the heat transfer in heat exchangers and pipelines can be reduced.

Equipment and processing plant design, cleaning, and disinfection regimens are the major tools available to the food industry to control the development of biofilms. Surfaces in meat processing facilities are frequently wet and biofilms can quickly form under wet conditions. Stainless steel, the material used for many food contact surfaces, is chemically and physiologically stable over a range of temperatures, and it is easy to clean. When the microtopographies of stainless steel surfaces are examined, they are seen to have many cracks and crevices that are good locations for bacteria to become attached. All areas of food processing environments including floors, walls, pipes, drains, conveyer belts, gaskets, and dead spaces are prone to biofilm formation. Similarly, materials that are commonly used in food processing facilities such as stainless steel, aluminum, nylon, Teflon, rubber, and plastic can become colonized by biofilms.

The development and structure of biofilms depend on many factors, both intrinsic and extrinsic, including the species of bacteria in the consortium, the temperature, pH and nutrient status of the environment, and the flow conditions of fluids that contact surfaces. In the food industry, because cleaning and disinfection are frequent, mature biofilm structures or continuous bacterial films are not often observed on equipment surfaces; however, microcolony development with or without EPS is common. Whether these attached bacteria constitute a biofilm is debated. Although not showing the

typical characteristics of biofilms, these microcolonies are important to the food industry because they can exhibit increased resistance to antimicrobial treatments within only a few hours of establishment; and cleaning and disinfection regimens must be designed to remove them completely. It is hypothesized that this resistance might, in part at least, result from a reduction in surface area exposed to antimicrobial treatment as a result of the attached surfaces not being in contact with the applied chemicals. Further research is required in this area, with particular emphasis on contamination of damp surfaces in food processing areas. In hard to access areas and areas that are infrequently cleaned, mature biofilms, with microbial cluster embedded in EPS and water channels, are observed.

The best approach to control biofilm development is prevention of their establishment. Equipment should be designed so that areas that are hard to access and clean and areas that allow bacteria to accumulate, such as dead ends, corners, valves and joints, are avoided. Good water drainage should be assured and proper attention should be paid to the welding, material, and surface finishing on both exposed and nonexposed surfaces. All surfaces should be nonporous and smooth, without pits or crevices. Glass is a hard, smooth, and corrosion-resistant material but its applications are limited since it breaks easily. Stainless steel is easy to clean but the surface is easily damaged. Rubber surfaces deteriorate. Surfaces in food establishments must be cleaned, but regimens that cause corrosion and produce topographical defects should be avoided. Defects and roughness of surfaces have greater effect on the ease with which a surface can be cleaned than the type of finish. Since biofilm development occurs when moisture is present, maintenance of dry conditions will aid in prevention of their establishment.

Even with proper equipment design and attention to surface characteristics and integrity, the likelihood of preventing biofilm development is slim. Cleaning and disinfection programs necessarily remain the main strategy for controlling surface contamination. Since bacterial adhesion is initiated soon after a surface is conditioned, regular cleaning and disinfection at short intervals are required to prevent firm bacterial attachment to surfaces and the formation of mixed species biofilms. Cleaning at short intervals will help to prevent sporulation by spore-forming bacteria, which is of particular importance to the dairy industry, but might become important to the meat industry as the involvement of psychrophilic (cold-loving) and pathogenic clostridia in meat spoilage becomes more common.

One major factor militating against elimination of biofilms is that they can be reseeded with a very small number of cells. It is almost impossible to remove all cells associated with a biofilm. Pouring liquid onto a surface will erode a biofilm but a negligible number of the biofilm cells will be detached. Any cleaning and sanitation strategy must ensure that the biofilm bacteria are dead, not just dislodged. Surfaces should be cleaned prior to sanitation and cleaning processes must break up or dissolve the EPS so that sanitizers can make contact with the viable bacteria within the biofilm. Some materials used for cleaning are surfactants or alkaline products that suspend or dissolve food residues by decreasing surface tension, emulsification of fat, and denaturation of proteins. Acid products can also be used if the surfaces are coated with precipitated minerals or much food residue. If it is practicable, exposure of the biofilm to high temperature can reduce the requirements for physical cleaning. The type of cleaning process must not cause aerosols. For example, when mechanical force is applied using scrubbing and brushing, or pulsed laser beams are used, bacteria can become airborne. Such processes, including use of high pressure sprays, are being replaced by foam or gel cleaning.

Bacterial cells within biofilms are highly resistant to antimicrobials. Even after a surface is cleaned, ridding the surface of viable cells is difficult. There are several mechanisms that have either been shown or are hypothesized to cause the elevated resistance of biofilm bacteria. Penetration of antimicrobials into the biofilm can be slow or the antimicrobial can be neutralized in the outer layers of the biofilm. The rate of diffusion might be slower than the rate at which the antimicrobial is inactivated, as is observed with resistance of biofilms to chlorine. The growth rate of bacteria deep in the biofilm is decreased and cells are in a 'quasi-dormant' state in which their resistance to biocides is increased. Older biofilms are more resistant to biocides than are younger ones. Genes controlling adaptive stress responses are expressed and biofilm cells are able to sense challenges from antimicrobials. *Salmonella enteritidis* isolated from biofilms had increased resistance to heat and chemicals when compared to planktonic cells. Repeated exposure of biofilm cells to antibiotics was shown to cause an increase in EPS synthesis in the biofilm. Cells, termed persister cells, that are phenotypically variant, can develop. These cells do not grow and do not die when challenged with antimicrobials. In the medical field, such cells are considered to be largely responsible for persistent infections resulting from biofilms. Their resistance is explained by their production of cellular toxins that block cellular processes such as translation and, so, render the cells resistant.

Prevention and control of biofilms are topics of active research. At this time, there is no means known of preventing or controlling the development of biofilms without adverse side effects. Almost all materials will support the formation of biofilms. Some current efforts are focussed on the possibility of incorporating antimicrobial agents into either the construction materials themselves or surface coatings. Much work has been concerned with biomedical applications, but application in the food industry will likely follow and has been the focus of some studies. Laboratory studies in which fungicides are incorporated into flooring materials have been reported, but no results of long-term studies have been published. Covalent coupling of silicone rubber implants with quaternary ammonium coatings, coating surfaces with silver, preconditioning surfaces with surfactants and adsorption of nisin onto food contact surfaces are all in the experimental phase. In food processing, the importance of the 'house microflora' and the possibility that the presence of bacteriocin-producing bacteria and other endogenous strains might influence the establishment of biofilms are being studied. Novel approaches to prevention, removal, and inactivation of biofilm bacteria include the development of enzyme-based cleaners; various combinations of enzymes, detergents, surfactants, and phenolic antimicrobials; use of bacteriophages; interruption of quorum sensing; and manipulation of nutrient availability.

The formation of biofilms is a unique, complex, and important biological phenomenon that enables many bacteria to survive and grow in interactive communities and in environments that are hostile to them. Biofilms can be advantageous, but in many parts of the food processing industry, they are considered to be biofouling agents responsible for problems with food spoilage, food safety, deterioration of equipment, and compromised efficiency of equipment. Our knowledge of the mechanisms that contribute to the formation, development and maintenance of biofilms is limited and improved control over biofilms will depend on significant advances in the field.

See also: Microbial Contamination: Decontamination of Processed Meat. Equipment Cleaning. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Packaging: Technology and Films

Further Reading

Annous, B.A., Fratamico, P.M., Smith, J.L., 2009. Quorum sensing in biofilms: Why bacteria behave the way they do. Journal of Food Science 74, R24–R38.

Bai, A.J., Rai, V.R., 2011. Bacterial quorum sensing and food industry. Comprehensive Reviews in Food Science and Food Safety 10, 184–194.

Bryers, J.D., Ratner, J.P., 2004. Bioinspired implant material befuddle bacteria. American Society of Microbiology News 70, 232–237.

Carpentier, B., Cerf, O., 1993. Biofilms and their consequences with particular references to hygiene in the food industry. Journal of Applied Bacteriology 75, 499–511.

Gandhi, M., Chikindas, M.L., 2007. *Listeria*: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology 113, 1–15.

Kumar, C.G., Anand, S.K., 1998. Significance of microbial biofilms in the food industry: A review. International Journal of Food Microbiology 42, 9–27.

Marchand, S., De Block, J., De Jonghe, V., *et al.*, 2012. Biofilm formation in milk production and processing environments; Influence on milk quality and safety. Comprehensive Reviews in Food Science and Food Safety 11, 133–147.

Mettler, E., Carpentier, B., 1998. Variations over time of microbial load and physicochemical properties of floor materials after cleaning in food industry. Journal of Food Protection 61, 57–65.

Shi, X., Zhu, X., 2009. Biofilm formation and food safety in food industries. Trends in Food Science and Technology 20, 407–413.

Simões, M., Simões, L.C., Vieira, M.J., 2010. A review of current and emergent biofilm control strategies. LWT – Food Science and Technology 43, 573–583.

Stoodley, P., Saur, K., Davies, D.G., Costerton, J.W., 2002. Biofilms as complex differentiated communities. Annual Reviews in Microbiology 56, 187–209.

Van Houdt, R., Michiels, C.W., 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. Journal of Applied Microbiology 109, 1117–1131.

Zotolla, E.A., Sasahara, K.C., 1994. Microbial biofilms in the food processing industry – Should they be a concern? International Journal of Food Microbiology 23, 125–148.

Relevant Websites

http://www.ift.org/~/media/Knowledge%20Center/Science%20Reports/Scientific%20Status%20Summaries/Editorial/editorial_0209_feat_biofilms.pdf
Institute of Food Technologists.

https://www.google.ca/search?q=biofilm+formation&oq=biofilm+formation&aqs=chrome..69i57j0l5.4979j0j8&sourceid=chrome&espv=210&es_sm=122&ie=UTF-8#q=biofilm+formation+and+food+safety
Manitoba Agriculture, Food and Rural Development.

BIOMETHANE PRODUCTION AND CLEANUP

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Glossary

Biodigestor A natural system that uses anaerobic digestion in a series of processes by which microorganisms break down the biodegradable material in absence of oxygen. It is used for industrial purposes in managing waste and releases energy.

Global warming Refers to an unequivocal and continuing rise in the average temperature of Earth's climate system.

Greenhouse effect A natural process that warms the Earth's surface. When the Sun's energy reaches the Earth's atmosphere, some of it is reflected back to space and the rest is absorbed and reradiated by greenhouse gases.

Methanogen Any of various archaea that is capable of producing methane from the decomposition of organic material.

Ruminal defaunation Elimination of all the protozoa (defaunation) from the rumen is a standard method of studying their overall effect.

Introduction

Global warming is the result of heat absorption by certain gases in the atmosphere and downward reradiation of some of the heat that helps to regulate the temperature of the planet. The worldwide trends of carbon dioxide have shown an increase over time, leading to an enhanced greenhouse effect and hence global warming due to its high absorption of infrared radiation. Methane is another important greenhouse gas that is produced mostly from agricultural activities and livestock. On 17 November 2003, the National Oceanic and Atmospheric Administration reported that the concentration of the potent greenhouse gas methane in the atmosphere was leveling off and it appears to have remained at this 1999 level. The Intergovernmental Panel on Climate Change (IPCC) in 2007 acknowledged that methane concentrations have plateaued, with emissions being equivalent to removals. These changes in methane atmospheric dynamics have raised questions about the relative importance of ruminant livestock in global methane.

It is a matter of concern that methane is increasing in the atmosphere at approximately 1% per year, 30% ($\sim$80 million tonnes) of which is from ruminants. This problem was a principal goal of discussion in different governments around the world, and the first assessment report of the IPCC, issued in August 1990, served as the basis for negotiation on the United Nations Convention on Climate Change. In 1995, a second report was finished and in the year 2001 approved the third IPCC report. The conclusion of the first global scientific consensus notes the importance of 'human activities' as a factor in global climate change in the past 50 years. The agreement of world political leaders was to reduce levels of greenhouse gases through the Kyoto Protocol, which entered into force on 16 February 2005. The participating countries must demonstrate that actions and progress are being made to reduce emissions of six main gases that induce the greenhouse effect, which are: carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF_6). Strategies have been established to reduce emissions of the gases in the following sectors: (1) energy, (2) transport, (3) industry, and (4) agriculture. Livestock is of interest in this article, considering aspects in the selection of animal breeds, food ingredients that produce less methane, and excreta management.

Methane Production in Livestock

Gas emission from ruminants is a direct consequence of fermentation performed by ruminal microbes due to the type of digestive process by which carbohydrates are broken down by microorganisms and methane is released as a by-product of enteric fermentation. Methane production in ruminants during the process of fermentative digestion and metabolism represents a loss of food energy, amounting to approximately 8% of gross energy at maintenance level of intake and 20% of the metabolizable energy. It is very important to know the factors associated with and the mechanisms of methane production. The methanogenic energy loss is high, particularly in low-quality roughages, such as a straw-based diet. Straw is the principal feed for vast majority of ruminants in developing countries and methane production is inversely related to propionate production, i.e., if propionate increases there is a decrease in methane production and vice versa. Evidently, the largest proportion of methane is produced from the poor-quality roughage feeds, because acetic acid is the principal fermented product of poor-quality feed. However, methane production can be changed by changing forage:concentrate ratio and the type of concentrate; moreover, forage:concentrate ratio can substantially influence methane production.

An adult bovine on an average releases between 70 and 120 kg of methane per year, and the negative effect on the climate of methane is 23 times higher than the effect of CO_2. Therefore, the release of approximately 100 kg methane per year for each bovine is equivalent to approximately 2300 kg CO_2 per year. According to the Food and Agriculture Organization (FAO), agriculture is responsible for 18% of the total release of greenhouse gases worldwide and cattle breeding is taking a major factor for these greenhouse gas emissions. Worldwide, there are approximately 1.34 billion cattle, and meat production was estimated at 275 million tons. Experts predict that by 2050 nearly twice as much meat will be produced as today, for a projected total of more than 465 million tons; a Japanese study showed that producing a kilogram of beef leads to the emission of greenhouse gases with a global warming potential equivalent to 36.4 kg of CO_2. Livestock now use 30% of the Earth's entire land surface, mostly permanent pasture but also including 33% of the global arable land used to producing feed for livestock. In 2010, at least 60% of meat was produced in developing nations, and Latin America released 11.7% of global emissions of gases that warm the atmosphere, mostly through rural activities. The Kyoto project includes the integration of many industrial countries, but the developing countries have not participated fully in the protocols of greenhouse gas emissions.

Factors Related to Methane Production in Ruminants

Animal welfare and environmental protection are increasingly important production factors. Consumers require that meat should be produced in systems that they consider animal friendly. On the environmental side, slurry-based systems for feedlots may be favoured because it is suggested that they provide lower ammonia (NH_3) and greenhouse gas (GHG) emissions than other systems. Housing systems must be found that take the animals' welfare into consideration while controlling emissions of NH_3 and GHG. Emissions from feedlots have been intensively researched, but the data are still limited and there are several differences in production systems. Feedlot systems, however, are not finely differentiated within GHG reporting; one single emission factor is given for all straw-based systems, due to limited data availability, and mitigation measures are not proposed. Figure 1 summarizes the factors associated with the animal, the dietary composition, and some modifying additives that reduce methane emissions.

Animals

There are approximately 1.34 billion large ruminants in the world; these are one of the largest methane sources, producing approximately 80 million metric tons of methane annually. Increasing the productivity of ruminants could lessen CH_4 emission per unit of meat. Specifically, in the developing countries, genetic potential of animals for production is not expressed due to under or improper nutrition; so CH_4 emissions per unit of meat produced could be decreased with proper feeding. Little information is available on opportunities to mitigate enteric CH_4 via animal genetics. Steers with a low residual feed intake (LRFI) have a higher digestibility than steers with a high residual feed intake (HRFI). LRFI cattle potentially have lower energy losses as CH4 when allowed access only to high quality pastures compared with HRFI. Residual

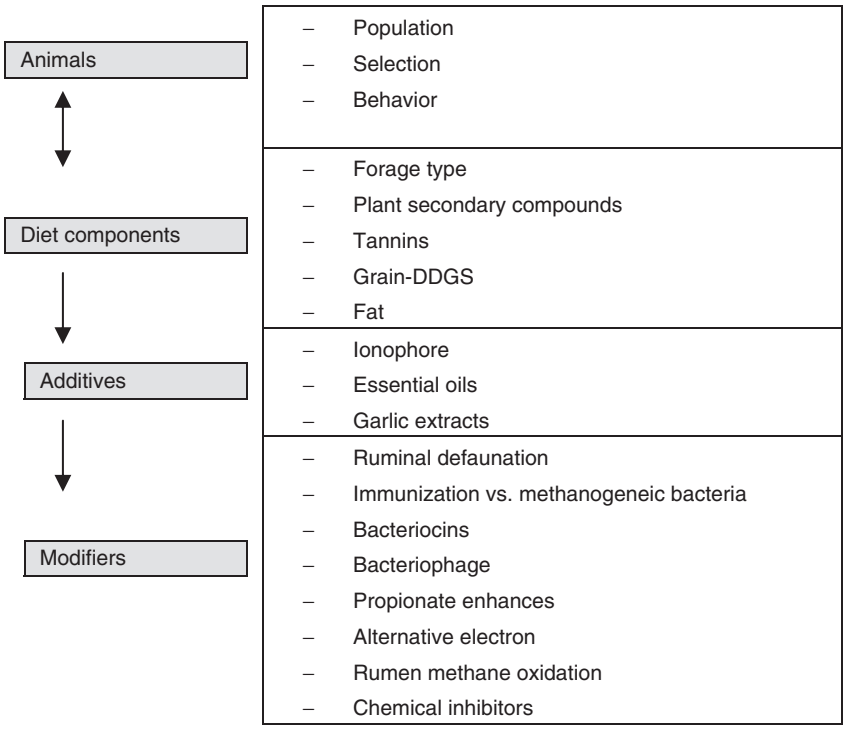

Figure 1 Schematic presentation of potential factors that reduce methane emissions in ruminants.

feed intake has been shown to be moderately heritable (0.4). An almost doubled total volatile fatty acid (VFA) concentration is also reported in rumen fluid of cow compared with HRFI steers, indicating more microbial activity fermentation and higher methane production in LRFI steers. However, improvements can be made through selection on associated trails, such as feed intake or RFI or through selection on CH_4 predicted from feed intake and diet composition. A study conducted in lactating cows found a large variation in dry matter intake (DMI) and predicted enteric CH_4 emission among first-lactation cattle; this study concludes that a classic selection program could reduce predicted methane emission by 11% and 26% in 10 years.

Diet Components

Cattle production systems have diets that are composed of forage (including grass and legumes), silage, supplements, or complete diets that provide their nutritional requirements. Cattle fattened in feedlots are fed small amounts of hay or straw supplemented with grains, silages, or other ingredients in order to increase the energy density of the diet. Decreasing the fiber content of forages has been found to reduce methane production. Particularly, some legume forages have been shown to decrease CH_4 production, which has been explained by the presence of condensed tannins, low fiber content, high DM intake, and faster passage from rumen. Saponins, such as *Sapindus saponaria* and *Sesbania sesban*, have potential to reduce CH_4 production through a direct effect on methanogen bacteria and protozoal number. Addition of 3–5% of the diet as tallow or oils has been found to decrease the methane production by 2–3%.

Additives

These are a group of feed ingredients that can cause a desired animal response through nonnutrient roles, such as pH, growth, or metabolic modifiers to reduce emissions of methane. Ionophores, such as monensin, included in diets at a dose of 24–35 mg kg^{-1} diet decreased CH_4 production by 4–10%, but the use of ionophores has been banned in the European Union. Furthermore, there are a number of reports available showing a decline of methane production as a result of garlic extracts, peppermint oil, essential oils from *Thymus* or *Origanum* plants, methanol and ethanol extracts of *Foeniculum vulgare* and *Syzygium aromaticum*, etc. The majority of the products cited have been evaluated on *in vitro* trials, but a great deal of research is still to be done *in vivo*, in order to consider the economic benefit of reducing methane production using these additives.

Modifiers

There is a wide interest in investigating the effect of rumen defaunation (elimination of protozoa) to reduce CH_4 production. Protozoa are indirectly involved in methane production due to their close symbiotic relationship with methanogens, which allows interspecies hydrogen transfer between them. Methanogen vaccines are other alternative for mitigating CH_4 emissions. Australian researchers have reported one study from immunization of sheep with whole-cell preparations of methanogens that showed a 7.7% reduction in CH_4 production; this observation suggests that vaccinating ruminants against methanogens may reduce the activity of ruminal methanogens. The use of a bacteriocin produced by *Streptococcus* sp. and therapy with bacteriophages are other alternative methods to suppress CH_4 production. Also, the addition of organic acids, such as malate and fumarate, increases propionate production, with a stoichiometric decrease in H_2 availability for CH_4 production, although other studies have concluded that supplementing diets with these acids at the levels required to decrease CH_4 emissions would be expensive.

Manure Management to Reduce Methane Emission and Using Techniques to Generate Energy

Livestock manure has been estimated to contribute 17.5 million tons of methane (CH_4) and 3.7 million tons of nitrous oxide (N_2O) to the atmosphere each year. The majority of methane emissions come from large swine and dairy farms that manage manure as a liquid. The US EPA expects that methane emissions from livestock manure will grow to 4.6 million tons in 2020 in the United States. But worldwide, the increase is expected to be particularly high in Africa (43%), Central and South America (30%), non-OECD Asia (39%), and the Middle East (10%). Interestingly, despite the rapidly increasing methane contribution from these countries, the OECD is expected to remain the top emitting region even in 2030. This increase in methane emissions is primarily due to the increasing use of liquid and slurry manure management systems that generate methane. The use of these systems is associated with the trend toward larger farms with higher, more concentrated numbers of animals. However, cost-effective technologies are available that can stem this emission growth by recovering methane and using it as an energy source; these technologies, commonly referred to as anaerobic digesters, decompose manure in a controlled environment and recover methane produced from the manure.

Anaerobic digestion that utilizes manure for biogas production is one of the most promising uses of biomass wastes, because it provides a source of energy while simultaneously resolving ecological and agrochemical issues. Anaerobic digestion involves bacterial fermentation of organic substances in the absence of free oxygen and the fermentation leads to the breakdown of complex biodegradable organics in a four-step process referred to as a three-phase process: (1) hydrolytic phase (step 1), (2) acid phase (steps 2 and 3), and (3) methane phase (step 4) (Figure 2). If the process is properly controlled in reactors so that it proceeds optimally through these stages, the principal end product, the biogas, contains 70% (by volume) of methane gas, the rest being carbon dioxide and traces of ammonia, hydrogen sulfide, and hydrogen. This biogas, which is a convenient and clean fuel, can either be used directly with or without the removal of carbon dioxide or be converted into electricity with the help of suitable generators.

The digesters are mixed mechanically to ensure uniform digestion. Each system attempts to maximize methane generation from the manure and the recovered methane can fuel engine generators to produce electricity or boilers to produce

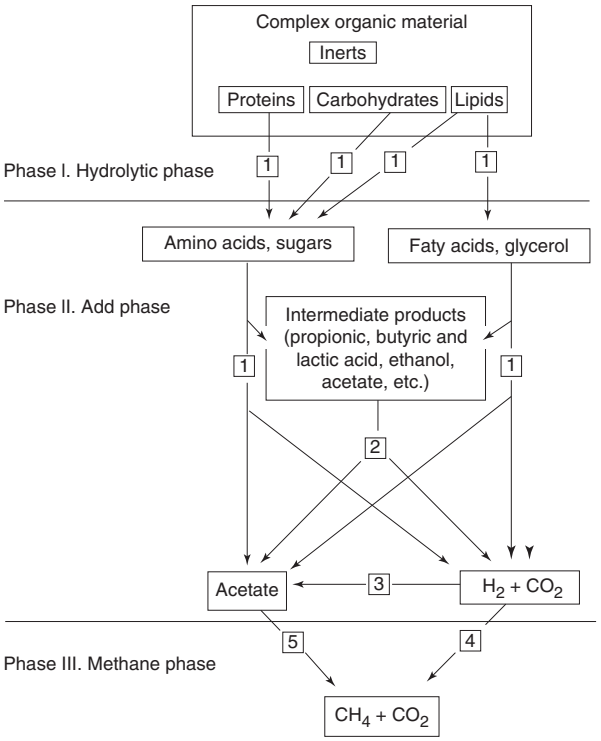

Figure 2 Steps associated with anaerobic digestion of organic materials. The bacteria involved are: (1) hydrolytic and fermentative, (2) hydrogen-producing acetogenic, (3) hydrogen-consuming acetogenic, (4) carbon dioxide reducing, and (5) aceticlastic methanogenic. Adapted from Tauseef, S.M., Premalatha, M., Abbasi, T., Abbasi, S.A., 2013. Methane capture from livestock manure. Journal of Environmental Management 117, 187–207.

heat and hot water. Electricity generation for on-farm use can be a cost-effective way to reduce farm operating costs. The economic feasibility of electricity generation usually depends on the farm's ability to use the electricity and the waste heat on-site. Digesters also reduce foul odor and can reduce the risk of groundwater and surface water pollution. Manure decomposes very rapidly when climate conditions encourage bacterial growth and so methane generation is greater in warm regions and lower in cool regions. Methane generation takes place in the volatile solids portion (VS) of the manure. The composition of the VS portion depends on livestock type and diet, so both affect the quantity of methane that can be produced per kilogram of VS in the manure. This quantity is referred to as 'Bo' and is measured in units of cubic meters of methane per kilogram of VS (m^3 CH_4 per kg VS). Methane production also depends on the type of manure management system (dry or liquid) used. Liquid/slurry systems use concrete tanks and lagoons to store flushed and scraped manure. Liquid management systems use water to facilitate manure handling. In addition, unmanaged manure from animals grazing on pasture falls into this category. Dry systems include solid storage, dry feedlots, deep pit stacks, and daily spreading of the manure. For dry systems, wet climates have higher emissions than arid climates, although emissions in either case are very low compared with wet systems.

Conclusion

Methane emissions caused by livestock and manure are high and these are the biggest contributors among the anthropogenic sources of the global warming gas methane. A number of technologies, such as animal selection, use of diet components, additives, or modifiers, are emerging to reduce methane production. The use of livestock manure is an alternative renewable source of energy and use of biodigesters can improve environmental conditions.

See also: Manure/Waste Management: Manure Management. Nutrition of Meat Animals: Ruminants. Quality Management: Farm Level: Safety and Quality of Beef. Slaughter-Line Operation: Cattle. Species of Meat Animals: Cattle

Further Reading

Beauchemin, K.A., McGinn, S.M., Petit, H.V., 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Canadian Journal of Animal Science 87, 431–440.

Food and Agriculture Organization of the United Nations (FAO), 2013. Mitigation of greenhouse gas emission in livestock production. Available: http://www.fao.org/docrep/018/i3288e/i3288e.pdf (accessed 03.01.14).

Guan, L.L., Nkrumah, J.D., Basarab, J.A., Moore, S.S., 2008. Linkage of microbial ecology to phenotype: Correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiology Letters 288, 85–91.

Hass, Y., de Windig, J.J., Calus, M.P.L., *et al.*, 2012. Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. Journal of Dairy Science 94, 6122–6134.

Hess, H.D., Beuret, R.A., Lotscher, M., *et al.*, 2004. Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with Sapindus saponaria fruits and Cratylia argentea foliage. Animal Science 79, 177–189.

Intergovernmental Panel on Climate Change, 2006. IPCC guidelines for national greenhouse gas inventories. IGES, Hayama, Kanagawa, Japan (Online). Available at: http: //www.ipcc-nggip.iges.or.jp/public/2006gl/index.html (accessed 10.04.13).

Jones, F.M., Phillips, F.A., Naylor, T., Mercer, N.B., 2011. Selection for LRFI cattle for grazing may reduce in CH4 emissions through any part of the season when cows have access to high quality pastures. Animal Feed Science and Technology 166–167, 302–307.

McAllister, T.A., Newbold, C.J., 2008. Redirecting rumen fermentation to reduce methanogenesis. Australian Journal Experimental Agriculture 48, 7–13.

Molano, G., Renard, T., Clark, H., 2003. The effect of level of feeding and forage quality on methane emissions by wether lambs. Proceedings of the 2nd Joint Australia and New Zealand Forum on Non-CO_2 Greenhouse Gas Emissions from Agriculture. In: Eckard, R., Slattery, B. (Eds.), Cooperative Research Centre for Greenhouse Gas Accounting. Australia, Canberra: Cooperative Research Centre for Greenhouse Gas Accounting, pp. F14.

Nkrumah, J.D., Okine, E.K., Mathison, G.W., *et al.*, 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of Animal Science 84, 145–153.

Patra, A.K., 2012. Enteric methane mitigation technologies for ruminant livestock: A synthesis of current research and future directions. Environmental Monitoring and Assessment 184, 1929–1952.

Qin Ng, W.P., Lam, H.L., Ng, F.Y., *et al.*, 2012. Waste-to wealth: Green potential from palm biomass in Malaysia. Journal of Cleaner Production 34, 57–65.

Tauseef, S.M., Premalatha, M., Abbasi, T., Abbasi, S.A., 2013. Methane capture from livestock manure. Journal of environmental management 117, 187–207.

United States Environmental Protection Agency (EPA), 2013. Livestock Manures. Available at: http://www.epa.gov/ (accessed 12.12.13).

Wahlquist, Å.K., 2013. Eating beef: Cattle, methane and food production. Asia Pacific Journal of Clinical Nutrition 22, 16–24.

Relevant Websites

http://ccap.org/policy-actions-to-mitigate-methane-in-latin-america/
Center for Clean Air Policy.

http://www.fao.org/agriculture/lead/themes0/climate/en/
Food and Agriculture Organization of the United Nations.

http://www.mla.com.au/Research-and-development/Research-programs-and-projects/Environment/National-livestock-methane-program
Meat & Livestock Australia.

BIOPRESERVATION

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Glossary

Bacteriocidal A compound that is lethal to bacteria and exposure at the appropriate concentration results in cell death.

Bacteriophages Bacteria-specific viruses used to control the growth of undesirable bacteria in foods.

Bacteriostatic A compound that prevents a bacterial cell from dividing but does not kill the cell.

Diacetyl A volatile organic compound produced by certain strains of lactic acid bacteria that has a strong buttery flavor.

Lactic acid bacteria A diverse group of Gram-positive bacteria that share a common feature that they can only ferment sugars to acids and cannot obtain energy using respiration.

Modified atmosphere packaged meat (MAP) MAP is used to extend the shelf-life of fresh meat by changing the concentrations of gases in the packaged product. Gases such as CO_2 and N_2 are added to lower the concentration of O_2 in the packed product.

Starter culture A meat starter culture contains one or more strains of lactic acid bacteria that are added during a meat fermentation to ensure consistency in desirable flavor and sensory outcomes.

Introduction

Biopreservation exploits the antimicrobial activities of some microorganisms to inhibit the growth of spoilage and pathogenic microbes in foods. This biological approach seeks to minimize the addition of chemical additives to foods, such as nitrite, sodium chloride, and organic acids. Research in the field of biopreservation for meat products remains active because it is perceived that natural methods of preservation are desirable. Most research on biopreservation has focused on the antagonistic activities of lactic acid bacteria against spoilage and pathogenic bacteria. However, in the past decade, the use of bacterial viruses (bacteriophages) to eliminate pathogenic bacteria from foods has gained considerable attention.

Lactic Acid Bacteria and Their Antimicrobial Activities

The role of lactic acid bacteria in meat fermentations is well established and is grounded in centuries of traditional artisan sausage production, where the fermentation imparted desirable sensory qualities and increased the shelf life of the sausage. For example, lactic acid bacteria, such as *Lactobacillus sakei* and *Lactobacillus curvatus*, play predominant roles in the production of traditional fermented meats, such as salami and other dry-fermented sausages, by producing acids during fermentation and lowering the pH. The lower pH creates a stressful environment for pathogenic and spoilage bacteria, thereby inhibiting their growth. Owing to their importance in meat fermentations, substantial research effort has focused on the development of specific lactic acid bacteria starter cultures for the production of fermented meat products with consistent desirable sensory qualities. In addition to these traditional fermentations, lactic acid bacteria are known to be the dominant bacterial flora in vacuum-packaged or modified atmosphere-packaged fresh meat and meat products during storage. The lactic acid bacteria are a metabolically diverse group of fermentative bacteria. Some of them produce metabolites that confer off-odors and flavors on fresh meats, whereas others spoil meat products. Some do not affect the sensory qualities of meat products even when they are present in high numbers. The nonspoilage lactic acid bacteria are well suited for biopreservation of meat products, as they can be added to the products without noticeably affecting the sensory qualities of the foods. The antagonistic activities of lactic acid bacteria against other bacteria in foods have been attributed to several mechanisms that include:

- Production of organic acids, such as lactic acid and acetic acid, and reduction of meat pH.
- H_2O_2 production.
- Sequestration of nutrients required by competing organisms.
- Production of antibacterial bacteriocins.
- Production of other metabolites with antimicrobial activities, such as diacetyl and reuterin.

The various antimicrobial activities are expressed at different levels by different species and strains of lactic acid bacteria. Furthermore, the antagonistic activities can vary from bacteriostatic (growth inhibiting) to bactericidal (lethal) and

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might affect a broad range of bacteria or only a limited, sometimes narrow, group of organisms. The acidifying effects of lactic acid bacteria are exploited for the preservation of fermented meat products, such as dry sausages. Now, rather than relying on bacteria naturally present on meat, selected strains of lactic acid bacteria are commonly used as starter cultures for fermentation. The production of bacteriocins by starter strains is currently receiving much attention because of the potentially wide use of those organisms and their products for biopreservation of meats.

Organic Acid Production

Lactic acid bacteria produce organic acids by fermentation of carbohydrates. Homofermentative lactic acid bacteria produce mainly lactic acid, whereas the heterofermentative species produce acetic as well as lactic acid and, to various extents, other acids such as formic, citric, and maleic. The antimicrobial activities of organic acids are primarily associated with the undissociated forms of the acids. For this reason, the inhibitory activities of organic acids are affected by the pH values of foods; i.e., their antimicrobial effects increase with decreasing pH. The inhibitory activities are mainly due to the following:

- Reduction of pH with an increase of the concentration of H^+ in a food. The pH can be reduced to values near or below the minima for growth of pathogens and potent spoilage organisms.
- Reduction of the intracellular pH of bacteria. The undissociated acid is relatively lipophilic and is, therefore, able to penetrate the bacterial membrane to release H^+ in the cytoplasm. Then, in order to survive, the bacteria have to use energy to transport H^+ out of cells. This causes energy depletion and cell stress.

Because the antimicrobial activities of organic acids increase as the pH decreases, their activities are greater in fermented sausages of pH 4.5 than in cooked meat products of pH 6.3. It is, however, possible to achieve some inhibitory effects by adding organic acids to products while maintaining a high pH.

Hydrogen Peroxide Production

In the presence of oxygen, some lactic acid bacteria produce H_2O_2 during fermentation of carbohydrates. To mitigate the toxic effects of H_2O_2, the bacteria produce enzymes, such as catalase, that break down the H_2O_2. The efficiencies of these enzyme systems vary among lactic acid bacteria, and the rate of production of H_2O_2 can be greater than the rate of its degradation. Then, the amount of H_2O_2 in the meat environment increases. H_2O_2 is inhibitory to bacteria because of its strong oxidative effect on cell membrane lipids and proteins. However, the production of H_2O_2 by bacteria in meats can pose sensory problems by causing green or brown discoloration of products and lipid oxidation that gives rise to rancid flavors.

Nutrient Competition

The growth of bacteria within foods depends on the amounts of nutrients available to them and various factors intrinsic and extrinsic to the food matrix, such as NaCl concentration, temperature, etc. that affect their growth. If a product is inoculated with high numbers of lactic acid bacteria capable of growth in the product, and conditions are such that the concentrations of nutrients are or become growth limiting, the growth of other bacteria using the same nutrients can be inhibited.

Bacteriocins

Bacteriocins are classified on the basis of their, often relatively narrow, range of target bacteria and their molecular compositions. Bacteriocins of relevance for biopreservation are comprised of chains of several amino acids that have undergone chemical modification. Owing to their proteinacious nature, bacteriocins can be inactivated by proteases. Bacteriocins produced by lactic acid bacteria have been known since the 1930s, when certain *Lactococcus lactis* strains (formerly known as N-streptococci) were shown to produce a compound with high antimicrobial activity. This compound is now known as nisin, which is one of the most-studied bacteriocins produced by lactic acid bacteria. The antimicrobial activities of bacteriocins are often limited to closely related lactic acid bacteria. However, some lactic acid bacteria produce bacteriocins that act against broad ranges of Gram-positive bacteria which can include the foodborne pathogens *Listeria monocytogenes* and *Staphylococcus aureus*. Nisin is effective against those organisms, and it inhibits germination of spores of *Bacillus* and *Clostridium* species. Gram-negative bacteria such as *Escherichia coli*, *Yersinia enterocolitica*, and *Salmonella* species are usually not affected by the bacteriocins produced by lactic acid bacteria. The resistance is, in part, due to the outer membrane of Gram-negative bacteria, which is not present in Gram-positive organisms. The outer membrane serves as a barrier to the entry of bacteriocins into cells of the Gram-negative bacteria and thus prevents the bacteriocins from accessing their cellular targets. However, if the bacteriocin is added together with a chemical agent that disrupts the outer membrane, for example, trisodium phosphate or a chelating agent such as ethylenediaminetetraacetic acid, the Gram-negative bacteria can become sensitive to some bacteriocins.

The bacteriocins produced by lactic acid bacteria are classified according to their primary structures and amino acid compositions. The three recognized classes are:

1. The lanthionines. These are peptides containing modified amino acids. The best-characterized bacteriocin of this group is nisin, which is produced by several *Lactococcus lactis* strains.
2. The small nonlanthionine bacteriocins. These are peptides composed of unmodified amino acids. The antilisterial bacteriocin pediocin is a member of this group.
3. The large, heat-sensitive bacteriocins. These are inactivated within 10–15 min at 60–100 °C. Only a few have been described, such as helviticin J and caseicin 80.

Bacteriocin Production

Lactic acid bacteria produce mainly Class I or Class II bacteriocins, all of which consist of between 20 and 60 amino acids. They are heat stable, hydrophobic, and cationic.

Synthesis and secretion of nisin and other lanthionines (Class I) involves the products from several genes, in addition to the structural gene. In contrast, synthesis and secretion of Class II bacteriocins generally requires just four genes. The lanthionines require genes coding for the prepeptide, transport proteins for secretion of the prepeptide, proteins involved in modification of the peptide, immunity proteins, and regulatory proteins for gene expression. The Class II bacteriocins require only genes that code for the prepeptide, two transport proteins, and one immunity protein.

The production of bacteriocins often occurs during exponential growth of the organism, although the conditions required for production can differ. For example, some strains require a specific substrate for production of a bacteriocin, whereas others produce the bacteriocin only while growing within a narrow pH range. The temperature at which a culture is incubated can affect bacteriocin production. Increasing amounts of sodium chloride can decrease and addition of glucose to a culture can increase bacteriocin production by some organisms.

Antimicrobial Activities of Bacteriocins

Bacteriocins can have either bacteriocidal or bacteriostatic effects on the sensitive bacteria. It is generally thought that bacteriocins act in two stages. In the first stage, the bacteriocin binds to a receptor on the surface of the sensitive strain. In the second stage, the bacteriocin penetrates the cell membrane, making small holes in it. Small molecular weight components leak from the attacked cell, and ultimately energy depletion causes cell death. However, recent research suggests that the antimicrobial actions of at least some bacteriocins are more complex. Thus, some bacteriocins can apparently enter the cytoplasm to interact with the cell's deoxyribonucleic acid (DNA) or essential proteins, thereby interfering with the proper functioning of the cell.

Some bacteriocins, such as the lanthionines nisin and lacticin 3147, are active against a broad range of Gram-positive bacteria. Nisin has been shown not only to be active against vegetative cells but also to prevent the outgrowth of bacterial spores. Other bacteriocins are active against only a narrow range of bacteria that are closely related to the organism that produces the bacteriocin. For example, some of the lactococcin bacteriocins are active against only strains of *Lactococcus* species. If bacteriocin-producing strains are used to increase shelf life, it is advantageous to use strains producing a bacteriocin with broad antimicrobial activity. However, if the purpose of biopreservation is to limit the growth of a specific spoilage bacterium, then use of a strain producing a bacteriocin that affects a narrow range of susceptible organisms which include the target strain might be appropriate.

The antimicrobial activities of bacteriocins can vary with food product composition. Thus, it has been shown that sodium chloride concentration, presence of fats and emulsifiers, storage temperature, and proteins in the food matrix as well as growth rate and growth phase of the target organism can affect the antimicrobial activities of bacteriocins.

Bacteriocin Resistance

Development of bacteriocin resistance by the targeted bacteria is an important issue for the effective use of bacteriocins for biopreservation. The cold-tolerant pathogen *Li. monocytogenes* is often the target for bacteriocins used for biopreservation of ready-to-eat meats. The bacteriocin resistance in *Li. monocytogenes* is well documented. When *Li. monocytogenes* is exposed to pediocin-like bacteriocins, resistance against these bacteriocins develops with a frequency between 10^{-6} and 10^{-3}. Similarly, nisin resistance in *Li. monocytogenes* has been shown to occur with a frequency between 10^{-9} and 10^{-2}.

In a few studies, a large number of strains of *Li. monocytogenes* were tested for resistance to bacteriocins. These studies showed that 1–3% of the strains were resistant to the pediocin-like bacteriocins but less than 1% were resistant to nisin. However, some strains had an enhanced tolerance to nisin, although they were not completely resistant.

Development of bacteriocin resistance can be due to various changes in the target organism. In some cases resistance develops as a result of changes to the cell membrane, in which the phosphotransferase system for uptake of sugars might be involved.

The occurrence of resistance against bacteriocins must be evaluated when practical applications of bacteriocins are considered. The risk of bacteriocin resistance is one of the reasons for not using bacteriocins as the sole preservative in a meat product. Biopreservation should be regarded as one component of a multiple hurdle approach to controlling pathogenic and/or spoilage bacteria in foods. In addition to directly enhancing control, the use of multiple barriers to bacterial growth and survival should help to minimize the risk of target organisms developing resistance to bacteriocins.

The Application of Bacteriocins to Meats

Bacteriocins can be incorporated into meats by various means. A bacteriocin-producing strain can be added to the meat, the same as a starter culture is added during meat fermentation. Alternatively, the bacteriocin can be purified from cultures of the bacteriocin-producing strains and added to meat preparations, applied directly to meat surfaces, or incorporated into packaging films that contact meat surfaces.

Traditionally, starter cultures have been used in the meat industry for production of fermented meats, with the cultures contributing to the texture, flavor, and preservation of the final products. Because bacteriocin-producing lactic acid bacteria can inhibit the growth of pathogens and spoilage organisms, there is increased interest in adding bacteriocin-producing lactic acid bacteria cultures to meat products, such as cooked, cured meat and sausages that are sliced and then packaged under vacuum or a modified atmosphere. Many patents for the use of bacteriocins and bacteriocin-producing strains of bacteria for preservation of meats have been filed. Companies selling starter cultures and other ingredients for use in foods are now marketing a variety of protective lactic acid bacteria cultures for inhibition of *Li. monotytogenes* in cooked, sliced meat products or Enterobacteriaceae that cause putrid spoilage

of fresh and processed meats. The effects of these cultures are often due to not only bacteriocins but also other antimicrobial actions of the starter cultures.

Direct addition of bacteriocins is possible because antimicrobial food ingredients containing bacteriocins, such as nisin and pediocin, are now commercially available. Examples of such products include Alta, Perlac 1911, Microgard, Chrisin, and Nisaplin. (Wesman Foods, OR, USA; Quest International, Naarden, the Netherlands; Chr. Hansen, Horsholm, Denmark; Danisco, Denmark). Manufacturers of packaging films are investigating the incorporation of active bacteriocins into edible and inedible films that can be used by the meat industry. This is an emerging technology that will require continued research to enhance the efficacies of antimicrobial films.

The intrinsic qualities of meat products can substantially affect bacteriocin activities and limit the means by which bacteriocins or bacteriocin-producing cultures can be added to meat. Fermented meats, cooked sliced meats, and fresh meats must then be considered separately with respect to the potential for use of bacteriocins in their biopreservation.

Fermented Sausages

Fermented sausages undergo rapid decreases in pH during fermentation, accompanied by development of desirable color, taste, and flavor. Lactic acid bacteria, such as pediococci and lactobacilli, are responsible for the decrease in pH and the subsequent acidic taste of the products. Micrococci, staphylococci, and yeasts are responsible for the development of desirable product color and additional flavors.

Starter cultures widely used in meat fermentation are strains of *Pediococcus acidilactici*. Use of a bacteriocin-producing strain of *P. acidilactici* was found to reduce the number of *Li. monocytogenes* by >3 log units, whereas a bacteriocin-negative variant reduced the concentration of the organism by <1 log unit. Various lactobacilli have also been shown to decrease the numbers of *Li. monocytogenes* in fermented sausages. Cultures containing one or more bacteriocin-producing starter strains for meat fermentations are available commercially.

Cooked Meats

Listeria monocytogenes can be introduced into cooked meat products after cooking and during peeling, slicing, and packaging of the product. The addition of a pediocin-producing strain of *P. acidilactici* at numbers of 10^7 colony-forming units (CFU) per gram inhibited the growth of *Li. monocytogenes* on vacuum-packed wiener and frankfurter sausages stored at 4 °C for 60 days. In the nonbiopreserved controls, *Li. monocytogenes* grew by up to 10^6 CFU g^{-1} during that time. However, this inhibition was not necessarily due to the production of a bacteriocin, as similar inhibition occurred when the products were inoculated with a bacteriocin-negative strain of *P. acidilactici*. The addition of lower numbers of the bioprotective culture (10^3–10^4 CFU g^{-1}) delayed but did not completely inhibit the growth of *Li. monocytogenes*.

Another organism suitable for inhibiting *Li. monocytogenes* in cooked, sliced meat is *Leuconostoc carnosum* 4010, cultures of which are commercially available. The effects of this organism, which produces the bateriocin leucocin, in a broad range of meat products have been documented. Cultures of the organism were applied to meat products as they were sliced, by nozzles placed next to the blade of the slicing equipment. The addition to slices of 10^7 *Le. carnosum* 4010 per gram of product completely inhibited the growth of *Li. monocytogenes* during storage at both 5 and 10 °C (Figure 1). This kind of antilisterial effect can be obtained with various bacteriocin-producing lactic acid bacteria. If meat is inoculated with high numbers of *Li. monocytogenes*, the addition of a protective culture will often cause a decrease in the number of *Li. monoctyogenes* due to the activity of the bacteriocin.

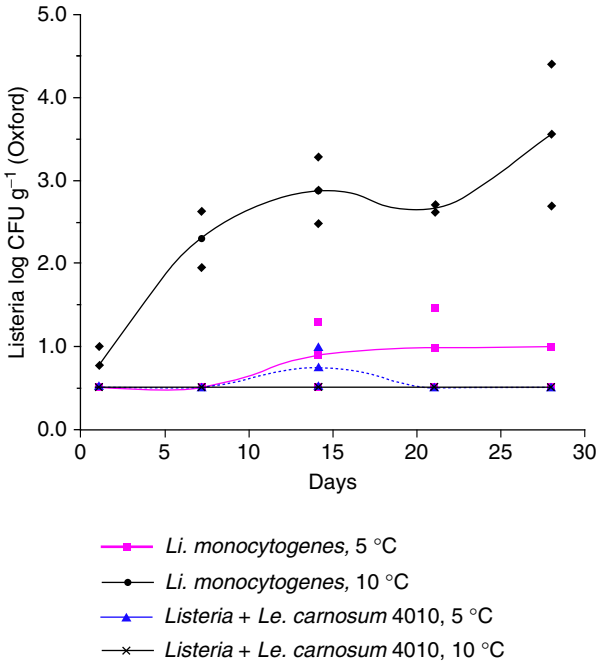

Figure 1 Inhibition of *Li. monocytogenes* in sliced ham biopreserved with *Le. carnosum* 4010. The product is sliced on a small-scale industrial slicer and the protective culture is added through two nozzles placed close to the knife. The product is packed in 20% CO_2/80% N_2 and stored at 5 °C and 10 °C. $n = 2$ for all controls only inoculated with *Li. monocytogenes*. For biopreserved series, $n = 2$ at days 1, 7, and 14 and $n = 10$ at days 21 and 28.

The use of nonbacteriocin-producing lactic acid bacteria has also shown promising effects for inhibition of *Li. monocytogenes* in sliced meat products and frankfurters. Organisms that give such effects include several strains of the species *Lactobacillus sakei*. These strains have been evaluated for antilisterial effect as well as sensory effects. The results showed that these strains are suitable for use as protective cultures in cooked, sliced meat products.

Fresh Meat

Several qualities unique to fresh meat can interfere with its biopreservation. Proteolytic and other enzymes found in the muscle tissue can remain active in raw meat. These can degrade bacteriocins and thus reduce the antimicrobial effect of the

bioprotective culture. Furthermore, other meat proteins might interact with and inactivate bacteriocins. Such proteins include glutathione, which can inactivate nisin by binding to the bacteriocin. Two other factors related to the microbial community of fresh meat can also limit bacteriocin efficacies. First, most fresh meat stored in air is spoiled by Gram-negative bacteria, and these organisms generally are not sensitive to the bacteriocins produced by lactic acid bacteria. Second, fresh meat contains a diverse bacterial community, so a bioprotective culture must be capable of competing with many different bacteria if it has to become the dominant organism throughout the entire storage period.

Biopreservation of fresh meat is potentially more effective when used in conjunction with vacuum packaging or packaging under an oxygen-depleted modified atmosphere. Vacuum-packed meat can be stored under refrigeration for several weeks. During this period, anaerobic or facultative anaerobic microorganisms proliferate on the surface of the meat. In the majority of cases, low spoilage potential flora of lactic acid bacteria will develop. However, vacuum-packed beef is sometimes spoiled due to the growth of psychotropic Enterobacteriaceae or clostridia or lactic acid bacteria of relatively high spoilage potential. Thus, some strains of *Lactobacillus sakei* are known to spoil vacuum-packed beef by producing sulfurous compounds. The growth of these organisms can be prevented by inoculating beef with *Leuconostoc gelidum* when the meat is vacuum packed. Vacuum-packaged beef can also be spoiled by psychotrophic *Clostridium* species, notably *Clostridium estertheticum*. Apparently, the growth of these strains can be inhibited by the use of biopreservative cultures. The growth of and spoilage of beef by *C. estertheticum* were prevented by addition of *Le. carnosum* 4010 to the surface of the meat before vacuum packaging. However, the use with fresh meats of additives, including biopreservative cultures, is not allowed in many countries. One possible application is the use of cultures with marinated meats because additives are permitted with this type of product.

Fresh sausages that are packaged under modified atmospheres and distributed at chilled temperature have a relatively short shelf life. Application of bioprotective cultures that have been carefully selected not to generate off-flavors can prolong the shelf life of this particular kind of sausage. Successful use of protective cultures requires trials and sensory assessment of their use with each product type, as a product might contain components that affect the culture with, ultimately, undesirable effects on the sensory quality of the product.

Bacteriophage Control of Bacteria in Meats

Bacteriophages are viruses that infect bacteria. They can be classified as lytic, if they invariably cause lysis of the infected host cell, or lysogenic, if they can integrate into the host genome and remain dormant (Figure 2). Bacteriophage host ranges are generally limited to specific bacterial species or, in some instances, to specific bacterial strains within a species. Lytic bacteriophages were recognized as potential antimicrobial agents by F. d'Herelle, in 1917. Before the discovery and subsequent use of antibiotics, bacteriophages were considered to offer a practicable means of treating bacterial infections in humans. The increase in antibiotic-resistant bacterial infections has led to a resurgence in research on the use of phages to treat bacterial infections. Several biotechnology companies are now working on the commercialization of phage therapy. Although most

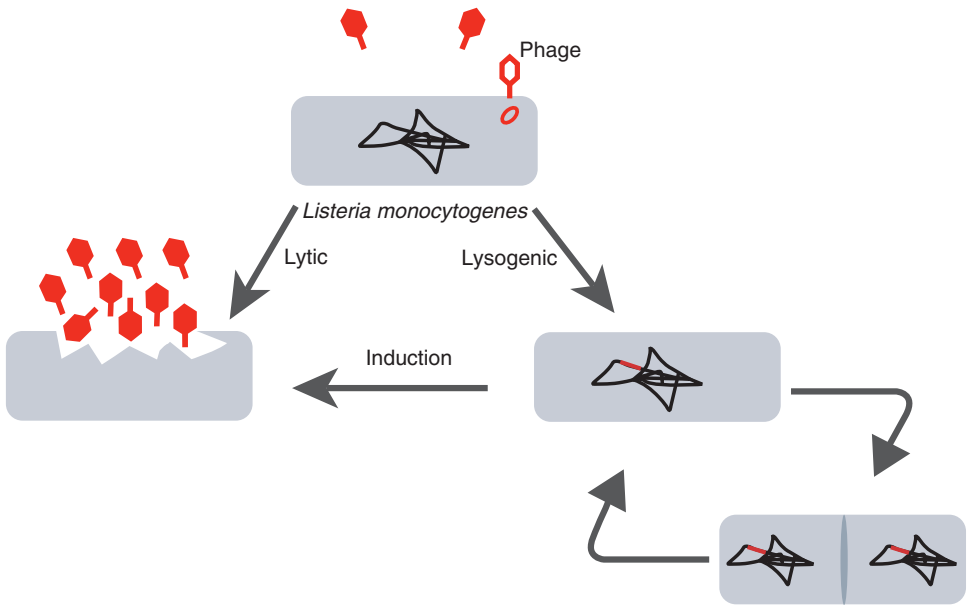

Figure 2 Bacteriophage infection begins with attachment of the phage to the host cell surface and subsequent injection of the phage nucleic acid into the host cell. The lytic life cycle results in the rapid production of progeny bacteriophage, lysis of the host cell, and phage release. The lysogenic life cycle occurs when the phage DNA integrates into the host genome, in which it is replicated and maintained in the host population during normal bacterial cell division. Induction occurs infrequently. Induced phage replicates rapidly apart from the chromosome, with consequent host cell lysis and release of progeny phage.

research on the use of bacteriophage as antimicrobials focuses on clinical applications, the use of bacteriophage as a possible means to reduce or eliminate pathogenic bacteria in foods is an emerging area of biopreservation.

The US regulatory authorities have approved the use of a phage formulation specific for *Li. monocytogenes* as an additive for ready-to-eat meats and poultry products. Consequently, commercial preparations of phages are being marketed for use in meat to control *Li. monocytogenes*. Bacteriophage preparations for use against other meatborne pathogenic bacteria, including *Salmonella* and *E. coli O157:H7*, continue to be developed with some being commercially available.

There has been only limited study of the use of bacteriophages for control of bacterial spoilage of meat. Laboratory studies have examined bacteriophage control of *Pseudomonas* spp., *Brochothrix thermosphacta*, *Le. gelidum*, and *Serratia liquefaciens*. The use of *Pseudomonas*-specific phages in trials with raw chilled beef suggested that addition of the phage extended the retail shelf life of meat by limiting bacterial growth and bacterial-associated lean tissue discoloration. However, the restricted host range of bacteriophages might limit their use for controlling spoilage, which is the result of the activities of multiple bacterial species and strains.

As with development of resistance to bacteriocins, bacteria can develop resistance to bacteriophage infection. Resistance is readily observed in controlled experiments but is seen to a lesser extent in commercial trials. To mitigate problems with resistance, bacteriophage cocktails can be used. These can contain several different bacteriophages that each use a different mechanism of cell attachment. The probability of the emergence of resistance to all components of such cocktails should be small. Ideally, the phages used as biopreservatives should be strictly lytic, because if a phage is lysogenic, development of bacteriophage resistance in the host will be promoted. Moreover, there may be other complications associated with gene exchange between host and lysogenic phage DNAs.

The interactions between bacteriophages and populations of host bacteria are complex. The efficiency of infection and cell death can be significantly influenced by the ecology of the meat. Thus, phages' concentration, threshold density of bacterial host cells, the specific meat matrix, and the storage conditions can all affect the efficacy of bacteriophage infection. Much remains to be understood about the ecology of phages in meat systems and appropriate methods for industrial application of phages before they can be routinely used as biopreservative agents for meats.

Consumer Acceptance of Biopreservation

The use of preparations containing bacteriocins or bacteriophages for meat preservation must be approved by relevant regulatory authorities. In some countries, lactic acid bacteria are considered generally recognized as safe (GRAS) organisms, which means that they can be used without any further approval, and in one instance an *Li. monocytogenes* bacteriophage has been accorded GRAS status. Bacteriocin-producing lactic acid bacteria are widely distributed and are often naturally present in fermented foods or vacuum-packaged foods, including meat products. Bacteriocin-producing lactic acid bacteria are isolated from approximately 50% of vacuum packs of sliced meat products. Such findings indicate that bacteriocin-producing lactic acid bacteria and bacteriocins are frequently consumed when vacuum-packaged meat products that have not been biopreserved are eaten. Acknowledgment of bacteriocin-producing lactic acid bacteria as usual components of meat products would likely facilitate consumer acceptance of the use of such organisms as food additives. The extent to which consumers accept the use of bacteriophage as food additives remains unclear. Consumers will probably have to be provided with appropriate information about the nature of bacteriophages and their effects on bacteria before the use of bacteriophage in foods is widely accepted.

See also: Fermentation. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Hurdle Technology; *Listeria monocytogenes*; Prions; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Viruses; Yeasts and Molds; *Yersinia Enterocolitica*. Packaging: Modified and Controlled Atmosphere; Vacuum. Sausages, Types of: Dry and Semidry; Emulsion; Fresh. Spoilage, Factors Affecting: Microbiological; Oxidative and Enzymatic

Further Reading

Aymerich, T., Picouet, P.A., Monfort, J.M., 2008. Decontamination technologies for meat products. Meat Science 78, 114–129.

Cocolin, L., Dolci, P., Rantsiou, K., 2011. Biodiversity and dynamics of meat fermentations: The contribution of molecular methods for a better comprehension of a complex ecosystem. Meat Science 89, 296–302.

De Vuyst, L., Vandamme, E.J., 1994. Bacteriocins of Lactic Acid Bacteria: Microbiology, Genetics and Applications. Glasgow: Blackie Academic and Professional.

Galvez, A., Lopez, R.L., Abriouel, H., Valdivia, E., Omar, B.N., 2008. Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. Critical Reviews in Biotechnology 28, 125–152.

Gill, A.O., Holley, R.A., 2003. Interactive inhibition of meat spoilage and pathogenic bacteria by lysozyme, nisin and EDTA in the presence of nitrite and sodium chloride at 24 degrees C. International Journal of Food Microbiology 80, 251–259.

Greer, G.G., 2005. Bacteriophage control of foodborne bacteria. Journal of Food Protection 68, 1102–1111.

Guenther, S., Huwyler, D., Richar, S., Loessner, M.J., 2009. Virulent bacteriophage for efficient biocontrol of Listeria monocytogenes in ready-to-eat foods. Applied and Environmental Microbiology 75, 93–100.

Hagens, S., Loessner, M.J., 2010. Bacteriophage for biocontrol of foodborne pathogens: Calculations and considerations. Current Pharmaceutical Biotechnology 11, 58–68.

Hugas, M., 1998. Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. Meat Science 49, S139–S150.

Joerger, R.D., 2007. Antimicrobial films for food applications: A quantitative analysis of their effectiveness. Packaging Technology and Science 20, 231–273.

Kaur, G., Malik, R.K., Mishra, S.K., *et al*., 2011. Nisin and Class IIa bacteriocin resistance among *Listeria* and other foodborne pathogens and spoilage bacteria. Microbial Drug Resistance 17, 197–205.

Lacroix, C. (Ed.), 2011. Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation. Cambridge: Woodhead Publishing.

Rose, N.L., Sporns, P., Stiles, M.E., McMullen, L.M., 1999. Inactivation of nisin by glutathione in fresh meat. Journal of Food Science 64, 759–762.

Stiles, M.E., 1996. Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek 70, 331–345.
Zhou, G.H., Xu, X.L., Liu, Y., 2010. Preservation technologies for fresh meat – A review. Meat Science 86, 119–128.

Relevant Websites

Bacteroicins in Food Products

http://www.danisco.com/product-range/antimicrobials/
Danisco.

Bacteriophage in Food Products

http://www.intralytix.com/Intral_Food.htm
Intralytix.
http://www.micreosfoodsafety.com/en/profile-mission.aspx#.UpNsdeDynP0
Micreos.

Bioprotective Cultures

http://www.chr-hansen.com/products/product-areas/meat-cultures/our-product-offering.html
Chr. Hansen.

BIOTECHNOLOGY IN MEAT ANIMAL PRODUCTION

Contents

Cloning

DN Wells, AgResearch Ltd., Hamilton, New Zealand

Glossary

Cloned animals Genetically identical animals that occur naturally in some phyla or that can be artificially produced using a variety of manipulation methods, including nuclear transfer.

Embryonic stem cells An undifferentiated cell type commonly isolated from the inner cell mass tissue of blastocyst-stage embryos. *Bona fide* embryonic stem cells can be maintained indefinitely in culture. With the appropriate induction signals, they can potentially differentiate into any somatic cell type of the body.

Nuclear (or epigenetic) reprograming Resetting deoxyribonucleic acid methylation, chromatin modification, and, ultimately, gene expression, in a differentiated somatic cell nucleus so that normal embryogenesis occurs following nuclear transfer.

Nuclear transfer The method of reconstructing a one-cell embryo from an enucleated oocyte (cytoplast) and the nucleus of a donor cell (karyoplast) to generate an embryo and ultimately a cloned animal.

Oocyte An unfertilized mammalian egg.

Pluripotent A cell that has the potential to differentiate into all three germ layers of an organism: endoderm, mesoderm, and ectoderm. However, pluripotent cells cannot develop into a fetus or adult animal themselves because they contribute poorly to the extraembryonic tissues, such as the placenta in the case of mammals.

Totipotent The ability of a single cell to divide and produce all of the differentiated cells in an organism, including the extraembryonic tissues in the case of mammals.

Introduction

A number of embryo micromanipulative techniques can be used to generate cloned livestock, the most significant of which is nuclear transfer (NT). This method involves reconstructing embryos using recipient oocyte cytoplasm and donor nuclei from individual cells. Thus, each cloned animal shares the same nuclear genetics as the original donor cell. The donor cells may be derived from embryos or somatic tissues, most notably from adults. The efficiency of NT, especially with somatic cells, is quite low, principally because of epigenetic errors in reprograming gene expression of the differentiated donor nucleus back to a totipotent state. As a consequence, aberrant patterns of gene expression during embryogenesis are believed to contribute to the cumulative losses and abnormal phenotypes observed with clones throughout development. Nevertheless, NT can produce some livestock with normal production characteristics. However, until nuclear cloning efficiency improves to levels comparable to sexual reproduction, the acceptability and utility of the technique is limited. Perhaps the best opportunity for the cloning of adults in agriculture, especially in the sheep and beef industries, is the production of teams of cloned sires from highly proven, superior progeny-tested males as an effective means of disseminating genetic gain following widespread natural mating. Cloning from embryonic cells is best combined with genomic selection, utilizing markers predictive of animal phenotype. Unique opportunities exist in both the resurrection of cloned animals following post-slaughter carcass assessment to preserve valuable genetic resources and the introduction of precise genetic modifications into the genomes of livestock via cell-mediated transgenesis.

Methods of Cloning

Animal cloning comprises a series of reproductive techniques to produce genetically identical animals. In mammals, there are four main methods:

1. blastomere separation;
2. embryo bisection;

3. embryonic stem cells combined with embryo complementation; and
4. NT.

The first two methods involve manipulating early embryos *in vitro* just a few days after fertilization (Figures 1(a) and (b)). Individual embryonic cells (blastomeres) from cleavage-stage embryos up to the four-cell stage are all undifferentiated and 'totipotent.' This means that each cell has the identical developmental potential to form offspring (following transfer back to the reproductive tract) in the same manner as the original zygote. The separated cells simply continue to divide according to their original developmental program to form a new organism. Individual blastomeres at the eight-cell stage, however, have only one-eighth the normal number of cells by the time of embryo compaction in the morula, which is insufficient for the resulting embryo to be viable. Alternatively, embryos at the morula or blastocyst stages (corresponding to days 5 and 7 postfertilization in cattle and comprising approximately 32–120 cells, respectively) can be cut into two equal halves using a microsurgical knife. The genetically identical animals produced by these manipulation methods are limited, however, to twins (or sometimes quadruplets) and mimic the processes that sometimes occur spontaneously in nature to produce monozygotic individuals. Nevertheless, embryo splitting has been integrated into multiple ovulation and embryo transfer breeding schemes to increase the number of progeny from matings between genetically superior dams and sires.

The third methodology is not currently applicable in livestock because definitive embryonic stem cells have yet to be isolated in these species. Embryonic stem cells are immortal, undifferentiated cultured cells that have been derived from embryonic blastomeres in mouse and human. Because embryonic stem cells are capable of differentiating into derivatives of all three embryonic germ lineages, but contribute poorly to placental cell types, they are defined as pluripotent. The potential of utilizing the extraordinary cellular plasticity of embryonic stem cells to produce cloned animals has been demonstrated in mice. In this species, a small group of embryonic stem cells can subsequently develop to form all of the tissues of the fetus and newborn when complemented with suitable helper cells, such as tetraploid embryonic cells, which develop into the placenta but are excluded from the fetus (Figure 1(c)).

NT is the most dramatic method of cloning because of the potential to produce extremely large numbers of clones that are genomic copies of selected individuals. Here, it is the nucleus, rather than the cell, that is totipotent. NT is essentially a replacement of nuclear deoxyribonucleic acid (DNA) between a recipient oocyte (an unfertilized egg) and a donor cell from the organism being cloned (Figure 2). The basic method was first developed in amphibians in the 1950s in order to examine the nuclear equivalence of increasingly differentiated cell types. It is noteworthy that although these pioneering studies revealed the totipotency of nuclei from larval stages, researchers were not successful in producing mature amphibians cloned from adults. In mammals, initial studies in the 1980s were restricted to undifferentiated embryonic cell types. Collectively, the data at that time suggested that cloning was more successful in those species that activated the embryonic genome later in embryogenesis, with a spectrum from mice (being most difficult), sheep and cattle (intermediate), to amphibians (relatively easy). Early mammalian experiments demonstrated the importance of coordination between the cell cycle stages of the recipient and donor cells for successful cloning. This understanding culminated in a revolution occurring in the NT field in 1996 with the birth of 'Dolly' the sheep, accomplished by Drs Keith Campbell and Ian Wilmut from the Roslin Institute in Scotland. Dolly will be forever famous as the first animal to be cloned from an adult cell. This breakthrough overturned a dogma concerning nuclear totipotency and consequently has opened new avenues of research and opportunities for agriculture and biomedicine.

Nuclear Cloning Methodology

The production of nuclear clones is a multistep process that essentially generates an entire organism with the same nuclear genetics as a selected donor cell using the technique of NT. Although there are species-specific requirements, the typical steps in the standard cloning methodology are summarized below (Figure 2):

1. Cloned animals are genomic copies of the donor cells used for NT. These can come from a variety of sources and broadly include embryonic and somatic cell types; hence the terms embryonic and somatic cell cloning, respectively. Embryonic cloning may utilize undifferentiated blastomeres or embryonic stem cells (pluripotent cultured cells derived from embryonic blastomeres; however, these have not yet been definitively isolated in livestock species). Various somatic cell types with differing degrees of differentiation derived from fetuses or biopsies obtained from selected adults can be used for NT either directly, or after variable lengths of *in vitro* culture. These primary cell cultures can be easily frozen and thawed, which provides flexibility and large numbers of cells. The optimal somatic cell type has yet to be identified. The inherent developmental plasticity of adult stem cell populations has not consistently resulted in superior cloning efficiencies, but terminally differentiated cell types typically result in very low cloning efficiency. For convenience, ill-defined dermal skin fibroblasts have commonly been used to clone adults following cell culture from an 'ear punch.' Both viable cells and intact nuclei can be obtained shortly after death (before DNA degradation), enabling suitable cell types to be isolated from carcasses (Figure 2). Furthermore, cloned animals have also been generated from tissue frozen without cryoprotectant solutions. Cultured somatic and embryonic cells can also be genetically modified *in vitro* and performing NT with these cells has many advantages in the production of transgenic animals (see Section Transgenic Animals).
2. The actual NT process begins with the enucleation of mature, unfertilized oocytes arrested at metaphase of the second cell division of meiosis. These can be obtained either by recovering oocytes from the reproductive tract a few hours following ovulation or more commonly, after *in vitro* maturation of oocytes obtained by aspirating follicles on ovaries collected from commercially slaughtered, sexually

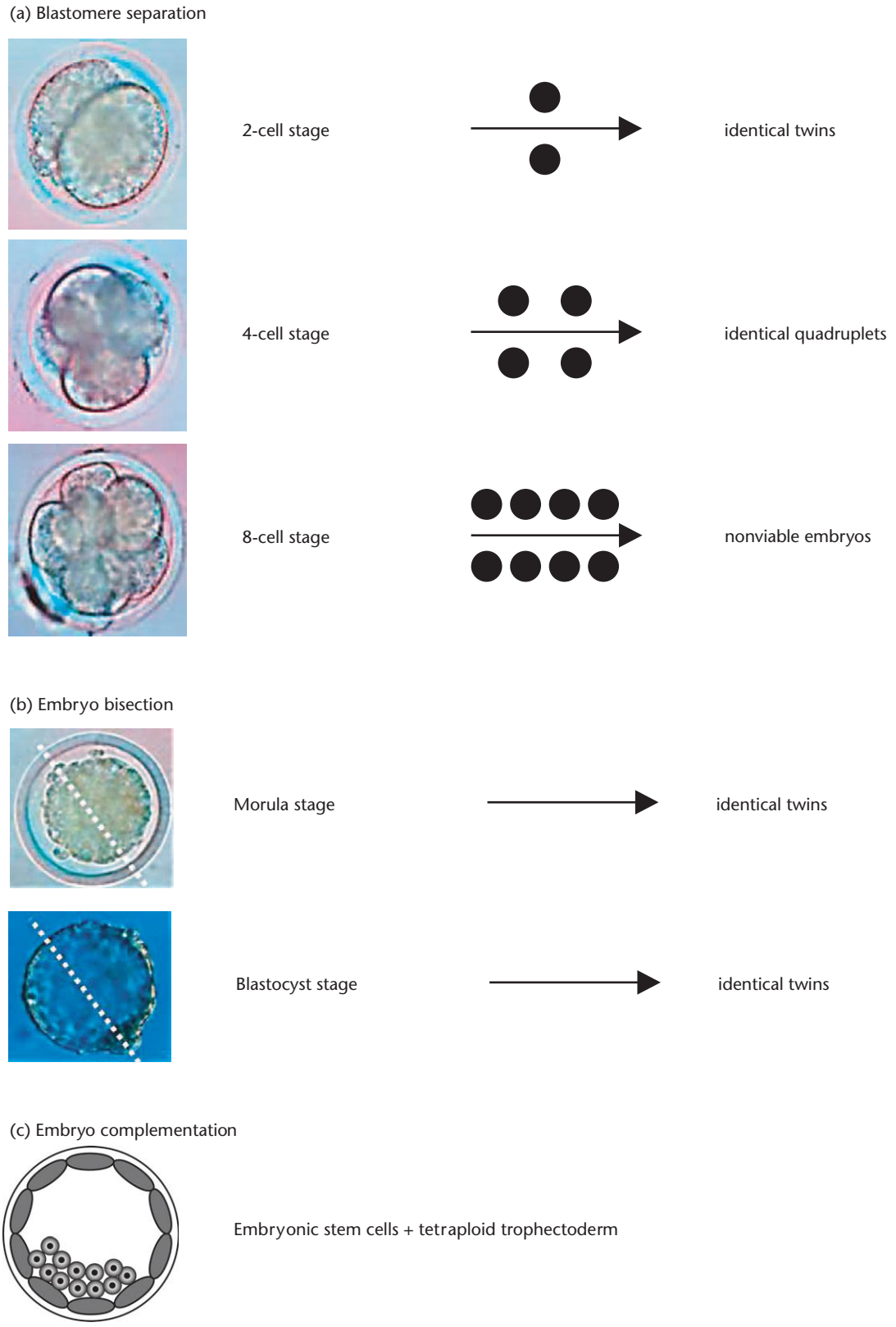

Figure 1 Cellular cloning. (a) Early cleavage-stage embryos can be separated, with each cell having the potential to produce an offspring. (b) Later preimplantation stages may be bisected, with each half possessing sufficient cells to potentially generate an animal. (c) Mouse embryonic stem cells are pluripotent (being able to contribute to differentiated derivatives of all three embryonic germ lineages) and can develop the entire fetus, but need the support of tetraploid cells in the reconstituted blastocysts to subsequently produce the placenta.

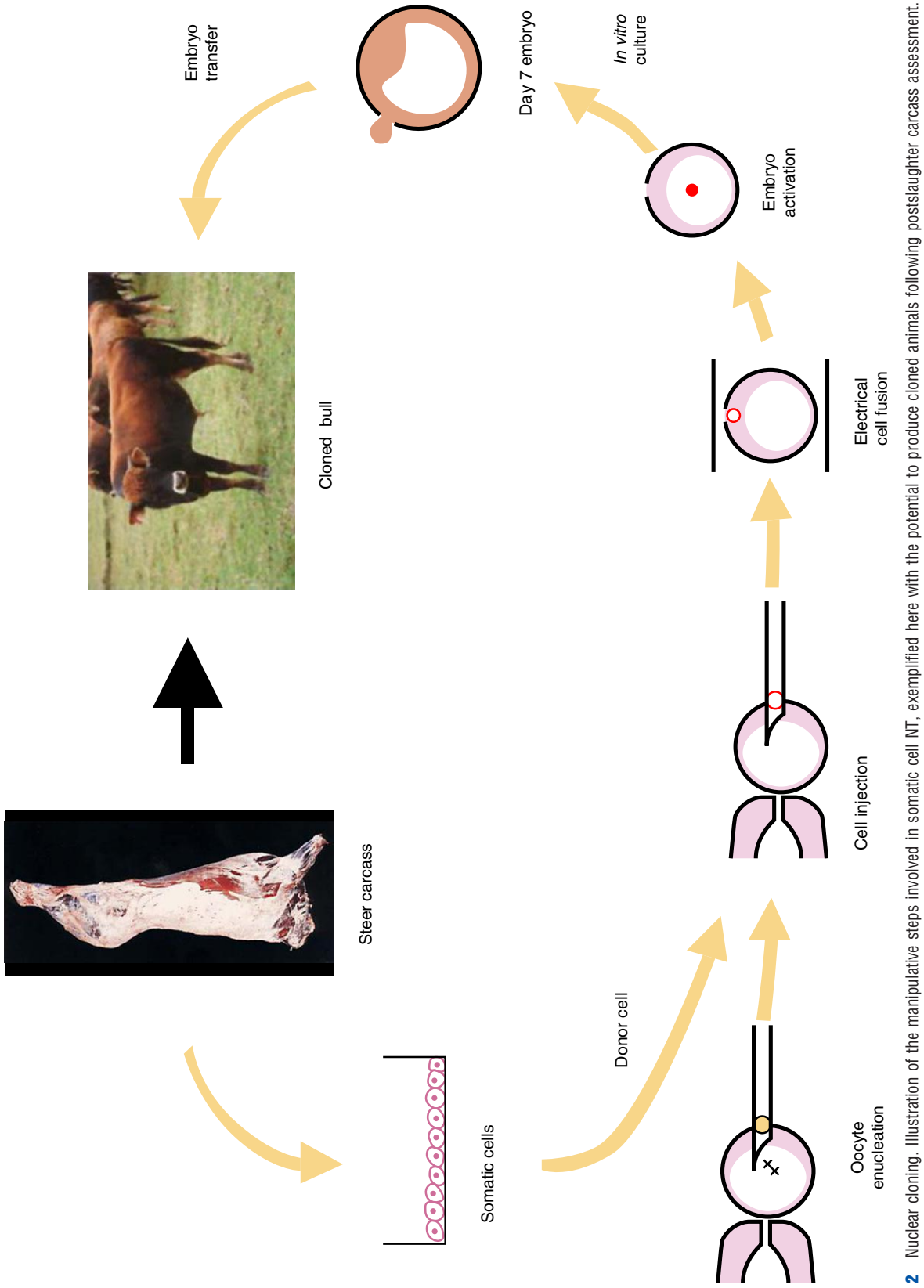

Figure 2 Nuclear cloning. Illustration of the manipulative steps involved in somatic cell NT, exemplified here with the potential to produce cloned animals following postslaughter carcass assessment. The donor cells used for NT can be obtained from a variety of somatic or embryonic types.

mature females. The enucleation process involves manipulation of each oocyte (diameter approximately 170 μm in cattle) in the microscope with finely controlled microsurgical instruments to physically aspirate the metaphase chromosomes (incorporating the oocyte's own nuclear DNA) and the extruded first polar body. Thus, the nuclear genetic material of the oocyte is removed, resulting in what is termed a cytoplast (a cell containing only cytoplasmic material). The mitochondrial DNA within the cytoplasm of the oocyte remains present (see Section Genetic and Phenotypic Identity of Clones).

3. A single donor cell is then injected underneath the outer zona pellucida (a protective membrane surrounding the oocyte and early embryo up to the blastocyst stage) and adjacent to the cytoplast membrane. Alternatively, the donor nucleus can be isolated and directly injected into the cytoplasm (circumventing fusion in step 4).
4. The cytoplast and the donor cell couplet are then correctly aligned in a fusion chamber comprising two parallel electrodes and fused together in a direct current electrical field. Thus, the genetic information contained within the nucleus of the donor cell enters the cytoplast. This is the essence of the term 'nuclear transfer,' whereby the genetic information of the oocyte is removed and replaced with that from the donor cell. Immediately following this embryo reconstruction, the donor nucleus has the opportunity to be 'reprogramed' following molecular exchanges between factors present in the oocyte cytoplasm and the donor chromatin (see Section Complete Reprograming).
5. The reconstructed one-cell embryos are then artificially activated using either chemical reagents or electrical pulses, in order to initiate embryonic development. This is *in lieu* of fertilization and ideally should mimic the repetitive intracellular calcium oscillations of the correct amplitude, frequency, and duration that simulate sperm activation.
6. Following activation, the cloned embryos are typically cultured *in vitro* in a species-specific medium to allow early development to be assessed. Culture systems that direct cloned embryos along appropriate metabolic pathways have improved development.
7. Blastocysts of suitable quality (after 7 days of culture in sheep and cattle) are then transferred to the uteri of synchronized recipient females for development to term and the eventual birth of cloned offspring that possess the same nuclear genetics as the original donor cells.

Current Efficiency of Nuclear Transfer

NT-derived clones have been produced from an ever-increasing range of species that includes laboratory mice, the major domesticated mammalian meat-producing species (including cattle, buffaloes, sheep, pigs, and goats), but not yet in poultry species. Successful cloning is influenced by many factors, one of which is the degree of differentiation of the donor cell. Expressed as the percentage of cloned embryos transferred into recipients that result in healthy offspring surviving into adulthood, the efficiency of embryonic cloning is approximately one order of magnitude higher than that of somatic cell cloning (30–40% vs. 1–15%). Specifically at AgResearch, the proportion of one-cell cattle embryos reconstructed with somatic donor cells that develop into transferable-quality blastocysts (40%) is comparable to that following *in vitro* fertilization. However, despite initial day 50 pregnancy rates being similar to those achieved with sexual reproduction (60%), the proportion of transferred cloned embryos that result in viable calves at weaning (15%) is only one-third that following *in vitro* fertilization (45%). This highlights the high rates of gestational loss that raise serious animal welfare concerns with the present cloning technology.

Complete Reprograming

One critical aspect allowing normal embryogenesis following NT is correct reprograming of the pattern of gene expression in the donor nucleus to a state comparable to that in a fertilized zygote. This involves epigenetic modifications to the organization of the donor chromatin to switch off the expression of those genes specific for the differentiated phenotype of the donor cell, and for the appropriate embryonic genes to be reactivated in the correct tissues, in the correct abundance and at the correct times to enable the cloned embryo and fetus to grow and develop normally. Our understanding of this highly orchestrated process is still evolving. Faithful expression of key embryonic genes occurs more reliably with undifferentiated embryonic cells as opposed to somatic donor cells. A functional measure of complete reprograming is evidenced by those surviving cloned animals that do display normal physiology, behavior, growth, reproduction, livestock production characteristics, and lifespans. However, even in apparently normal clones, detailed gene expression analyses indicate aberrations in up to 4% of genes implying that development is rather tolerant of subtle epigenetic differences.

Incomplete Reprograming

In the majority of reconstructed embryos, there is increasing evidence of epigenetic errors in reprograming following NT, leading to abnormal patterns of DNA methylation, chromatin modification, and X-chromosome inactivation, culminating in the misexpression of both imprinted and nonimprinted genes. The pattern of mortality and abnormalities observed following NT presumably reflects the inappropriate expression of various genes whose harmful effects are exerted at different stages of development. Moreover, sublethal aberrations that occur early in embryo or fetal development may impair subsequent health in adulthood. There is a wide spectrum of phenotypic outcomes, ranging from those that are lethal to those that are more benign and may not compromise the health and welfare of the cloned animal. As expected, the incidence and severity of the abnormalities are increased in somatic cell clones compared to embryonic clones because of the greater difficulties in reprograming a more differentiated nucleus. Understanding the molecular mechanisms involved in reprograming will ultimately improve cloning efficiencies.

The consequences of incomplete reprograming following somatic cell NT are commonly higher rates of pregnancy loss, complications during parturition, and higher postnatal losses.

Placental Abnormalities

A failure of the placenta to develop and function correctly is a common feature amongst cloned pregnancies. There are species differences in the patterns of loss during gestation but in cattle, typically 50–70% of pregnancies at day 50 are lost during the remainder of gestation compared with only 0–5% with artificial insemination. Of particular concern are the losses in the second half of gestation, especially the occurrence of hydroallantois. The accumulation of excess fluid in the allantois can be four times greater than normal. The natural occurrence of hydroallantois is rare; however, in cattle typically 25% of those cows pregnant at day 120 of gestation with clones develop the clinical syndrome. Research is underway to identify nonviable pregnancies much earlier to lessen the welfare burden. Ultimately, improved reprograming should solve this problem and increase the success.

Parturition Difficulties

Intervention is often necessary to deliver cloned offspring, as gestation length is typically prolonged and birthweight of clones can be 25% heavier than normal. Corticosteroid therapy has been used successfully to aid fetal maturation and increase the occurrence of normal vaginal delivery and subsequent rearing. Although not completely natural, this approach toward controlled parturition is feasible and acceptable on farm.

Postnatal Viability

The viability of cloned offspring at delivery and survival into adulthood depends on species, and is particularly reduced in sheep and cattle but less so in pigs and goats. The range of pathologies includes neonatal respiratory distress, defects in the cardiovascular, urogenital, neural, and skeletal systems, as well as increased incidence of chronic umbilical and lung infections. The cell cycle stage of the donor nucleus at the time of NT affects postnatal viability. Cloned calves derived from quiescent adult donor cells (in G_0 phase of the cell cycle) have significantly greater viability up to weaning compared with those derived from proliferating cells (in G_1 phase of the cell cycle; 80% vs. 50%).

Long-Term Consequences

Although clones can be physiologically normal and apparently healthy at birth, or require some time postnatally to adjust to normal homeostasis, other reports indicate abnormal clone-associated phenotypes that become apparent during juvenile and adult life. These have included obesity and shortened lifespan in mice and compromised immune systems in cattle. The incidence of these anomalies depends on the particular species, genotype, sex, cell type or specific aspects of the NT, and culture protocols used. This emphasizes the need for detailed long-term scientific studies on the health and longevity of cloned animals, especially livestock with greater biological lifespans than mice. In cattle, the current data indicate lower survival of clones between birth and 2 years of age (ranging between 47% and 80%). Although farmed livestock rarely get the opportunity to live to their biological limit, long-term viability remains an important issue with premature aging possibly affecting production characteristics, such as meat quality. The cloned offspring syndrome is a continuum, in that lethality or abnormal phenotypes might occur at any phase of development, depending on the degree of dysregulation of key genes. Even apparently normal clones can misexpress many genes without resulting in any obvious phenotype. This indicates that development is rather tolerant of some stochastic variation, at least up to a threshold, beyond which it presumably predisposes animals to some pathological condition.

Figure 3 The offspring of clones produced following sexual reproduction appear phenotypically normal, suggesting that any epigenetic errors are largely corrected during gametogenesis. This lamb resulted from the natural mating between a cloned ram (left) and a cloned ewe (center) and lacked the typical pregnancy and health complications associated with its cloned parents. Reproduced with permission from Wells, D.N., 2003. Cloning in livestock agriculture. Reproduction Supplement 61, 131–150.

Transgenerational Effects

Although there are problems in the cloned generation stemming from incomplete reprograming, the offspring of surviving clones produced following sexual reproduction appear completely normal (Figure 3). This has been most clearly demonstrated in mice; where XY male and XO female clones from the same embryonic stem cell line were mated together and produced phenotypically normal offspring, thus excluding the possibility of transmission of deleterious recessive genetic or epigenetic mutations. This indicates that the abnormal phenotypes in clones are largely epigenetic in origin and that these errors appear to be corrected during gametogenesis. This has been substantiated following the analysis of DNA methylation in the sperm of cloned bulls and provides initial confidence in those applications of NT that capture the potential of breeding from genetically elite clones.

Genetic and Phenotypic Identity of Clones

Unlike monozygotic twins, NT-derived animals are not strictly 'true clones' and there is the expectation of greater phenotypic differences among members of a clonal family (a set of NT clones derived from the same source of donor cells) than implied from the broad heritability of specific traits. There are

additional genetic, epigenetic, and environmental differences potentially contributing to variations in phenotype between clones. For instance, NT clones might possess different mitochondrial DNA derived from the recipient oocytes (if obtained from different maternal lineages). Moreover, mitochondrial DNA heteroplasmy might exist, where each cell comprises two mitochondrial genotypes, with the majority from the recipient oocyte and a minor contribution from the fused donor cell. This might have subtle but measurable effects on some livestock and meat production characteristics and could be put to advantage by combining favorable nuclear and maternal lineages. Thus, nuclear clones might at best be genomic copies of the donor cells. However, possible nonlethal point mutations or other chromosomal rearrangements in the genomic DNA of individual donor cells or which arise during embryo culture would create clones that might not even be faithful genomic copies of the donor animal. The majority of these genetic differences are likely to be inconsequential, similar to most spontaneous mutations. Epigenetic variations between clones arise from alterations in the patterns of gene expression during *in vitro* culture (of individual donor cells or embryos) and from stochastic reprograming of the donor genome following NT. Alternative patterns of mosaic X-chromosome inactivation would also be expected in cloned females. Finally, each donor nucleus/embryo/cloned animal is exposed to different environmental influences from the individual recipient oocyte cytoplasm, maternal uterus in each surrogate female, and during postnatal development. All these factors contribute to potential variations in phenotype (and genotype also in some cases) within a clonal family and deviations from the original founder animal. Despite this, the variation in livestock production traits within a clonal family would be less than the herd or population distribution, and would be dependent on the heritability of the trait. In practice, this is observed in cloned dairy cows where the composition of the milk from cloned cows is very similar to the milk of the original donor cow.

Food Safety

Despite the subtle phenotypic variability in surviving clones affecting livestock production traits, the composition of food products derived from cloned livestock is within the normal range and has been demonstrated safe to consume from animal feeding trials. It is important to note that, in most agricultural applications of cloning technology, considerably greater numbers of sexually derived progeny of clones enter the food chain, rather than the primary clones themselves.

Cloning Applications in the Meat Industry

Potential opportunities of cloning include increasing and disseminating genetic gain, the conservation of livestock genetic resources, and the production of transgenic animals. Many of these opportunities are not commercially viable until cloning efficiencies improve markedly.

Increasing Genetic Gain

Cloning might be appropriately integrated into existing breeding schemes to aid the phenotypic evaluation of selected animals, and thus contribute to additional increases in the rates of genetic progress without any increase in the rate of inbreeding. Effective breeding programs require the accurate identification of superior livestock in the nucleus population before the subsequent dissemination of their genes into the commercial population using various assisted reproductive technologies. Genomic selection strategies that allow for the identification of favorable genes or single nucleotide polymorphism markers that correlate with production will aid in selecting desirable genotypes in the future. However, actual performance might remain uncertain unless markers have exceptional predictive value for polygenic quantitative traits.

NT could be used to produce many small clonal families from selected animals. The phenotype of each family could then be determined by evaluating individual members in a variety of environmental conditions, and thus enhance genetic progress by increasing the accuracy of selection. This would also enable easier identification of genotype × environmental interactions. The evaluation of clonal families requires fewer clones per family compared with the number of offspring per parent in progeny-testing schemes to give the same accuracy of selection. This is because the clones in a family are all of the same genotype and will average out the environmental influences. However, the ideal number depends on the trait, with those of lower heritability benefiting from additional accuracy and, hence, larger family sizes. At a fixed number of animals evaluated in a breeding scheme (clones or progeny), clonal testing possibly enables greater selection pressure to be exerted by measuring more cloned families or genotypes. Another possibility is the use of lines of cloned dams in sire-proving schemes, to reduce the cost and increase the accuracy of selecting elite males for the artificial insemination industry.

The rate of genetic gain would be further enhanced by evaluating clones produced from selected embryos or embryonic cell lines to avoid the genetic lag of up to three generation intervals associated with the cloning of adults. These cell lines could be derived from embryos previously screened as superior by genomic selection following matings within nucleus breeding herds. With beef animals, for example, lines of cloned cattle could be generated and specific meat quality characteristics directly measured by harvesting some clones within each line. In those clonal lines that perform favorably, the remaining cloned animals could be used for breeding. In addition, other clones could be readily produced by thawing the appropriate frozen cells and using NT to release a larger number of the desirable animals to the industry. An extension of this is to identify carcasses with genetically superior meat characteristics shortly after harvest and to clone animals from recovered cells either for breeding or commercial meat production and, therefore, rescuing these valuable genetics (Figure 2).

Dissemination of Genetic Gain

Efficient cloning could enable the rapid dissemination of superior genotypes from nucleus breeding flocks and herds, directly to commercial producers (Figure 4). By multiplying the most superior clonal families, it has been estimated that in the dairy industry this might provide a substantial increase in

Figure 4 The application of nuclear cloning to produce multiple copies of genetically superior livestock. Somatic cells collected from the ovarian follicles of the donor cow provided the nuclear genetics to produce the 10 cloned calves.

Figure 5 The integration of cloning in the conservation of rare farm animal genetic resources. The cow in the background was the last surviving individual of the Enderby Island cattle breed, adapted to harsh sub-Antarctic conditions. Assisted reproductive technologies have been used to produce a bull calf (right), following transvaginal ovum pick-up and *in vitro* fertilization with frozen–thawed sperm available from one of nine, now dead, Enderby Island bulls, and a cloned heifer calf (left) that is a genetic duplicate of the cow. Further assisted reproduction has contributed to increasing the genetic diversity of this cattle breed. This work was supported by both AgResearch and the New Zealand Rare Breeds Conservation Society.

genetic merit for individual producers in a single generation equivalent to 15 times the annual genetic gain achieved with conventional progeny-testing schemes. The rate of genetic gain then matches that of the nucleus population until the next outstanding individuals are identified and clonally disseminated. Whole genotypes could be provided that are ideally suited for specific product characteristics or environmental conditions. Outstanding F1 crossbred animals, or composite breeds with otherwise complicated and expensive breeding strategies, could be cloned to maximize the benefits of both heterosis and greater uniformity within the clonal family without segregation of alleles following breeding. Cloned genotypes could be disseminated by the controlled release of selected lines of elite live animals for breeding or on a larger scale by the transfer of frozen/thawed cloned embryos. The embryo costs need to be relatively low, although a premium should be expected for high value known genetics of proven performance compared with semen from a progeny-tested sire that only provides half the genes and an unknown performance in the individual offspring. The marketing of cloned embryos would be an alternative to artificial insemination, but needs to be cost effective and as successful in terms of pregnancy rates, with the infrastructure and technical expertise required for extensive embryo transfer on farms, if it is to be adopted. It is noteworthy that even less complex reproductive technologies, such as *in vitro* fertilization are difficult to implement in low-cost pastoral agriculture systems and require excellent husbandry and management practices to succeed. However, if the cloned genotypes generate either novel or value-added products for which farmers receive a premium, then there might be greater economic incentive for technology adoption.

The first major commercial opportunities for cloning in agriculture will be the production of small numbers of cloned animals with superior genetics for breeding. Ideally, these would be cloned sires from highly proven, superior progeny-tested males for widespread dissemination of their elite genetics following natural breeding or, alternatively, increased semen production for artificial insemination. If cloned sires are faithful genomic copies of the original donor, this application avoids confounding issues with the transmission of mitochondrial DNA (which is only maternally inherited) and phenotypic differences arising from environmental influences, as they only need to transmit haploid copies of the donor's genome in the form of sperm. Importantly, initial results indicate that any subtle epigenetic errors in the clones are corrected via gametogenesis with resulting offspring being apparently normal. In sheep and beef industries, the use of teams of cloned sires, representing multiple copies of genetically elite males, for widespread natural mating could substitute for artificial insemination to effectively disseminate superior genes more widely throughout the population. Artificial insemination is poorly adopted in these more extensive farming systems because it is often expensive and inconvenient for farmers, quite unlike the situation in dairy industries where it has been a very successful technology to disseminate genetic gain.

Animal Conservation

Cloning can be integrated into assisted reproductive strategies to conserve rare farm animal genetic resources that should not be lost from the global gene pool (Figure 5). As most of the genetic variation in a livestock species resides in the various different breeds, the demise of indigenous or traditional breeds represents a very significant loss of biodiversity and limits any future opportunities to capture as yet unappreciated traits. More important than cloning per se, is the cryopreservation of somatic cells from rare breeds of livestock. The cryobanking of this genetic material would provide an insurance policy against further losses of diversity or possible extinction. NT could then be used to produce a clone of a deceased animal using a previously cryopreserved cell, and thus reintroduce its genetics back into the live breeding

population. Moreover, the collection and cryopreservation of somatic tissues and cells is more straightforward than the specialized and expensive protocols required for gametes and embryos. Even for conventional agriculture, it is prudent to cryopreserve cells from genetically elite animals in case of accidental death or disease.

Transgenic Animals

NT is one of several methods available to produce transgenic animals. This cell-based approach, however, has a number of distinct advantages, including: (1) the ability to introduce specific genetic enhancements to an existing genetically superior background using cells from an animal of chosen performance and sex (especially important for agricultural applications); (2) efficient production of transgenic offspring (even with current NT methods); (3) the ability to produce lines of transgenic animals rather than individual founders; (4) the potential to introduce a more extensive range of genetic modifications to the cells cultured *in vitro*; and (5) all of the transgenic animals should transmit the genetic modification through the germline rather than be sexual mosaics.

Although it is still commonplace for introduced genes (either from the same species or a different species) to be integrated at a random location within the genome, methods of site-specific integration enabling 'gene targeting' in somatic cells of livestock have been successful at some loci. Gene targeting also allows for either the functional deletion of an undesirable gene on an otherwise favorable genetic background, or to precisely alter the nucleotide sequence of a particular gene to improve a particular function in the resulting protein. However, gene targeting in primary somatic cells is considerably less efficient than with embryonic stem cells (at least, in mice). This should prompt renewed interest in attempting to isolate these pluripotent cells in livestock species with the concomitant advantage of greater NT efficiency as demonstrated in mice. The combination of NT and gene targeting has the potential to be far more precise, extensive, and rapid in terms of genetic progress than what can be achieved with traditional breeding and other available transgenic methods. For agricultural applications, cloned-transgenic males, homozygous for the trait of interest, could be used for artificial insemination or natural mating to rapidly introduce the desired genetic change into the population.

Conclusions

To develop an acceptable NT technology with wide applicability, solutions to the problem of cloning abnormalities must be found. It is recognized that the health and well-being of clones must be equal to that of conventional animals. This goal will be accomplished by improvements to the various steps of the NT process and from a greater fundamental understanding and control of reprograming. Current evidence indicates that the abnormal clone phenotypes are largely due to epigenetic errors, which are corrected during gametogenesis and are unlikely to be transmitted to sexually produced progeny. This is encouraging for the potential of cloned sires to effectively disseminate genetic gain through natural breeding or artificial insemination.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding

Further Reading

Bortvin, A., Eggan, K., Skaletsky, H., *et al.*, 2003. Incomplete reactivation of Oct4-related genes in mouse embryos cloned from somatic nuclei. Development 130, 1673–1680.

Campbell, K.H., Loi, P., Otaegui, P.J., Wilmut, I., 1996. Cell cycle co-ordination in embryo cloning by nuclear transfer. Reviews in Reproduction 1, 40–46.

Chavatte-Palmer, P., Camous, S., Jammes, H., *et al.*, 2012. Review: Placental perturbations induce the developmental abnormalities often observed in bovine somatic cell nuclear transfer. Placenta 33 (Suppl.), S99–S104.

Hiendleder, S., Zakhartchenko, V., Wolf, E., 2005. Mitochondria and the success of somatic cell nuclear transfer cloning: From nuclear-mitochondrial interactions to mitochondrial complementation and mitochondrial DNA recombination. Reproduction, Fertility and Development 17, 69–83.

Jullien, J., Pasque, V., Halley-Stott, R.P., Miyamoto, K., Gurdon, J.B., 2011. Mechanisms of nuclear reprogramming by eggs and oocytes: A deterministic process? Nature Reviews Molecular Cell Biology 12, 453–459.

Latham, K.E., 2005. Early and delayed aspects of nuclear reprogramming during cloning. Biology of the Cell 97, 119–132.

Mizutani, E., Yamagata, K., Ono, T., *et al.*, 2012. Abnormal chromosome segregation at early cleavage is a major cause of the full-term developmental failure of mouse clones. Developmental Biology 364, 56–65.

de Montera, B., El Zeihery, D., Muller, S., *et al.*, 2010. Quantification of leukocyte genomic 5-methylcytosine levels reveals epigenetic plasticity in healthy adult cloned cattle. Cellular Reprogramming 12, 175–181.

Murphey, P., Yamazaki, Y., McMahan, C.A., *et al.*, 2009. Epigenetic regulation of genetic integrity is reprogrammed during cloning. Proceedings of the National Academy of Sciences, USA 106, 4731–4735.

Niemann, H., Tian, X.C., King, W.A., Lee, R.S., 2008. Epigenetic reprogramming in embryonic and foetal development upon somatic cell nuclear transfer cloning. Reproduction 135, 151–163.

Oback, B., Wells, D.N., 2007. Donor cell differentiation, reprogramming, and cloning efficiency: Elusive or illusive correlation? Molecular Reproduction and Development 74, 646–654.

Rideout, 3rd, W.M., Eggan, K., Jaenisch, R., 2001. Nuclear cloning and epigenetic reprogramming of the genome. Science 293, 1093–1098.

Shimozawa, N., Ono, Y., Kimoto, S., *et al.*, 2002. Abnormalities in cloned mice are not transmitted to the progeny. Genesis 34, 203–207.

Solter, D., 2000. Mammalian cloning: Advances and limitations. Nature Reviews in Genetics 1, 199–207.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., Campbell, K.H., 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385, 810–813.

Genetically Modified Organisms in Meat Animal Production

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Glossary

Chimera An animal composed of at least two different cell types. Usually these animals are made from injecting an embryonic stem cell into a blastocyst stage embryo.

Embryonic stem (ES) cell Stem cells derived from the inner cell mass of a blastocyst which are able to differentiate into all embryonic tissues.

Genetically engineered A cell or animal that has had the sequences of bases within its deoxyribonucleic acid (DNA) purposefully rearranged.

Genetically modified organism (GMO) An animal that is genetically engineered by addition of a gene (transgenic), or removal of a gene (knockout).

Lentivirus From the Retroviridae family, unique ability among retroviruses of being able to infect nondividing cells.

Pronuclear injection The use of a fine needle to inject DNA into the nucleus of an unfertilized egg.

Ribonucleic acid (RNA) interference Means of gene expression within the cell where small pieces of RNA are utilized to target degradation or translational inhibition of specific genes.

Short hairpin RNA (shRNA) A sequence of RNA that makes a tight hairpin turn that can be used to silence target gene expression via the RNA interference (RNAi) pathway in the cell.

Transgenic An animal that has a gene from another species inserted into its genome.

Zygote Earliest developmental stage of the embryo formed after fusion of male and female gametes.

Introduction

A genetically modified organism (GMO) is an organism in which genetic material is altered by genetic engineering technology. GMO may sound futuristic; however, one is already surrounded by foods derived from GMO products, as they have been present in one's food supply for decades. For example, a large portion of the various crops one consumes is GMO. In contrast, animals considered as GMO cannot enter the food supply without food and drug administration (FDA) approval. Moreover, the cost for producing transgenic animals is very expensive, thus application of transgenic animals is very limited. Dramatic advancement in the technologies used for transgenic animal production may allow one to see animal-based GMOs that can enter the food chain. A discussion of the status of GMOs in the meat food supply will be provided at the end of the article as it is still controversial and under debate.

Methods to Produce Transgenic Animal Production

The first transgenic animal was reported in 1980. Transgenic mice were born by introducing foreign deoxyribonucleic acid (DNA) into a mouse pronucleus; a procedure termed pronuclear (PN) injection. This technology permits introduction of foreign DNA into an embryo at the single cell stage. If the DNA is integrated into the zygote's genome, then it is replicated with the rest of the DNA. Thus all the cells of the embryo and hence all the cells of the resulting animal contain the genetic modification. This technology is still used to produce transgenic animals; however, there are some disadvantages of using this technology. PN injection can neither control the number of copies that are integrated nor the site of integration, thus there is less control over the expression of the transgene. In addition, founder animals often exhibit mosaicism, where the animal is made up of both modified and unmodified cells because the foreign DNA often integrates after the one-cell stage. Various transgenic animals have been produced using PN technology but the efficiency of creating a founder animal with the genetic modification represented in the germ cells is lower in other species compared to mice.

Transgenic mouse production became more practical with the development of embryonic stem (ES) cells and gene targeting. ES cells are derived from early embryos and can contribute to various cell types including germ cells when transferred into host embryos. Because of the availability of ES cells, genetic modification can be performed *in vitro* and genetically modified ES cells then introduced into host embryos. If the modified ES cells contribute to the germline, then this method can result in the production of chimeric mice. Transgenic mice with the desired genetic modification can be produced by simple mating. When gene targeting technology is combined with the ES cells, transgenic mice with specific genetic modifications can then be produced. This approach is very effective and has become the standard protocol to produce knock-out mice. However, this is unique to mice and rats because to date there have been no ES cells derived that can be used for this approach in other species.

Because of a lack of ES cells, transgenic animal production has been limited in species other than mice. This was changed when the first mammal was cloned from a somatic cell. The production of such sheep as Megan and Morag (first animals cloned from differentiated cells), the now famous Dolly (first animal cloned from an adult cell line) and Molly and Polly (first transgenic animals produced using somatic cell nuclear

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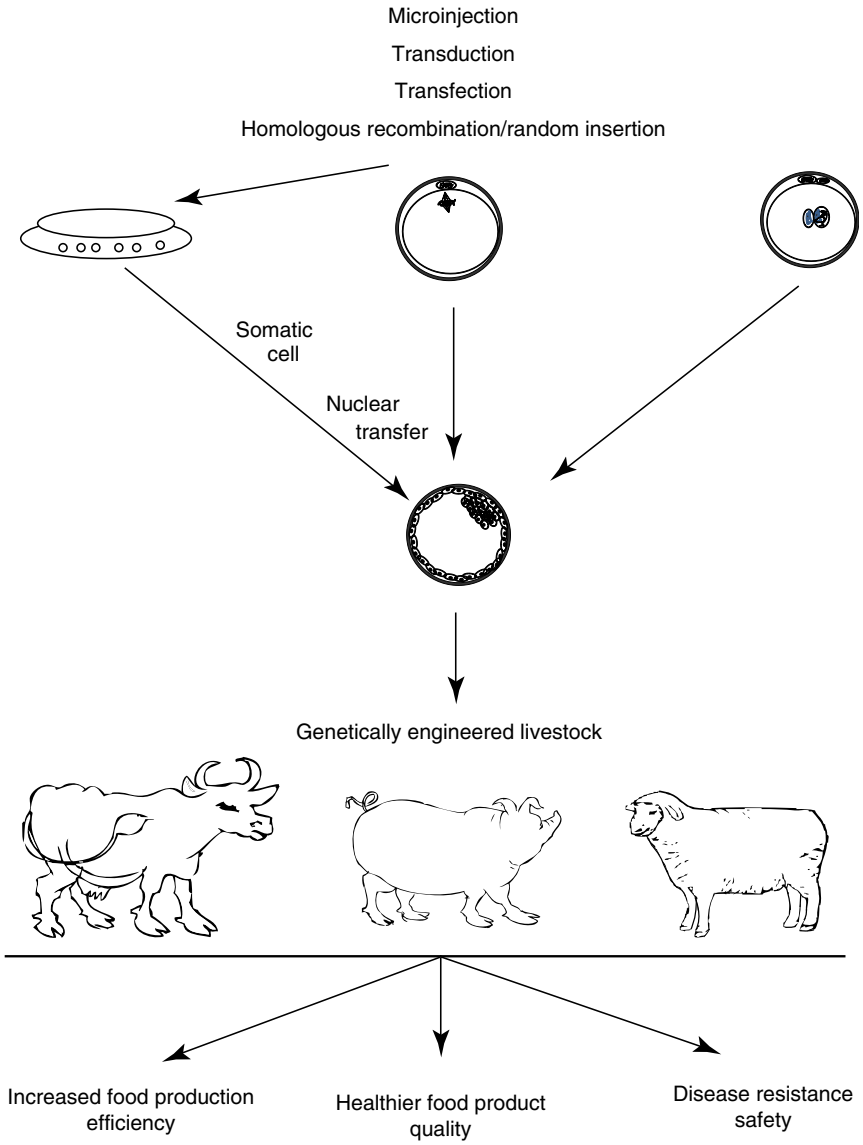

Figure 1 Schematic showing how genetic engineering can result in an early embryo that will produce an animal (cow, pig, and sheep) with the genetic modification. Such genetic modifications can increase the efficiency of meat production, make a healthier product, or result in animals that are more resistant to disease.

transfer (SCNT)) opened the door to genetic modifications that could be conducted in somatic cells *in vitro* which could then be used to produce a transgenic animal. Now SCNT technology is routinely used to produce transgenic animals in many species. However, the efficiency of genetic modification in somatic cells and cloning is extremely low, leading to the development of alternative means of transgenic livestock production.

One of these technologies is the use of a virus to deliver foreign DNA into an embryo. Certain viruses, such as retroviruses, can effectively transduce cells, oocytes and embryos when exposed to the cell surface. When oocytes were exposed to viral vectors containing foreign DNA, transgenic animals were produced in livestock animals such as cattle and pig. Advancements in this technology include the use of replication incompetent lentiviral vectors, which transduce both dividing and nondividing cells and are heritable through the germline. This method has been shown to be effective in producing transgenic cattle and swine. In addition, viral vectors have been used to generate specific genetic modifications in somatic cells that were in turn used to produce transgenic animals through SCNT. Viral vectors are certainly a way to produce transgenic animals but the viral vector can only deliver limited size of DNA fragment into the target cells (Figure 1).

Recent studies show a new possibility of producing a transgenic animal with a specific genetic modification. Zinc finger nucleases (ZFN) can be used to induce a random mutation in a specific location in zebrafish and rat embryos. ZFNs have ability to recognize specific sequences on chromosome and induce double strand break (DSB) at that location. Cells will not survive with the DSB, thus a repair mechanism is activated that results in a mutation during the repair process.

The report in the rat was a significant development because it showed that specific genetic modification could be induced in the mammalian embryonic state. It also suggested that this could be successfully utilized in other species. However, when ZFN has been introduced into pig zygotes, it showed promising results *in vitro* but there have been no viable piglets produced from the embryos. It is unknown why ZFNs are effective only in the zygotes of certain species. Application of ZFNs in transgenic animal production raises some questions on the definition of transgenic animals. In theory, ZFNs can produce transgenic animals without any trace of foreign DNA because the mutation is caused from the internal mechanism. Because of the uniqueness it will be interesting to see the future of ZFNs in transgenic animal production.

There are various methods to produce transgenic animals but there are challenges and limits in each method. Currently, production of transgenic animals on a large scale is not practical due to technological obstacles and cost. Therefore, most of transgenic animals that have been produced have been for research purposes. However, the technology provides a new avenue for improvement of livestock and specifically meat production, with the advantage of making significant genetic progress while decreasing the generation time that's normally needed with selective breeding methods.

Use of Genetically Engineered Animals in Meat Production

The ability to genetically modify food animals in order to enhance meat production provides new opportunities for livestock improvement beyond what selective breeding can achieve. Genetic modification includes not only the addition or deletion of an existing gene, but also the possibility of adding a gene from another species or utilizing already existent cellular pathways in novel ways; such as ribonucleic acid interference (RNAi). Adding a gene to improve meat quality was first investigated in 1990, when an additional copy of the growth hormone gene was introduced into swine. Earlier reports using mice demonstrated an effective increase in muscle growth using this method. However, an inability to control expression level of the gene caused researchers to halt further work with this modification. The advent of SCNT in sheep in 1997 provided a new means of modifying gene expression by allowing modification of a somatic cell line *in vitro* prior to producing embryos for transfer. This renewed interest in gene modification, paved the way for production of animal models that could someday be used to improve meat quality and production.

Current Examples of Genetic Engineering of Animals

In 2006, genetic modification was introduced to produce swine that express a gene isolated from *Caenorhabditis elegans* called *Fat-1*. The *Fat-1* gene's codons were engineered to be optimized for use in mammals prior to introduction into the pig. This gene is an *n*-3 fatty acid desaturase that converts *n*-6 fatty acids to *n*-3 fatty acids, more commonly known as omega-3 fatty acids. Omega-3 fatty acid content of red meat such as pork and beef is normally quite low because beef and swine lack an omega-3 fatty acid desaturase gene, and methods currently used to increase the omega-3 fatty acid content, such as alteration of feed ration formulations, have shown only nominal increases in content. The health benefits of consuming meat high in omega-3 fatty acids have been widely recognized. The pigs GMO produced that contain the *Fat-1* gene had elevated levels of omega-3 fatty acids in their muscle. Therefore this modification has great potential for improving the quality of the meat that is produced, and possibly the health of the animal. In 1997, the genetic modification behind the double muscling phenotype in Belgian Blue cattle was identified as a mutation in the myostatin gene, which produced an inactive protein. This mutation resulted primarily in hyperplasia (increase in number) of the muscle fibers, essentially doubling the muscle produced by one individual. Another effect of myostatin inactivation was a decrease in fat deposition, producing a leaner meat. However, this also reduced the amount of marbling in the meat; which, in many cultures, made it less desirable in taste and tenderness. Additionally, increased birth weights of the offspring contribute to dystocia in these animals, often requiring cesarean section delivery of calves. Hemizygous mutations in both mice and cattle demonstrate that the double muscling effect is dose dependent, resulting in an approximate 50% increase in muscling. This suggests that modulating the level of myostatin expression may allow us to benefit from this modification without the negative side effects seen in breeds such as Belgian Blue cattle. In 2011, researchers at Texas A&M University reported the production of calves expressing a short hairpin RNA (shRNA) targeting the myostatin gene. shRNAs work through the RNAi pathway and provide a method of decreasing the expression of a gene without eliminating its expression altogether. Other examples of increasing meat production through the use of genetic engineering include the development of transgenic trout and salmon, the latter of which will be discussed in the Section Food Safety/Approval of Genetically Engineered Animals.

Genetic modifications could also be extended to protection and improvement of the animal itself, increasing productivity and decreasing incidence of disease. One example of this is the *PrP* gene, a mutation of which results in bovine spongiform encephalopathy. Although elimination of animal-born feedstuffs such as bone and blood meal from livestock rations has dramatically reduced both the potential and the actual incidence of this disease in the US, four isolated cases in the US have still been confirmed, and spontaneous mutation of the endogenous PrP protein is still a concern. In 2006, RNAi was utilized to reduce PrP protein in goats, and then work by Richt *et al*. in 2007 produced cattle in which the *PrP* gene was ablated. Analysis of the cattle revealed that they were not only were phenotypically and immunologically normal, but that their cells also are resistant to prion propagation *in vitro*. Another example of how genetic modification can be used to improve animal health is the introduction of the lysostaphin gene into the mammary glands of cattle. This gene is used as a defense mechanism by *Staphylococcus* stimulants to guard it against other invading bacteria, including *Staphylococcus aureus*. *Staphylococcus aureus* infections in dairy cattle are extremely hard to cure, and infected animals usually end up being culled.

Expression of the lysostaphin gene in the mammary tissue of transgenic cattle effectively eliminated infection by *S. aureus* when challenged with a intramammary dose.

Another example affecting the quality of the animal product is that of people that are allergic to cow's milk. The most common immunogene is β-lactoglobulin. A transgene has been introduced into a calf that encodes a gene that produced an RNA that pairs up with the transcript for β-lactoglobulin. This pairing recruits double stranded RNA destruction pathways in the cell and destroys the RNA encoding the β-lactoglobulin. Thus β-lactoglobulin is not produced in the milk. The milk from these animals would not be immunogeneic.

Future Direction of Genetic Engineering

Genetic engineering provides a means of improving food production not only through increased muscle mass or disease resistance, but opens the door to a multitude of possibilities and allows us to decrease the time it would take to accomplish these changes through selective breeding. Consider not only increasing muscle mass, but the marbling, tenderness, or other carcass characteristics. In addition to improving disease resistance and animal health, genetic modifications could be implemented to decrease the immunogenicity of the product, increase feed efficiency and reproductive performance, increasing overall efficiency in food production and ultimately reducing food costs. The major limitation for the type of genetic modification that can be done is the imagination of the scientist. If a protein is the problem, or if a protein can solve the problem or enhance the product, then genetic engineering can probably provide a solution. The limits that exist preventing these ideas and animals from contributing to the production of quality animal products are generally not scientific, nor even economic. Although there can be considerable cost involved with the production of genetically engineered founder animals, subsequent costs are no different from any other genetically superior animal as the genes can be transmitted to subsequent generations through natural mating. The major hurdle preventing use of these genetic modifications to improve the food supply is regulatory impediments.

Food Safety/Approval of Genetically Engineered Animals

Numerous studies have reported improved growth, disease resistance, and improved qualities of carcass composition due to genetic engineering of food animals (see above examples). Unfortunately, none of these traits currently are permitted in the food supply. In contrast, genetically engineered plants have been readily accepted into and form an integral part of the food supply. Why are animals different? In 2009 the FDA issued their final guidance on regulating genetically engineered animals. They state that "the Federal Food, Drug and Cosmetic Act (FFDCA) defines 'articles (other than food) intended to affect the structure or any function of the body of man or other animals' as drugs." ….Thus "Developers of these animals must demonstrate that the construct and any new products expressed from the inserted construct are safe for the health of the genetically engineered animal and, if they are food animals, for food consumption." The final guidance was updated in 2011. Embedded in this guidance for most genetically engineered food animals is a requirement for an Investigational New Animal Drug application with the FDA. In addition, the FDA deems 'unsafe' any genetically engineered animal that has not been approved. For the new animal drug to be approved a New Animal Drug Application (NADA) must be approved.

The NADA has 14 points that must first be addressed in the text, and then there are 7 steps required to gain approval: (1) product identification, (2) molecular characterization of the construct, (3) molecular characterization of the genetically engineered animal lineage, (4) phenotypic characterization of genetically engineered animal, (5) genotypic and phenotypic durability assessment, (6) the food/feed safety and environmental safety assessments, and (7) effectiveness/claim validation. Finally there are postapproval responsibilities. So there is a clear path to approval. However, to date no genetically engineered animal has been approved for entry into the human food supply.

Since Investigational New Animal Drug Applications and NADAs are confidential information, it is not clear how many genetically engineered animals are under consideration at the FDA. One application that has become, at least partially, public information is for the Aqua Bounty salmon. These Chinook salmon have a growth hormone gene from Atlantic salmon that causes them to grow much faster and reach a marketable size in half the time as compared to wild-type salmon. While at one time it was thought that fish would be the first genetically engineered animal to navigate the approval process, there is still no approval even though Aqua Bounty appears to have provided FDA with all the information requested in 2010. There were even 2 day-long meetings (one public) evaluating the application. Unfortunately science does not always win arguments, as Congressional representatives introduced bills in Congress to ban or label AquAdvantage™ salmon. The reasons behind the opposition appear to be based on antitechnology rather than safety or economics.

Investment capital is needed for chaperoning any genetically engineered animal through the FDA regulatory process. However, if an entrepreneur evaluates the potential for approval of any of the genetically engineered animals above, he must ask himself at least two important questions. The first is "what will approval cost?", and the second is "how long will it take?" Even though these technologies have a potential to improve human health, productivity, or carcass composition, and they might make economic sense if they could be approved, an investor is not likely to take a risk on a product that may never be approved, or cost so much that the investment could not be recouped. It is not clear what will need to be done to gain approval of genetically engineered animals to enter the food supply. The longer this takes, the more likely the development of the technology will stall.

What may be needed is a product(s) that the consumer demands. One example may be pork with high levels of omega-3 fatty acids, or hypoallergenic milk, as described above. Another example may be food! The human population continues to increase and at some point the supply of food

will be limiting. When people are hungry, then they will demand increased productivity regardless if it is genetically engineered or not.

See also: Carcass Composition, Muscle Structure, and Contraction. Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Human Nutrition: Nutraceuticals. Meat, Animal, Poultry and Fish Production and Management: Disease Control and Specific Pathogen Free Pig Production. Microbiological Analysis: DNA Methods. Microbiological Safety of Meat: Viruses. Nutrition of Meat Animals: Pigs

Further Reading

Golding, M.C., Long, C.R., Carmell, M.A., Hannon, G.J., Westhusin, M.E., 2006. Suppression of prion protein in livestock by RNA interference. Proceedings of the National Academy of Sciences of the USA 103 (14), 5285–5290.

Hammer, R.E., Pursel, V.G., Rexroad, Jr., C.E., *et al.*, 1985. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 315 (6021), 680–683.

Lai, L., Kang, J.X., Li, R., *et al.*, 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nature Biotechnology 24 (4), 435–436.

Laible, G., Alonso-González, L., 2009. Gene targeting from laboratory to livestock: Current status and emerging concepts. Biotechnology Journal 4 (9), 1278–1292.

Lee, K., Prather, R.S., 2013. Cloning pigs by somatic cell nuclear transfer. In: Cibelli, J., Gurdon, J., Wilmut, I., *et al.* (Eds.), Principles of Cloning, second ed. Academic Press, pp. 245–254. Chapter 19.

Prather, R.S., Lorson, M., Ross, J.W., Whyte, J., Walters, E., 2013. Genetically engineered pig models for human diseases. Annual Review of Animal Biosciences 1, 12.1–12.17.

Rogers, C.S., Stoltz, D.A., Meyerholz, D.K., *et al.*, 2008. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. Science 321 (5897), 1837–1841.

Stotish, R., 2012. AquAdvantage salmon: Pioneer or pyrrhic victory. Abstracts from the UC Davis Transgenic Animal Research Conference VIII, August 7–10, 2011. Transgenic Research 21, 913–914.

Tessanne, K., Golding, M.C., Long, C.R., *et al.*, 2012. Production of transgenic calves expressing an shRNA targeting myostatin. Molecular Reproduction and Development 79 (3), 176–185.

Wall, R.J., Powell, A.M., Paape, M.J., *et al.*, 2005. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. Nature Biotechnology 23 (4), 445–451.

Whyte, J.J., Prather, R.S., 2011. Genetic modifications of pigs for medicine and agriculture. Molecular Reproduction and Development 78, 879–891.

Whyte, J.J., Prather, R.S., 2012. Zinc-finger nucleases to create custom-designed modifications in the swine (sus scrofa) genome. Journal of Animal Science 90, 1111–1117.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., Campbell, K., 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385 (6619), 810–813.

Relevant Website

http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf
Food and Drug Administration.

BOAR TAINT: BIOLOGICAL CAUSES AND PRACTICAL MEANS TO ALLEVIATE IT

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Glossary

Detection methods The methods for measuring boar taint in pig tissues.
Enterohepatic circulation The circulation of biliary acids, bilirubin, drugs, or other substances from the liver to the bile, followed by entry into the small intestine, absorption by the enterocyte, and transport back to the liver.
Genomics A discipline in genetics that applies recombinant deoxyribonucleic acid (DNA), DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes (the complete set of DNA within a single cell of an organism). The field includes efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping.
Single-nucleotide polymorphism (SNP, pronounced snip; plural snips) A DNA sequence variation occurring when a single nucleotide – A, T, C, or G – in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes.

Introduction

Male meat-producing animals were traditionally castrated to increase the proportion of fat in carcasses and to reduce aggressive and sexual behavior. As consumers' preferences changed to require mainly lean meat, the lower production costs of intact males led to the elimination of castration for some species. However, young male piglets are still routinely castrated in many countries, without anesthesia or analgesia, in order to raise them to heavy market weight without the risk of boar taint, an unpleasant smell and taste from heated pork products. However, the situation is changing, at least in Europe (Australia and New Zealand adopted the no-castration policy in the 1980s); entire male production has dramatically increased in the past couple of years in countries such as the Netherlands and Germany. Moreover, anesthesia and/or analgesia are also increasingly used. Yet, the major production chains in Europe have signed a declaration where they commit themselves to stop castration by 2018.

Advantages and Disadvantages of Using Entire Males for Pork Production

Castration prevents boar taint, but intact males (entire males, boars) have better feed efficiency, nitrogen retention, and lean gain compared with castrates, which typically results in significant economic gains to producers, processors, and integrated companies. These advantages can be summarized as follows:

- Boars have up to 13% better growth rate as compared with that of castrates.
- Boars consume up to 9% less feed as compared with castrates.
- Boars have up to 14% better feed conversion (to live weight) as compared with that of castrates.
- Boars have up to 20% improvement in leanness as compared with castrates.

As a result, the overall output of nitrogen in manure is less with intact males than with castrates. Not castrating also reduces labor costs, along with reduced death losses due to castration-related infections and temporary decreases in growth performance usually seen following castration.

The lower adipose tissue content of meat cuts from entire males also makes them potentially more visually appealing to consumers. There are also some reports that the meat is redder and has increased ability to retain moisture. In addition, the higher levels of polyunsaturated fatty acids in fat and muscles and the higher protein content in carcasses from entire males might indicate nutritional advantages of this meat for consumers compared with that from castrates. However, the processing quality of fat can be worse in entire males, because it is softer and less resistant to oxidation and it might negatively affect production of bacon from pork bellies. Extreme leanness can also result in a lack of cohesiveness between backfat layers and the underlying muscles. This is important as modern commercial genotypes are lean and, therefore, feeding diets with a high content of unsaturated lipids increase the cohesiveness problem. The low intramuscular fat content, which can affect the flavor and texture of the meat, may be of some concern in intact males, if it is lower than the 2–3% level recommended for optimum sensory quality. Intact males also have a 2–2.5% lower dressing percentage than gilts and castrates and higher bone content than the other sexes.

A major driving force for using intact males is the growing animal welfare concerns associated with castration. Several EU countries have banned surgical castration (even with anesthetic) and some major retailers have decided not to sell pork from castrates. Avoiding surgical castration without anesthetic, undoubtedly, improves the welfare of the animals in the short term, although as the males approach sexual maturity, they exhibit more aggressive and sexual behavior than exhibited by castrates. The increased fighting occurring between the animals can lead to an increased incidence of DFD (dark, firm, and dry) meat, skin damage, carcass bruising, and poorer growth performance. Special care must be exercised during the preslaughter handling of intact males, for instance, avoid mixing of unfamiliar animals, optimize lairage time, and use good handling practices to reduce the amount of stress on the animals.

Avoiding castration without risking boar taint would also lower the costs of swine breeding programs because the nonselected animals could be sold for meat at a normal market price. Controlling boar taint without surgical castration would, therefore, have many potential benefits for both the pork production systems and consumers acceptance of pork products.

Boar Taint: Description and Causes

Boar taint occurs in meat from entire male pigs and makes it undesirable for sensitive consumers. Two substances are primarily responsible for boar taint: androstenone (5α-androst-16-ene-3-one) and skatole (3-methylindole). Other chemicals that might also contribute to a lesser degree to off-odor in meat include androstenols, indole, and 4-phenyl-3-buten-2-one. Aldehydes and short-chain fatty acids may also either promote the perception of skatole and androstenone or be responsible for the development of off-flavors.

Cutoff levels that define a limit between untainted and tainted samples have been proposed for androstenone and skatole from sensory assessments by trained panels. Suggested cutoff levels for skatole are 0.20 or 0.25 ppm, whereas cutoff levels for androstenone range between 0.5 and 1 ppm. A large EU study including six different countries found that 30% and 11% of carcasses from boars were above the cutoff levels for androstenone and skatole, respectively. The incidence of boar taint was quite variable, due to the different breeds, slaughter weights, and production systems used. Because of the large variation in the incidence of boar taint, and because of the variety of culinary habits between countries, the acceptability of meat from intact males can be quite inconsistent in consumer surveys. Consumers in France, Germany, Spain, and Sweden were critical of pork from entire male pigs; consumers in Denmark and Holland were negative to the odor but not to the flavor, whereas consumers in the UK were not critical at all. In all other cases outside the UK, substantially, more consumers would be dissatisfied with pork from entire male than from gilts, with the difference of 10.2% versus 6.1% for odor and 6.3% versus 2.4% for flavor.

Androstenone produces a urine-like odor, whereas skatole causes a fecal- or manure-like odor and a bitter taste. People react very differently to boar taint, depending on their sensitivity. As many as 99% of consumers are sensitive to skatole, whereas the percentage of individuals that are insensitive to androstenone ranges from 11% to 30% for women compared with 24–37% for men, depending on geographic location. Among those who are sensitive to androstenone, a small minority likes the odor, whereas the majority perceives it as very unpleasant. Some of this variation may be due to differences between individuals in the expression of the human odorant receptor, ORD7D4, which is involved in human perception of sensitivity to androstenone.

Synthesis and Metabolism of Androstenone and Skatole

Androstenone is a steroid produced in the testis as the boar nears puberty and is released into the blood stream (Figure 1). It is removed by a specific binding protein in the salivary gland and released into the saliva, where it acts as a pheromone to induce a mating response in estrous sows and regulate reproductive development in gilts. Owing to its hydrophobicity, androstenone also accumulates in adipose tissue causing an off-odor when heated. Androstenone is metabolized in the liver through both Phase I (hydroxylation) and Phase II (conjugation) reactions.

Skatole is produced by bacterial degradation of tryptophan in the hindgut, with tryptophan first converted into 3-indoleacetic acid, which is subsequently converted into skatole. It is absorbed from the gut, metabolized in the liver, and then partially excreted with the urine and partially deposited in the fatty tissue. The amount of skatole produced is primarily regulated by the availability of tryptophan and the composition and activity of the intestinal bacteria. A major source of tryptophan for skatole production is from the turnover of the gut mucosa cells. Skatole is produced in equivalent amounts in the gut of both male and female pigs but is poorly metabolized and eliminated by some males, so it accumulates in fat. High activities of cytochrome P450 2E1 (CYP2E1) and CYP2A in mature male pigs are usually associated with low skatole accumulation in fat, whereas low enzyme activities can result in both high and low skatole accumulation, depending on the intensity of skatole production.

Factors Affecting the Accumulation of Boar Taint Compounds

Androstenone biosynthesis is controlled by the same mechanism as that of other testicular steroids, namely, through the activation of the hypothalamic–pituitary–gonadal axis. Thus, during puberty, androstenone levels drastically increase simultaneously with other testicular steroids. Skatole levels also increase at puberty, possibly due to the decrease in hepatic metabolism caused by the increase in testicular steroids.

The accumulation of both androstenone and skatole in fat is affected by genetic factors, and distinct breed differences in the levels of these compounds have been identified in a number of studies. Between 5% and 8% of purebred Hampshire, Yorkshire, and Landrace boars have high concentrations of androstenone in fat, whereas 50% of Duroc intact males have high concentrations. Fat skatole levels also differ among breeds. Genetic selection for animals with low boar taint should be possible due to the relatively high heritability (range from 0.25 to 0.87) of fat androstenone. Likewise, the heritability of skatole

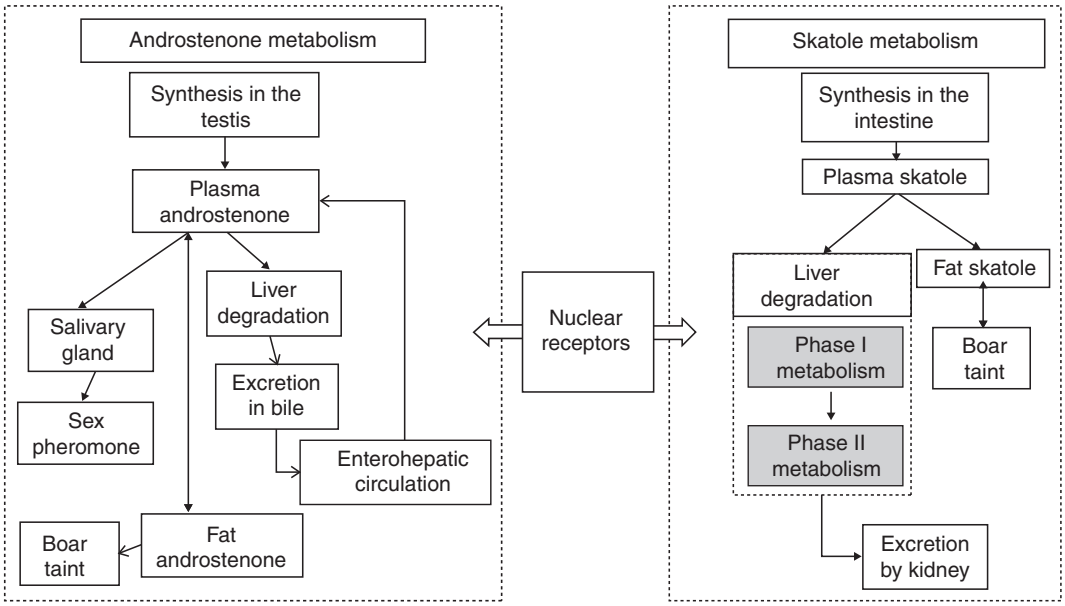

Figure 1 Summary of the metabolic pathways of androstenone and skatole. Modified from Zamaratskaia, G., Squires, E.J., 2009. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal: International Journal of Animal Bioscience 3, 1508–1521, with permission from Cambridge University Press.

is 0.55 for Landrace and 0.23 for Duroc and a positive genetic correlation between skatole and androstenone of 0.36 for Landrace and 0.62 for Duroc has been reported. This is likely a consequence of the interactions of androstenone and its metabolites with skatole metabolism. The accumulation of both skatole and androstenone can also be affected by dietary and management factors as described below.

Alternatives to Castration for Control of Boar Taint

Castration under anesthetic and/or analgesic is not a long-term solution. Indeed, economically viable methods (anesthesia with CO_2 or lidocaine or analgesia) are insufficient to avoid pain, whereas the combination of anesthesia during the surgical procedure and analgesia for a few days postsurgery is not economically feasible in most countries.

Given that steroids are mostly regulated by synthesis and release of the gonadotropins from the anterior pituitary gland during puberty, androstenone levels increase with age/weight. Therefore, slaughter at younger age and lower weight might minimize the risk of boar taint. However, this does not entirely eliminate boar taint, even when reducing slaughter weight to as low as 75 kg live weight, because the time of puberty can differ markedly among breeds and among individuals within the same breed. From an economic point of view, further reduction of slaughter weight is not an attractive alternative for producers or for processing companies.

Castrating pigs at 2 or 3 weeks before harvest reduces androstenone content in fat to levels similar to those in castrates and gilts, but surgical castration of older animals cannot be used in practice. Antagonists or agonists for GnRH can be used to interfere with gonadotropin production, but this is not an option in many countries including the EU. Attempts have been made to remove androstenone by active immunization against the steroid, but it was still deposited in the body tissues. However, immunization against GnRH is effective (see Section Immunoneutralization).

Sorting of semen according to sex is possible but not yet available for routine use in pigs. The use of flow cytometry is far too time consuming because it takes 10 h to produce a 150 million sperm semen dose (i.e., a typical commercial semen dose contains between 1 and 3 billion of sperm cells). There is, however, ongoing research to isolate sex-specific proteins on the sperm cell surface. The technology will produce specific molecules that bind together X-chromosome-bearing (female) sperm cells, leaving unbound Y-chromosome-bearing (male) cells free to be filtered from the sample. The bound or 'agglutinated' female cells can then be deagglutinated for immediate incorporation into a conventional diluter, resulting in a dose of sexed semen. By depositing the sperm directly into the uterus (intrauterine or deep intrauterine insemination), the necessary number of sperm cells can be reduced. This technique is more complicated than the conventional insemination technique where the sperm is deposited in the cervix, and it will require special training of personnel.

Use of Tainted Meat

Heat processing can reduce the levels of boar taint in processed meat products because both androstenone and skatole are volatile. Cooked products, such as cooked hams, luncheon meat, frankfurters, and cooked sausages, are typically acceptable unless they are prepared from very strongly tainted meat. Other ways of utilizing tainted meat include dilution with nontainted material and use of flavor-masking substances such as spices. Moreover, the actual sensory recognition of taint is much less pronounced in pork products that are consumed cold.

There is a great need for rapid online/at-line detection in abattoirs for identifying carcasses with unacceptable levels of boar taint compounds that might make them unsuitable for the fresh meat or high-quality markets. The 'boiling test' or 'soldering iron test' involves heating the fat sample, which causes volatilization of androstenone and skatole that can be detected by an operator. This is currently used at commercial level by some major operators in the Netherlands and Germany, but effective detection differs between operators and fatigue of the sensory response develops quickly. Objective methods are, therefore, needed. The most successful method used thus far is the spectrophotometric method for skatole used in some Danish slaughter plants. This is a 'skatole equivalent' method as it measures both skatole and indole. The limitation of this method is that androstenone is not measured at all and no more than 180 samples per hour can be tested. Research and development efforts are currently being devoted to finding operational and objective-automated sorting methods that will work at acceptable line speeds.

Provided that an effective sorting method can be developed, it is generally considered that producing entire males and using the tainted meat for processing would be economically viable if the proportion of tainted carcasses was less than 5%.

Strategies to Control Boar Taint

Effective Dietary and Management Methods to Control Skatole and Androstenone

There is a wide variation among different animals in fat skatole levels at slaughter depending on the rate of skatole production, intestinal transit time, intestinal absorption, and hepatic metabolism. Feeding and rearing factors affect skatole-producing bacteria in the hindgut. Including fermentable carbohydrates, such as inulin, sugar beet pulp, high-amylase barley, and raw potato starch, in swine diets decreases the production of skatole by the gut microflora and, consequently, lowers skatole levels in fat. This has been proposed to be due to decreased turnover of cells lining the gut, that act as a source of available tryptophan, or changes in the activity of the microflora in the intestinal tract. Skatole production can also be decreased by using various antibiotics to alter the gut microflora. Undigested carbohydrates increase fecal wet and dry weight and decrease intestinal transit time, which reduce the rate of skatole absorption from the large intestine. Withholding feed on the evening before slaughter has also been shown to reduce fat skatole levels. Skatole can also be absorbed from the manure, so dirty pigs of any sex can have high skatole levels in fat. Raising pigs on slatted floors decreases skatole levels compared with animals on concrete, likely because the animals are less dirty. Thus, a proper control of the environment and diet may reduce fat skatole levels to acceptable levels by decreasing the production of skatole.

Androstenone production is controlled by the sexual maturity of the boar, so diet does not affect the production of androstenone. Steroid hormones, including androstenone, are metabolized in the liver by a two-phase process involving first hydroxylation reactions and then the formation of conjugates by sulfation or glucuronidation. These conjugates can enter the bile and be delivered to the small intestine, where they can be deconjugated by the intestinal bacteria to produce the free steroids, which are absorbed into the portal venous blood from the enterocytes; this is known as the enterohepatic circulation. It is shown that including binding agents as dietary additives can reduce the accumulation of androstenone in fat (Figure 2). In these studies, boars were fed diets supplemented

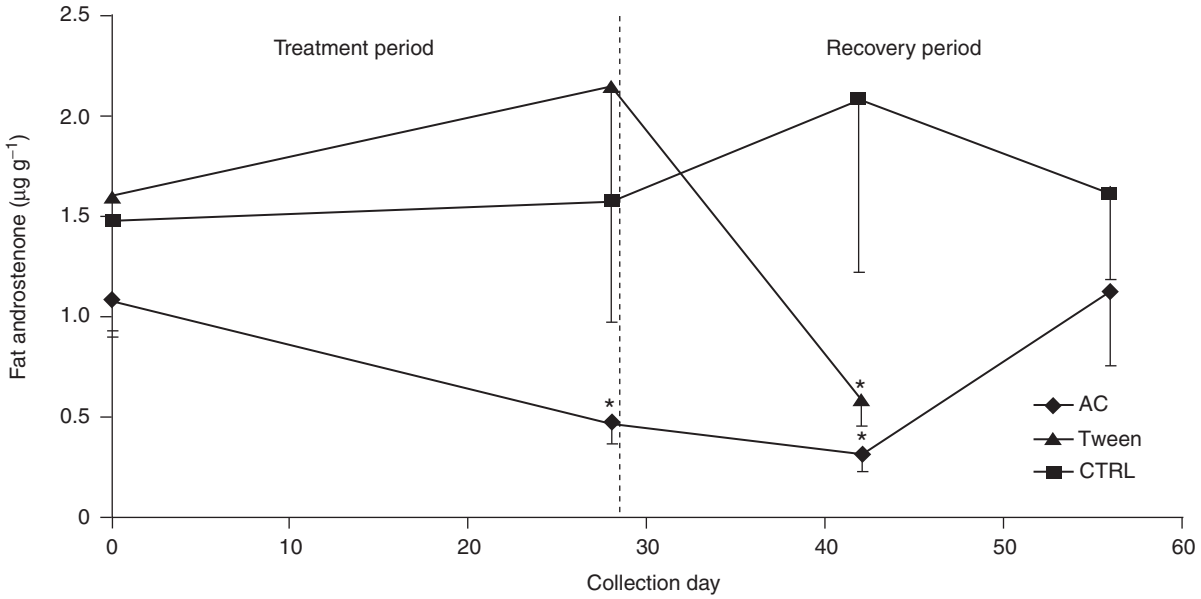

Figure 2 Profile of androstenone concentrations in fat of boars treated with activated carbon (AC, —◆—; $n=13$), (Tween, —▲—; $n=13$) and boars fed a control diet (CTRL, —■—; $n=11$). Values are plotted as means ± SE, *indicates values significantly different ($P<.05$) from values at day 0. Modified from Jen, K., Squires, E.J., 2011. Efficacy of non-nutritive sorbent materials as intestinal-binding agents for the control of boar taint. Animal: International Journal of Animal Bioscience 5, 1814–1820, with permission from Cambridge University Press.

with either 5% activated carbon or 5% tween-60 (a polysorbate that acts as a surfactant and emulsifier) for 28 days followed by either 14 or 28 days of recovery. Feeding activated carbon significantly reduced the levels of androstenone by day 42 in fat compared with day 0. Fat androstenone levels also decreased between day 28 and day 42 following treatment with tween-60. Levels of fat androstenone for control boars remained high and constant throughout the experiment. This technology is now being tested under commercial production conditions.

Immunoneutralization

Active immunization against gonadotropin-releasing hormone (GnRH), so called immunocastration or immunoneutralization, involves the administration of a modified form of GnRH to provoke the development of anti-GnRH antibodies, which bind to GnRH to prevent the stimulus for secretion of pituitary LH. Thus, a subsequent reduction of testicular steroid secretion occurs, resulting in reduced androstenone levels, size of reproductive organs, sperm numbers, and even aggressive behavior. The reduction of skatole and indole levels to low or undetectable levels in immunocastrated pigs is most likely due to enhanced metabolic clearance by the liver after suppressed steroid production, as occurs in surgically castrated pigs. IGF-1 concentrations in plasma were also reduced in immunocastrated pigs, which might affect skatole production. Therefore, immunocastration allows the production of heavy male pigs with reduced boar taint and aggressive behavior. However, the main concern about immunocastration is uncertainty about consumer acceptance of immunocastrated pork, and the adoption of Improvac™ has been slow in several EU countries and in North America. In an Australian consumer survey, consumers were positive toward the use of immunocastration, without knowing much about the background of off-odor in pork. However, the Australian pork industry has been utilizing intact males for several years and, at least to some extent, the Australian consumers are more used to potential boar taint than consumers from other regions.

To immunize pigs, two doses of Improvac™ (2 ml of 200 $\mu g\ ml^{-1}$ GnRH conjugate) are given at an interval of at least 4 weeks, with the second dose given 4–6 weeks before harvest. This allows pigs to retain the performance advantages of intact males until a few weeks before harvest, when immunologically castrated. The vaccine is administered at the base of the ear with a special vaccinator designed to prevent accidental needle sticks. Accidental self-injection of the workers performing the vaccination may, however, be a concern.

Compared with intact males, immunocastrated pigs grow faster, have a similar or slightly decreased feed efficiency, and have a higher fat content in their carcass (Table 1). This is attributable to increased feed intake, which may be due to the sharp reduction in levels of estrogen, which is known to reduce feed intake. Compared with surgically castrated pigs, immunocastrated pigs may grow faster or grow at a similar rate but consistently have improved feed efficiency and decreased fat content. This is attributable to the positive effects of androgens secreted from the testes before immunization.

An economic model to assess the costs and returns of using Improvac™ in the US market suggests an additional income of US$5.48/pig for immunocastrates compared with surgical castration. The economic implications of castration and immunocastration in the EU have also been studied.

Table 1 Effect of vaccination with Improvac™ on performance of male pigs in eight studies

Number of animals per treatment	*Growth rate*	*Feed efficiency (Gain/feed)*	*Fat content*
100	121 (119)	103 (115)	114 (85)
60	110 (103)	–	–
28	109	96	116
270	(101)	–	(98)
47	113 (115)	95 (105)	103 (98)
23	(99)	(108)	(92)
24	(110)	(110)	(92)
48	113	95	115

Note: Results are expressed as percentage of the control intact males and/or percentage of surgical castrates (between brackets).
Source: Modified from Prunier, A., Bonneau, M., Von Borell, E.H., *et al.*, 2006. A review of the welfare consequences of surgical castration in piglets and evaluation of non-surgical methods. Animal Welfare 15, 277–289.

Development of Genetic Markers for Low Boar Taint Pigs

The use of genetic markers to produce lines of pigs that are free of boar taint but otherwise grow as normal boars is a potential long-term solution to raising entire male pigs for pork production. Although the heritability of both androstenone and skatole is moderate to high, previous attempts to select for pigs with low boar taint have resulted in reproductive problems. The development of specific genetic markers for boar taint would minimize these negative effects on reproduction. Marker-Assisted Selection programs would produce lines of pigs that have a decreased genetic capacity to accumulate androstenone and skatole in fat while maintaining the normal levels of testicular steroids and would thus grow as normal boars.

One approach to developing genetic markers for boar taint is to investigate polymorphisms, usually single nucleotide polymorphisms (SNPs), in candidate genes. Candidate genes can code for key enzymes in the metabolic pathways that regulate the synthesis and degradation of boar taint compounds and should not involve other pathways such as anabolic steroid metabolism. A number of studies have shown differences in expression of candidate genes encoding enzymes involved in the metabolism of boar taint compounds, but only a few studies have reported SNPs in these genes that are correlated with levels of boar taint. A recent report from Norway compared a large number of SNPs to boar taint in Duroc and Norwegian Landrace breeds. They found significant marker effects for fat androstenone in Duroc, but not in Landrace, and significant marker effects for fat skatole in both breeds. Individual markers explained from 2.5 to 16.3% of the total variation in the traits.

The University of Guelph studies conducted by E.J. Squires and his colleagues over the past 20 years allowed for development of genetic markers for low boar taint based on SNPs in candidate genes that were selected on the basis of functional

studies. The ultimate goal was to identify the causative mutations in the most important genes, and then use these markers in breeding programs to develop lines of pigs that were free or had reduced boar taint but otherwise grew as normal boars. These studies characterized the metabolites and enzymes of the metabolic pathways involved in the secretion and degradation of the boar taint compounds. The team developed a database of approximately 1300 animals representing eight different lines, comprising six breeds (Duroc, Hampshire, Landrace, Large white, Pietrain, and Yorkshire, $n=76–219$), which were used for the discovery and validation of SNP markers. For SNP discovery, the sequences of candidate genes from pools of DNA obtained from animals from the extremes of the boar taint phenotypes in each line were compared. All animals in the database were genotyped for each SNP and an association analysis was conducted for each SNP with the amount of boar taint from androstenone and skatole in the carcass. Approximately 80 effective SNPs in 28 candidate genes for boar taint were identified. The number of significant SNPs across lines varied from 5 to 17 and from 3 to 16 for skatole and androstenone, respectively (Tables 2 and 3). In addition, 65% of the 80 effective SNPs were associated with both skatole and androstenone, which corroborates with the reported moderate positive genetic correlation between these two boar taint compounds. It is predicted that application of the markers to produce pigs homozygous for the favorable alleles would decrease fat skatole levels by 20–53% and fat androstenone by 26–61%, depending on the genetic breed and/or line.

It was also determined that none of these markers were associated with negative effects on production traits (backfat thickness, loin muscle depth, front leg score, rear leg score, subjective live muscle/conformation score, or average daily gain).

The discovered 80 SNP marker set was also validated in six lines of pigs obtained from a different source. The number of significant SNPs across lines varied from 5 to 13 and from 2 to 10 for skatole and androstenone, respectively, with 12 SNPs associated with both skatole and androstenone. Across all lines, the SNPs explained an average of 51% (range 33–73%)

Table 2 Summary of marker effects for skatole

Breed	*Number of effective markers*	R^2	*Favorable allele frequency*	*Current geometric mean*[a]	*Mean with favorable allele*[b]	*% Change*
Duroc	13	0.59	0.05–0.95	40.9	24.0	−41.2
Hampshire	17	0.42	0.28–0.85	97.5	54.4	−44.2
LWxDuroc	5	0.09	0.06–0.87	96.5	77.6	−19.6
Landrace	13	0.42	0.02–0.76	59.2	35.0	−40.9
Large White	10	0.18	0.15–0.74	72.2	48.2	−33.3
Pietrain	12	0.45	0.10–0.88	63.4	29.6	−53.3
Sire Line	9	0.33	0.03–0.92	42.1	22.2	−47.2
Yorkshire	10	0.19	0.30–0.91	24.8	15.9	−35.7

[a]The geometric mean is what is obtained when the data are back transformed.
[b]Mean with favorable allele is the mean level of skatole that would be found if the animals all had the favorable allele for each marker, that is, if the markers were used to select for low skatole levels.
Note: The R^2 represents how well the markers explain the levels of skatole.
Source: Modified from Squires, E.J., Schenkel, F.S., Mitchell, C.E., Walling, G.A., 2011. Development and validation of genetic markers for low boar taint. Plant and Animal Genomics XIX Conference Jan 2011. Available at: www.intl-pag.org/19/abstracts (accessed 07.11.13).

Table 3 Summary of marker effects for androstenone

Breed	*Number of effective markers*	R^2	*Favorable allele frequency*	*Current geometric mean*[a]	*Mean with favorable allele*[b]	*% Change*
Duroc	11	0.41	0.03–0.96	1.38	0.67	−51.6
Hampshire	3	0.13	0.21–0.81	0.79	0.31	−60.9
LW-Duroc	16	0.35	0.06–0.78	2.14	1.23	−42.7
Landrace	14	0.32	0.05–0.79	0.52	0.29	−44.1
Large White	5	0.08	0.07–0.96	0.55	0.38	−31.3
Pietrain	12	0.51	0.10–0.94	0.35	0.18	−47.3
Sire Line	10	0.27	0.09–0.74	0.88	0.54	−38.6
Yorkshire	7	0.16	0.17–0.92	0.55	0.41	−26.0

[a]The original data was log transformed to make it normally distributed. The geometric mean is obtained by back transforming log transformed numbers.
[b]Mean with the favorable allele is the mean levels of androstenone that would be found if the animals all had the favorable allele for each marker, that is if the markers were used to select for low androstenone levels.
Note: The R^2 represents how well the markers explain the levels of androstenone.
Source: Modified from Squires, E.J., Schenkel, F.S., Mitchell, C.E., Walling, G.A., 2011. Development and validation of genetic markers for low boar taint. Plant and Animal Genomics XIX Conference Jan 2011. Available at: www.intl-pag.org/19/abstracts (accessed 07.11.13).

of the skatole variance and an average of 50% (range 24–74%) of the androstenone variance. This confirmed that the discovered markers were effective in different lines of pigs and demonstrated their potential for wide applicability in pig breeding. For instance, the effect of the discovered markers, estimated in reference populations, could be used for calculating genomic-based estimated breeding values for the boar taint compounds. Then these estimated breeding values could be incorporated into overall selection indices for selecting young animals for breeding purposes.

Further Reading

Gunn, M., Allen, P., Bonneau, M., *et al.*, 2004. Welfare aspects of the castration of piglets. Scientific report of the Scientific Panel for Animal Health and Welfare. The European Food Safety Authority Journal 91, 1–18.

Jen, K., Squires, E.J., 2011. Efficacy of non-nutritive sorbent materials as intestinal-binding agents for the control of boar taint. Animal: An International Journal of Animal Bioscience 5, 1814–1820.

Prunier, A., Bonneau, M., Von Borell, E.H., *et al.*, 2006. A review of the welfare consequences of surgical castration in piglets and evaluation of non-surgical methods. Animal Welfare 15, 277–289.

de Roest, K., Montanari, C., Fowler, T., Baltussen, W., 2009. Resource efficiency and economic implications of alternatives to surgical castration without anaesthesia. Animal 3 (11), 1522–1531.

Squires, E.J., Schenkel, F.S., Mitchell, C.E., Walling, G.A., 2011. Development and validation of genetic markers for low boar taint. Plant and Animal Genomics XIX Conference Jan 2011. Available at: www.intl-pag.org/19/abstracts (accessed 07.11.13).

Zamaratskaia, G., Squires, E.J., 2009. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal: An International Journal of Animal Bioscience 3, 1508–1521.

Relevant Websites

http://www.boartaint.com/
Information on boar taint from Pfizer Animal Health.

http://www.thepigsite.com
5M Publishing.

http://boars2018.com/preventing-boar-taint/
Web site for the conference "Boars headed for 2018" devoted to voluntarily ending surgical castration of pigs in Europe by January 1st, 2018.

http://w3.rennes.inra.fr/pigcas/
Website for PIGCAS project which provides information on pig castration that will support EU policy.

The specific objectives are:

Objective 1: to collect information on the attitudes of relevant stakeholders.

Objective 2: to collect information on the practice of pig castration.

Objective 3: to evaluate research work and other information, in order to examine the various alternatives to surgical castration without anesthesia and derive research priorities.

Objective 4: to integrate the collected information and evaluation in a report providing support for the EU policy.

BY-PRODUCTS

Contents

Edible, for Human Consumption

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Introduction

The yield of edible by-products from animals varies tremendously, depending on species, sex, live weight, fatness, and methods of collection. In general, the total by-products range from 10% to 30% of the live weight for beef, pork, and lamb, and from 5% to 6% of the live weight of chickens (Table 1). The yield of edible by-products including blood and organs in cattle averages 12%, in sheep 14%, and in hogs 14% (if pork rinds are also included).

Biologically, most noncarcass material is edible if the product is cleaned, handled, and processed appropriately. What is considered edible in one geographical region though, may be considered inedible in another. Red viscera, sometimes called 'variety meat' or 'fancy meat', include the liver, heart, kidney, 'white' offal (intestines and stomach), and also blood and trimmings. Because of people's customs, religions, palatability, and reputation of products, variety meat by-products are usually limited to liver, heart, kidney, tongue, and thymus plus other sweetbreads, brain, tripe, and sausage casings. However, additional items are salvaged or used in many cultures. Although animal harvest has increased over time, the use of edible by-products for human consumption has declined.

Noncarcass material is usually separated into categories of decreasing value, such as:

1. Hides: In most cases considered nonedible.
2. Main edible ingredients: Variety meat, sausage meat, cheeks, head trimmings, and pork rinds.
3. Pet food.
4. Agricultural animal feed materials such as blood, lungs, spinal cord, breast fat, bones, and some of the stomachs. However, owing to bovine spongiform encephalopathy (BSE), tissue from ruminants in the US is no longer utilized in rumen feed and is banned as feed for farm animals in the European Union (EU).
5. Fertilizer.
6. Raw material for biogas production.

The category in which tissue is placed depends not only on potential utilization but also on market demand. Many edible by-products are downgraded in price because of a nonprofitable market. This, combined with their usually high nutritional value, makes them an economical buy, particularly in countries with low incomes; therefore, there is a large international trade in these products. If these products are not salvaged, they pose a tremendous environmental pollution problem. Fortunately, many cultural groups prepare interesting and delicious variations of variety meats and are often large consumers of these products. Variety meats are usually different from skeletal tissue in structure, proximate composition, and functional and sensory properties, and they usually have excellent nutritional value as shown by the protein, fat, mineral, and vitamin contents in Tables 2–4. In general, variety meats can be higher in cholesterol than muscle tissue (Table 5). These tissues are more perishable than muscle tissue because of their higher glycogen content and lack of fat covering. Therefore, they should be cooled quickly after slaughter, handled in a hygienic manner, and cooked as soon as possible.

Products

Liver

In market-weight animals, beef liver averages 5 kg, veal liver 1.5 kg, lamb liver 1.4 kg, and pork liver 1.4 kg (3.2 kg for a mature sow). Pork liver can be identified by the connective tissue structure, which has a 'nutmeg' (or Morocco leather) appearance. Livers are removed on the harvest floor, and the gallbladder and bile duct are removed. The liver is washed, drained under refrigeration, and quickly chilled. The liver may be sent to a retail store in this condition, or the capsula fibrosa first might be removed with a knife or with a mechanical skinner. Livers may be frozen but they become softer, and their quality decreases with increasing storage temperature. To increase storage time, vacuum-packaging can be used. Increasing

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Table 1 By-product yield from various species

By-product	*Percentage of live weight*			
	Beef	*Hog or pig*	*Lamb*	*Chicken (1.4–2.3 kg)*
Blood	2.4–6	2–6	4–9	
Dried blood	0.7			
Brain	0.08–0.12	0.08–0.1	0.26	0.2–0.3
Breast fat	0.07			
Cheeks	0.03–0.32			
Chitlings (in Europe chitterlings)	0.06			
Cracklings	3.0	2.2		
Ears	0.02			
Edible kill fat (edible fat removed on the slaughter floor)	1–7	1.3–3.5	12	
Feet	1.9–2.1	1.5–2.2	2.0	
Gizzard				1.9–2.3
Gullet	0.03	0.1		
Hanging tender	0.19			
Head		5.2	6.7	
Head and cheek meat	0.32–0.4	0.54–0.6		
Head trimmings	0.03			
Heart	0.3–0.5	0.15–0.35	0.3–1.1	0.3–0.8
Intestines		1.8	3.3	
Jowl		2.7		
Kidney	0.07–0.24	0.2–0.4	0.3–0.6	
Large blood vessels	0.07			
Lips	0.1–0.24			
Liver	1.0–4.5	1.1–2.4	0.9–2.2	1.6–2.3
Lungs	0.4–0.8	0.4–0.85	0.7–2.2	0.7
Omasum	0.38			
Abomasum (maw)	0.48			
Pancreas	0.06	0.1	0.2	
Pizzle (penis of male animal)	0.18			
Rendered edible fat	2–11	12–16	9	
Rennet	0.23			
Skirt	0.15–0.3	0.4–0.5	0.5	
Spinal cord	0.3			
Spleen	0.1–0.27	0.1–0.16	0.1–0.4	0.15
Stick trimmings	0.48	0.21		
Tail	0.1–0.25	0.1		
Tongue	0.25–0.5	0.3–0.4		
Tripe (stomach)	0.75	0.6–0.7	2.9–4.6	
Weasand (muscle tissue of the oesophagus)	0.04–0.09	0.05		

Source: Data from Gerrard, F., Mallion, F.J., 1977. The Complete Book of Meat. London: Virtue Press; Ockerman, H.W., 1975, 1983, 1996. Chemistry of Meat Tissue, eighth, tenth and eleventh eds. Columbus, OH: The Ohio State University; Ockerman, H.W., Hansen, C.L., 1988. Animal By-Product Processing. Chichester, UK: Ellis Horwood; Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic; and Romans, J.R., Jones, K.W., Costello, W.J., Carlson, C.W., Zeigler, P.T., 1985. The Meat We Eat, twelfth ed. Danville, IL: Interstate.

bacterial counts (10^6 g^{-1}) and pH values below 6 often indicate spoilage.

Liver is considered one of the most nutritious parts of the animal and is often used as a source of vitamin B_{12} and vitamin A. Liver is usually thinly sliced and cooked by a variety of techniques, or it may be minced and incorporated into many dishes, loaves, spreads and sausages, such as braunschweiger (often 50% liver), liver cheese, liver loaf, liver mush, liver paste, liver paste with truffles, liver pudding, liver sausage, liver spread, and liverwurst (often a minimum of 30% liver). These sausage items frequently contain 2.5% salt, up to 3.5% nonfat dried milk, 0.3% onion powder, 0.25% sugar, sodium nitrite, sodium erythorbate, white pepper, allspice, cloves, sage, marjoram, nutmeg, and ginger. The batter mixture is stuffed into a casing and cooked in water to an internal temperature of 67 °C and then cooled in ice water.

Heart

In market-weight animals, a beef heart averages 1.4 kg, veal heart 227 g, pork heart 227 g, and lamb heart 113 g. Hearts are removed on the harvest floor and trimmed of cartilages and fat; they are slashed for inspection and to remove clotted blood. The hearts are then washed, drained under refrigeration, and chilled. Hearts are less tender than liver and require moist cooking for extended periods. They may be diced and added to stews or minced and added to other meat for added

Table 2 Range of composition of beef variety meat per 100 g of raw edible portion

	Protein (g)	*Fat (g)*	*Ca (mg)*	*P (mg)*	*Fe (mg)*	*Na (mg)*	*K (mg)*	*Thiamin (mg)*	*Riboflavin (mg)*	*Niacin (mg)*	*Vitamin B_6 (mg)*	*Pantothenate (mg)*	*Biotin (g)*	*Folacin (g)*	*Vitamin B_{12} (mg)*	*Vitamin A (IU)*	*Ascorbic acid (mg)*
Brain	10.4–11.5	8.6	10	312	2.1–2.4	125	219	0.07–0.23	0.22–0.26	3.0–4.7	0.10–0.26	2.5	2.0–6.1	4–12	4.7–10.9	Nil	16.6–23.0
Heart	14.9–28.5	3.6–20.0	5	195–230	4.0–4.9	86–95	193–320	0.19–0.68	0.80–1.02	6.3–9.5	0.23–0.43	1.2–2.3	2.0–7.3	2–110	8.0–13.7	Trace–3.0	2.0–7.6
Kidney	15.3–24.7	2.6–6.7	10–11	219–230	5.7–7.4	176–180	225–230	0.28–0.38	1.90–2.55	5.4–7.9	0.32–0.44	3.4	24.0–92.0	41–77	8.5–31.0	264–880	8.9–15.0
Liver	19.0–22.9	3.8–7.8	6–8	352–360	6.5–7.0	81–136	281–320	0.23–0.28	2.78–3.30	12.8–21.0	0.74–0.94	5.5–8.3	33.0–100.0	81–330	65.0–110.0	12 709–105 032	2.6–31.0
Pancreas	17.6–27.1	7.3	8	216–330	2.8–8.4	67	276	0.14	0.34–0.55	3.1–5.8	0.20	3.8	14.0	–	4.8–5.0	Nil	13.7–14.0
Tongue	15.3–22.2	10.4–14.6	6–8	170–182	2.1–2.9	73	197–250	0.12–0.17	0.28–0.49	3.9–4.9	0.13–0.31	2.0	1.0–3.3	4–7	3.8–7.0	Nil	3.1–7.0
Veal liver	19.2–21.5	4.7–7.3	7–8	333–360	8.0–8.8	73–93	281–330	0.20–0.52	2.70–3.30	11.4–16.5	0.30–0.54	6.0	39.0–75.0	46–240	100.0	13 530–22 500	18.0–36.0

Source: Data from Anon, 1976. The Nutritive Value of Meat and Other Protective Foods. Chicago: The National Live Stock and Meat Board; Ockerman, H.W., 1975, 1983, 1996. Chemistry of Meat Tissue, eighth, tenth and eleventh eds. Columbus, OH: The Ohio State University; Ockerman, H.W., Hansen, C.L., 1988. Animal By-Product Processing. Chichester, UK: Ellis Horwood; Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic; Paul, A.A., Southgate, D.A., 1978. McCance qnd Widdowsons. The Composition of Foods, fourth ed. London: HMSO; USDA, 1963. Composition of Foods (Agricultural Handbook No. 8). Washington, DC: Agricultural Research Service; and US Export Federation (No date). Variety Meat from the USA – A Buyers' Guide, second ed. Denver, CO: US Export Federation.

Table 3 Range of composition of pork varietly meat per 100 g of edible portion

	Protein (g)	*Fat (g)*	*Ca (mg)*	*P (mg)*	*Fe (mg)*	*Na (mg)*	*K (mg)*	*Thamin (mg)*	*Riboflavin (mg)*	*Niacin (mg)*	*Vitamin B_6 (mg)*	*Pantothenate (mg)*	*Biotin (g)*	*Folacin (g)*	*Vitamin B_{12} (mg)*	*Vitamin A (IU)*	*Ascorbic Acid (mg)*
Brain	10.3–12.2	8.6–9.2	10	312	2.6–2.4	125	219	0.16–0.23	0.26–0.28	4.3–4.4	0.19	2.8	–	6.0	2.2–2.8	Nil	13.5–18.0
Heart	16.8–23.5	2.7–4.4	3–6	131–220	3.3–4.8	54–80	106–300	0.13–0.16	0.81–1.24	6.6–9.6	0.29–0.39	2.5	4.0–18.0	2–4	2.4–8.0	Trace–106	3.0–5.3
Kidney	15.4–25.4	2.7–3.6	8–11	218–270	5.0–6.7	115–190	178–290	0.26–0.58	1.70–1.90	7.5–9.8	0.55	3.1	32.0–130	72	6.6–14.0	130–230	14.0–14.2
Liver	18.9–21.6	2.4–6.8	6–10	356–370	19.2–21.0	73–887	271–320	0.28–0.31	3.00	14.8–16.4	0.68–0.69	0.9	27.0	110–212	25.0–26.0	Nil–10 900	13.0–25.3
Pancreas	28.5	4.0–15.0	–	–	18.9	–	–	0.11	0.46	3.5	–	4.6	–	–	6.5–7.0	Nil	15.0–15.3

Source: Data from Anon, 1976. The Nutritive Value of Meat and Other Protective Foods. Chicago: The National Live Stock and Meat Board; Ockerman, H.W., 1975, 1983, 1996. Chemistry of Meat Tissue, eighth, tenth and eleventh eds. Columbus, OH: The Ohio State University; Ockerman, H.W., Hansen, C.L., 1988. Animal By-Product Processing. Chichester, UK: Ellis Horwood; Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic; Paul, A.A., Southgate, D.A., 1978. McCance qnd Widdowsons. The Composition of Foods, fourth ed. London: HMSO; USDA, 1963. Composition of Foods (Agricultural Handbook No. 8). Washington, DC: Agricultural Research Service; and US Export Federation (No date). Variety Meat from the USA – A Buyers' Guide, second ed. Denver, CO: US Export Federation.

Table 4 Range of percentage of fatty acids in beef and pork organ fats

Fatty acid	*Liver*		*Heart*		*Kidney*		*Brain*		*Spleen*	
	Beef	*Pork*	*Beef*	*Pork*	*Beef*	*Pork*	*Beef*	*Pork*	*Beef*	*Pork*
$C_{10:0}$	–	–	0.1	0.3	0.1	0.1	–	–	–	Tr
$C_{12:0}$	Tr	–	0.1	0.2	0.1	0.1–0.3	–	–	Tr–0.3	Tr–0.4
$C_{13:0}$	Tr	0.1	–	–	–	–.1	–	–	–	–
$C_{13:1}$	Tr	–	–	–	–	–	–	–	Tr	–
$C_{14:R}$	–	–	0.2	–	0.1	0.1	0.2	Tr	–	0.1
$C_{14:0}$	0.8–1	0.5–0.8	1–2	0.2–2	2.0	1	0.4–1	0.3–0.4	1–2	1–2
$C_{14:1}$	0.7	0.2	0.2	–	0.4	0.1	–	–	–	Tr
$C_{15:R}$	0.5	–	0.2	–	0.7	–	0.2	–	–	–
$C_{15:0}$	0.7	0.5	0.3	–	0.8	0.1	2	0.1	0.4	0.1
$C_{15:1}$	2	–	0.3	–	0.4	0.4	–	1	0.7	Tr
$C_{16:R}$	–	0.7	0.6	0.3	–	–	0.2	–	–	–
$C_{16:0}$	12–15	12–16	12–16	14–20	14–22	18–21	12–16	12–16	18–24	18–22
$C_{16:1}$	1–4	0.8–1	2–4	2–3	1–4	2–3	1–2	1–2	3	2–4
$C_{16:2}$	–	–	–	–	–	–	0.8	–	0.8	–
$C_{17:0}$	1	0.7	0.9	0.6	0.9	0.4	0.7	0.5	2	2
$C_{17:1}$	3.7	1.2	2.3	0.1	0.9	0.3	2.8	1.1	1.0	2.3
$C_{18:0}$	15–25	17–19	14–21	19–14	15–25	13–19	10–22	18–27	13–15	13–20
$C_{18:1}$	12–19	13–28	19–29	21–40	18–29	30–40	16–30	12–35	23–31	23–29
$C_{18:2}$	9–10	10–12	7–16	9–18	5–12	5–8	0.2–2	1–2	7	7–8
$C_{18:3}$	3.2	0.8–1	2	2–5	0.3–2	1.7	4	1–3	2	2
$C_{19:0}$	0.3	0.7	0.6	2	2	0.6	0.6	1	1	3
$C_{20:0}$	4	1	2	2	0.4	0.1	2	0.4	2	1
$C_{20:1}$	–	–	–	Tr-4	–	Tr-6	Tr-2	2	–	1
$C_{20:2}$	4	2	2	0.8	–	0.4	–	–	–	2
$C_{20:3}$	–	3	0.8	1	–	0.7	0.6	0.7	–	–
$C_{20:4}$	6–8	10–12	1–8	1	2–10	2–3	8.0	5–8	5.2	2.4
$C_{20:5}$	7	3	4	0.1	–	0.4	–	–	–	0.4
$C_{21:0}$	–	–	–	–	0.7	–	2	–	–	0.3
$C_{21:3}$	–	–	–	–	–	–	–	–	2	–
$C_{22:0}$	–	–	2	0.7	0.6	0.4	1	0.6	–	2
$C_{22:4}$	–	–	–	–	–	–	1	–	1	–
Saturated	37.3–39.0	32.1–38.3	30.0–46.0	26.6–40.9	31.5–56.5	32.00–43.8	23.3–48.4	22.6–46.1	33.3–46.8	33.3–50.6
Unsaturated	61.0–62.7	61.7–67.9	54.0–70.0	59.1–73.4	43.5–68.5	56.2–68.0	51.6–76.7	53.9–77.4	53.2–66.7	49.4–66.7

Note: Tr, trace; R, clockwise rotation around the asymmetric carbon.

Source: Data from Renon, P., Comi, G., Cantoni, C., Persiani, G., 1980. Acidi grasi del grasso d'organi di animali domestici. Industrie Alimentari 19(6), 507–510 and USDA, 2003. USDA Nutrient Data Laboratory. Available at: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (accessed 18.01.04).

flavor. Heart cavities may be stuffed with dressing or parsley and then roasted. Hearts may be sold fresh or frozen or used in processed luncheon meat to add high-quality protein and color.

Tongue

Beef tongue of a market-weight animal averages 1.7 kg (long cut 2.3–3.1 kg and short cut 1.6–2.3 kg) and may be white, black, or variegated and may frequently have black spots. Veal tongue averages 0.7 kg, pork tongue 0.3 kg, and lamb tongue 0.2 kg. Tongues are removed on the harvest floor, trimmed, washed, drained under refrigeration, and chilled. The tough outer membrane of the tongue can be removed easily after blanching and a short submersion in boiling water. Tongues are rather tough and require long-time moist heat cooking. In addition to fresh availability, tongues may be pickled (corned, and often requiring soaking before cooking), smoked or canned in an agar solution, or used in a perishable jellied product. This can be manufactured by curing, water cooking, mincing, adding gelatin, seasoning, stuffing or placing in molds, and chilling, or it can be potted by fine mincing, mixing with seasoning and then canned and processed. Tongue also can be used as an ingredient in luncheon meat. Tongues might be brine cured by a long-immersion cure or artery cured using the two lingual arteries at the base of the tongue. This is usually followed by placement in a cover pickle for several days. Tongues are thinly sliced and can be served hot or cold, often with garnishes or with sweet or sour sauce, horseradish, mustard sauce or other spicy sauces or dressings and might also be added to casseroles and salads.

Kidney

A pair of beef kidneys is lobed (15–25 lobes) and is contained in the kidney knob (suet and fat). An average kidney weight is 0.4–0.6 kg each. Veal kidney is similar to beef kidney, except that it is smaller (340 g) and is sometimes left as part of the

Table 5 Cholesterol content

Variety meat	*Treatment*	*Cholesterol (mg per 100 g meat)*
Brain	Raw	>2000
Brain and beef	Raw	1672
Brain and pork	Raw	2195
Heart and beef	Raw	140
Heart and beef	Cooked	270
Heart and pork	Raw	131
Kidney and beef	Raw	285–375
Kidney and pork	Raw	319
Kidney	Cooked	800
Lard	Rendered	95–240
Liver and beef	Raw	300–354
Liver and beef	Cooked	435
Liver and calf	Cooked	435
Liver and lamb	Cooked	435
Liver and pork	Cooked	435
Liver and pork	Raw	301
Spleen and beef	Raw	263
Sweetbread	Raw	260
Tongue	Raw	180
Tripe	Raw	95
Muscle: Beef, pork, and lamb	Raw	59–79

Source: Data from Ockerman, H.W., 1975, 1983, 1996. Chemistry of Meat Tissue, eighth, tenth and eleventh eds. Columbus, OH: The Ohio State University; USDA, 1963. Composition of Foods (Agricultural Handbook No. 8). Washington, DC: Agricultural Research Service; and USDA, 2003. USDA Nutrient Data Laboratory. Available at: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (accessed 18.01.04).

loin to produce veal kidney chops. Sheep kidney has a single lobe and averages 57 g. It is sometimes left in the carcass to produce kidney chops or English lamb chops, but most often it is sold whole. The pork kidney is single lobed, weighs 113 g in a market-weight animal, and is usually sold whole. The kidneys might remain with the carcass until it is cut. After its removal and removal of capsule membrane and fat, the kidney is soaked in water, dried and chilled. Kidneys may be used as ingredients in meat casseroles, stews, or pies. Beef kidney should be cooked in water or braised. Lamb and veal kidneys are tenderer than beef kidneys and may be broiled or wrapped in bacon and cooked on a skewer.

Sweetbreads

Sweetbreads are harvested from calves, lambs, and young cattle. Two different organs and three different tissues located in these animals are called 'sweetbreads.' A yellowish-white lobulated thymus consists of two parts: One portion (0.05–0.23 kg) is located in the cervical region in the neck adjacent to the trachea and is called 'neck bread,' 'neck sweetbread,' 'throat sweetbread,' or 'throatbread'; the other portion (0.05–0.1 kg) is in the thorax region and is called 'heart bread' or 'heart sweetbread.' The thymus tissue is large and active during animal growth (only available from young calves and lambs) but degenerates and is replaced by fibrous tissue after the animal matures. During harvest, it is separated from surrounding tissue, washed, dried under refrigeration, and then chilled. The thymus sweetbread is tender but very perishable and should be frozen, precooked (simmered in acid water), or used immediately. The membrane should be removed.

The brownish-yellow lobulated pancreas is called 'gut bread' or 'stomach sweetbread' and weighs approximately 170 g in market-weight beef animals and 85 g in sheep and pigs. It is also trimmed, washed, drained under refrigeration, and then chilled.

Sweetbreads may be scrambled (often with eggs), reheated in sauce, breaded and deep-fat fried, used in salads, or coated with butter and broiled.

Tripe

Beef tripe is produced from the first (rumen, paunch) and second (reticulum, honeycomb) stomachs of cattle. It is referred to as plain (3.2 kg) and honeycomb (680 g), respectively. Sheep stomach can be processed similar to beef stomach and will yield approximately 1 kg of tripe. Pork stomach can also be processed and will yield approximately 1.2 kg of tripe. The omasum (bible) from beef or lamb is difficult to clean, deteriorates quickly, and is not usually used for human food. Brown, almost furry, 'raw unscalded' beef tripe is the paunch that has been cold-water flushed to remove the contents. After scalding, cream-colored, denuded tripe is made from the paunch (rumen), which is then washed in running water, with hot water in a rotating machine with continuous flushing or with diluted soda water (limewater), and then soaked in tap water. The dark internal lining is scraped to remove the mucosa. The clean stomach is converted into tripe by cutting to size and pickling in salt brine, or by cooking and pickling in a weak salt and vinegar brine. Tripe may be precooked (usual form) in water, sometimes fully cooked, and may be packed in vinegar, pickled or canned.

Types of beef tripe products available include the following:

- Tripe cooked: Scalded tripe, cooled, drained, and then cooked to increase firmness.
- Tripe cooked and bleached: Cooked tripe that is bleached and neutralized.
- Mountain chain beef tripe: Dark cream-colored, muscular pillars from mature cattle that is scalded or treated with additives.

Types of pork stomach available include the following:

- Whole unscalded: Light to medium brown, inverted, cleaned, and trimmed; the lining might be removed.
- Scalded form: Cream to light brown, inverted, cleaned and trimmed, and scalded; the lining might also be removed.

Precooked tripe requires additional salt-water cooking and is often served with sauces or dressings or used in meat casseroles, stews, or pies. Because tripe is delicately flavored, it is often combined with tomato sauce, buttered and broiled, covered with dressing and baked, dipped in butter and sautéed, or combined with a thick soup.

Brains

Beef brains weigh 454–482 g, veal and pork brains 113–127 g, and lamb brains 127–142 g. They are removed on the harvest floor. Brains are less popular than in the past because of the

possible connection of nervous tissue with BSE ('mad cow' disease). Cattle brains belong to the 'specified risk material', which in the US and EU must be removed from bovine carcasses at harvest and incinerated. Other species' brains are available with the outer membrane retained or removed and usually briefly soaked in water, drained under refrigeration, and chilled. Brains are very perishable and should be used immediately, precooked (which makes removal of membrane easier and firms the tissue to make slicing easier), or frozen. Brains are tender, and are often thinly sliced, dipped in butter or flour, and deep-fat fried. They can also be broiled, sautéed, braised, cooked in liquid, or broken into pieces and scrambled (often with eggs).

Oxtail

Beef tails are removed from the carcass between the second and third coccygeal vertebrae (in some areas between the sacral and coccygeal vertebrae) and quickly chilled. It is usually available in the tipped and trimmed form, which weighs 0.8–1 kg and has the excess fat removed and three or more of the end posterior coccygeal vertebrae removed. Oxtail is usually browned and then simmered until the meat is tender, that can easily be removed from the bone, which sometimes occur before serving. It can be combined with other soup ingredients and its rich flavor adds taste and texture to soups.

Stock

Veal, lamb, pork, or cracked beef bones; cooked or uncooked and sometimes combined with meat scraps, can be converted into soup stock. Lamb and pork bones produce distinct and strong flavors and should be used in lamb and pork dishes. Stock is economical and adds nutrition and flavor to cooked products. To produce stock, bones are usually combined with vegetables, covered with hot water and placed in an oven for roasting until the bones turn brown. Fat is separated and the bones are combined with additional vegetables covered with water and simmered in the water, and then skimmed. The stock is then strained and cooled; it can be refrigerated or frozen. It is used with cooking in meat dishes, soups, vegetable dishes, sauces, or gravies.

Meat Extract

Meat extract can be produced by pressing or by cold water soaking, but the most popular procedure is rapid boiling of meat that is to be canned. The juice from these procedures is concentrated into an extract. Other edible meat products can also be used as starting ingredients. The yield and quality of the extract will be influenced by the type of raw material (sex, cut, postmortem age, type of tissue or bones, size of cut, and fat content), the length of time used in cooking, the number of times fresh product is cooked in the same soup, and treatment (open pan and holding time) during evaporation of the soup. When bones or meat are boiled, the juices are combined with meat wash water and it is often economical to boil new lots of bones or meat two or more times in the same liquid. The soup is skimmed to remove fat and filtered to remove particles and suspended solids. It is then boiled to coagulate protein, refiltered, concentrated by vacuum evaporation, and then reheated in open pans. First- and second-class extracts often contain 16% or 19.5% moisture, 44% or 40% minimum organic soluble material, 7% or 6% creatine, 1.5% maximum water-insoluble material, 25% maximum ash, 4.05% maximum salt, 0.01% maximum saltpetre, and 0.01% maximum copper.

Extracts are also produced in the solid form and are the foundation for various fluid extracts and bouillon cubes, broths, 'teas,' and soups.

Trimmings

Beef outside skirt is the thin, free portion (wing) of the diaphragm muscle with the tedious skin tissue (pleura) remaining, which is separated from the liver with a knife. In beef animals, it normally weighs 1.9–2.5 kg. After removal from the carcass, it is soaked in water, drained and chilled and then a portion of the fat and membrane material is removed. It is used in comminuted meat products.

'Hanging tender' is the thick portion (pillar) of the diaphragm muscle next to the spinal column, which is treated similarly and has similar usages to the outside skirt. In beef animals it usually weighs 0.3–1 kg.

'Beef weasand' (gullet) is the smooth-muscle lining that surrounds the oesophagus from the larynx to the first stomach (paunch). It is separated with a knife on the harvest floor. The fat and membrane, as well as the fibrous internal membrane, is removed, washed, drained, dried under refrigeration, and chilled. It is used in emulsion-type products.

Other trimmings such as 'beef cheek meat,' 'beef tongue trimmings,' 'beef meat from tongue trimmings,' 'beef head meat,' 'beef lips,' 'pork cheek meat,' 'pork snouts – lean in,' 'pork snouts – lean out,' 'pork head meat,' 'pork skirt,' and 'pork hanging tender' can be used in sausage production. The terminology for some of these products would include the following:

- Beef cheek papillae: The muscle including the lining of the mouth that is external to upper and lower jaw bones from the tip of the mouth back to the parotid salivary glands; might also include the muscle inside the lower jaw bone. It has none of the external lip remaining.
- Beef cheek papillae off ('nut' or 'kernel'): The beef cheek papillae in which the papillas' lining has been removed by trimming.

Pork Jowl

The jowl is separated from the pork carcass by a cut at the first or second cervical vertebrae and lymph nodes are removed. It is placed in water, drained under refrigeration, and chilled. Jowls are often cured like bacon.

Pig Tail

The tail of pig carcasses is removed between the fourth and fifth caudal vertebrae, washed, drained under refrigeration and chilled. Tails may be mild brine-cured or used as jelly stock for brawn (headcheese).

Pigs' Feet ('Trotters')

Feet are cleaned on the harvest floor after scalding by pulling the toenails and removing the skin and hair between the toes. The feet are then scraped free of hair, washed, and chilled. For dry-cured ham, the feet are separated at the middle of the hock joint. For other uses of hams, more part of the feet is removed. The hind foot is usually not used for human food because it contains little muscle. However, sometimes small meaty portions are removed and pickled and are called 'tid-bits.' The fore foot (0.5–0.7 kg per foot) is removed at the junction of the fore shank bone and the foot bone (vague). It is often used for food because it contains more muscle (46% raw). The fore shank or pork hocks (lower shank portion of picnic shoulder) can be sold fresh or frozen or can be used for pickled pigs' feet, boned and used for sausage, or used to produce a jelly stock for brawn-like products. Pickled pigs' feet are hot- or cold-cured with salt and nitrite, heated or reheated in water (sometimes vinegar-acidulated water), hot showered, cold-water chilled, and often boned. Large feet are usually pickled boneless or semiboneless, and smaller feet might be split ('split foot') or boneless. They are then placed in 35-grain vinegar, packed in jars that are filled with 45–55-grain vinegar (pH must be below 4.5 to be shelf-stable) containing salt, ascorbic acid, and spices or condiments. The product is then usually held for 2 weeks before selling, for continuous pickling.

Chicken or Duck Feet, and Chicken Paws

Chicken feet (4 toes, feet 30–45 g each; Fang zhao, Phonix claws, and chicken claws) and duck feet, are the shank portion of the chicken or duck leg. Poultry contains only part of the ankle bones and the industry uses the word 'hock' to describe the ankle region and 'hock joint' refers to ankle joint. Chicken paws are chicken feet cut off at the ankle. Almost all the edible portion consists of skin and tendons and many small bones (often removed before serving). The feet are mostly cartilage, which makes them very gelatinous. Chicken feet are washed and yellow skin and hard nail are removed that contain no bruises and are usually frozen. They are popular in the Orient (often selling for a higher price than the breast), and Central and South America. They have little flavor but are often deep fried or boiled or steamed, which makes them puffy before being stewed and simmered in a sauce flavored with fermented black bean paste and sugar. Marinated feet are simmered with soy sauce, sichuanse peppercorn, clove, garlic, star anise, cinnamon, and chili flakes but might also be flavored with rice vinegar and chili. They are often eaten as a beer snack, snack (like peanuts), cold dish, soup, or a main dish. The US exported approximately 500 000 metric tons in 2008. Duck feet, which are also popular are often flavored with mustard, and served with vinegar, green peppers, and crushed garlic.

Jellied Products

Jellied products such as headcheese (brawn), souse, and scrapple use high-collagen meat sources in their production. These include pork skins, head trimmings, or gelatin. Tissue often utilized in production of these products includes cured pork tongues, hearts, cheeks, ears, snouts, or pork skin gelatin; sometimes nonfat milk is also incorporated. The product is seasoned, hot-water cooked, cubed, rind minced, mixed, stuffed into natural or artificial casings, recooked in water, cold-water chilled, and placed in a cooler for a final chill. Sometimes the jellied product is cold-smoked or washed in vinegar. Because the product is very perishable, it may also be canned.

Haggis

Haggis is made from calf and sheep hearts, lungs, and livers with oatmeal added. It is heavily seasoned and cooked in a sheep's stomach.

Intestines

Pork large intestines and stomach are collected at harvest and cleaned; they are cooked, often with sauce, and are referred to as chitlings in the US and chitterlings in Europe. In some areas, a small portion of beef small intestine is also used. The intestines and other areas of the digestive track of cattle, pigs, or sheep are cleaned and some inner and outer layers are usually removed, salted, and used as sausage casings.

Testicles

Beef testicles (a bull testicle weighs 0.2–0.3 kg; calf's 'mountain oysters' are smaller) are the complete gland with the epididymis removed and chilled. Rams' testicle weight is 0.2–0.3 kg, lambs' testicles are smaller; and boar testicles weigh 127 g. They are often thinly sliced, dipped in a batter of flour (breaded), and deep-fried.

Pork Skins

Pork rind is utilized as a binding product for jellied products, for the production of gelatin, to prepare a popped snack item, or as a preemulsion for sausage production. Pigskins can also be tanned to produce leather products. Popped pork rind (bacon rind, 'skeens') are green belly skins, green backfat skins, green ham skins, or cured and smoked bacon skin removed from the respective cuts, usually producing a skin with as little as 6 mm of fat. The rind is then cut into 1.3–21.5 cm squares. Next, it can be cooked and allowed to cool and drain and then placed in a wholesale package. It is later taken out and processed in hot oil so that it puffs up or expands to a much larger volume with a crisp texture. The puffed product can receive salt and several types of seasoning to give a variety of flavors. These puffed skins are less hygroscopic than most puffed snack items. Rinds that are to be used in sausage emulsion are cooked, minced and combined with pork fat, water, and emulsifying or stabilizing agents such as soy protein isolate or sodium caseinate to produce the preemulsion, which is used as is or sometimes dried (in granular form). Either type can be used hot or chilled and incorporated in the chopping stage of sausage manufacturing.

Blood

Blood is the first product obtained at harvest. Beef will yield 10–12 l and sheep 1.5 l. In some countries, blood is utilized as human food, usually in sausage. To extend the shelf-life for a

few days in areas without adequate refrigeration, high levels of salt might also be added. Typically, blood from healthy animals is free from microorganisms, but it is necessary to obtain it in as sanitary a fashion as possible. The product is mixed with other meat ingredients, stuffed into natural or artificial casings or moulds and water-cooked, chilled and normally cold-smoked, and then rechilled. Blood is usually limited to 0.5–2% in a sausage product. Above this level, it has a negative effect on color and flavor. In Europe, blood is often stabilized with citrate and collected. It is sequentially separated and the plasma fraction is used in processed meat products as a high-protein ingredient. The red corpuscles are often frozen and used as mink feed. Blood can be separated into corpuscles (dense blood, primarily red blood cells) and plasma (yellowish liquid) by centrifugation. Plasma can be incorporated at the rate of 10% to replace some of the meat and water in sausage. Anticoagulated or defibrillated beef blood might be cured and strained, and used to form an emulsion when it is mixed with chopped, cooked, ground, defatted pork skins and subsequently added to the sausage mixture. Some products have a high proportion of whole blood, such as black pudding and blood (black) sausage. Blood not collected in a sanitary manner is used to make feed for young pigs.

Spleen

Beef spleen will weigh 0.9–1.4 kg, pig spleen 170 g, and sheep spleen 57–85 g. After removal and trimming, the spleens are soaked in water, drained, dried, and then chilled. Before use, the connective tissue covering is removed. Spleen can be fried, used in a pie as melt or used for flavoring or in blood sausage. To remove the gristle-like texture, it is sometimes processed through a mechanical deboner with a desinewing head. The use of spleen in sausages is often limited to less than 10%.

Poultry Giblets

The heart, liver, and gizzard (split, emptied, washed, and lining removed) and sometimes the neck are cleaned and wrapped in paper, bags or film and inserted into the bird's body cavity. They might be washed, salted and wrapped in aluminum foil, and cooked with poultry. Giblets can also be simmered in salted water until tender. They can then be ground and added to crumbled bread or cornbread, or cooked with rice, to produce stuffing. Milk and cooking broth might be added to minced giblets to form gravy. Livers can be fried with bacon or onions to produce a flavorful product. Owing to reduce the risk of *Salmonella* cross contamination, most poultry in Europe is sold without giblets. Poultry livers, hearts, and gizzards are packed in consumer packs (150–300 g) and sold frozen in retail stores.

Mechanically Separated Meat

Mechanically separated meat (MSM) (species) is obtained by forcing bone and attached meat through a sieve using high pressure to separate the soft tissue (meat and some calcium dust) from hard tissue (bone and connective tissue). The soft tissue was declared (1996) to be safe for human consumption by United States Department of Agriculture/Food Safety and Inspection Service. It can be labelled trimmings or ground (species) and must be in the ingredient list as mechanically separated (species).

MSM is the most widely used system in the US and the product where it is used most commonly is in hot dogs, which can contain no more than 20% of mechanically deboned pork. Compared to hand deboned tissue, protein is lower, and fat content is higher than hand deboned, because of the higher content of bone marrow (increases red color) and lower content of connective tissue (improves tenderness), and with a calcium content that is higher (the US diets are low in calcium), and fluoride is higher but most of the fluoride is discarded with the bone.

MSM (species), mechanically recovered/reclaimed meat (MRM), and mechanically deboned meat (MDM) are all processed by the same system and the only difference is the starting material. MSM (species) gives information on the species used, whereas MRM and MDM do not give information on species.

MDM (could contain beef), owing to concerns (2004) with BSE, it is considered inedible (the US, the UK, and the EU).

In chicken or turkey (MSM species) franks, the quantity of mechanically deboned poultry is unlimited and may contain chicken or turkey skin and fat in proportion to a turkey or chicken carcass. These techniques salvage a lot of soft, edible, highly nutritious meat that does not contain connective tissue that would otherwise essentially be wasted. As the world population grows we cannot afford discarding nutritious products that can be salvaged.

See also: By-Products: Hides and Skins; Inedible. Ethnic Meat Products: Middle East. Minced Meats. Processing Equipment: Battering and Breading Equipment; Smoking and Cooking Equipment. Sausage Casings

Further Reading

Anon, 1976. The Nutritive Value of Meat and Other Protective Foods. Chicago: The National Live Stock and Meat Board.
Anon, 1998. The Guide to Identifying Meat Cuts. Chicago: National Cattleman's Beef Association and National Pork Producers Council.
Gerrard, F., Mallion, F.J., 1977. The Complete Book of Meat. London: Virtue Press.
Ockerman, H.W., 1975, 1983, 1996. Chemistry of Meat Tissue, eighth, tenth and eleventh eds. Columbus, OH: The Ohio State University.
Ockerman, H.W., Hansen, C.L., 1988. Animal By-Product Processing. Chichester, UK: Ellis Horwood.
Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic.
Paul, A.A., Southgate, D.A., 1978. McCance and Widdowsons. The Composition of Foods, fourth ed. London: HMSO.
Renon, P., Comi, G., Cantoni, C., Persiani, G., 1980. Acidi grasi del grasso d'organi di animali domestici. Industrie Alimentari 19 (6), 507–510.
Romans, J.R., Jones, K.W., Costello, W.J., Carlson, C.W., Zeigler, P.T., 1985. The Meat We Eat, twelfth ed. Danville, IL: Interstate.
US Export Federation, No date. Variety Meat from the USA – A Buyers' Guide, second ed. Denver, CO: US Export Federation.
USDA, 1963. Composition of Foods (Agricultural Handbook No. 8). Washington, DC: Agricultural Research Service.
USDA, 2003. USDA Nutrient Data Laboratory. Available at: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (accessed 18.01.04).

Hides and Skins

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Introduction

Humans have utilized animal skins throughout recorded history, and nomadic people still utilize them for shelter, clothing, weapons, and food containers. In spite of competition from synthetic materials, many quality items still demand the wearing ability, moisture–vapor transfer, and insulating properties of leather.

Animal hide is a very significant proportion (4–12%) of the live weight of an animal (Table 1) and is one of the most valuable by-products that can be converted into a variety of items. A few examples are shown in Table 2. Other uses of animal skin include food, cosmetics, and medical prosthetics.

Trade in Hides and Leather

A significant percentage of US cattle hides enter the export market. Many types of pigskin have other uses and often are not salvaged for leather; this market, therefore, has a large potential for growth. Tanning of fish skins has also made fish leather available. Developing countries account for the manufacture of approximately 45% of the world's leather products. For example, China now has 10 000 tanning enterprises employing 1 million workers. Table 3 indicates that 88% of US footwear is derived from other countries. Table 4 illustrates leather movement and Table 5 shows its origins and destinations, showing that leather indeed is a global product.

Classification

Hides are classified according to weight, whether or not the hide is branded and location of the brand, sex, fatness, defects, and skill of removal (Tables 6 and 7). Sheep pelts are graded according to wool length. The leather industry has its own terminology, and glossaries can be found on the Internet at sites listed under Further Reading.

Hide Composition

The skin of living animals forms a physical protective barrier. The thickness of the skin varies with species, age, sex, and body region. It is is composed of three major layers: the surface pigmented epidermis, the underlying connective tissue corium, and the deep subcutis (Table 8).

The chemical composition of the skin (Table 9) varies with the type and age of animal, sex, fat level, and hide treatment. In general, the hide is low in fat and minerals and high in

Table 1 Hide yield weights and yields as a percentage of animals' live weights

Type of animal	*Range of hide yield (lbs)*	*Percentage of live weight (%)*
Cattle	5.1–5.8	Average 7.0
Average using hide stripper	4.0–6.0	Average decrease of 2% or 5%
Herford	8.5	
Angus	7.5	
Shorthorn	6.5	
Charolais, bull, 15 months old	8.5	
Charolais, bull, 20 months old	8.3	
Charolais, bull, 30 months old	6.7	
Good stear	6.6–7.6	
Poor stear	6.4–7.8	
Good heifer	5.1–7.9	
Branded cow	6.6–7.7	
Canner, cutter	5.7–6.8	
Bull	6.7–7.5	
Bologna bull	7.0–8.1	
Sheep		
Sheep and lamb (wool + skin)	11.0–11.7	
Swine		
Pig, vertical drum skinner	3.0–8.0	
Boar	10.0–12.0	

Source: Reproduced from Bengtsson, O., Holmqvist, O., 1984. By-products from slaughtering. Fleischwirtschaft 64 (3), 334–336; Judge, M.D., Salm, C.P., Okos, R.M., 1978. Hog Skinning Versus Scalding. Arlington, VA: AMI Foundation, pp. 155–164; Lawrie, R., 1981. Development in Meat Science – 2. London: Applied Science Publishers; Minnoch, J.K., Minnoch, R.M., 1979. Hides and Skins. Ithaca, NY: National Hide Association; Romans, J.R., Ziegler, P.T., 1974. The Meat We Eat. Danville, IL: Interstate; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

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Table 2 Examples of some cured and tanned uses of hides, skins, or pelts and their by-products

Portion of hide, skin, or pelt	*Examples of finished products*
Cattle hide by-products	
Cured and tanned hides	Sole, upper, linings and heels for leather shoes, rawhide, bags, athletic equipment, belting, upholstery, harness, saddles, etc.
Corium layer	Picking bands, textile shuttle holders and passers, reconstituted collagen sausage, casings, cosmetics products, and collagen products
Tail hair	Paint brushes and upholstery padding (no longer used much)
Body hair	Felting, plaster retardant, etc.
Inside of ear hair	Imitation camel hair brushes
Hide trimmings	Tankage, fertilizer, glue, and inedible gelatin
Hide fat	Tallow
Calf skin	Light-weight leather, fabric trimmings, drumheads, gloves, etc.
Hog skin by-products	
Pig skin	Gloves, belts, razor straps, shoe uppers, inner-soles, upholstery, shoe counters, sausage, pork rinds, edible gelatin, glue, etc.
Trimmings	Dog chews
Hair	Upholstery padding (not used much anymore), felting, and plaster retardant
Bristles	Brushes
Sheep pelt, by-products	
Wool	Blankets, gloves, clothing, carpets, upholstery fabric, lanolin, etc.
Slats (skin after wool or fleece is removed)	Shoe and slipper uppers and lining, hat sweat bands, fancy shoes, gloves, garments, sporting goods, chamois, book bindings, diplomas, etc.
Hair sheep	Small pneumatics, diaphragms, and bellows
Pelts (wool or fleece left on) and trimmings	Heavy coat material, moutons, shearlings, glue, and tankage
Horse hide by-products	
Cured and tanned hides	Shoe sole and uppers, gloves, sporting goods, luggage, belts, harness, saddles, etc.
Domesticated land and water buffalo hide by-products	
Cured and tanned hides	Shoe sole and uppers, fancy leather goods, luggage, handbags, and buffing wheels
Goat and kid	
Cured and tanned hides	Shoe uppers and linings, gloves, fancy leather, handbags, and book bindings
Deer and elk hide by-products	
Cured and tanned hides	Shoe uppers, clothing, gloves, moccasins, and mukluks
Kangaroo hide by-products	
Cured and tanned hides	Shoe uppers, diaphragms, and bellows
Exotic and fancy leathers	
Aquatic group	Frog, seal, shark, walrus, and turtle leather
Land group	Camel, elephant, ostrich, emu, rabbit, and pangolin leather
Reptile group	Alligator, crocodile, lizard, and snake leather

Source: Adapted from Clemen, R.A., 1927. By-products in the Packing Industry. Chicago, IL: University of Chicago Press; Ockerman, H.W., 1996. Chemistry of Meat Tissue. Columbus, OH: Department of Animal Science; Tanners' Council of America, 1983. Dictionary of Leather Terminology. Washington, DC: Tanners Council of America; Leather Industries of America Research Laboratories, 1991. Dictionary of Leather Terminology. Cincinnati, OH: University of Cincinnati; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

protein (collagen), which increases as the hides are converted to leather. Hair is composed almost entirely of the protein keratin (6–10% of total hide protein).

Hide Harvesting

The quality of leather depends on techniques used for hide removal (flaying) and the processing that takes place in the harvest facility. The operations performed there are hide removal, preservation, fleshing, trimming, selection and grading, storage, and shipping.

Hide removal from cattle, sheep, and goats is accomplished by two basic techniques: the knife-skinning technique, using either conventional skinning knives or air-driven reciprocating knives, or the hide-puller technique. Another technique used in some countries pumps compressed air between the hide and the carcass to cause hide separation. Today in most modern plants, hides are pulled, because they can be removed by less-skilled labors, and the pulling technique results in less hide damage, lower manpower requirements per animal, less carcass contamination, and an increase of 2% in carcass yield because the stripper (or puller) pulls the hide from the fell rather than from the carcass itself, and this reduces shrinkage of the hide.

Table 3 World leather production, imports, and exports

	Footwear (million pairs)	*Hides/skins (million)*	*Bovine hides, raw (1000 t)*	*Cattle hides, raw (1000 m^2)*	*Sheep/goats hides (1000 m^2)*	*Sheep/goats (1000 t)*	*Percentage (%)*
World hides		283	5750			636	
US cattle hides		36	896				12
World production of footwear	3900						
US production of footwear	143						
US imports of footwear[a]	1089 (US$10.4 million)						
US exports of footwear	23 (US$0.4 million)						
Percentage of US utilized footwear that is imported							88
China, cattle hides		31	597			526	11 (world)
Brazil, cattle hides		28–38	647				10–13 (world)
Brazil sheep/goats hides		7.3 (US$900 million)					
India, hides		23	407			71	8 (world)
Russian Federation, cattle hides		13	521				
Argentina		13	256				6 (world)
Australia		9				37	
Mexico		6					2 (world)
Ukraine		6					2 (world)
France, hides		6 (cattle)			4 150		42 (export)
Germany, hides		4 (cattle)					48 (export)
Italy		4 (cattle)			46 550		59 (export), 36 (EU), 1 (world)
Columbia		4 (cattle)					1 (world)
Canada		4 (cattle)					1 (world)
New Zealand		4 (cattle)				41	1 (world)
Spain				28 300	20 850		39 (export)
Portugal				9 593	1 299		19 (export)
UK				11 500	3 200		63 (export)
EU-15			643	248 087	77 068		60 (export)

[a]US imports of footwear (nonrubber) from: China 45%, Taiwan 13%, Korea 12%, Brazil 10%, Indonesia 5%, Italy 4%, Thailand 3%, and Spain 4%.

Source: Adapted from US Department of Commerce, 1987. Footgear Retailers of America. Washington, DC: United States Department of Commerce; FAO, 2002. Commodity Market Review. Rome: FAO; International Labour Organization, 1992. Recent Development in the Leather and Footgear Industry and Notes on Proceedings of Fourth Tripartite Technical Meeting for the Leather Footgear Industry. Geneva: ILO; Leather Industries of America, 1996. US Leather Industry Statistics. Washington, DC: Leather Industries of America; United States Hide, Skin and Leather Association, 1997. Census. Washington, DC: United States Hide, Skin and Leather Association; Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co; and Euroleather, 2003. Sectoral Data. Available at: http://www.euroleather.com/sector.htm (accessed 15.07.12).

Pigs are often scalded in hot water (57–71 °C) containing chemicals to aid in hair and scurf removal until the hair slips, and dehairing occurs mechanically in a dehairing machine ('polisher') for 10–15 s. This machine scraping action, along with hot (60 °C) water spray, removes the hair. Difficult to remove hair is hand-scraped with bell scrapers and shaved, and the remaining hair is often singed with a gas flame. However, the skin (rind) is then not generally used for leather production because it has been denatured by heat, which causes the protein in the hair follicles to denature and result in 50% of pigskin being unsuitable for upper shoe leather. Therefore, the skin (rind) often stays on the carcass and is cooked as part of the meat joint (crackling), is used as filler in meat processing, or converted into gelatin. Pigskin that has been dehaired at a temperature less than 58 °C can be removed from the carcasses or a portion of the carcasses (e.g., belly) and used for production of leather (e.g., Puppy leather). In some countries, a covering during scalding protects the skin that is to be pulled. The Wolverine skinner pulls this portion of the carcass through a knife that separates the pigskin from the carcass. The pigskin goes through a fleshing machine, which removes all but approximately 3% of the fat. The skin is then refrigerated and shipped to a tanner.

Table 4 US leather imports and exports

	Imports		*Exports*		
	Value (US$1000)	*Percentage (%)*	*Bovine (1000 t)*	*Value (US$1000)*	*Precentage (%)*
Bovine/equine: full grain or grain split – upholstery	314 372	28.8			
Parts of seats for motor vehicles/cut to shape	145 474	13.3			
Bovine/equine: full grain or grain split – fancy	118 957	10.9			
Bovine/equine: other upholstery	57 322	5.2			
Bovine/equine: wet blue, other	39 926	3.6			
Bovine – vegetable pretanned	33 859	3.1			
Bovine/equine, upper leather	31 554	2.9			
Bovine/equine, other fancy	29 664	2.7			
Sheep/lamb: other fancy	29 282	2.6			
All other categories	288 549	26.4			
Parts of seats for motor vehicles/cut to shape				211 354	24.3
Bovine/equine: wet blue, not split				94 167	10.8
Bovine/equine: other-upholstery				83 804	9.6
Bovine/equine: full grain or grain split – upper, split				113 431	13.0
Calf and kid upper leather				54 719	6.2
Bovine/equine: wet blue split grains				50 991	5.9
Bovine/equine: wet blue-split, not grained				31 676	3.7
Bovine/equine: parchment fancy				25 929	2.9
All other categories				203 920	23.6
Totals	1 088 959	100	510	869 991	100
Gloves	294 549				
Baseball gloves	182 828				
Wearing apparel	909 598				
Handbags	1 044 973				
Luggage	2 392 925				
Other leather products	555 275				

Source: Adapted from Leather Industries of America, 1996. US Leather Industry Statistics. Washington, DC: Leather Industries of America; Leather Industries of America Research Laboratories, 1997. Dictionary of Leather Terminology. Cincinnati, OH: University of Cincinnati; United States Hide, Skin and Leather Association, 1997. Census. Washington, DC: United States Hide, Skin and Leather Association; Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co; and FAO, 2002. Commodity Market Review. Rome: FAO.

A Townsend skinner separates the skin and some of the fat from other tissues and is often used to remove the skin from dehaired ham, loin, or belly, and the skin and some adhering fat is used for pork rinds, cracklings, or gelatin production. The gelatin industry in the United States uses approximately 50% of pork skins. Some undenatured pork skins are used in human burn/grafting after the hair has been removed by a laser. In some countries, pigskin is eaten, whereas in others pigskin is used as filler in sausages and meat pies.

Mechanical pulling of pork hides (dehiding) is gaining popularity (96% of all pigs are, e.g., dehided in Japan) because of the energy and labor savings and the fact that the carcasses chill more quickly, which reduces the risk of producing pale, soft, and exudative pork. This technique produces hides that are useful for leather production and for medical use (burn treatment). The mechanically pulled pig hides are fleshed, salted, and shipped to tanners.

Hide Curing

The hide after removal is quickly cooled or treated with a bactericidal spray or acidic or caustic treatment. Then, it is cured to remove excess water and to arrest bacterial and enzymatic decomposition. With pigskin, traditional salt curing does not work well; solvent dehydration may be used, or the uncured hide may go directly to the tanning operation (usually chrome).

Drying in areas with low relative humidity may be used to preserve hides or, more commonly, salt may be used as the curing ingredient. There are four basic techniques for curing: air-drying, salt-pack, mixer, and raceway curing.

The oldest salt curing method is the salt-pack technique (**Table 10**). Salt-pack curing is simply a flesh side-up stack (1–1.3 m) of hides with approximately a unit of salt per unit of hide spread evenly over the flesh side of each hide. This salt level controls bacterial growth and draws moisture out of the hides. Preservatives are often used with salt-pack curing; 1% sodium fluoride (NaF – should not be inhaled) or 1% naphthalene ($C_{l0}H_8$) plus 1% boric acid (H_3BO_3), based on the weight of salt, have been used successfully. Other salt additives might be zinc oxide (ZnO) or sodium metabisulfite ($Na_2S_5O_2$) in various combinations. Cattle hides in the stack are usually allowed to cure for 20–30 days.

Brine curing reduces curing time from 30 days (salt-pack curing) to 24 h. It is desirable to flesh hides first because fat retards salt penetration and moisture removal. The mixer curing method (hide processor) is often used, particularly in smaller plants. The mixer looks and operates much like the familiar cement mixer. It utilizes a saturated salt solution, or

Table 5 US foreign trade in cattle hides[a]

	Percentage imports (%)[b]	*Percentage exports (%)*[c]
Canada	93.1	
Mexico	4.0	
Ireland	1.2	
Costa Rica	0.4	
Germany	0.3	
UK	0.3	
Dominican Republic	0.1	
Sweden	0.1	
Others	0.5	
Korea, Republic		39.2
Taiwan		14.1
Japan		11.7
Mexico		10.4
People's Republic of China		8.3
Canada		5.7
Italy		2.6
Hong Kong		2.4
Thailand		2.2
Others		3.4
Total	100.0	100.0

[a]The majority of leather is now tanned in developing countries owing to strict environmental regulations in the developed countries.
[b]Percentage of total US$74 436 648.
[c]Percentage of total US$1 125 072 253.
Source: Adapted from Leather Industries of America, 1996. US Leather Industry Statistics. Washington, DC: Leather Industries of America; Leather Industries of America, 1987. US Leather Industry Statistics. Washington, DC: Leather Industries of America; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

fresh salt may be added at 20–24% of the weight of hides. A chlorinated lime or similar bacterial or mold deterrent is also often utilized. The hides are rotated in the mixer for 6–12 h. When hides are removed from the mixer they are wet. They pass through a wringing machine to squeeze out the excess liquid or are hung to drain by dripping.

Raceway curing is the most common method of curing hides (Tables 11 and 12). The raceway-shaped tank ('raceway vat') is agitated by two overhead paddle wheels that circulate the brine and keep the hides moving. The raceway is filled with saturated brine together with other additives (Table 13), for example, 0.3% (of hide weight) of sodium fluoride (NaF). This method requires approximately four units of saturated brine for each unit of green hide. Hides are cured in the raceway for 16 h. When hides are removed from the curing raceway, moisture is removed as with brine curing. A proper brine-cured hide should contain $<40\%$ moisture with a salt saturation of $>85\%$. Pit curing or vat curing is a modification of raceway curing and salt-packing. The hides are salted and the pit is flooded with saturated brine (Table 14). This curing technique requires 24–33 h. Owing to the environmental impact of sodium chloride, potassium chloride (KCl) can be used instead. Nonsalt methods of cattle hide curing are also available. These include sodium sulfite/acetic acid; solvent processing (acetone at pH 4.5–5.0, ether–alcohol, or ether–alcohol and ether–ester); biocide curing, which has been utilized in South Africa due to restrictions on salt; electron beam radiation; refrigeration (but freezing is not an alternative); and chrome tanning, at least to the blue stock stage immediately after harvest.

Fleshing

Fleshing is necessary to produce quality hides. The fleshing machine removes 9–12 kg fat, flesh, hair, and manure per cattle hide. The fleshing operation may be done before or after curing. Fleshing residue and hide trimmings are also utilized by the by-products industry.

Trimming

Trimming can be accomplished before or after fleshing and therefore before or after curing. Hides are trimmed to remove parts that have no value as leather. A knife is used to remove ears, ear butts, snouts, lips, scrotal sac, udders, tail, head skin, fat, and muscle tissue from the side of the head and ragged ventral edges.

Sorting

Sorting of hides after curing is done on the basis of sex, weight, and branding. Basic categories of hides include steer hides, both native (unbranded) and branded; heifer; and cow and bull hides. Bulls produce the thickest hides, which are used for production of shoe sole leather. Steer and heifer hides tend to be thicker than cow hides but thinner than bull hides and are often used for shoes and boots. Cow hides are usually the thinnest. Mature bovine hides are used to produce garments, purses, and gloves. Hides are also graded for quality into categories 1, 2, or 3. The difference in grade is based on number and type of defects, which include scratches, cuts, branding, mange, insects, and animal diseases. Further Reading includes sources for more complete listings of hide damage and defects during storage and shipment.

Graded hides are next sprinkled with approximately 450 g of 'safety salt' to avoid deterioration during storage and shipment. Hides are folded, flesh side out, and tied to form a bundle. The bundles are then stacked to a maximum height of 1 m to squeeze out excess moisture and stored to await shipment. Under cool conditions, the hides can be stored for 1 year, but under hot and humid conditions, hides can be stored only for a few months.

In some countries, unsalted hides are chrome tanned at the slaughter plant to produce a 'wet blue' moist product. These hides can be stored for months. Another possibility for eliminating salt curing is treating with sodium sulfite (Na_2SO_3) and acetic acid (CH_3COOH) and holding them in a closed system. Hide-shipping containers are usually sprayed with an insecticide before loading.

Quality of Cure

Hides and skins are normally evaluated for quality of cure by determining the moisture (volatile material) and salt (or ash)

Table 6 Packer cattle hide selection (North America)[a]

Selection	*Description*	*Range of net weight (lbs)*[b]	
		Conventional	*Trimmed and fleshed*
Slunk	Unborn calf		
Light calfskin		Less than 9	
Heavy calfskin		9–15	
Kidskin		15–25	
Overweight kidskin		25–30	
Heavy native steer	Steer hide free of brands	58 and above	47 and above
Light native steer	Steer hide free of brands	48–58	39–47
X-light native steer	Steer hide free of brands	30–48	23–39
Heavy butt-branded steer	Steer hide branded one or more times behind the break in flank	58 and above	47 and above
Butt-branded steer	Steer hide branded one or more times behind the break in flank	30 and above	23 and above
Heavy Colorado or side-branded steer	Steer hide branded one or more times forward of the break in flank	58 and above	47 and above
Colorado or side-branded steer	Steer hide branded one or more times forward of the break in flank	30 and above	23 and above
Light-branded steer	Steer hide branded one or more times	30–58	23–47
Heavy Texas steer or branded steer	Steer hide branded one or more times	58 and above	47 and above
Texas steer or branded steer	Steer hide branded one or more times	30 and above	23 and above
Heavy native cow and heifer (plump)	Hide from female bovine free of brands	53 and above	43 and above
Light native cow and heifer (plump)	Hide from female bovine free of brands	30–53	23–43
Heavy native cow and heifer (thin or spready)	Hide from female bovine free of brands	53 and above	43 and above
Light native cow and heifer (thin or spready)	Hide from female bovine free of brands	30–53	23–43
Branded cow and heifer	Hide from female bovine branded one or more times	53 and above	43 and above
Light branded cow and heifer (plump)	Hide from female bovine branded one or more times	30–53	23–43
Heavy native bull	Hide from bull free of brands	58 and above	
Heavy branded bull	Hide from bull branded one or more times	58 and above	

[a]Germany (Switzerland and Austria similar), South America, North Africa, South Africa, West Africa, East Africa, Asia Minor, China, Japan, India, Pakistan, Thailand, Indonesia, Australia, and New Zealand have different nomenclatures and weight ranges.

[b]Multiply by 0.454 to convert into kilograms.

Source: Adapted from Leather Industries of America and US Hide, Skin and Leather Association, 1985. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Leather Industries of America and US Hide Skin and Leather Association; Leather Industries of America and US Hide, Skin and Leather Association, 1993. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Leather Industries of America and US Hide Skin and Leather Association; Price, J.F., Schweigert, B.S., 1971. The Science of Meat and Meat Products. San Francisco: WH Freeman; Tanners' Council of America, 1972. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Tanners Council of America; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

content. Hide moisture of less than 40% indicates excessive drying and protein denaturation and that of more than 48% indicates an inadequate cure.

Tanning

When cured hides arrive at the tannery, they are retrimmed and split along the backbone. Hides are graded and sorted into uniform size, weight, and type so that the tanning operation can be adjusted.

Soaking

'Soaking' is utilized to restore moisture that was removed for control of bacterial growth. Soaking flexes and softens hides and is accomplished with water, wetting agents (detergents), and disinfectants. The soaking process usually requires 8–20 h for reabsorption of needed water. The last step of soaking is washing the hides free of dirt, manure, salt, and blood.

Dehairing

The dehairing process is chemical in nature; however, mechanical dehairing equipment is sometimes used after hair has been chemically loosened. The most common chemical depilatory agents are a saturated solution of calcium hydroxide ($Ca(OH)_2$; hydrated lime) and sodium sulfide (Na_2S) or sodium bisulfide (NaHS) at a pH of 12.5. Other mixtures used to remove hair might include milk of lime (CaO) fortified with sodium sulfide (NaS), sodium bisulfide (NaHS), arsenic sulfide (As_2S_2), or dimethylamine ($(CH_3)_2NH$). Another formulation uses 30% water, 6–12% sodium sulfide, 2–3% sodium

Table 7 Grades of shearlings or sheep pelts (USA)

Grade	*Wool length (inch)*[a]
Number 4	Bare to 1/8
Number 3	1/8–1/4
Number 2	1/4–1/2
Number 1	1/2–1
Fall clip	1–2
Wool pelts	1 1/2

[a]Multiply by 2.54 to convert into centimeters.

Source: Adapted from National Hide Association, 1979. Hides and Skins, third ed. Prepared by Education Committee (Whitney, E.A. Chairman). Sioux City, IA: National Hide Association; Price, J.F., Schweigert, B.S., 1971. The Science of Meat and Meat Products. San Francisco: WH Freeman; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

bisulfide, and 4% hydrated lime. Dimethylamine sulfate is also often used and allows reduction of lime and sulfide concentrations. If hair is to be saved, a weak solution and a low temperature is used, and only the hair roots are loosened. In 2–4 days, hair is collected, washed with water, rewashed in water (100 parts) with acetic acid (1 part), and dried. More concentrated solutions that have a higher pH (>11.5) and are applied at higher temperatures can be used. Hair can be totally dissolved in a few hours. Pigskin requires higher concentrations (4–5%) of sulfide than cattle hides. If all of the hair is not removed by the chemical reaction, the dehairing machine removes the remainder. Enzymes can also accomplish dehairing and dewooling. This technique has the advantage that the hair comes out by the roots, and it yields more hair and a cleaner hide grain.

Table 8 Skin layers and two methods of splitting hides (USA)

Side	*Skin*	*Leather*	
		Five layers	*Four layers*
Hair-side (hair and oil glands)	Epidermis, pigmented, thin	Buffing	
	Grain layer and papillary	Machine buff	Top grain
	Corium, dermis, derma, cutis vera, connective tissue, and greatest part of hide	Deep buff	Deep buff
		Split	Split
Flesh-side (softer)	Subcutis, attachment, filled with fat	Slab	Slab

Source: Adapted from Moulton, C.R., Lewis, W.L., 1940. Meat Through the Microscope, revised ed. Chicago, IL: Institute of Meat Packing, The University of Chicago; Price, J.F., Schweigert, B.S., 1971. The Science of Meat and Meat Products. San Francisco: WH Freeman; Tanners' Council of America, 1983. Dictionary of Leather Terminology. Washington, DC: Tanners Council of America; Leather Industries of America Research Laboratories, 1991. Dictionary of Leather Terminology. Cincinnati, OH: University of Cincinnati; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Table 9 Chemical composition of hides

Type and age of animal	*Area (m^2)*	*Moisture (%)*	*Protein (collagen, keratin, elastin, and reticulin) (%)*	*Fat (%)*	*Ash (phosphorus, sodium, potassium, arsenic, magnesium, and calcium) (%)*
Average slaughter cattle	3.70–5.00	62–70			1.0
Mature cattle hide, without hair		65	30.0		
Very fat animal				10–12	
Wet cattle hide		83.0	15.7	0.2	0.1
Air-dried cattle hide		9.1	89.9	0.2	0.8
Newborn calf	0.75–1.40	67.9	30.8	1.0	1.0
Three-month-old calf		66.0	31.0	1.6	1.4
Two-year-old steer		61.2	35.0	3.2	1.1
Four-year-old steer		55.6	38.2	6.0	1.1
Old cow		60.2	36.0	3.1	1.1
Sheepskin	0.45–0.95			30–50	
Goatskin	0.55–0.85	60.0		3–10	
Angora goatskin	0.95–1.20			6–17	
Pigskin		37.0	14	30–50	
Cured cattle hide		39–48	41		11–16 (including tanning metals)

Source: Adapted from Aten, A., Innis, R.F., Knew, E., 1955. Flaying and Curing of Hides and Skins as a Rural Industry. Rome: FAO; Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA; Henrickson, R.L., Turgut, H., Rao, B.R., 1984. Hide protein as a food additive. Journal of American Leather Chemists 79, 132–145; Moulton, C.R., Lewis, W. L., 1940. Meat Through the Microscope, revised ed. Chicago, IL: Institute of Meat Packing, The University of Chicago; Leather Industries of America and US Hide, Skin and Leather Association, 1993. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Leather Industries of America and US Hide Skin and Leather Association; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Table 10 Pack-salting, requiring a minimum time of 30 days and yielding 75–85% of green cattle hide weight

Weight of hides (kg)	*Operation or activity*	*Composition or change*
Green		
100	Receive hides from slaughter	62–70% water
30–35% of weight is hides		
100	Trim ears, snout, and tail	3% loss; 3 kg/trim to rendering
	Salt hides into pack 1.2–1.5 m (4–5 feet) tall; little pitch for short hair; 15 cm (6 in.) spread for long hair	0.5–3 kg of rock salt (40% no. 1 rock salt and 60% no. 2 rock hair, salt) per 1 kg of hide
97	Cure in pack for 30 days; 10–16 °C (50–60 °F)	15–17% net loss in weight; 25–35 kg loss in water; 6–13 kg uptake of salt; and 13–17 kg loss of salt to sewer[a]
Cured		
82.5	Take hides from pack, inspect, and bundle	Reclaim 60% original salt used and mix with new salt or discard and use all new salt
82.5	Move hides to storage or load for shipment	–
82.5	Deduct tare allowance, 3% salt, 1.5% manure	–
79	Net shipping weight to tannery	12–16% salt; 35–45% water; and 40–50% hide substance

[a]Most modem processor recycle excess brine and do not discharge it. Also the use of evaporation ponds decreases discharge to sewers.

Source: Adapted from Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA; Minnoch, J.K., Minnoch, R.M., 1979. Hides and Skins. Ithaca, NY: National Hide Association; Romans, J.R., Ziegler, P.T., 1974. The Meat We Eat. Danville, IL: Interstate; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Table 11 Agitated brine-curing of unfleshed cattle hides, requiring a minimum of 3 days and yielding 78–82% of green hide weight

Weight of hides (kg)	*Operation or activity*	*Composition or change*
Green		
100	Receive hides from slaughter	65–70% water 30–35% of weight is hides
100	Trim ears, snout, and tail	3% loss; 3 kg trim to rendering
97	Wash hides	2% loss; 2 kg blood and manure
95	Move hides to raceway	4 kg of brine per 1 kg of hide, maintain brine at 94–97° salimeter
95	Cure in moving brine for 24 h	15–17% net loss in weight; 20–25 kg loss in water; 8–12 kg uptake of salt; 10–18 kg loss of salt to sewer[a]
Cured wet		
	Remove from brine and drain on horses, 48 h	Loss of excess brine
79	Remove from horses, inspect, add 1 kg fine salt, bundle	1 kg uptake of salt
79	Move hides to storage or load for shipment	10–15% salt; 40–45% water; 35–45% hide substance

[a]Most modem processors recycle excess brine and do not discharge it. Also the use of evaporation ponds decreases discharge to sewers.

Source: Adapted from Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA; Minnoch, J.K., Minnoch, R.M., 1979. Hides and Skins. Ithaca, NY: National Hide Association; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Some leather is tanned with the hair or wool remaining on the hide (e.g., shearling leather) that is produced from sheepskins (uniformly clipped to short lengths). Either chrome or the vegetable process can tan this type of product, but the wool must be cleaned and degreased. Weaker tanning solutions for longer lengths of time are used.

Some hides such as kidskins, sheepskins, and pigskin contain a large quantity of fat (Table 9). Degreasing is done by warming hides in water and pressing them in a hydraulic press, followed by washing and rinsing. In some cases, hides are washed with surface-active or emulsifying agents (i.e., quaternary ammonium salts of higher fatty alcohols).

Deliming

The deliming process consists of a washing step in which ammonium sulfate ($(NH_4)_2SO_4$)) or ammonium chloride (NH_4Cl) and sometimes trisodium phosphate (Na_3PO_4) or sulfuric acid (H_2SO_4) are added to convert the remaining lime into water-soluble compounds. Ammonium chloride penetrates faster than sulfate and results in softer leather. Ammonium sulfate is used for most shoe upper leather because it produces firmer leather with more temper. These deliming operations lower the pH to 8–9, which reduces the alkaline swelling of the hide appropriate for enzymatic bates.

Table 12 Agitated brine-curing of fleshed cattle hides, requiring a minimum of 2 days and yielding 62–68% of green hide weight

Weight of hides (kg)	*Operation or activity*	*Composition or change*
Green		
100	Receive hides from slaughter	65–70% water, 30–35% of weight is hides
100	Trim ears, snout and tail	3% loss; 3 kg trim to rendering
97	Wash hides	2% loss; 2 kg blood and manure
95	Flesh and demanure with machine	12–18% loss; 12–15 kg fleshing to rendering; 1–3 kg manure
80	Trim pattern	3–4% loss, 3 kg trimming to rendering
77	Move hides to raceway	4 kg of brine per kg of hide, maintain brine at 94–97° salimeter
77	Cure in moving brine for 24 h	15–17% net loss in weight; 20–25 kg loss in water; 7–10 kg uptake of salt; 10–15 kg loss of salt to sewer[a]
Cured wet		
	Remove from brine and pass through wringer	Loss of excess brine
65	Inspect, add 1 kg fine salt, bundle	1 kg uptake of salt
65	Move hides to storage or load for shipment	12–15% salt; 40–50% water; 35–45% hide substance

[a]Most modern processors recycle excess brine and do not discharge it. Also the use of evaporation ponds decreases discharge to sewers.

Source: Adapted from Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA; Minnoch, J.K., Minnoch, R.M., 1979. Hides and Skins. Ithaca, NY: National Hide Association; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Table 13 Some bactericides used in tanning (hides to leather)

Bactericide	*Parts per 100 parts of salt*
Sodium fluoride (NaF)	2
Sodium silicofluoride (Na_2SiF_6)	2
Zinc chloride ($ZnCl_2$)	0.5
Mixture of:	
Soda ash (Na_2CO_3)	2
Naphthalene ($C_{10}H_8$)	1

Source: Adapted from Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Bating

Bating removes the alkaline dehairing chemicals and other nonleather substances. Bates digest and dissolve the noncollagenous protein constituent of skin and are inactive on collagen. Bates are enzymes of bacterial, fungal and plant, or animal origin. Bating makes the hide softer, less harsh, and cleaner. Bates also remove the glue-like material between the collagen fibers that would result in hard and 'tinny' leather. The bating operation may last from a few hours to 16 h. A strong bating action is used when making soft leather and a light bating action is used in making less flexible leather. Antiseptics, such as sodium fluoride, sodium pentachlorophenol (C_6HCl_5ONa), or β-naphthol ($C_{10}H_8O$), are sometimes added. Many bates are a mixture of deliming materials and various enzymes, so that deliming and bating can be conducted simultaneously. After bating, the pelts are rewashed to remove undesirable, digested nonleather material.

Pickling

Pickling (which can be skipped if hides are to be tanned immediately after bating) places the hides in acid (pH < 3). The hides require a pH reduction in order to accept the tanning materials (e.g., chrome), which are not soluble in an alkaline environment. Sulfuric acid (H_2SO_4) is the most common acid employed, but other acids can also be used. The pickling procedure starts with the addition of salt. This prevents swelling (acid swelling) by tying up excess moisture. The pickling takes only a few hours.

Tanning

Tanning converts the collagen fibers of skin into stable, nonputrescible leather. Chrome tanning is the most popular (90%) method of tanning because it can be accomplished quickly and produces leather with desirable physical and chemical properties (long wearing and heat resistant). Chrome plays a role in hide preservation, acts as a hide stabilizer to temperature extremes, and is a well-fixed, nonleaching tanning material. Disposal of chrome is something of a problem, even though chromium in leather is Cr(III) and not the toxic Cr(VI). Accordingly, the Environmental Protection Agency does not consider the waste as toxic. A chromium salt (e.g., sodium bichromate, $Na_2Cr_2O_7$) reacts with a reducing sugar (maltose, $C_{12}H_{22}O_{11}$) and sulfuric acid (H_2SO_4), which reduces the chromium salt to basic chromic sulfate ($Cr(OH)SO_4$, called 'chrome'), which is then added to the hide at 1.5–3%. A sodium salt of a chlorinated phenol preservative (0.02–0.1%) is sometimes added. The pH of the drum contents is increased ('basification') to 3.4–3.6 by adding sodium bicarbonate ($NaHCO_3$, baking soda) or other alkalis, which increase the affinity of the collagen to the chrome. The theory of chrome tanning is that cross-linkage is accomplished by bonding the various chrome ions with free carboxyl groups in the collagen side chains. The liming helps in exposing additional carboxyl groups by chemical hydrolysis of amine side chains. The tanning operation requires 4–6 h and the resultant bluestock is now called leather. It is preserved against putrefaction and its thermal stability has been raised. Hides are now 'in the blue.'

Table 14 Pit-curing of fleshed cattle hides, requiring a minimum of 3 days and yielding 62–68% of green hides weight

Weight of hides (kg)	*Operation or activity*	*Composition or change*
Green		
100	Receive hides from slaughter	65–70% water and 30–35% of weight is hides
100	Trim ears, snout and tail	3% loss; 3 kg trim to rendering
97	Wash hides	Wash – hides and manure
95	Flesh and demanure with machine	12–18% loss; 12–15 kg fleshing to rendering; 1–3 kg manure
80	Trim pattern	3–4% loss; 3 kg trimming to rendering
77	Salt hides down into pit 1.2–1.5 m (4–5 feet) deep; flood with saturated brine	0.5 kg of salt (no. 1 rock salt) per 1 kg of hide
77	Cure in pit (still brine) for 48–55 h; drain pit for 24–33 h	15–17% net loss in weight; 20–25 kg loss in water; 7–11 kg uptake of salt; and 10–15 kg loss of salt to sewer[a]
Cured		
65	Remove from pit, inspect, and bundle	Reclaim excess salt
65	Move hides to storage or load for shipment	12–16% salt; 35–45% water; and 40–50% hide substance

[a]Most modern processors recycle excess brine and do not discharge it. Also the use of evaporation ponds decreases discharge to sewers.

Source: Adapted from Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA; Minnoch, J.K., Minnoch, R.M., 1979. Hides and Skins. Ithaca, NY: National Hide Association; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Vegetable tanning (see Retanning below) is a slower process that takes several months and produces firmer leather with more water resistance. Zirconium (Zr) can be processed into ZrO_2 and, along with silica and under acidic conditions (pH <2), can tan skin rapidly.

A small percentage of skins are tanned by the alum (aluminum potassium sulfate, $AlK(SO_4)_2$) process, sometimes called 'tawing' process, to obtain pure white or sole-grey leather. The oil method using 'fish' oil (cod, whale, seal, or shark) is used to produce soft leather for moccasin or chamois leather. Other tanning methods include the formaldehyde procedure (formalin solution for white and washable leather), the glutaraldehyde method (for producing light-tan washable shearling bed pads), the calgon method (5% sodium hexametaphosphate at pH 2.8), the quinone technique, tungsten tanning, the aluminum method, the iron technique, and the silica procedure.

Wringing/Setting

The purpose of wringing or setting, sometimes called 'sammy,' is to remove excess moisture, smooth the grain, and remove wrinkles from the hide. The machine that accomplishes this is similar to the previously described wringing machine.

Splitting and Shaving

The purpose of splitting and shaving is to adjust leather thickness. Hide thickness (sometimes 8 mm or more) can vary from animal to animal as a result of age and can also vary between different areas of the skin on the same animal. The major portion of the thickness adjustment is accomplished by splitting with a horizontal band saw that contains a sharp flexible knife instead of saw teeth. The hide is fed through the machine with the grain (outer) portion up and this is the portion that is sized. Adjustable feed rolls above and below the knife control the ultimate thickness of the grain side of the hide to 0.25 mm. The underside (flesh layer) is called a 'split' (it does not contain any grain) and is often thick enough to be used for suede.

The two most popular methods of splitting hides can be found in Table 8.

Retanning

Hides are often 'retanned' using the combined desirable properties of more than one tanning agent. Retanning stabilizes chrome from leaching, imparts softness and body, and, in some cases, bleaches the chrome blue color. The more popular retanning agents are vegetable extracts and syntans. Vegetable extracts are some of the originally used tanning agents extracted from trees and shrubs with water and heat. Tannin (tannic acid, $C_{76}H_{52}O_{46}$) is the active agent in vegetable extracts and is found in more than 300 species of plants. Vegetable tanning results from hydrogen bonding between the tannin phenolic groups and the peptide bonds of protein chains. Frequently, 50% (by weight) of tannin is incorporated into hides.

Vegetable retanning results in solidity, body, and more uniformity in chrome-tanned leather. This is important in pigskin to modify the difference in temper of various parts of the skin.

Syntans are synthetic chemicals generally produced by condensing aromatic sulfonic acids or phenols with formaldehyde. They can also be of the acrylic resin type. Syntans are used to produce softer leather and to produce white or pastel shades because the syntan has a bleaching effect on the blue-green chrome-tanned leather.

For retanning, hides are washed and neutralized with a mild alkali to adjust the pH for the retanning material selected (e.g., pH 5 for vegetable tanning). After the retanning agent is added, the process usually takes 1–2 h.

Dyeing/Coloring

Dyeing (coloring) of leather is often preformed. There are hundreds of different dyestuffs and auxiliary products, and leather is usually dyed with blends to achieve the desired color. The tanner uses pH control to affect the affinity of the dyestuff for the fibers.

Useful properties of dyes can be classified into various categories:

- Acid dyes: penetrate readily, bright colors.
- Aniline types: combine with skin fibers.
- Basic dyes: surface dyeing, brilliant shades.
- Direct dyes: surface dyeing, deep shades.
- Metallized dyes: level dyeing, subdued colors.

The aim of coloring is not only to produce the right strength (contraction) and shade (hue or dullness) but also to produce a color that will resist fading, will not bleed, and can be dry-cleaned or washed.

Fatliquoring

Fatliquoring is used to adjust the firmness or softness of leather by lubricating the internal fibers and to increase the tensile strength of leather. The basic ingredients ('sponging' compounds) are vegetable, fish, mineral, or animal oils, such as neatsfoot oil; glycerin and related fatty substances; soaps; egg yolk; and sometimes waxes or clay and chemical reagents to improve water solubility or emulsifiers that disperse the nonpolar oils. Some fatliquoring materials are highly sulfated oil blends (e.g., sulfated castor oil). Fatliquoring increases softness by breaking down thick fibers into smaller ones. Anionic fatliquoring substances are prepared from mixtures of sulfated or sulfonated oil; cationic fatliquors are blends of alkylated long-chain amines mixed with raw oils. By selection of the type and amount of fatliquor, various degrees of softness can be achieved. Pigskin usually requires more fatliquor than cattle skins.

Setting Out

The 'setting out' process smoothes and stretches the leather and compresses and squeezes out the excess moisture and grease. This is accomplished on a machine, like a fleshing machine with a blade designed to exert pressure and to smooth the grain. This compresses the leather and results in a product with 60% moisture.

Drying

Drying removes all but the equilibrium moisture. There are four different drying methods that influence the characteristics of leather. The simplest drying method is 'hanging,' in which the leather is hung and often passed through a large drying oven. Another method is called 'toggling,' in which the skins are stretched and attached to a perforated metal frame by fastening hooks called toggles. An additional skin is then fastened to the other side of the frame. These frames are then placed in a drying oven.

A popular drying technique is called 'pasting,' in which hides are actually pasted to large stainless-steel, porcelainized steel, or glass plates and are placed in a drying oven (60–66 °C, 40% relative humidity) for 4–6 h. Another drying technique uses 'vacuum dryers.' After drying, the skins contain 10–12% moisture.

Conditioning

Conditioning or 'wetting back' involves introducing controlled amounts of moisture. The moisture is applied by shower-like nozzles, and the hides are then stacked and covered with a moisture-proof material to 'mull' for approximately 16 h. The moisture level is raised to 25%.

Staking

'Staking' (mechanical massaging) is done to soften the leather and to make leather more pliable, in combination with fatliquoring. This primarily governs the final temper. The combination of pulling and rolling by the staking machine applies a great deal of mechanical stress and flexes the fibers.

Buffing

Buffing involves smoothing the grain surface by light mechanical sanding to improve the appearance. Brushes, jets of air, or vacuuming removes leather dust.

Table 15 Physical properties of shoe-upper leather

Property	*Value*
Tensile strength (MPa)	15.3–37.5
Elongation at break (%)	29.5–73.0
Stitch tear strength (N cm^{-1})	1280–2275
Thickness (mm)	1.5–2.4
Bursting strength (kN cm^{-1})	1.1–24.5
Apparent density (g cm^{-3})	0.6–0.9
Real density (g cm^{-3})	1.4–1.6
Heat resistance	Shrinks depending on moisture; anhydrous decomposition at 160–165 °C

Source: Adapted from United States Military Specification (undated) MIL-L-3122; Wilson, J.A., 1927. International Critical Tables of Numerical Data, Physics, Chemistry and Technology, pp. 250–254. New York: McGraw-Hill; Conabere, G.O., Hill, R.A., 1948. Progress in Leather Science, 1920–1945. London: British Leather Manufacturers' Research Association; Roddy, W.T., Echerlin, R., Jansing, J., 1948. Quartermaster General's Research Reports on Leather Technology: April 15. Washington, DC: US Army Quartermaster; Roddy, W.T., Jacobs, J., Jansing, J., 1949. The water resistance and other physical and chemical characteristics of an Army upper leather. Journal of the American Leather Association 44, 308; Kanagy, J.R., 1965. In: O'Flaherty, F., Roddy, W.T., and Lollar, R.M. (Eds.), The Chemistry and Technology of Leather, vol. IV, pp. 369–416. New York: Reinhold; Kremen, S.S., Lollar, R.M., 1951. A study of the moisture relationship and thermal properties of skin and leather. Journal of the American Leather Association 46, 34; Kirk-Othmer, 1981. In: Martin, G. (ed.), Kirk-Othmer Encyclopedia of Chemical Technology, third ed. vol. 14, pp. 200–230. New York: Wiley; and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Finishing

Finishing is the application of film-forming materials (polymeric impregnates) that provide abrasion and stain resistance, enhance color (finishing material; transparent to opaque), add body, alter tactile properties, and make an easy care leather. The type of finish is determined by the type of skin and its ultimate use.

Coating materials include the following:

- Acrylate polymers (basic structure acrylic acid, $C_3H_4O_2$).
- Albumin, blood (see 'albumin, egg').
- Albumin, egg (egg white: 53% C, 7% H, 16% N, 2% S).
- Butadiene polymers (basic structure butadiene, C_4H_6).
- Casein (cow's milk protein, 54% C, 7% H, 16% N, 22% O, 1% P, and 1% S).
- Isinglass (fish glue).
- Linseed oil (glycerides of linolenic, linoleic, oleic, stearic, palmitic, and myristic acids).
- Nitrocellulose ($Cl_2Hl_6N_4O_{18}$).
- Polyurethane (basic structure is urethane, $NH_2COOC_2H_5$).
- Acrylic-urethane copolymer.
- Shellac (resinous excretion of an insect).
- Vinyl polymers (basic structure is vinyl acetate, $C_4H_6O_2$).
- Wax (vegetable fat expressed from fruit).

After the finishing material is applied, it is dried in a tunnel that is heated with steam or infrared units. Maximum results are obtained when several finish coats are applied with intermittent drying.

Planning

'Planning' smooths the grain surface or produces a varied grain texture and is conducted on presses. The leather can also be embossed in which pressure is applied by engraved plates that produce a permanent pattern.

Area Measurement

Because leather is sold by area, it is measured with a planimeter. This employs fingers that sense the leather as it passes through a machine and sums the total area. The final step is grading for uniformity of thickness, color, and defects.

Physical Properties of Leather

Leather has many unique and valuable physical properties (Table 15), most of which can be attributed to its internal

Table 16 Tanning steps and what occurs at each stage

Tanning step	*Explanation*
Flaying	Hide removal: Knife, puller, and air pressure used
Hide curing	Temporary protective (from bacteria and enzymes) treatment for hides, traditionally by NaCl, sometimes drying
Fleshing	Removes attached adipose tissue (can also be done prior to curing, soaking or liming)
Trimming	Removes odd-shaped, unworkable areas of the hide
Sorting	According to sex, weight, branding, and defects
Storage/shipment	Safety salt and insecticides are often used
Soaking	Removes salt, blood; restoration of moisture
Liming/dehairing	Removes hair, epidermis, some soluble proteins; traditionally uses $Ca(OH)_2$, and Na_2S or sulfides, NH_3; enzymes can also be used
Splitting	Diverts bottom layer into food use (e.g., sausage casings)
Deliming	Removes alkali (traditionally using $(NH_4)_2SO_4$)
Bating	Removes noncollagenous materials enzymatically
Pickling	Lowers pH (using salt, then H_2SO_4) for reception of tanning chemicals
Tanning	Preserves from putrescence; imparts thermal stability; 90% of hides tanned with salts of Cr(III)
Wringing/setting	Removes excess moisture
Trimming	Removes unusable perimeters; generates Cr-containing waste
Splitting	Lower layer destined to suede, top layer to grain leather
Shaving	Adjusts thickness, generates Cr(III)-containing waste
Retanning	(Minerals, vegtans, and syntans) Adds body, softness, bleaches colour, and stabilizes Cr from leaching
Coloring	Dyeing (with acid, metallized, direct, and basic dyes)
Fat-liquoring	Lubricates for flexibility, softness (lipid derivatives; emulsifiers)
Setting out	Removes excess moisture, stretches, smoothing of grain surface
Drying	Removes all but equilibrium moisture
Conditioning	Introduces controlled amounts of moisture for softness
Staking	Mechanical flexing for softness
Buffing	Sanding of grain surface; generates Cr(III)-containing waste
Finishing	Impregnates and coats with polymeric materials for abrasion and stain resistance, adds body, colour effects, gloss and handling properties and makes the leather easier to care for
Planning	Smooths grain and produces varied grain texture
Area measurement	Leather is sold by area

Source: Adapted from Marmer, W.N., 1996. Preservation and Tanning of Animal Hides. American Chemical Society Symposium Series 647. Washington, DC: American Chemical Society; New England Tanners Club (1994); and Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

structure. Leather has high tensile strength, which makes it one of the strongest flexible sheet materials. Leather also has very high tear strength, because the fibers are random in orientation and do not have a fixed tear path. Pigskin, however, is much weaker in tear resistance and cannot be used in some products. The elongation (maximum stretching without breaking) of leather can be controlled (15–73%) by selecting the tanning and fatliquoring processes. Leather also has excellent flexibility over a wide temperature and moisture range, making the product suitable for harsh environments. Leather also provides a safety feature through its puncture resistance, which contributes to its long wearing. Leather can absorb and transmit moisture (breathability; pigskin is very good at this), has the ability to cool in hot weather and insulate in cold weather, and can block wind, all of which make it an ideal material for garments and shoes. Leather can also be molded; it retains its other desirable properties even after being permanently deformed into new shapes, which is one of its most significant properties for making shoes. This combination of properties makes leather a unique and very useful material.

Tanning Effluent and Waste

Information on these topics can be located in the Further Reading.

Summary

A summary of the tanning process and what each step accomplishes is given in **Table 16**.

See also: By-Products: Edible, for Human Consumption; Inedible

Further Reading

Aten, A., Innis, R.F., Knew, E., 1955. Flaying and Curing of Hides and Skins as a Rural Industry. Rome: FAO.

Bengtsson, O., Holmqvist, O., 1984. By-products from slaughtering. Fleischwirtschaft 64 (3), 334–336.

Biedermann, K., Neck, H., Neher, M.B., Wilhelmy Jr., V., 1962. A technical–economic evaluation of four hide curing methods. Agricultural Economic Report No. 16. Washington, DC: Marketing Economics Division, Economic Research Service, USDA.

BLC Leather Technology Centre, 2003. Improving Hide Defects. Available at: http://www.blcleathertech.com (accessed 15.07.12).

Clemen, R.A., 1927. By-products in the Packing Industry. Chicago, IL: University of Chicago Press.

Conabere, G.O., Hill, R.A., 1948. Progress in Leather Science, 1920–1945. London: British Leather Manufacturers' Research Association.

Eurohides, 2003. Leather Glossary. Available at: http://www.kenscottpouches.com/leatherbook.htm (accessed 15.07.12).

Euroleather, 2003. Sectoral Data. Available at: http://www.euroleather.com/sector.htm (accessed 15.07.12).

FAO, 1994. World Statistical Compendium for Raw Hides and Skins, Leather and Leather Footgear 1974–1992. Rome: FAO.

FAO, 2001. Commodity Problems. Available at: http://www.fao.org/docrep/meeting/003/Y0590e.htm (accessed 15.07.12).

FAO, 2002. Commodity Market Review. Rome: FAO.

Hague, H.M., 1949. Leather. Washington, DC: Tanners' Council of America.

Henrickson, R.L., Turgut, H., Rao, B.R., 1984. Hide protein as a food additive. Journal of American Leather Chemists 79, 132–145.

International Labour Organization, 1992. Recent Development in the Leather and Footgear Industry and Notes on Proceeding of Fourth Tripartite Technical Meeting for the Leather Footgear Industry. Geneva: ILO.

Judge, M.D., Salm, C.P., Okos, R.M., 1978. Hog Skinning Versus Scalding Arlington, VA: AMI Foundation. pp. 155–164.

Kanagy, J.R., 1965. In: O'Flaherty, F., Roddy, W.T., Lollar, R.M. (Eds.), The Chemistry and Technology of Leather, vol. IV. New York: Reinhold, pp. 369–416.

Kirk-Othmer, 1981. In: Martin, G. (Ed.), Kirk-Othmer Encyclopedia of Chemical Technology, third ed. vol. 14. New York: Wiley, pp. 200–230.

Kremen, S.S., Lollar, R.M., 1951. A study of the moisture relationship and thermal properties of skin and leather. Journal of the American Leather Association 46, 34–40.

Lawrie, R., 1981. Development in Meat Science – 2. London: Applied Science Publishers.

Leather Industries of America, 1987. US Leather Industry Statistics. Washington, DC: Leather Industries of America.

Leather Industries of America, 1996. US Leather Industry Statistics. Washington, DC: Leather Industries of America.

Leather Industries of America and US Hide, Skin and Leather Association, Skin and Leather Association, 1985. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Leather Industries of America and US Hide Skin and Leather Association.

Leather Industries of America and US Hide, Skin and Leather Association, Skin and Leather Association, 1993. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Leather Industries of America and US Hide Skin and Leather Association.

Leather Industries of America Research Laboratory, 1991. Dictionary of Leather Terminology. Ohio: University of Cincinnati.

Leatherindia, 2003. Leather Glossary. Available at: http://www.leatherindia/com/leatherglossary.htm (accessed 15.07.12).

Leather Tanning, Environmental Protection Agency (EPA). Available at: http://www.epa.gov/ttnchie1/ap42/ch09/final/c9s15.pdf (accessed 15.07.13).

Marmer, W.N., 1996. Preservation and Tanning of Animal Hides. Washington, DC: American Chemical Society. American Chemical Society Symposium Series 647.

Mavlyanov, S.M., Islambokov, S.Y., Ismailov, A.I., Dalimov, D.N., Abdulladzhanova, N. G., 2001. Vegetable tanning agents. Chemistry of Natural Compounds 37, 1–24.

Moulton, C.R., Lewis, W.L., 1940. Meat Through the Microscope, revised ed. Chicago, IL: Institute of Meat Packing, The University of Chicago.

National Hide Association, 1979. Hides and Skins, third ed. Prepared by Education Committee (Whitney, E.A. Chairman). Sioux City, IA: National Hide Association.

New England Tanners Club, 1984. Leather Facts, second ed. Peabody, MA: New England Tanners Club.

Ockerman, H.W., 1996. Chemistry of Meat Tissue. Columbus, OH: Department of Animal Science.

Ockerman, H.W., Hansen, C.L., 2000. Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Price, J.F., Schweigert, B.S., 1971. The Science of Meat and Meat Products. San Francisco: WH Freeman.

Roddy, W.T., Echerlin, R., Jansing, J., 1948. Quartermaster General's Research Reports on Leather Technology: April 15. Washington, DC: US Army Quartermaster.

Roddy, W.T., Jacobs, J., Jansing, J., 1949. The water resistance and other physical and chemical characteristics of an army upper leather. Journal of the American Leather Association 44, 308–315.

Romans, J.R., Ziegler, P.T., 1974. The Meat We Eat. Danville, IL: Interstate.

Scott, K., 2003. A Glossary of Leather Terminology. Available at: http://www.kenscottpouches.com/leatherbook.htm (accessed 15.07.13).

Tanners' Council of America, 1972. Trade Practices for Proper Cattlehide Delivery. Washington, DC: Tanners Council of America.

Tanners' Council of America, 1983. Dictionary of Leather Terminology. Washington, DC: Tanners Council of America.

Tanning, 2011. Wikipedia. Available at: http://en.wikipedia.org/wiki/Tanning (accessed 15.07.12).

United States Hide, Skin and Leather Association, Skin and Leather Association, 1997. Census. Washington, DC: United States Hide, Skin and Leather Association.

United States Military Specification (undated) MIL-L-3122.

US Department of Commerce, 1987. Footgear Retailers of America. Washington, DC: United States Department of Commerce.

Wilson, J.A., 1927. International Critical Tables of Numerical Data, Physics, Chemistry and Technology New York: McGraw-Hill, pp. 250–254.

Inedible

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Introduction

Inedible meat by-products have a prehistoric significance that was established long before the advent of the meat industry. As animal protein (meat) was acquired primarily by hunting and fishing for food purposes, the inedible portions were left for nature's disposal or used for clothing, housing, fuel, and other life-support functions. In the early days of the modern meat industry, the inedible meat and fat remaining after hide removal were likewise disposed of by, most often, dumping into trenches or rivers. The earliest nonedible usage of animal by-products (ABPs) was probably as fertilizer. American-Indians placed fish with seeds when planting corn. Also, the protein and mineral fraction that remained following the removal of a portion of fat was generally disposed of by spreading over crops. The modern world is more environmentally sensitive and dense population centers made this practice unacceptable. This evolution began in the nineteenth and early twentieth centuries, and it was during this period that rendering was adopted as a means of processing animal raw materials via cooking and fat extracting. Fat was then used in soap making and candle making in this era. The protein fraction was not used extensively until the early 1900s. A recorded benefit of the use of digester tankage animal protein was documented in 1901 by Professor C.S. Plumb of Purdue and later of The Ohio State University (OSU). He published research conducted with swine showing dramatically increased growth rate by including animal protein ingredients as a supplement to ear corn. This research was so important that a university building was named after him at OSU. Since that important discovery, the literature has rapidly indicated the benefits of animal protein and fat ingredients for all livestock, poultry, domestic animals, and aquaculture species.

In recent years, bovine spongiform encephalopathy (BSE) – mad cow disease – and other transmissible spongiform encephalopathies (TSEs) have become endemic throughout regions of the world. The infectious agent in BSE is believed to be a specific type of misfolded protein called prion. Prions are not destroyed even if the beef containing prion is cooked. This ruminant disease is transferable to humans by consumption of contaminated tissue. Current regulations have mandated modifications in processes and precautionary principles to prevent animal and human diseases. Currently, regulations for the utilization of ABPs in North America differ from those in Europe as well as in other parts of the world. The United States has prohibited use of mammalian-derived proteins in any feed for cattle or other ruminant animals, with specific exemptions (21CFR589.2000), since 1997. Downer cattle are also no longer permitted in the food chain.

The European Union (EU) has issued Regulation (EC) No. 1774/2002 on the use of ABPs. Detailed requirements for the processing and use of ABPs are currently divided into three categories. Meat and bone meal (MBM) is at present not allowed as feed for farm animals. MBM produced from category 3 ABP materials can be used for pets (companion animals). The conditions for rendering ABPs are also specified in the EU regulation, including maximum particle size, temperature, time, and pressure for heat treatment. All category 1 ABPs must be incinerated, whereas category 2 ABPs can be used, for example, as fertilizer or for landfill. The categories according to the EU regulation are defined in Table 1. Regulations and directives that govern the processing and utilization/disposal of animal coproducts are under constant review with regard to possible changes as dictated by current issues affecting individual countries; thus, changes can be expected.

Table 1 Basic categories of animal by-products (ABPs)

Category 1 ABPs	*Category 2 ABPs*	*Category 3 ABPs*
Animals suspected of transmissible spongiform encephalopathy	Manure or digestive tract content from mammals	Animal waste fit for human consumption
Specified bovine spongiform encephalopathy (BSE) risk material (including dead ruminants)	Material from treatment of wastewater (category 2 and 3 plants)	Nonruminant blood, hides, and skins, which have passed antemortem inspection
Material from treatment of wastewater (category 1 plants)	Products containing veterinary drug residues	Shells, hatchery by-products, and cracked eggs with no sign of clinical disease
Pet, zoo, and circus animals	Farmed animals that have died or been killed on the farm	Raw milk from healthy animals
Experimental animals	Fish with clinical signs of disease	Food destined for animal consumption
Products from animals with residues of prohibited substances or environmental contaminants		Fish and fish offal
International catering waste		Blood, hides, hooves, feathers, and hair from animals with no sign of disease
		Catering waste, including used cooking oils

The Office International des Epizooties is structured to evaluate science, to assess risk and, to develop policies that affect animal health on a global basis. Future direction and policy will, undoubtedly, be supplied by this organization, which currently represents 175 members. It is considered by most authorities as the 'World Organization for Animal Health.' The incidences of TSEs as well as all emerging diseases and ethnic and ethical issues will continue to dictate specific geographical policies.

Processed ABPs are generally classified as either animal proteins or fats. This article discusses the products within these major classifications.

Inedible By-Products

Production and processing of edible meat and poultry result in an inedible fraction. Edibility is a relative term and is determined by a number of factors, including consumer acceptance, demographics, regulatory requirements, economics, tradition, hygiene, and religious beliefs. The inedible raw materials from animal production consist of hides, skin, hair, feathers, hooves, horn, feet, heads, bone, toe nails, blood, organs, glands, intestines, muscle, fat, egg shells, and whole diseased carcasses. Using approximations, these tissues comprise 50% of the live weight of cattle, 40% of the live weight of pigs, and 30% of the live weight of broilers. As further processed, prepackaged, and table-ready meat products are being brought to the marketplace, the inedible portions have increased in relation to the original weight of the animal. In recent years, global regulations and directives have arisen as precautionary principles for adding specific animal tissues categorized as inedible fractions. These trends are expected to continue and to contribute even more inedible tissue to the by-product pool. The only exception to this trend is mechanical deboning, which converts soft tissue (attached to the bone and bone marrow) and extracted gelatin from bones into the edible category.

World production of ABPs is not known in exact quantities due to the inconsistency of terminology, specifications, processing, and end usage.

Processing

Several processes are utilized to handle or dispose of inedible raw animal tissues. The most frequently referenced are burial, landfill, and composting as well as burning (on pyres), incineration, or rendering. Rendering, composting, and biogas production are the only processing methods that recycle and provide value to the by-product tissues, as the other processes are disposal procedures. Rendering and incineration are the only processes in which temperature-, time-, and pressure-controlled procedures and other controlled process procedures are used in treating raw animal tissue. The rendering process separates fat from the proteinacious and mineral components, removes most of the moisture, and inactivates microorganisms and parasitic organisms. The rendering process completely decharacterizes the muscle, connective, structural, organ, and adipose tissues. The resulting tissue is a rich granular-type substrate and fats with specific nutritional components that have no resemblance to the original raw material. Biosecurity questions arise with all disposal procedures that do not incorporate sterilization principles provided by the controlled application of heat or other treatments. The UK Department of Health provides a summary of potential health risks for various methods of handling ABPs (Table 2). This summary associates a risk factor with the most common methods of handling ABPs. Rendering remains the primary method for processing ABPs in most countries.

In 2009, the United States produced and harvested approximately 9 billion head of livestock as well as 8.9 billion poultry and a growing number of fish for the aquaculture industry. This makes animal food production one of the largest economic industries within the United States. Animal production is similarly important in most other countries. Animal protein and fat are important components of human diets.

Table 2 Summary of potential health risks for various methods of handling animal by-products

Disease/hazardous agent	*Exposure of humans to hazards from each option*				
	Rendering	*Incineration*	*Landfill*	*Pyre*	*Burial*
Campylobacter, *Escherichia coli*, and *Listeria*	Low	Low	Moderate	Low	High
Salmonella and *Bacillus anthracis*	Low	Low	Moderate	Low	High
Clostridium botulinum and *Leptospira*	Low	Low	Moderate	Low	High
Mycobacterium tuberculosis var. *bovis* and *Yersinia*	Low	Low	Moderate	Low	High
Cryptosporidium and *Giardia*	Low	Low	Moderate	Low	High
Clostridium tetani	Low	Low	Moderate	Low	High
Prions for BSE and Scrapie	Moderate	Low	Moderate	Moderate	High
Methane and CO_2	Low	Low	Moderate	Low	High
Fuel-specific chemicals and metal salts	Low	Low	Low	High	Low
Particulates, SO_2, NO_2, and nitrous particles	Low	Moderate	Low	High	Low
PAHs and dioxins	Low	Moderate	Low	High	Low
Disinfectants and detergents	Low	Low	Moderate	Moderate	High
Hydrogen sulfide	Low	Low	Moderate	Low	High
Radiation	Low	Moderate	Low	Moderate	Moderate

Abbreviations: BSE, bovine spongiform encephalopathy; PAH, polyaromatic hydrocarbons.

The pig population in China in 2007 was 462 million, nearly seven times that of the United States. China is also the largest producer of aquaculture foods. Projection for the next 30 years suggests that the world will need to produce 250% more meat, milk, and eggs than it currently produces. This production challenge requires adapting new technology and production practices.

On a global scale, modern efficient rendering facilities are concentrated in countries and regions possessing strong and well-established animal production industries. In the United States, the current annual quantity of inedible raw material generated from animal processing is approximately 23.4–24.3 billion kg. World or country data for individual countries are not readily available. An estimate of EU production is 16.1 million tonnes of animal by-products annually, of which 14.3 million tonnes are sourced from fit-for-human-consumption sources and 1.8 million tonnes from unfit-for-human-consumption sources. Argentina, Australia, Brazil, and New Zealand collectively process another 11 million tonnes of ABPs per year. In China, the rendering process is virtually nonexistent, and the inedible portion derived from each animal species is considerably lower than in other parts of the world. The protein, mineral matter, fat, and water derived from specific ABP materials are outlined in Table 3.

The Rendering Process

The rendering process uses ABPs from meat production. All ABPs are the direct result of animal food production and originate from the animal food production chain, for example, slaughterhouses (abattoirs), meat processing plants, butcher's shops, supermarkets, and livestock rearing.

The rendering process comprises a number of processing stages as depicted in Figure 1. The raw material is received at the installation in regulated, closed transportation units. Processing is usually initiated within a short time following receipt. Preparation for rendering involves prebreaking to reduce the particle size, which is important to provide proper heat penetration during the cooking process. The material is then heated under controlled conditions for specific monitored times.

EU regulations require that mammalian by-product materials are processed to 133°C for at least 20 min without interruption at a pressure (absolute) of at least 300 kPa produced by saturated steam. Sterilization by pressure cooking is not required for nonmammalian (poultry) material. Pressure cooking is not a standard practice in the United States and some other countries. Thus, processing specifications and the equipment used vary between installations and facilities.

Table 3 Reference data for animal carcasses and slaughterhouse by-product use. The sum of protein, mineral matter, fat, and water portions need not be 100%, as there are other ingredients in the substances mentioned, for example, starch, nucleic acid, and raw fibers. The figures serve only as a guide, as they depend on the actual composition of the raw material

Raw material and finished wastewater (condensed vapor)	*Quantity*	*Protein matter*		*Minerals*		*Fat*		*Water products*	
	(kg)	*(%)*	*(kg)*	*(%)*	*(kg)*	*(%)*	*(kg)*	*(%)*	*(kg)*
Animal carcasses	1000	15	149	4	38	12	118	68	683
Animal meal	240	62	149	16	38	12	29	5	12
Animal fat	90	0	0	0	0	99	89	1	1
Condensed vapor (wastewater)	670	0	0	0	0	0	0	100	670
Slaughterhouse waste (red meat)	1000	9	90	2	20	14	137	74	739
Animal meal	150	60	90	13	20	12	18	5	8
Animal fat	120	0	0	0	0	99	119	1	1
Condensed vapor (wastewater)	730	0	0	0	0	0	0	100	730
Bones	470	40	188	40	188	12	56	5	24
Bone meal	470	40	188	40	188	12	56	5	24
Animal fat	90	0	0	0	0	99	89	1	1
Condensed vapor (wastewater)	440	00	0	0	0	0	0	100	440
Blood	1000	12	123	1	7	0	3	87	867
Blood meal	140	88	123	5	7	2	3	5	7
Condensed vapor (wastewater)	860	0	0	0	0	0	0	100	860
Hair	1000	28	255	1	6	2	21	72	718
Hair meal	300	85	255	2	6	7	21	6	18
Condensed vapor (wastewater)	700	0	0	0	0	0	0	100	700
Poultry waste	1000	12	124	2	21	18	181	66	663
Poultry meal	190	65	124	11	21	12	23	6	11
Animal fat	160	0	0	0	0	99	158	1	2
Condensed vapor (wastewater)	650	0	0	0	0	0	0	100	650
Feathers	1000	28	281	1	7	2	23	69	690
Feather meal	330	85	281	2	7	7	23	6	20
Condensed vapor (wastewater)	670	0	0	0	0	0	0	100	670

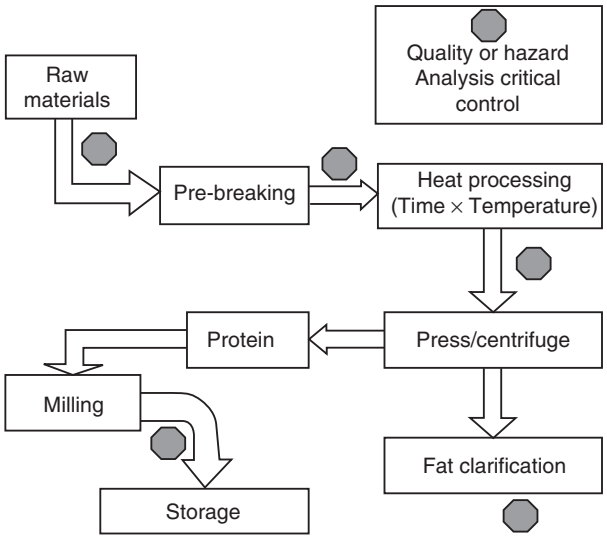

Figure 1 The basic rendering process.

Although the heat-processing vessels vary between batch and continuous systems, the process provides the sterilization function and also separates the fat from the nonfat material. Batch systems (dry rendering cookers) use vats of varying sizes that are surrounded by a steamheated jacket in the pressure cooking process. The newest installations use continuous cylindrical containers in which material is preheated and conveyed through the heating vessel in a continuous flow calibrated to meet the required temperature and time. In facilities requiring pressure treatment, the material is held in a pressure chamber at 300 kPa at the end of the cooking phase.

In either case, systems rely on heat to release fat from the cells of the fatty tissues, either in the absence (dry rendering) or the presence (wet rendering) of added water/steam. In dry rendering, the prebroken fatty tissues are heated in a steam-jacketed vessel to disintegrate the fat cells, release the melted fat, and drive off moisture. Most batch cookers for dry rendering are equipped with rotating agitators, which may be steam heated. Agitation aids in heat transfer in both batch and continuous systems while also serving as part of the conveyor action for the continuous systems. Dry rendering can be achieved at atmospheric pressure, under vacuum, or most commonly, at elevated pressure.

Cooking times vary but average approximately 2.5 h, during which the content is heated and sterilized and water is evaporated. After discharge from the cooking vessel into a percolator, which allows free fat to drain, the material passes to an expeller or centrifuge for further defatting. For efficient fat removal, a solvent extraction plant is sometimes used. This can reduce the fat content in the residue to 10–5%, but it is not often used today because of the explosive potential of the solvent. The cooked proteinaceous residue, most often referred to as cracklings or crax, is then cooled and milled to produce MBM.

Processing of Feathers and Pig Hair

Processing of feathers and pig hair first releases keratin, an indigestible protein, by hydrolysis. The hydrolyzed protein is then dried to produce a digestible, high-protein meal, which could be sold separately before the European ban on the use of certain animal proteins in animal feed, but today it is normally mixed with other types of meal and used as a protein concentrate.

Pig hair and feathers are treated separately, as the conditions (temperature and time) for a suitable hydrolysis of the two products are different. A more powerful heat treatment is required to 'open' pig hair.

The processing can be done in batches in dry rendering cookers, where the keratin-containing material is exposed to high temperature (135–145 °C) and pressure for 30–60 min. The pressure is then released and the product is dried and milled. This can eliminate the requirement for mechanical dewatering.

Special rendering equipment is also available for the continuous hydrolysis of feathers and hair. The material is transported in small batches to a compression chamber, where it is preheated, and then to the hydrolysis unit, where it is treated with direct steam under suitable pressure conditions for a shorter period (normally 10–15 min). The hydrolyzed material leaves the reactor at the bottom. Part of the water is then removed in a decanter. An evaporative unit is used for concentrating the liquid phase. The dewatered product is dried separately or with other rendering products.

Feather processing produces high sulfide emissions in the waste water. Removing hydrogen sulfide is important, because sulfide can impair the activity of the activated sludge and thus the biological treatment process.

Statutory Requirements for Rendering

Regulatory practices concerning ABPs are not consistent between countries. The EU has very severe restrictions. Regulations are altered frequently as a result of geographical incidences of specific animal and human diseases. Permitted disposal methods also differ between countries. Even the process of rendering is interpreted and regulated differently. As an example, an EU directive requires mammalian-derived raw material to be processed at 133 °C, 300 kPa pressure for 20 min. Processing conditions incorporating pressure treatment generally lower the nutritional value of resulting protein meals, except for those tissues with high keratin content. The rendering processes are consistent as a time–temperature treatment for the inactivation of microbiological organisms (bacterial, viral, protozoan, and parasitic).

Raw materials processed by the rendering industry are known to contain significant numbers of microorganisms. Many are potential foodborne pathogens, including a large number of *Salmonella* serovars. Clinically normal animals presented for slaughter and processing harbor microbial organisms, especially within their digestive systems. The presence of digestive tissue and intestinal content, other contaminated tissue from processing, and fallen stock contribute to raw materials with a high microbiological content. This is illustrated by the data for isolation from raw material representing multiple species of food animals as shown in Table 4.

Table 5 illustrates the efficacy of properly managed time–temperature processes for rendering. Temperatures exceeding the thermal death values for specific microorganisms and exposure to excessive temperature are correlated with lowered nutritional values, especially of protein and amino acids. Although research has shown that rendering lowers the infectivity of the prion, the agent most commonly believed to be the cause of the TSEs, it is not inactivated by any of the currently available rendering processess (see section Introduction for more information on BSE). Thus, rendering methods have never been capable of completely inactivating TSEs, nor has any other industrially described operational process.

Table 4 Microbiological isolations of foodborne pathogens from raw material at 17 US/Midwestern rendering establishments during two periods of sampling: winter and summer[a]

Organism	*Number of isolates/number of samples including replicates*			
	Winter	*Summer*	*Total*	*Percentage*
Clostridium perfringens[b]	30/42	30/42	60/84	71.4
Listeria spp.	33/42	31/42	64/84	76.2
Listeria monocytogenes	4/42	3/42	7/84	8.3
Campylobacter spp.	19/42	6/42	25/84	29.8
Campylobacter jejuni	15/42	2/42	17/84	20.0
Salmonella spp.	37/42	34/42	71/84	84/5
Coliforms[b]	42/42	42/42	84/84	100

[a]Some establishments operated more than one rendering line. Sampling occurred twice at each visit for each line, hence the denominator is based on 21 lines sampled twice each visit = 42.
[b]Characterization of the bacterial organisms and aerobic plate counts were not possible owing to the nature of the material.
Note: Fats and Proteins Research Foundation, Inc., Directors Digest no. 312.

Table 5 Microbiological isolations of foodborne pathogens from crax at 17 US/Midwestern rendering establishments during winter and summer[a]

Organism	*Number of isolates/number of samples, including replicates*			
	Winter	*Summer*	*Total*	*Percentage*
Clostridium perfringens[b]	0/42	0/42	0/84	0
Listeria spp.	0/42	0/42	0/84	0
Listeria monocytogenes	0/42	0/42	0/84	0
Campylobacter spp.	0/42	0/42	0/84	0
Campylobacter jejuni	0/42	0/42	0/84	0
Salmonella spp.	0/42	0/42	0/84	0
Coliforms[b]	0/42	2/42	2/84	2.4

[a]Some establishments operated more than one rendering line. Sampling occurred twice at each visit for each line, hence the denominator is based on 21 lines sampled twice each visit = 42.
[b]Other organisms were isolated and recorded as laboratory observations but not identified within the scope of this pilot study.
Note: Fats and Proteins Research Foundation, Inc., Directors Digest no. 312.

Industrial Uses of Animal By-Products

Although the significant historical utilization of ABPs has been for feed ingredients, they have also been used in a variety of industrial applications. Rendered animal fat has been used to provide fuel sources and to make soap for more than 2000 years. These uses have declined in nearly all countries with the increased use of petroleum-based and synthetic ingredients. To illustrate this point, during the 1950–60s, more than 75% of all tallow production was directed to the soap-making industry. Today, the figure is approximately 6–7%. Nevertheless, usage has expanded into other areas, such as hand and body lotions, creams, and other cosmetic and bath products. Several hundred categories of chemical processes and formulations use animal-derived fatty acids. These include rubber and plastic polymerization, heavy metal salts, fabric softeners, lubricants and plasticizers, and oleochemical compounds. Gelatin, collagen, and glycerin have been basic ingredients for surfactants, paints, varnishes, adhesives, antifreeze, cleaners, polishes, and numerous pharmaceuticals. During the past few years, animal fats have proven to be a potentially valuable resource as an alternative fuel. Their use as a biofuel (biodiesel or burner fuel) has significantly lowered pollutant emission compared with the combustion of petroleum-based products. Thus, the industrial applications for nonfood, nonfeed by-products have provided an important market for utilizing inedible ABPs. It is projected that the conversion of unused animal tissues into value products will become increasingly important to the world's huge meat and poultry industry.

Animal By-Product Feed Ingredients

Animal protein supplements and fats have long been utilized by the feed compounding industry, primarily for their protein (amino acid) content, mineral content (calcium and phosphorus), and energy contributions. More than 125 individual ABPs are listed in the Association of American Feed Control Officials 2002 Ingredient Manual, but only 11 are major high-volume ingredients. This listing includes 46 different animal protein ingredients. The major protein ingredients are MBM (meat meal (MM)), poultry meal, hydrolyzed feather meal (HFM), blood meal, blood and plasma meal, and fish meal. Similarly, five primary categories comprise the major quantity of animal fats, which are tallow, choice white grease (CWG) (lard), yellow grease, poultry fat, and fish oil. All of these ingredients have different nutrient and product specifications. They all have specific contributions when used as components of livestock, poultry, companion animal, and aquaculture diets. The term nutrient is applied to any food constituent or group of constituents of the same general chemical composition that aids in the support of life. Protein, carbohydrates, fat, minerals, and vitamins are the generally recognized classes of nutrients. ABP ingredients not only contribute most to protein, fat, and mineral nutritional requirements of animals but also provide several sources of fat-soluble and B vitamins. The nutrient qualities complement other feed ingredients and particularly the feed grains, which are primary carbohydrate sources. During early development of the modern nutrient requirement and nutrient allowance data, MBM provided unidentified growth factors

(UGFs) that contributed to unexplained improvements in growth, feed utilization, reproduction, and disease prevention. Most of the UGFs have now been identified as specific vitamins, amino acids, or microminerals that can be included in diets from synthetic or refined sources. However, both modern commercial and experimental diets exhibit improved performance when animal by-products are included in the diets under a variety of management and production practices. Several antinutritional compounds and contaminants associated with other ingredient classes are not problematic with ABPs. Specifically, these include trypsin inhibitors, goitrogenic compounds, gossypol, mycotoxin/aflatoxins, glucosinolates, tannis, lectins, phytates, lathyrism, oxalates, alkaloids, and cyanogens. In addition, animal proteins do not contain oligosaccharides and other nonstarch polysaccharides that have been shown to alter gut viscosity and to reduce the digestibility of fats and proteins.

The animal feed and ingredient industries are major users of rendered animal fats and proteins. The subsequent text addresses the specific characteristics and specifications for each of the basic animal fats and protein ingredients.

Table 6 Commodity trading standards for tallow and grease

	Titer (min)	*FFA (min)*	*MIU*	
			Basis	*Max*
Tallow				
Extra fancy	42.0	2	1	
Fancy	40.5	4	1	
Bleachable fancy	40.5	4	1	2
Prime	40.5	6	1	2
Edible	42.5		1	1
Dark tallow				
Special	40.5	10	1	3
1	40.5	15	2	4
3	40.5	20	2	4
2	40.0	35	2	4
Grease				
Choice white (all hog)	36	4	1	2
Yellow (feed fat)	36	15	2	4

Abbreviations: FFA, free fatty acids; MIU, moisture, impurities, and unsaponifiables.

Major Animal-Derived Fats

Inedible Tallow

Tallow is primarily derived from rendered beef tissue but can contain other animal fat as well. In terms of total volume and economic value, tallow is one of the most important animal fats. The term 'inedible' does not define any specific grade or specification other than that the rendering or processing was not conducted under food regulatory supervision. Edible animal fat in the United States can be rendered only in food-grade plants under inspection of the US Department of Agriculture. Most countries have similar requirements. Tallow is accompanied by many grades, specifications, and criteria depending on its end use. Titer is a basic specification requiring solidification above 40 °C (104 °F) after saponification. In contrast, greases solidify below this temperature. Fat quality is determined by hardness, color, moisture, impurities, stability, and free fatty acid (FFA) content. For reference, the commodity trading standards for tallow and greases are included in Table 6. These specifications place an emphasis on titer and FFA content in determining grade and value. For soap production, 'hard fats' or fats of high titer make soap of hard textures, whereas lower titer fats make softer textured soap. Fats with higher FFA contents have a greater glycerin loss in the soap-making process, which lowers their value.

These specifications, however, do not have the same influence on the value of fat utilization when used as feed ingredients. The primary benefit of using fats in animal diets is their energy contribution. Fats provide the most concentrated energy of all food/feed materials, containing approximately 37 kJ of energy per gram. As a general rule, fat provides at least 2.25 times the energy content supplied from the same weight of corn. In some formulations, management conditions, and species, the difference may be as high as 3.8 times the energy of feed grains.

Tallow is used extensively as a feed ingredient. Chemically, animal feeding fats are triacylglycerols, whose structure consists of 1 unit of glycerol and 3 units of fatty acids. The fatty acids are actually the components that give the respective fats their individual characteristics. Table 7 provides fatty acid profiles for the respective mammalian-derived fats. Most fatty acids found in natural fats vary in chain length between 8 and 24 carbon atoms. Feeding fats are predominantly of chain lengths between 14 and 18 carbon atoms. Fatty acids that contain double bonds are termed 'unsaturated' (the number of double bonds is indicated after the number of carbon atoms; thus, $C_{16:3}$ is a 16-carbon fatty acid with 3 double bonds). Conversely, structures without double bonds are termed 'saturated' fatty acids. As the carbon chain length increases in saturated fatty acids, the melting point increases. In other words, they possess higher titer and are thus 'harder.' A comparison of various animal fats, marine oils, and vegetable oils is provided in Table 8.

Tallow can be categorized as a saturated fat with its primary fatty acid profile that consists of palmitic (16:0), stearic (18:0), and oleic (18:1) acids. These qualities position tallow as the most appropriate feed ingredient for use in ruminant diets. When dairy cattle diets are fortified with tallow at 0.45–0.90 kg per cow per day, a routine positive milk production response is achieved. Studies have demonstrated a 6% improvement in feed efficiency incorporating tallow in feedlot cattle rations. Tallow has been utilized most recently in finishing diets for swine and in sow diets to improve pork quality. Scientists continue to explore the benefits of tallow as a feed supplement for improving feed efficiency; reducing feed dust; preventing segregation of ingredients; improving meat quality; and slightly modifying fatty acid profiles in meat, milk, and egg products as well as numerous expanded uses in both the industrial and feed ingredient industries.

Choice White Grease

CWG is commonly the inedible fat derived from swine. As described for tallow, an inedible designation for fats derived from swine is determined by a similar procedure. Edible fat from swine

Table 7 Properties of fats and greases[a]

Test	*Chicken fat*	*Yellow grease*	*Choice grease*	*White tallow*
Fatty acid profile (% relative)				
$C_{8:0}$	<0.10	<0.10	<0.10	<0.10
$C_{10:0}$	<0.10	<0.10	<0.10	<0.10
$C_{11:0}$	<0.10	<0.10	<0.10	<0.10
$C_{12:0}$	<0.10	<0.10	<0.10	<0.10
$C_{14:0}$	0.57	0.70	1.57	2.73
$C_{14:1}$	0.26	0.14	0.36	0.50
$C_{15:0}$	<0.10	0.11	0.26	0.43
$C_{15:1}$	<0.10	<0.10	<0.10	<0.16
$C_{16:0}$	22.76	14.26	22.04	22.99
$C_{16.1}$	8.37	1.43	5.03	2.86
$C_{16.2}$	<0.10	<0.10	<0.10	<0.10
$C_{16.3}$	<0.10	<0.10	<0.10	<0.10
$C_{16.4}$	<0.10	<0.10	<0.10	<0.10
$C_{17.0}$	0.11	0.33	0.63	1.35
$C_{I7.I}$	0.12	0.23	0.43	0.75
$C_{I8.0}$	5.36	8.23	9.95	19.44
$C_{18.1}$	42.07	43.34	42.45	41.60
$C_{I8.2}$	17.14	26.25	13.17	3.91
$C_{I8.3}$	1.07	2.51	0.97	0.49
$C_{I8.4}$	0.22	0.47	0.29	0.36
$C_{20.0}$	<0.10	0.33	0.14	0.14
$C_{20.1}$	0.45	0.48	0.56	0.33
$C_{20.2}$	0.20	<0.10	0.19	0.10
$C_{20.3}$	0.19	<0.10	0.12	<0.10
$C_{20.4}$	0.45	<0.10	0.34	<0.10
$C_{20.5}$	<0.10	<0.10	0.11	<0.10
$C_{21.5}$	<0.10	<0.10	<0.10	<0.10
$C_{22.o}$	<0.10	3.50	<0.10	<0.10
$C_{22.1}$	<0.10	<0.10	<0.10	<0.10
$C_{22..2}$	<0.10	<0.10	<0.10	<0.10
$C_{22.3}$	<0.10	<0.10	<0.10	<0.10
$C_{22.4}$	<0.10	<0.10	<0.10	<0.10
$C_{22.5}$	<0.10	<0.10	<0.14	<0.10
$C_{22.6}$	<0.10	<0.10	<0.22	<0.10
$C_{24.0}$	<0.10	<0.12	<0.10	<0.10
$C_{24.I}$	<0.10	<0.10	<0.10	<0.10
Unknown components	0.56	0.72	1.03	1.96
MIU analysis				
Moisture and volatiles	0.12	0.38	0.24	0.17
Insoluble impurities	0.08	0.06	0.29	0.12
Unsaponifiable matter	0.51	0.42	0.73	0.30

[a]Data from Woodson-Tenent Laboratories, Memphis, TN, USA.
Abbreviation: MIU, moisture, impurities, and unsaponifiables.

is most commonly labeled lard, although edible lard may likewise be used as a feeding fat. The composition, characteristics, and consistency of lard vary greatly according to the part of the animal or tissue from which it is extracted (Tables 7 and 8) and the composition of the fat consumed. Both lard and CWG possess titers of less than 40. CWG contains less saturated fatty acids than tallow does and a more even distribution among the respective C16 and C18 chain length fatty acids.

CWG and lard are quite similar in composition. Their use has been primarily for feed or food, although several important industrial applications utilize these fat sources. Industrial dependence or applications are not as extensive as for tallow. CWG as feeding fat is generally directed to swine, poultry, and companion animal diets, although it is not restricted to these species. Piglet diets routinely contain from 5–8% fat, with animal fat being a primary source and CWG most often being the preferred source. A substantial database exists to predict a 2% improvement in feed efficiency for each 1% supplemental fat incorporated into swine growing-finishing diets. The most common formulation level is 2–5% supplemental fat. The use of fat in late gestation and lactation is a very common feeding strategy. Feeding fat to sows before and during lactation increases both milk fat percentage and total milk yield, thus increasing the survival potential of the litter. Enhanced energy intake during lactation provides reproductive benefits that include shorter return to oestrus and increased sow longevity. The beneficial effects of fats may be more pronounced in environments of elevated temperatures.

Table 8 Fatty acid composition of common feed animal fats, fish oils, and vegetable oils

Lipid source	*IFN*[a]	*Percentage of total fatty acids*																
		14:0	*16:0*	*16:1*	*18:0*	*18:1*	*18:2 n – 6*	*18:3 n – 3*	*18:4 n – 3*	*20:1*	*20:4 n – 6*	*20:5 n – 3*	*22:1*	*22:5 n – 3*	*22:6 n – 3*	*Σn – 6*	*Σn – 3*	*n – 3/n – 6 ratio*
Animal fat																		
Beef tallow	4-08-127	3.7	24.9	4.2	18.9	36.0	3.1	0.6	–[b]	0.3	–	–	–	–	–	3.1	0.6	0.19
Pork fat	4-04-790	1.3	23.8	2.7	13.5	41.2	10.2	1.0	–	1.0	–	–	–	–	–	10.2	1.0	0.10
Poultry fat	4-09-319	0.9	21.6	5.7	6.0	37.3	19.5	1.0	1.1	0.1	–	–	–	–	–	19.6	1.0	0.05
Fish oils																		
Anchovy		7.4	17.4	10.5	4.0	11.6	1.2	0.8	3.0	1.6	0.1	17.0	1.2	1.6	8.8	1.3	31.2	24.0
Cod liver	7-01-994	3.2	13.5	9.8	2.7	23.7	1.4	0.6	0.9	7.4	1.6	11.2	5.1	1.7	12.6	3.0	27.0	9.0
Capelin	7-16-709	7.9	11.1	11.1	1.0	17.0	1.7	0.4	2.1	18.9	0.1	4.6	14.7	0.3	3.0	1.8	12.2	6.78
Channel catfish, cultured		1.4	17.4	2.9	6.1	49.1	10.5	1.0	0.2	1.4	0.3	0.4	–	0.3	1.3	12.7	3.2	0.25
Herring, Atlantic	7-08-048	6.4	12.7	8.8	0.9	12.7	1.1	0.6	1.7	14.1	0.3	8.4	20.8	0.8	4.9	1.4	17.8	12.71
Herring, Pacific		5.7	16.6	7.6	1.8	22.7	0.6	0.4	1.6	10.7	0.4	8.1	12.0	0.8	4.8	1.0	15.7	15.7
Menhaden	7-08-049	7.3	19.0	9.0	4.2	13.2	1.3	0.3	2.8	2.0	0.2	11.0	0.6	1.9	9.1	1.5	25.1	16.73
Redfish		4.9	13.2	13.2	2.2	13.3	0.9	0.5	1.1	17.2	0.3	8.0	18.9	0.6	8.9	1.2	19.1	15.92
Salmon, sea caught		3.7	10.2	8.7	4.7	18.6	1.2	0.6	2.1	8.4	0.9	12.0	5.5	2.9	13.8	2.1	31.4	15.00
Vegetable oil																		
Canola	4-06-144	–	3.1	–	1.5	60.6	20.2	12.0	–	1.3	–	–	1.0	–	–	20.2	12.0	5.94
Coconut	4-09-320	16.8	8.2	–	2.8	5.8	1.8	–	–	–	–	–	–	–	–	1.8	0.0	0.0
Corn	4-07-882	–	10.9	–	1.8	24.2	58.0	0.7	–	–	–	–	–	–	–	58.0	0.7	0.01
Cottonseed	4-20-836	0.8	22.7	0.8	2.3	17.0	51.5	0.2	–	–	–	–	–	–	–	51.5	0.2	0.04
Linseed	4-14-502	–	5.3	–	4.1	20.2	12.7	53.3	–	–	–	–	–			12.7	53.3	4.2
Palm		1.0	43.5	0.3	4.3	36.6	9.1	0.2	–	0.1	–	–			–	9.1	0.2	0.02
Peanut	4-03-658	0.1	9.5	0.1	2.2	44.8	32.0	–		1.3	–	–		–	–	32.0	0.0	0.0
Safflower	4-20-526	0.1	6.2	0.4	2.2	11.7	74.1	0.4		–	–	–	–	–	–	74.1	0.4	0.0
Soybean	4-07-983	0.1	10.3	0.2	3.8	22.8	51.0	6.8		0.2	–	–	–		–	51.0	6.8	0.13
Sunflower	4-20-833	–	5.9	–	4.5	19.5	65.7	–	–	–	–	–	–	–	–	65.7	0.0	0.0

[a]IFN, International Feed Number.

[b]Dash indicates that measurements were taken but no values were detected.

The literature abounds with examples of the utilization of CWG, lard, or pork fat for various applications in feed formulation and production. Similarly, the importance of pork to the global supply of animal protein foods indicates that lard and CWG will remain a source of these fats for both feed and food purposes.

Poultry Fat

Poultry fat is derived from the slaughter and processing of chickens, turkeys, ducks, and other avian species. The majority of poultry fat is derived from broiler chickens. The available quantity has increased, and continues to rise, as further processing increases. As with further processing of other major categories of meat, this practice leaves more tissues for rendering at the processing sites and less as table waste. The fats rendered from poultry are of the lowest titer and have the highest ratio of unsaturated fatty acids compared with the saturated fatty acid components of the fats of any other species. A fatty acid profile is included in Table 7.

Poultry fat is used in domestic pet diets. Fat extracted from feathers has a high content of cholesterol and is a resource for the pharmaceutical industry.

Yellow Grease

Yellow grease is a very misunderstood product with respect to its source and utilization. It is a category of fat that evolved from the practice of renderers assuming their responsibility to collect, process, and utilize used cooking oils and restaurant greases as part of their raw material resources. Traditionally, fat/grease acquired from restaurants was of animal origin (tallow and lard), but with the controversy surrounding health effects of saturated and unsaturated fatty acids, the major components of frying media are now plant oils. These changes were made despite a lack of sound empirical data and the fact that most taste panels preferred foods prepared in animal fats. These major changes had drastic effects on the tallow and lard markets and brought about a new category of fats: feed-grade animal fat or yellow grease.

Yellow grease is best defined as a fat product that does not meet the definitions for animal fat, vegetable fat or oil, hydrolyzed fat, or fat ester. Like any other grade of fat, it must be sold on its specifications, which include the minimum percentage of total fatty acids, the maximum percentage of unsaponifiable matter, the maximum percentage of insoluble impurities, the maximum percentage of FFAs, and the amount of moisture. Most importantly, it must meet the Food and Drug Administration's established criteria for pesticides or other toxic chemicals.

The basic specifications for yellow grease are:

Total fatty acids (min)	90%
FFA (max)	15.0%
Moisture (max)	1%
Impurities (max)	0.5%
Unsaponifiable (max)	1%
Total MIU (max)	2%

Abbreviation: MIU, moisture, impurities, and unsaponifiables.

Although these are basic specifications, they are subject to negotiation between buyer and seller on a contract-by-contract basis. The presence of FFA in fats or ABPs was once considered an indication of rancidity. Questions are still raised regarding the utilization of fat sources with high FFA content as a feeding fat ingredient. A very reliable research database exists to indicate that FFA per se is not, on its own, a qualitative monitor for fat quality. Dr. Park Waldroup and coworkers at the University of Arkansas reported no difference in performance in broilers fed diets supplemented with fats with low or high (44.7%) FFA content. Dr. Richard Zinn of the University of California has likewise reported similar findings in feedlot cattle. In the studies (of growth performance) comparing 10% FFA yellow to 50% FFA, no differences were detected. Thus, a higher FFA content without the indicators of rancidity (rancid odor, palatability influences, or high peroxide values) does not affect the feeding value of yellow grease. There are specific industrial uses for which high FFA levels may become a factor, such as in the esterification process associated with biodiesel.

Yellow grease has many uses. It is a primary contributor to the total fats and oils used as feeding fats. It has several industrial end uses, including as a biofuel either in biodiesel or perhaps as a burner fuel. Its properties and its recyclable benefits make it anything but waste grease.

Fish Oils

Fish oils are produced during the processing of fish meal in a manner similar to the processing of other rendered products. The raw materials are derived from fishing specifically for species of fish for fish meal and fish oil production, such as menhaden, sand eel, and Norway pout. The increase in aquaculture production has resulted in an increase in use of both the fish oil and the meal derived as a by-product from seafood production and processing. Fish oil is used extensively in aquaculture diets for all species, providing specific fatty acids not available in other fats and oils. It is likewise a common ingredient in companion animal diets, especially for the feline species. Industrial, pharmaceutical, and nutraceutical uses have been replaced by other fats and oils, synthetic vitamins, and other products that in the past relied on fish oil.

Fish oils derived from different species vary considerably in their characteristics, so quoting specifications is difficult. Tables 8 and 9 detail the fatty acid content of various fish oils. Fatty acids designated as 'omega-3' and 'omega-6' (symbolized as n – 3 and n – 6 in Table 8) have received considerable support for their beneficial effects on human health.

Summary: Animal Fats

The inedible raw materials derived from producing and processing food animals provide high-energy products that are available for feed ingredient, industrial, and bioenergy uses. The available resources correlate with the numbers of animals processed for food and the recycling efficiency of used cooking and restaurant greases. These energy by-products are the direct result of livestock, poultry, and marine animal production. They are highly regarded as economically and socially beneficial adjuncts to the primary meat products.

Table 9 Principal fatty acid (percentages) of major marine oils of commerce[a]

		Ma	*SM*	*P*	*C*	*H*	*A*	*CL*	*MA*	*HM*	*NP*	*S*	*SA*
Myristic	C14:0	9	7	8	7	7	9	3	8	8	6	1	7
Palmitic	C16:0	20	15	18	10	16	19	13	14	18	13	16	15
Palmitoleic	C16:1	12	10	10	10	6	9	10	7	8	5	7	8
Oleic	C18:1	11	15	13	14	13	13	23	13	11	14	16	9
Eicosaenoic	C20:1	1	3	4	17	13	5	0	12	5	11	10	15
Eurucic	C22:1	0.2	2	3	14	20	2	6	15	8	12	14	16
Omega-3 fatty acids	C20:5	14	17	18	8	5	17	11	7	13	8	6	9
	C22:6	8	10	F9	6	6	9	12	8	10	13	9	9

[a]A, anchovy; C, capelin; CL, cod liver; H, herring; HM, horse mackerel; MA, mackerel; Ma, menhaden; NP, Norway pout; P, pilchard; S, sprat; SA, sand eel; SM, specifically processed marine oil (menhaden).

Major Animal-Derived Proteins

The primary animal products derived from processing inedible animal tissues are MBM, MM, blood meal, poultry meal, feather meal, hair meal, and fish meal. Tissues subjected to the rendering process are treated to remove the fat, then dried and milled to produce the respective animal protein products. The ingredients, or product specifications and definitions, are distinct for each product.

Meat and Bone Meal

The regulatory definition of MBM in the United States is the rendered product from mammalian tissues, including bone but exclusive of blood, hair, hoof, horn, hide trimmings, manure, and stomach and rumen contents. The definition describes materials that are used in the manufacture of MBM and, more importantly, what cannot be included. MBM, as defined, must contain a minimum of 4% phosphorus with a calcium level not exceeding 2.2 times the actual phosphorus level. Ingredients containing the same tissues but less bone content, and thus a lower phosphorus content, are defined as MM. Although regulations and directives vary worldwide and from country to country, MBM can be used nutritionally in all nonruminant species of livestock and poultry and as an aquaculture rations to provide protein, amino acids, phosphorus, calcium, energy, and other nutrients. Sourced (nonruminant) and processed raw material must be used in ruminant rations in the United States. The EU has suspended the feeding of MBM to all farm animals. It is important to consult the current regulations in each country of use.

Both MBM and MM have protein levels that generally meet or exceed 50%. The 1998 Nutrient Requirements of Swine (National Research Council) references MBM as containing 51.5% protein and MM as containing 54.0% protein. Standards for product specifications vary according to country of origin and must be referenced using peer-reviewed databases. Although no standard is required for MBM and MM products, guarantees for protein as well as minimum calcium and fat contents must be stated on the identifying label. Unfortunately, MBM and MM are often combined into common databases. This is illustrated in Table 10, showing data from 29 samples of MBM assembled by the Fats and Protein Research Foundation and analyzed at the University of Illinois. Although all of the products were identified as MBM, more than 50% were below the phosphorus minimum. Supplier guarantees are often much more reliable indicators of nutrient content than product names.

Blood Meal

Approximately 4–5% of the live weight of animals is collectable blood. Dried blood is high in protein and rich in the amino acid lysine; moreover, the amino acids are highly digestible. Procedures for processing blood into blood meal have been much improved during the past decade. Flash drying techniques provide digestibility of the essential amino acids that exceeds 90% and generally approaches 95%. Newer processes are available to separate the plasma (serum) from the red cell portion. Both products, when dried properly, are value-added ingredients utilized extensively in animal nursery diets. Measured in value per unit, blood meal comprises the highest of all of the commodity terrestrial animal meals. Measured in total quantities or final dried meal (total tonnes), blood meal comprises a relatively small amount compared with other ABP protein sources.

Poultry Meal

Poultry meal consists of the milled, rendered, and cleaned parts of the carcasses of slaughtered poultry. Inedible tissues comprising the raw materials include the heads, necks, feet, undeveloped eggs, intestines, and skeletal frames from which muscles have been removed. The completeness of muscle removal for boneless chicken meat varies somewhat. Similarly, several of the tissues listed above also have edible markets. Poultry meal is to be exclusive of feathers, with the exception of such amounts as might occur unavoidably with the use of good processing practices. A considerably higher quantity of inedible tissue from poultry processing is acquired from each carcass as trends advance for further processing and more table-ready foods.

Poultry meal is an excellent source of protein. It is a product used extensively in companion animal diets. Processing improvements have resulted in better poultry meal, as with most by-product ingredients. Improvements in digestibility have enhanced its usage in other animal species, such as in aquaculture and starting pig diets. Specifications vary slightly depending on source. Broiler chickens, laying hens, and turkeys comprise the major sources of poultry raw

Table 10 Average digestibility (%) for Meat and bone meal (MBM) received through the fats and proteins research foundation

Identity	*Sample no*	*Protein*	*Lysine*	*Tryptophan*	*Metonine and cystine*
MBM	1	69.13	68.60	67.34	61.55
MBM	2	66.43	71.72	73.46	64.74
MBM	3	66.03	65.57	67.33	61.45
MBM	4	73.84	76.07	72.40	68.41
MBM	5	60.63	57.05	61.65	51.99
MBM	6	76.24	77.75	73.95	69.51
MBM	7	73.00	78.20	76.74	69.27
MBM	8	74.00	75.24	72.68	71.29
MBM	9	75.59	79.35	68.75	73.25
MBM	10	67.52	67.64	74.23	61.56
MBM	11	55.68	50.10	53.62	57.36
MBM	12	75.19	77.74	74.11	71.32
MBM	13	72.59	77.24	78.20	68.56
MBM	14	78.95	82.48	76.23	70.45
MBM	15	74.28	80.46	78.27	65.54
MBM	16	60.48	63.83	53.06	52.52
MBM	17	76.92	78.30	72.32	70.43
MBM	18	69.66	68.38	69.74	67.94
MBM	19	69.83	76.31	76.67	68.05
MBM	20			No sample submitted	
MBM	21	65.45	67.47	63.98	65.74
MBM	22	59.46	61.42	63.04	49.42
MBM	23	65.91	71.26	71.91	61.78
MBM	24	65.67	63.38	57.19	57.11
MBM	25	65.56	66.34	66.92	66.18
MBM	26	68.05	69.57	72.04	65.07
MBM	27	70.13	76.59	79.73	77.72
MBM	28	62.95	63.42	65.18	63.68
MBM	29	63.74	70.14	76.59	65.29
MBM	30	68.80	73.58	67.11	46.56
Mean		68.68	70.87	69.70	64.3

Note: Prediction equations for amino acid (AA), Digestibility in meat and bone meals (MBM).
Source: Reproduced from Fat and Proteins Research, Inc. Directors Digest, 1997.

materials. However, they all yield similar final rendered products. Ducks and other less dominant avian species may show some variations.

Feather and Hair Meal

Feather meal is often referred to as HFM. Feathers contain a protein level averaging 87%, but the protein is in an undigestible keratin helix and special processing is necessary to break the disulfide-bonded helices. Pressure cooking is the most common processing procedure, although enzymes under experimental conditions have also been effective.

Although HFM is high in protein, the availability of essential amino acids, lysine, methionine, and histadine, is limited. However, the product is an excellent source of the sulfur-containing amino acid cystine as well as elemental sulfur and selenium. Its protein has limited degradation in the rumen of ruminant species, which allows its passage and digestion in the intestinal tract. HFM has emerged as an important ingredient for ruminant animals. Recognizing the contribution of bioavailable amino acids, HFM can be supplemented via synthetic sources or combined with other by-product ingredients to supply its deficiencies and thus can be a very useful feed ingredient. Hair meal derived from pig hair is a similar product, but it is produced in much lower quantities and requires even higher pressure and temperature for its production.

Fish Meal

Fish meal is acquired from the specialized industry that maintains fleets that harvest entire fish exclusively for processing into fish meal. Fish meal is also produced from the inedible portions of food fish. The protein derived from both sources is highly valuable to the feed and ingredient industry. Historically, fish meal has commanded premium prices compared with other animal protein sources and most plant sources. It has an excellent amino acid composition that is highly bioavailable. Fish meal is appreciated as a feed ingredient for its palatability-enhancing properties, especially in feline diets. Properties of fish meals vary with respect to the species of fish that comprise the raw material; there are species differentiation of respective and fish meals that are often included in the ingredient name. Some countries prohibit the use of fish meal for finishing pigs due to the risk of 'fishy' off-flavor.

There has been concern recently about the depletion of free harvested fish and thus over the future availability of fish meal.

Each year a higher proportion of seafood and shellfish produced by aquaculture replaces seafood and shellfish traditionally sourced from commercial fishing and the aquaculture industry is projected to continue its growth.

The processing of fish by-products from aquaculture production represents a supplementary income to producers and processors of fish for human consumption.

Summary

Inedible by-products resulting from the production and processing of meat for edible use comprise approximately 50% of total livestock, poultry, and aquaculture production. This article described the basic alternatives for maintaining a symbiotic utilization of both the edible and inedible fractions. Alternatives as well as challenges surrounding the utilization or disposal of raw inedible by-products are controversial. Requirements that this highly perishable material must be collected, processed, and utilized in a manner that satisfies ecological, economic, environmental, and biosecure methodology are paramount. These criteria must be scientifically validated. Rendering has provided a time–temperature process that has met these criteria for bacterial, viral, fungal, and parasitic organisms. The etiology of TSE has presented an enigmatic assessment yet to be resolved in terms of animal by-product processing and disposal. Realistically, the world is still trying to understand the biology of this complex group of diseases, which are unlike any other. This complicated group of diseases deserves the utmost scientific scrutiny.

All the protein, fat, minerals, and other compounds currently derived from the inedible fraction of food animals are the direct result of livestock and poultry production. Technologically advanced meat production does not produce meat, milk, eggs, and animal fibers without the associated production of inedible tissues. Changes in technology will inevitably alter the amount and nature of by-products. Response to these changes will be influenced by consumers, legislators, and myths/biases as well as scientific guidance and will, undoubtedly, bring many unpredictable developments and opportunities. It is easier to analyze the history than to predict the future. Historically, the performance of livestock agriculture would predict a strong future for animal-derived products to be utilized by both humans and animals.

See also: By-Products: Edible, for Human Consumption; Hides and Skins. Chemical Analysis: Raw Material Composition Analysis. Chemical Analysis for Specific Components: Micronutrients and Other Minor Meat Components. Chemistry and Physics of Comminuted Products: Nonmeat Proteins. Microbiological Safety of Meat: Prions; Viruses

Further Reading

Franco, D.A., Swanson, W., 1996. Original Recyclers. Alexandria, VA: The Animal Protein Producers Industry, The Fats and Proteins Research Foundation, and The National Renderers Association.

National Research Council, 1998. Nutrient Requirements of Swine. Washington, DC: National Academy Press.

Pearson, A.M., Dutson, T.R. (Eds.), 1992. Inedible Meat By-Products. New York, NY: Elsevier Applied Science.

Waldroup, P.W., *et al.*, 1996. Comparison of two blended animal-vegetable fats having low or high free fatty acid content. Journal of Applied Poultry Research 4, 41–48.

Zinn, R.A., 1988. Comparative feeding value of supplemental fat in finishing diets for feedlot steers supplemented with and without monensin. Journal of Animal Science 66, 213–227.

Zinn, R.A., 1989. Influence of level and source of dietary fat on its comparative feeding value in finishing diets for steers. Journal of Animal Science 67, 1038–1049.

Relevant Websites

http://www.fao.org/docrep/010/ai407e/ai407e03.htm
FAO.

http://www.ers.usda.gov/media/147867/ldpmz0901.html
USDA.

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CANNING

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Glossary

Anaerobes The microorganisms that are able to grow and proliferate in the absence of oxygen.
A_w The amount of water available in a food for enzymatic reactions or microbial growth.
Emulsion A two-phase system formed by a disperse phase (or droplets) in a continuous phase.
Gel A physical system in which water is trapped in a lattice, usually formed by proteins or carbohydrates.
Heat capacity The amount of heat necessary to increase the temperature of a given material.
Mesophiles The microorganisms that are able to grow and proliferate at moderate temperatures (usually between 15 and 30 °C).
Microaerophiles The microorganisms that are able to grow and proliferate in low oxygen concentrations.
Oxygen tension The pressure of oxygen on a surface.
Redox potential The positive or negative net charges in a system.
Thermal conductivity The ability of a given material to transfer heat.
Thermophiles The microorganisms that are able to grow and proliferate at high temperatures (usually above 40 °C).
Transport phenomena The physical mechanisms involved in heat and mass transfer.

Introduction

Heating is probably the most efficient preservation method for foods. The main objective of heating for preservation is to ensure the destruction of microbial populations; both vegetative cells and spores of pathogens are prevented from growing and, in relevant cases, from producing toxins. Depending on the expected shelf life, a specific food is subjected to a specific set of conditions. The severity and length of the heat treatment are the key factors to ensure sanitation. However, when thermal processing is too severe, it might impair product quality; so a compromise must be reached between sanitation and quality. If a food is cooked, only partial elimination of viable cells and enzymes might take place and additional means of preservation would be necessary. In the other extreme of heating, canning allows the destruction of virtually all viable cells. The result of the canning process is the production of self-stable foods with a considerably long shelf life without the need of applying any further preservation method. In addition, canning of meat allows its storage and transportation, otherwise a highly perishable food commodity in conditions under which no other preservation method could succeed. This is particularly true in high-temperature and high-humidity environments such as tropical zones. However, although this process may preserve chemical characteristics of food, canning is also likely to alter sensory and physical characteristics; in some cases this process changes the physical structure of meat products. In some cases, physical modifications are highly desirable, even in part of some processes, such as luncheon meat preparation, where an emulsion changes to a gel.

Microbial Population and Process Severity

From a microecological point of view, the events determining food sanitation and quality are contamination, colonization,

and metabolite production, either harmful to humans or able to change food characteristics. Once undesirable microorganisms are reduced as much as possible by any preservation method, particularly thermal technologies, it is necessary to avoid nullifying the effects of processing by preventing recontamination. This is achieved by packaging in hermetic containers or by the use of aseptic containers such as cans or flexible pouches; in both cases, measures must be taken to avoid recontamination. Finally, food distribution must be carried out by delaying the growth of surviving microorganisms. Thus, in addition to sufficient thermal processing to ensure food sanitation, other precautions must also be taken.

Canning is based on a heat gradient from a heating medium to the food inside the container. Thermal processing for canned meats focuses on reducing counts of *Clostridium botulinum*, the most dangerous agent, which produces a fairly heat-stable toxin. Reduction of spoilage populations is the second task of heat treatment. Any commercially sterile canned food is free of spoilage microorganisms such as *Bacillus stearothermophilus*, *Clostridium perfringens*, or spore-forming thermophiles such as *Clostridium sporogenes* if the storage temperature is approximately 40 °C. Heat treatment is severe enough to destroy *C. botulinum* or *C. perfringens* and gives a stable food without the need to apply special storage conditions.

Microbial communities in foods are dynamic; heterotrophic populations changing with time, depending on the presence of specific chemicals. Preliminary processing before canning modifies intrinsic food characteristics, particularly in rich substrates such as meats containing almost all necessary nutrients to support a wide variety of microbial populations. In raw meats (pork, lamb, or beef), the native microflora is dominated by *Acinetobacter*, *Aeromonas*, *Enterococcus*, *Moraxella*, *Pseudomonas*, and *Psychrobacter*. If raw meat is refrigerated before canning, the specific microbial associations are nonfermenting psychrotrophs such as *Pseudomonas*, *Alcaligens*, *Flavobacterium*, *Shewella*, and *Moraxella*. Cured meats represent an ecological niche for Gram-positives, such as *Micrococcus*, *Lactobacillus*, *Carnobacterium*, and *Brochothrix*. The main spoilage microorganisms in canned meats are *Clostridium thermosacarolyticum*, surviving at a very high temperature; *Pseudomonas* sp.; *Achromobacter*; mesophile *E. coli*; *Bacillus subtilis*; *Streptococcus thermophilus*; *C. perfringens*; and *Bacillus stearothermophilus*. Certain specific microorganisms are also likely to colonize a given meat species, such as *C. perfringens*, *Salmonella* spp., *Staphylococcus* spp., and *Campylobacter* spp. in raw poultry meat and poultry products. As heat treatments depend on a time–temperature relationship, process severity will be calculated on the basis of the degree of destruction necessary for an indicative microorganism. Therefore, the specific dominant microflora of a meat or meat product implies different processing parameters.

Thermal Resistance of Microorganisms

Although meat sanitation depends on destruction of specific microorganisms, particularly pathogens, shelf life extension depends on intrinsic food characteristics (mainly chemical composition) and external conditions (mainly temperature and nutrient availability). While vegetative cells are destroyed at 50–70 °C, spores can survive at much higher temperatures. Heat treatment conditions destroying *C. botulinum* and *C. sporogenes* result in a thermostable food with considerably long shelf life. However, for tropically preserved products expected to have a minimum shelf life of 1 year at 40 °C, destruction of sporulated thermophiles are of consideration. The destruction of food enzymes involved in spoilage reactions is also a criterion for theoretical considerations; usually process calculations for microbial destruction are also valid for enzyme inactivation. Because process calculations are specific for a given food product, some basic information is necessary, in addition to knowing the type of dominant or indicative microflora.

Meat Physicochemical Characteristics

Meat physicochemical characteristics are closely related to the types of microorganisms present. Resistance to heat processing and process efficiency depend on physicochemical characteristics, such as pH, water activity, food composition, and structure. Low-acid foods (pH > 4.5), such as most meats and meat products, need more intense heat treatment due to the possible presence of *C. botulinum* and production of toxins in canned meats. This pathogen, however, needs A_w 0.95–0.97 or higher to grow; therefore, reducing water activity which can also reduce process severity. Canned meat is a system totally void of oxygen. Because oxygen tension is decisive for certain strict anaerobes, such as *Clostridium* spp. or microaerophiles, these microorganisms can proliferate. Other pathogens, such as *Staphylococcus* spp., can grow in low oxygen concentrations. Finally, food composition drastically influences process severity extension. Carbohydrates, fats, and proteins are bad thermal conductors. Because meat contains protein and some fat, meat products need more severe treatments.

Expected Shelf Life

There are mainly four thermal processes applied to food materials, based on the severity of heat application and the expected wholesomeness duration of the food.

(1) Blanching, in which the process temperature is 65 °C, is applied to food materials to eliminate gas from tissues and to provide initial cleaning. It is used to inactivate enzymes in products receiving further heat treatment; (2) cooking, at approximately 85 °C, is applied to improve sensory characteristics, to moderately reduce microbial populations present in the food material, and to inactivate some enzymes. These two treatments are mostly related to cleaning and to improving food quality; (3) pasteurization is a thermal process per se; it destroys vegetative pathogen cells, but certain heat-resistant microorganisms and spores can survive. Typical pasteurization time–temperature regimens are 140–150 °C for 1–45 s or 70–73 °C for 15–20 s. Pasteurized foods have short shelf life even at refrigeration temperatures; (4) sterilization, to destroy vegetative cells as well as spores, sterilization processes must be applied. The shelf life of sterilized foods is considerably extended even without the application of additional preservation methods. Time–temperature regimes depend on the thermal

resistance of a given microorganism taken as an indicator, such as *C. botulinum* and *C. sporogenes* for meat products.

Heat and Mass Transfer during Canning

Thermal processing is basically an operation in which heat flows from a hot element (the heating medium of water or steam) through a barrier (a can) to a cold element (the food). Canning includes two transport phenomena: (1) heat transfer, where heat flows in direct proportion to the driving force and in inverse proportion to the resistance to flow; (2) mass transfer within the food material and brine and also as a result of reactions between food components (lipids, proteins, minerals, etc.). In products such as soups containing meat pieces, or sausages in brine, a combined heat and mass transfer mechanism takes place. Heat is transferred from fluid to and within the meat, and between the product and the heating medium, and mass transfer as water and nutrients diffuse within the can. Knowing the type and extent of the driving forces involved allows estimation of transport parameters in the system. Heat transfer obeys one of the following mechanisms: conduction, convection, or radiation.

The mechanisms involved in heat transfer during canning are conduction and convection. Radiation has no practical application in food canning; it only occurs in heating systems such as microwaves and infrared heating. Conduction occurs within a solid as a result of vibrations of adjacent molecules. In meat canning, conduction takes place in brines containing solid chunks, such as soups containing meat pieces, canned sausage in brine, and in gelled canned pastes, as in the case of luncheon meat. Conduction also varies among meat cuts within the same animal depending on the particular chemical composition.

Convection heating is mostly related to fluids; it takes place in foods such as soups, brine, milk, etc., as a result of movement of fluid of different densities when the fluid is heated or cooled. Convection can be accelerated if stirring is applied, thereby reducing the temperature difference. Therefore, to obtain the maximum heating rate during canning, condensed steam is the most efficient heating medium through a barrier (the can) to the cold fluid inside the can.

Thermal Properties of Meat Systems

Thermal properties establish the heat distribution within the product. These properties include transference properties (thermal conductivity and specific heat) and physical properties (density and product geometry). In addition, meat thermal properties are altered during heating as fat melts, proteins denature, and water evaporates. Cooking time decreases with increasing thermal conductivity, but increases with increasing product size and specific heat capacity. This is the case of thermal conductivity of meat emulsions, such as sausage, that increases with temperature and moisture content.

Average thermal conductivity of meats is 1.89 kJ h^{-1} mol^{-1} K^{-1}, but it also dependent on the direction in which heat is transferred. Thermal conductivity in lean beef at 78.5% water content and 0 °C, when thermal flow is perpendicular to the meat fibers is 1.75 kJ h^{-1} mol^{-1} K^{-1} whereas under the same conditions, if the flow is parallel to muscle fibers, conductivity is 1.79 kJ h^{-1} mol^{-1} K^{-1}.

Heating mechanisms might change in products that modify their physical characteristics during processing. In canned emulsions, such as luncheon meats and pâtés, where the product changes from semifluid to solid, the heating mechanisms change from convection to conduction. In a meat emulsion, the continuous phase is made of muscle fibers, connective tissue fractions, carbohydrates, and other additives; the disperse phase is made of fat droplets; the high fat content allows the spreadability of the product. It turns from an emulsion to a gel by protein unfolding and interlinking during heating. Convection occurs in fluids, whereas conduction is a heat mechanism for solids, which results in convection changing to conduction. As the transfer mechanism changes from convection to conduction during gelling of a meat emulsion, heating rate also varies. Thermal conductivity increases with process temperature and moisture content, whereas thermal properties change as fat melts, proteins denature, and water evaporates. The heat process is, therefore, a combination of convection and conduction.

Meat Canning Operations

Canned meat products include whole muscles, meat stews, luncheon meat, sausages, sauces with meat pieces, and paste products. Meat canning essentially includes three main operations: can filling, exhaustion, and heat treatment. Heat penetration is affected by the solid:liquid ratio as well as the distribution of solid within the can. Solid materials packed loosely are heated faster than closely packed material. In general, 30% of the can volume must be a liquid (brine or sauce) in order to allow good heat transfer. When pastes are filled in the can, it is important to ensure the absence of air bubbles, as heat transfer is less efficient in air and may create sterilization problems. Headspace, approximately 0.5% of the total can volume, must also be taken into consideration in thermal calculations.

Exhaustion is carried out by evacuation of air from the headspace and the bulk of the food. Exhaustion is necessary to achieve good heat penetration and the desired sterilization temperature. Air exhaustion also reduces the risk of promoting the growth of aerobes, particularly if the product is pasteurized, as are some luncheon meats. Exhaustion is generally carried out by vapor injection or by conveying the cans on a belt into an exhaustion chamber or tunnel in which they are heated at 85–95 °C, removing 90% or more of the air in the headspace; in both cases, the cans are then immediately closed. When the cans are cooled, partial vacuum is produced by condensation of the water vapor.

Heat Treatment

Heat treatment includes two cycles: heating and cooling. Heating is involved in microbial and enzyme inactivation, whereas cooling is applied for several reasons, such as ease of handling and reduction of the deterioration of sensory

characteristics. These two cycles are the core of canning operations. Process severity and length are calculated on the basis of several considerations, such as composition of the food material (i.e., heat sensitivity of the microbial population depending on the food composition); specific microbial population; and factors necessary to achieve a given shelf life, depending on transport and storage conditions, initial microbial load, and desired final microbial load; among other things.

Inactivation Parameters

To model a thermal process at an industrial level, several inactivation parameters have been developed as mathematical tools to obtain a time–temperature relationship, which is necessary to achieve a successful treatment.

D- and z-values

If a microbial population is subjected to temperatures slightly above the optimum for vegetative cell or spore growth, destruction occurs with cell concentration (c) decreasing with time (t) in direct proportion to instantaneous cell concentration (c) (equation 1).

$$\frac{dc}{dt} = kc \qquad [1]$$

That is, 90% of the microorganisms are destroyed in a given characteristic time interval if constant temperature is applied. The time interval is different for each microorganism, and is called the decimal reduction time (D). D-values are expressed at a given temperature (e.g., D_{120} for treatment 120 °C). This parameter enables the processor to compare the severity of various processes. The z-value is the temperature increase necessary to reduce D-values in 1/10.

F-value

Several events occur during thermal processing. First, temperature is increased from the ambient to the process temperature specific for the destruction of a microorganism chosen as the process indicator. This temperature is then held for a fixed time to achieve the desired lethality rate. At the end, the temperature is decreased. Each of these events has an effect on the entire process. F-values represent the severity of the whole process, summing the individual effects. To consider process severity in detail, it would be necessary to calculate the effect of temperature increase at every point of the container – an impracticable task. For this reason, the $F=1$ concept is applied; this is the lethality effect of heating at 120 °C for 1 min. Heat treatments are also calculated taking into consideration spore survival from two of the most damaging bacteria in meat products: *C. botulinum* and *C. sporogenes.* While heating at 120 °C for 1 min, the D-value for *C. sporogenes* is taken as a calculation basis. Finally, F_s is the sum of all F-values in each part of the container.

As heating is not homogenous throughout the can geometry, calculations are always performed considering the temperature rise where heating is slowest, that is, in the cold point when the sum of all lethal effects is F_c. The position of the cold point is determined by the dominant heat transfer mechanism, which, in turn, is related to the type of food material. When convection heating is the main mechanism involved, the cold point is on the vertical axis, close to the bottom end of the can. In conduction heating, the cold point is located in the geometric center of the container. Viscous meat materials cooked inside the can, such as luncheon meats, show the cold point close to the geometric center. The position of the cold point is also affected by can rotation in the retort. In static heating of liquid or semisolid products, such as meat pieces in brine, where the leading heat transference mechanism is convection, the cold point is one-third from the can bottom end. F_c is always lower than F_s as the heat effect in the center is always lower than in the rest of the container. Thus, F-values are related to the shelf life required for a given food and to the severity of the thermal process. Process lethality, or destruction rate per minute of a given microorganism at temperature T, is given by eqn [2]:

$$\log\left(\frac{t}{F}\right) = \frac{250-T}{z} \qquad [2]$$

where t=processing time and z=z-value as described before. Therefore, lethality can be calculated at a given temperature throughout the process.

Implications for Meat Quality

Process severity and length affect the quality of canned meats. Sometimes these changes are desirable, such as protein denaturation and development of aroma- and flavor-related compounds. In other cases, deleterious changes might occur. Changes due to microbial growth can take place before heat treatment if a delay between filling and sealing following heat processing occurs. Processing must not be delayed more than 20 min after closing the cans. When thermal processing is insufficient, microbial metabolism can promote can blowing, acidification, or the development of off-odors and flavors. Process conditions (time–temperature relationship) and the microbial quality of raw materials must be checked, as well as sanitation of the equipment, water supply, etc.

Microbial contamination can occur through leaks in can seams from cooling water lacking adequate sanitary characteristics. Changes can also be due to insufficient heating caused by formation of air packs within the retort. If the cans are tightly loaded in the retort, there is no free access of vapor or water to all parts of the container, resulting in irregular heating.

When cooling is not carried out at a suitably fast rate, growth of thermophiles occur. Canned meat products must be cooled to 35 °C, or lower if large can blocks are stocked. Recontamination after heat treatment is one of the commonest problems and a cause of can blowing. In addition to being due to gas production by microbial metabolism, can blowing may occur owing to reactions between meat components and tin if the can has defects in the covering enamel or minute failures (pinholes). The reaction produces hydrogen or stannous sulfide. Severe heating can also alter sensory characteristics, such as by fiber disruption, which promotes excessive tenderness or production of flavor-related compounds by Maillard reactions and oxidation of acids to aldehydes.

Physical alterations in the can itself are due to inadequate operation of the heating equipment, such as rapid increase in retort pressure, insufficient vacuum conditions, or excessive can filling. The presence of air in the can produces excessive internal pressure during heating as a result of expansion of the gas. Conversely, excessive exhaustion promotes can collapse.

Conclusion

Caning ensures the destruction of pathogens and spoilage microorganisms and allows foods to be handled and transported in an easy way. Canned products processing is calculated according to the expected shelf life: from semipreserves requiring further preservation methods, to full and tropical preserves, with a shelf life of 4 years at 25 °C and up to 1 year at 40 °C, respectively. Heat treatments are also applied to develop specific sensory characteristics or physical properties, as well as to improve the nutritional availability of several food components.

See also: Chemical Analysis: Raw Material Composition Analysis. Chemical Analysis for Specific Components: Curing Agents; Major Meat Components. Chemical and Physical Characteristics of Meat: Chemical Composition. Cooking of Meat: Flavor Development; Heat Processing Methods; Physics and Chemistry. Microbiological Analysis: Indicator Organisms in Meat; Standard Methods. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Hurdle Technology; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; Prions; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Yeasts and Molds; *Yersinia enterocolitica*. Modeling in Meat Science: Microbiology. Sausages, Types of: Cooked; Emulsion. Spoilage, Factors Affecting: Microbiological

Further Reading

Crang, A., Sturdy, M., Bowcott, J., 2006. Bottled and canned meat products. II. Processing requirements for domestic methods. Journal of the Science of Food and Agriculture 9 (9), 565–575.

Greer, G., 1989. Red meats, poultry and fish. In: MacKellar, R.C. (Ed.), Enzymes of Psychrotrophs in Raw Foods. Boca Raton, FL: CRC Press, pp. 268–292.

Guerrero Legarreta, I., 2006. Thermal processing of meat. In: Hui, Y.H., Culbertson, J.D., Duncan, S., *et al.* (Eds.), Handbook of Food Science, Technology and Engineering, vol. 4. New York: Marcel Dekker.

Guerrero-Legarreta, I., 2010. Canned Products and Paté. In: Toldrá, F. (Ed.), Handbook of Meat Processing. Ames, Iowa: Wiley–Blackwell, pp. 337–349.

Guerrero-Legarreta, I., García Barrientos, R., 2012. Thermal Technology. In: Aalhus, J., Cocolin, L., Guerrero- Legarreta, I., *et al.* (Eds.), Handbook of Meat and Meat Processing. New York: CRC Press, pp. 523–530.

Ju, J., Mittal, G.S., 2000. Relationship of physical properties of fat substitutes, cooking methods and fat levels with quality of ground beef patties. Journal of Food Processing and Preservation 24, 125–142.

Mittal, G.S., Blaisdell, J.L., 1984. Heat and mass transfer properties of meat emulsions. Lebensmittel Wissenschaft und Technologie 17 (2), 94–98.

Mittal, G.S., Usborne, W.R., 1985. Moisture isotherms for uncooked meat emulsions of different compositions. Journal of Food Science 50, 1576–1579.

Müller, W.D., 1990. The technology of cooked cured products. Fleischwirtschaft International 1, 36–41.

Pérez, M.G.R., Calvelo, A., 1984. Modeling the thermal conductivity of cooked meat. Journal of Food Science 49, 152–156.

Siripon, K., Tansakul, A., Mittal, G.S., 2007. Heat transfer modeling of chicken cooking in hot water. Food Research International 40 (7), 923–930.

Thumel, H., 1995. Preserving meat and meat products: possible methods. Fleischwirtschaft International 3, 3–8.

Zamudio, M., 2006. Microorganismos patógenos y alternantes. In: Hui, Y.H., Guerrero-Legarreta, I., Rosmini, M. (Eds.), Ciencia y Tecnología de Carnes. Mexico City: Noriega Editores, pp. 337–370.

Relevant Websites

http://www.aamp.com/
American Association of Meat Processors.

http://www.ampc.com.au/
Australian Meat Processor Corporation.

http://www.inspection.gc.ca/food/meat-and-poultry-products/eng/1300124955992/1300125034322
Canadian Food Inspection Agency. Meat and Meat Products.

http://www.fao.org/docrep/010/ai407e/AI407E08.htm
FAO – Heat treatment of Meat Products.

http://www.globalmeatnews.com/
Global meat news.

http://www.meathaccp.wisc.edu/validation/heat_treatment.html
University of Wisconsin-Madison.

http://www.fce-sid.com/?s_kwcid=TCI9244|canning%20food%20processing||SIbI6160661535
US FDA Food Canning Establishment (FCE) Registration and Process Filings (SID).

CARCASS CHILLING AND BONING

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Glossary

Aging Holding the carcass under refrigeration to improve tenderness.
Cold shortening The effect, on muscle and tissue, of chilling the carcass quickly after harvesting.
Electrical stimulation Passing electrical current through the carcass after harvesting.
Hot boning Removal of muscle from bone prior to chilling.
Scalding Placing the carcass in hot water to aid in hair removal from the pig or feather removal from birds.

Introduction

There are varying procedures for converting animal carcasses into meat items for consumers. A few of the more popular systems will be discussed, together with their effect on meat quality. Some of the factors that have a major influence on final meat quality are electrical stimulation, carcass chilling, salt addition, and carcass boning (*see* Tables 1–3). This article harmonizes many of the processing, boning, and chilling technologies.

Carcass Boning and Hot Boning

Carcass boning, or production of retail cuts containing bones, can be performed in the traditional manner on a chilled carcass for products designated for the retail steak and roast trade and for sausage manufacture, and ground meat (Table 2). Another boning technique is to utilize the prerigor hot carcass, from which the tissue is primarily used for meat or sausage manufacturing (Table 2). A third technique is carcass boning after some chilling of the warm product; this technique, which is used mainly for pig carcasses, economically produces a quality chop or roast product in a more cost-effective manner (Table 3). Because conventional boning is simple (Table 1) and covered elsewhere, this article will concentrate on the newer and less-understood hot-boning and rapid-chilling techniques. Equipment is now available that will hold the carcass stationary and will pull muscles downward to assist in hot boning.

Hot boning has potential advantages over cold boning of higher meat yields (1.4%), labor saving (20%, faster, 4 min/carcass), less weight loss during chilling (1.5% less), less drip loss in vacuum packages (0.1–0.6%), more uniform products, a darker color, reduced refrigerator space (50–55%), lower refrigeration costs (40–50%), shorter processing time (40–50%), lower transport costs (primals vs. carcasses), and superior water-holding capacity and emulsifying capacity. However, there are some problems associated with hot boning, such as shape distortion of cuts because the muscle is not attached to the bone, reduced flexibility in production, stricter hygiene requirements, increased temperature control, new cutting procedures, retrofitting of conventional cold-boning areas, retraining or hiring of new boning personnel, quality changes in cuts such as reduced tenderness often caused by cold and rigor shortening, alteration of color, and accelerated microbial growth. The major quality problem with hot boning is that substantial cold shortening may result in tougher (a major quality factor) meat with higher drip loss. Carcass chilling systems that maintain a meat temperature of 15–16 °C during rigor minimize tenderness reduction due to cold

Table 1 Extreme variation in chilling of beef carcasses

Traditional chilling	*Rapid chilling*
• 2–4 °C including aging for 5–21 days	• Electrical stimulation
• Tenderness increases with aging up to about 21 days	• –20 °C or –35 °C, air velocity 3.2 m s^{-1}, 10 h
• Shrinkage continues during aging	• 24 h postmortem at 2 °C
• Exposed muscles become darker on aging	• Aged for 6 days
• Mold growth can occur during prolonged aging	• Increased tenderness at 6 days, shorter time to obtain acceptable tenderness
	• 21 days no difference in tenderization
	• Reduced cooler time and shrink, slower rate of pH drop, increased perception of marbling, darker color, increased drip at retail, good meat quality

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Table 2 Examples of variation in boning and chilling beef carcasses

Traditional processing	*'Tenderay processing'*	*Lower-quality tissue processing*						*Manufacturing tissue processing*
Grain-fed young animals	Grain-fed young animals	Lower-quality grade animals, usually older animals	Lower-quality grade animals					Manufacturing grade animals
Slaughter – internal temperature 30–39 °C								
Chill at –4 to 5 °C for 36–48 h	Age at 16 °C, 85–90% RH, UV lights, 3 days, shrinkage 0.5%, shorten aging time	Electrical stimulation 5–10 min postmortem	Electrical stimulation at 30 min postmortem	Electrical stimulation at 30–40 min	Electrical stimulation at 45 min postmortem	Electrical stimulation 60 min postmortem	Electrical stimulation 60 min postmortem	Hot boning
		Electrical stimulation increases tenderness						
Aging 0–5 °C for 2–21 days	Cut steaks and roast	Hot process 3–4 h postmortem	Chill until 5 h postmortem	Hot process within 60 min postmortem	Hot process 2 h postmortem	Hot process 1 h postmortem	Hot process 2 h postmortem	Chop and mix with curing ingredients to retard bacterial growth
Cut steaks and roast Aging improves tenderness	Accelerates aging process	Vacuum pack subprimals Chill in ice water for 5 h	Wrap subprimals in plastic Freeze at –18 °C or condition at 10 °C for 72 h before freezing	Vacuum pack subprimals Age at 1–2 °C for 2 weeks	Loose wrap subprimals Chill 5 °C postmortem for 24 h postmortem	Vacuum pack subprimals Freeze–18 °C, or chill 5 °C for 46 h postmortem and then freeze	Vacuum pack subprimals Age 1–5 °C, until 6 days postmortem	Increase shelf life
		Age at 2 °C until 7 days postmortem	Cut steaks and roast	Cut steaks and roast	Cut steaks	Cut steaks and roast	Cut steaks and roast	
		Cut steaks and roast			Vacuum pack, chill 2 °C for 6 days postmortem or freeze			

Source: Reproduced from Wismer-Pedersen, J., 1989. Recent development in the meat industry. In: Trends in Food Science and Technology. Proceedings of the 2nd International Food Convention, Mysore, India, pp. 162–171.

Table 3 Dutch 'semihot' boned fresh pork

	Nonexported fresh pork	*Exported fresh pork*
Preboning	Blast chill –25 °C for 80 min or 45 min	Blast chill –25 °C for 80 min or 45 min
Equilibration	At 2 °C for 100 min or 135 min	At 2 °C for 100 min or 135 min
Semihot boned	Semihot boned	Semihot boned
Packaging	Vacuum	Vacuum
Temperature	1 °C	1 °C
Storage	12 days at 1 °C	Storage would occur during transport
Results	Similar appearance, sensory and bacteriological qualities as for conventional procedure	

Source: Reproduced from Van Laack, R.L.J.M., Smulders, F.J.M., 1989. Quality of 'semi-hot' and cold boned, vacuum-packaged fresh pork as affected by delayed or immediate chilling. Journal of Food Protection 52 (9), 650–654.

shortening. Hot boning is particularly susceptible to cold shortening and increased toughness when applied to small carcasses and carcasses with low fat cover, because under these conditions the deep areas can be chilled much faster. This increased toughness can be reduced by introducing a conditioning period (e.g., semihot boning, Table 3) of 4 h more until rigor has occurred (in beef sometimes as much as 24 h or more) before chilling. This procedure will still allow improvement in production economy and productivity and an increase in carcass throughput. To reduce the time for rigor mortis to occur, electrical stimulation immediately after slaughter is utilized for carcasses that are going to be hot boned. Electrical stimulation was first applied to lamb carcasses that were to be rapidly frozen.

Other Options of Hot Boning

Carcasses boned directly off the slaughter floor and placed in typical cartons and conventionally air blast frozen often cannot meet the required chilling rate requirements; therefore, modified hot boning is often utilized.

1. A modified hot-process system for beef carcasses involves removal of the lower-value cuts along with associated bone and fat. This product is preblended with salt and chilled for emulsion-type products or immediately processed by rendering or directly processed into fresh hot-boned meat. The remaining high-value cuts are chilled in the usual manner. The time required to chill the deep tissue of the hindquarter is not significantly different from that for conventional chilling, but this technique can be cost-effective for less-valuable cuts.
2. 'Australia' carcasses above 20 °C deep but temperature must be processed using a deep boning program. Usually, the carcasses are chilled several hours before boning (warm boning, boning on the curve, same day boning) until deep butt temperature is 30–36 °C. In the US, approximately 25% of pork is sold fresh, and the remainder is either sold as cured meat or sausage products. With leaner pork carcasses and with rapid chilling, cold shortening might become a problem. However, because normal postmortem metabolism in pork occurs rapidly, cold shortening and increased toughness are not likely to be a major problem. Whole-hog fresh sausage processors can utilize hot boning and rapid salting. This process almost doubles the sausage microbiological shelf life, because surface bacteria have not had time to grow in this favorable environment before the antimicrobial salt addition. Also, as the product is comminuted, toughness is not a problem. This technique results in a higher ultimate pH and consequentially increased water-holding and emulsifying capacities, which result in better eating quality.
3. Electrical stimulation of carcasses will lower the pH and speed up rigor mortis and enhance tenderness, which will reduce some of the disadvantages of hot boning.

Chilling

Chilling is critical for meat hygiene, safety, product shelf life, appearance, and eating quality. Chilling in air reduces carcass surface temperature, enhances carcass drying, and reduces the growth of bacteria.

Refrigeration equipment in large plants often consists of ammonia units. Medium to small plants often have a range of hydrochlorofluorocarbons (HCFCs, R-22, R-123, R-408A) and hydrofluorocarbon (HFCs, R-134a), because chlorofluorocarbons (CFCs, R-11, R-12, R-502, R-113, R-500) are now being phased out. All systems have three basic components: the compressor, the condenser, and the evaporators. The evaporators are most critical in relation to meat quality. They consist of finned tubes (coils) through which the refrigerant flows. Air is forced over the fins and through the cooler. Rapid chilling can be accomplished by utilizing low refrigerated temperatures. However, this will result in increased shrinkage of the carcass and condensation and freezing on the coils, which will require frequent defrost cycles. Minimum weight loss, higher humidity, and fewer defrost cycles can be accomplished by keeping the temperature differential as low as possible (or practicable) between the temperature of the air entering and exiting the coils. A practical air return temperature would be 10 °C as the cooler is being loaded with hot-boned meat or carcasses, and this should be reduced to 0 °C after 1–2 h. Air velocity has less effect on chilling rate than temperature and should be low (0.5 m s^{-1}) during loading of the cooler, higher (1–2 m s^{-1}) during maximum chilling, and low (<0.5 m s^{-1}) during holding.

An increase in air velocity and/or a decrease in temperature (both controllable) decrease chilling time. A limiting factor, however, is difficulty in removing heat quickly from the deeper tissues of carcasses. For traditional chilling of beef carcasses, a temperature of 5 °C or less (air velocity of less than 1 m s^{-1} and a relative humidity greater than 80%) is recommended, because the growth of both spoilage and pathogenic bacteria is greatly reduced at these temperatures. Aging (>5 °C) is a major factor for improving tenderness, and this is most often accomplished with the traditional (Tables 1 and 2) procedure. However, early consumption after slaughter of double-muscled

breeds is often recommended, but a longer aging time is necessary for most carcasses to obtain optimum tenderness. It is recommended that chilling regimes should always be optimized for the carcass types utilized.

Chilling of high-quality beef carcasses (young, grain-fed animals) is traditionally accomplished at a temperature slightly above freezing for a long period of time to produce the highest quality product. In areas where refrigeration is not available, hot boning is required before the tissue spoils microbiologically. For most hot-boned products, when electricity is available, electrical stimulation is used to accelerate rigor and to compensate for the reduced tenderness caused by muscle shortening during onset of rigor when the muscles are detached from the bones and chilled.

Rapid carcass chilling increases yield due to lower evaporation from the surface, and rapid drying of the carcass surface helps to reduce bacterial growth. Also, less shrinkage results in financial savings. If meat is removed from the carcass while the tissue is still hot and then cooled in vacuum packages, the chilling time is normally reduced by 50%. Commercial chilling equipment is capable of reducing the temperature of an 8 cm thick piece of meat from 40 to 2 °C in less than 8 h. It is often recommended that beef should be at a high temperature for as short a time as possible and muscles should enter rigor at 10–15 °C for optimum tenderness. Prerigor chilling has been conducted at −15 °C for 1 h and postrigor chilling at −5 °C for 10 h; however, this does result in some muscle freezing. Optimum rapid chilling of beef carcasses has been recommended at −15 °C (air velocity 0.5 m s^{-1} and a RH of 90%) for 1 h and then chilling up to 10 h postmortem at 0 °C. It has also been suggested that carcass quality can be improved by appropriately positioning evaporators and fans, room loading, and air-mixing devices.

Pale, soft, and exudative meat (PSE) in pork is a major problem that is genetically linked, but it can also be influenced by the chilling rate. Rapid anaerobic glycolysis produces lactic acid, which results in a rapid lowering of pH, and if this is combined with a high carcass temperature it often results in a denaturation of proteins followed by high moisture loss and increased shrinkage. An increase in the rate of chilling will lower the temperature rapidly and thus reduce the rate of glycolysis and pH fall, resulting in a reduced incidence of PSE. Scalding and singeing of the carcasses can also contribute to the PSE problem. Dehided carcasses, compared to scalded/dehaired carcasses, have an improved muscle color and water-holding capacity and often have lower bacterial counts. Electrical stimulation is not often used in the pork industry owing to the natural rapid onset of rigor mortis and the risk of producing PSE meat. Rapid chilling after slaughter is often employed in the pork industry to improve pork quality, produce a darker color, increase firmness, decrease the chilling loss, and increase the shelf life without altering tenderness. Chilling is often used as a critical control point (CCP) in pork slaughter hazard analysis and critical control point (HACCP) plans.

PSE is also becoming a problem for poultry pectoralis muscles for the same reason as in pork, which is an accelerated postmortem glycolysis while carcass temperature is still high, which results in protein denaturation and poor meat quality. PSE in poultry is often blamed on slow chilling rates.

Control of air temperature to ensure that carcass surface temperature requirements are met is the most important aspect of carcass chilling. This will vary according to the size of the carcass, glycolytic rate, fatness, and species. Achieving an even air flow at optimum velocity over all carcasses is also important to provide adequate and uniform heat transfer and minimum weight loss.

European Union regulations (95) stipulate that carcasses of cattle and horses must be cooled to a temperature less than 7 °C within 36 h and smaller slaughter animals within 24 h to decrease bacterial growth. There is some concern that this rapid chilling in some cases could result in cold shortening and increased toughness of beef.

Beef

Hot boning beef from the skeleton may reduce contamination with the central nervous system (CNS) tissue compared to splitting the spinal column. Beef carcass chilling time can be decreased by an increase in air velocity and decrease in temperature. Recommendations are often that the air velocity should be less than 1 m s^{-1} and the relative humidity should be greater than 80%. Besides the avoidance of cold shortening, aging is the main factor influencing beef tenderness. Some reports suggest that beef should be at a high temperature for as short a time as possible, and muscles should enter rigor at 10–15 °C for tenderness to be optimized.

Research has also shown that rapid chilling of beef carcasses at −5 to −10 °C (air speed 1–2 m/s, RH of 90–100%, chilling time 115–212 min followed by holding in a cooler at 0–4 °C) resulted in a significant decrease in meat tenderness as a result of cold shortening although weight loss and bacterial counts were lower. However, other reports suggest that beef carcass meat quality superiority can be gained by prerigor chilling at −15 °C (air velocity of 0.5 m/s and 90% relative humidity) for 1 h and further chilling at 10 h postmortem at 0 °C.

Delayed chilling ('tenderay process' – for meat held at 16 °C for 3 days at 2 °C for up to 15 days) can improve beef quality compared to conventional chilling and conditioning for all carcass grades. Because microorganisms grow rapidly at this temperature, ultraviolet lights are used to inhibit growth. Preventing the muscles from shortening by wrapping can prevent toughness.

Electrical stimulation can enhance the tenderness of beef, accelerate the rate and extent of tenderization, and reduce the difference between British and tropical breeds and produce a more consistently tender beef product. Recent work has shown that electrical stimulation produces beef carcasses that are more tender than nonstimulated carcasses, and the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) band pattern of myofibrils show a more rapid breakdown of troponin T and troponin I bands than for nonstimulated carcasses. In chilled beef carcasses, the relationship between pH and color is primarily influenced by the chilling temperature. Recent studies have shown drip as a consequence of tenderization (from denaturation of cytoskeletal proteins rather than myosin as in PSE); there is initially more drip with electrical stimulation but the total amount of drip does not increase for the same tenderness.

It has been reported that very fast chilling (−20 to −35 °C) of beef carcasses immediately after slaughter improves tenderness at 6 days but this improvement disappeared after 21 days of aging. The advantages of this extreme chilling regime are a significant reduction in chilling loss, a slower rate of pH fall, and an increased perception of marbling but it also resulted in a darker meat color and increased drip losses. An increased toughness and shorter sarcomere lengths have also been found with ultrarapid chilling (−30 °C for 30 min with an air velocity of 4 m s^{-1}) of beef carcasses, suggesting an increased risk of cold shortening.

Spray chilling of beef carcasses with an intermittent water mist (1 °C, intermittent for 4–16 h) reduces carcass shrinkage (reduced by 0.08 g per 100 g per hour of spraying) without compromising quality or increasing spoilage losses. However, there should be sufficient time after the end of spray chilling to prevent the carcass from having an undesirable pale color and a wet surface, which would increase bacterial growth and make boning more difficult.

Stretching prerigor muscles during postmortem chilling tends to increase the tenderness of the stretched beef muscles and is particularly helpful in conditions that might produce cold shortening. In the method an S-shaped hook is placed in the obturator foramen, and the carcass is hung from this hook, which causes the pelvic limb to hang in a horizontal position. 'Tender cut' involves severing the bones, connective tissue, and some muscles at the 10–11 vertebrae leaving only the thoracis lumborum intact, and a second cut in the round/sirloin junction behind the 5th sacral vertebra causes gaps after hanging of 12 cm and 3 cm, respectively, in those two cut areas. Both of these carcasses suspension methods have been reported to be useful for improving tenderness for rapidly chilled (susceptible to cold shortening) cattle carcasses.

Organic acid (lactic or acetic) sprays for both hot and chilled carcasses results in pathogen reduction. Decontamination processes, such as steam vacuuming, preevisceration carcass washing and organic acid rinsing, and hot water carcass washing are techniques that could be useful for improving the microbial quality of beef carcasses.

Lamb

Owing to lamb size, temperature falls during chilling can be rapid, particularly if there is little fat covering, and this means the carcasses are subject to cold shortening with such rapid chilling. Because of the propensity of lamb to cold shorten and toughen prerigor, the acceleration of rigor mortis through electrical stimulation has been incorporated into lamb processing, especially in New Zealand and Australia and, additionally, the long aging period during shipping ensures a reproducible tender product.

Pork

Approximately 25% of US pork is processed fresh and the remainder is either sold as cured meat or sausage products. Hot boning was first applied by Oscar Mayer to packer sows hams that were to be used for sausage manufacturing. With leaner pork carcasses and with rapid chilling, cold shortening could also be a problem with pork. However, because normal postmortem metabolism in pork occurs rapidly, cold shortening and increased toughness should not be major problems. Whole-hog fresh sausage processors can utilize hot boning and rapid salting, which can almost double the sausage microbiological shelf life because surface tissue bacterial numbers have not had time to grow in this favorable environment before the antimicrobial salt addition, and because the product is ground, toughness is not a problem. This technique results in a higher pH and, consequentially, increased water-holding and emulsifying capacities.

Comparison of dehided and scalded/dehaired pig carcasses indicates that 24 h after chilling, the dehaired carcasses had higher weight loss than the skinned carcasses. Skinned carcasses should have improved muscle color and water-holding capacity and normally have lower bacterial counts. The higher counts of dehaired carcasses are thought to be caused by high temperature and humidity around the scalding process, which would encourage bacterial growth. During the first 2 h of chilling, internal temperature reduction was more rapid for the skinned pork carcasses, but no difference was obtained in subsequent chilling. Additionally, pH or surface bacterial counts were not affected by subsequent chilling. Pork quality did not differ between the two procedures. The pH values of accelerated chilling (−32 °C for 100 min) of pork carcasses resulted in 0.15 higher pH value from 4.5 to 24 h postmortem. Fat trimming of the hot carcass can accelerate the chilling rate, reduce PSE, and can improve the hind leg muscle color, water-holding capacity, and firmness scores.

PSE (pale in color, soft in texture, and exudative or watery) in pork is a major problem that has been genetically linked, but the incidence of PSE also can be influenced by the chilling rate. A rapid accelerated anaerobic glycolysis produces excess lactic acid, which results in a rapid decline in pH, and if this is combined with a high carcass temperature, particularly at approximately 45 min postmortem, approximately 20% of the sarcoplasmic and myofibrillar proteins will be denatured, resulting in free moisture loss and increase shrinkage of the tissue. An increase in the rate of chilling will lower the temperature and reduce the rate (which is temperature dependent) of glycolysis and pH decline, resulting in less denaturation and reduced incidence of PSE. Scalding (60 °C) to aid in hair removal (used by 69% of US processors due to the speed of the operation) and singeing can also contribute to the PSE problem because both increase carcass temperature, which accelerates glycolysis and pH decline. This combined with high carcass temperature can also increase protein denaturation and the incidence of PSE. Rapid chilling after slaughter is often employed in the pork industry to improve pork quality, produce a darker color, increase firmness, decrease muscle separation, decrease shrinkage during chilling, and increase shelf life without altering tenderness. This accelerated chilling also slows the rate of glycolysis, which lowers temperature and thus reduces denaturation and PSE. Chilling is often used as a CCP in pork slaughter HACCP plans.

Electrical simulation is not normally used in the pork industry due to the natural rapid onset of rigor mortis in this species; however this may change as pork carcasses become leaner and more susceptible to cold shortening and increased

toughness. In some UK studies with electrical stimulation, the pork was more tender with a nonsignificant early appearance of drip (due to early tenderization) – stimulation is not currently used with pork in the UK, however.

To reduce incidence of PSE, it is recommended that pigs are rested 2 h before slaughter, that accelerated chilled be used, and that hot fat trimming of the carcasses be employed. Washing of live pigs and subsequently pork carcasses with cold water has been reported not to be an effective microbiological control measure. Rapid chilling is often used as a CCP. Rapid chilling of pork carcasses can reduce the combination of purge loss and cooking loss without affecting tenderness. Ultrarapid chilling (−30 °C, air velocity 4 m s^{-1}) can result in increased sheer force values, indicating an increased risk of cold shortening.

Spray chilling of pork carcasses results in no significance difference in muscle color values after 20 h of chilling; however, the surface of the skin does become lighter. Pork carcasses can be chilled in brine or slush ice and the temperature can control the degree of shell freezing. However, this technique is not commercially practical, but this procedure would require less time and possibly less energy if cold water or ice was used instead of traditional air chilling.

Injection of calcium chloride for in-plant marinating 24 h postmortem has been reported to enhance tenderness. Use of sodium bicarbonate has also been shown to increase pH, water-holding capacity, decrease tenderness, and to produce a darker muscle color. However, these are not common practices in the pork industry to date.

Poultry

Factors, such as delayed chilling, that lead to a rapid postmortem pH fall will reduce the quality of turkey breast meat. Improper slow chilling of turkey pectoral muscle causes accelerated postmortem glycolysis while the carcass temperature is still high, resulting in protein denaturation and poor meat quality (PSE-like). Evaporative chilling reduces total bacterial count on the external surface of the poultry carcass by approximately 0.5 log total viable count, but air chilling has shown little effect on total bacterial count. Total count of the visceral cavity is unaffected by the chilling method. However, some new plants are using air chilling to reduce bacterial counts.

Chicken breast muscle tenderness increases with aging time, but when sodium tripolyphosphate (STPP) was applied to plant-marinated chicken breast immediately after carcass chilling, the tissue was tougher than the controls. However, this increase in toughness can be avoided by aging for 2 h before treatment with STPP. Electrical stimulation has been successfully used in poultry.

Fish

Fish are also processed by a variety of procedures. Saltwater fish usually follow one of these routes: caught, filleted, frozen or iced on board the ship; caught, iced or frozen, and filleted on the shore; caught, frozen, shipped to destination, thawed, filleted; caught, eviscerated, iced or frozen, filleted on the shore or at the destination. Freshwater fish usually follow one of these routes: caught, filleted, iced or frozen; caught, iced or frozen, shipped to destination, filleted. Even though fish go into rigor quickly, the fish that are caught and then filleted, or small fish that are caught and quickly frozen usually are in a prerigor state when boned. Fish that are iced and filleted later are usually in a postrigor state when boned. Because fish are very susceptible to bacterial growth, quality is usually determined by handling hygiene and low temperature and much less by the order of processing.

See also: Automation in the Meat Industry: Cutting and Boning. Chemical and Physical Characteristics of Meat: Water-Holding Capacity. Cutting and Boning: Hot Boning of Meat; Traditional. Electrical Stimulation. Hazard Analysis Critical Control Point and Self-Regulation. Refrigeration and Freezing Technology: Applications; Freezing and Product Quality; Principles; Thawing. Tenderizing Mechanisms: Chemical; Mechanical. Tenderness Measurement

Further Reading

Hildrum, K.I., 1996. Meat science. In: Proceedings of the 42nd International Congress of Meat Science and Technology, Lillehammer: Norway, 289 pp.

Hildrum, K.I., Solvang, M., Nilsen, B.N., Froystein, T., Berg, J., 1999. Combined effects of chilling rate, low voltage electrical stimulation and freezing on sensory properties of bovine M-longissimus dorsi. Meat Science 52, 1–7.

Hopkins, D.L., Shaw, F.D., Baud, S., Walker, P.J., 2005. Effect of level of current during lamb carcass electrical stimulation on post-mortem muscle changes and meat quality. Proceedings New Zealand Society of Animal Production Abstract AB05051 2005.

Jay, J.M., 1984. Modern Food Microbiology, 2nd edn. New York, USA: Van Nostrand Company.

Rosenvold, K., North, M., Devine, C., *et al*., 2008. The protective effect of electrical stimulation and wrapping on beef tenderness at high *pre rigor* temperatures. Meat Science 78, 299–306.

Van Laack, R.L.J.M., Smulders, F.J.M., 1989. Quality of 'semi-hot' and cold boned, vacuum-packaged fresh pork as affected by delayed or immediate chilling. Journal of Food Protection 52 (9), 650–654.

Relevant Websites

www.daff.gov.au/__data/assets/pdf_file/0004/113944/2001_20.pdf
Australian Government, Department of Agriculture.

http://www.fao.org/docrep/004/t0279e/t0279e05.htm
Food and Agriculture Organization of the United Nations.

http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CCsQFjAA&url=http%3A%2F%2Fwww.meatupdate.csiro.au%2Fdata%2FChilled_meat_for_export_04-91.pdf&ei=BYaKUqrqJKHx2AWIqIGICw&usg=AFQjCNH2obXSdN9RB0BKDfR5JsplEahWtw&bvm=bv.56643336,d.b2I
Meat Industry Services.

CARCASS COMPOSITION, MUSCLE STRUCTURE, AND CONTRACTION

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Glossary

Motor unit A motor unit is made up of a single motor neuron as well as all the muscle fibers that neuron activates. A single motor neuron can control several hundred muscle fibers at a time, depending on the size and function of the muscle.

Muscle fiber Cylindrical, multinucleate cell composed of numerous myofibrils that contracts when stimulated. Also called muscle cell.

Myofibril Slender striated strand within skeletal muscle fibers, composed of bundles of myofilaments. Myofibrils occur in groups of branching threads running parallel to the cellular long axis of the fiber.

Sarcolemma Plasma membrane of the muscle cells.

Sarcoplasmic reticulum Special type of smooth endoplasmic reticulum found in smooth and striated muscle fibers whose function is to store and release calcium ions.

T-tubule Deep invagination of the sarcolemma which appears as a small tubule that runs transversely from the sarcolemma across the myofibril of striated muscle.

Introduction

In nature, the edible parts of an animal killed for food are not organized according to the requirements of consumers. Even after up to twelve centuries of domestication, the structure of animals used by humans as meat is still dependent on the strict requirements of the living animal to successfully seek food, overcome environmental hazards, and compete for the right to reproduce.

It is, therefore, unrealistic to separate an account of carcass structure and function from the function of the living animal. This is especially true because, following the death of animals, chemical processes that are essential for the survival of the whole organism continue by a process of anaerobic glycolysis for hours, and sometimes days. This article emphasizes that a meat carcass consists of structural elements, formed according to the requirements of the living animal. Structure is highly relevant to meat production, not only because the art of butchery is an anatomical process but also because most of the material making up a carcass has a mechanical role, forming an essential part of a living machine.

This article is, therefore, a description and an explanation of meat carcass anatomy, ranging in scale from grossly dissectible components to microscopic and molecular elements. Inspection of any comprehensive textbook of anatomy will show that the subject is burdened with considerable detail. It is hoped that by restricting this account to topics relevant to meat production, the information presented is no more than is needed to introduce, describe, and explain the carcass features that will be addressed in other articles.

Meat Carcasses

Following the slaughter of an animal for meat, certain parts of the whole animal are separated from the carcass (1). The procedure varies for different species and countries. For instance, a pig carcass usually includes the skin and head, and the cannon bones are left with the sheep carcass in some countries. With the exception of the skin in pigs (Figure 1c), a carcass contains only three tissues present in amounts significant for meat production. Although these are designated as 'tissues,' muscle, fat, and bone each consist of a variety of different cell types and extracellular material. The process of separation of carcass muscle, fat, and bone, whether by the butcher's knife or by consumer's knives and forks, is an anatomical one. The three carcass tissues are, therefore, defined as those that can be separated grossly with a knife. Fat in the total carcass, or in a particular part of it, can also be measured by solvent extraction. The proportions of each tissue in carcasses of cattle, sheep, and pigs, and typical ranges, are given in Table 1. Interestingly, the muscle:bone ratio is higher in fat sheep carcasses than in lean carcasses, which is not the case for beef and pork carcasses. The remaining structures, such as blood vessels, lymph nodes, nerves, tendons, ligaments, joint capsules, and fibrous sheets, such as the linings of the body cavities, seldom total more than 2% of the weight of a carcass.

Although the functions, and hence the carcass proportions, of the three major tissues are closely interrelated, it is convenient to divide this account into a discussion of each of them. Their growth characteristics are discussed in other sections.

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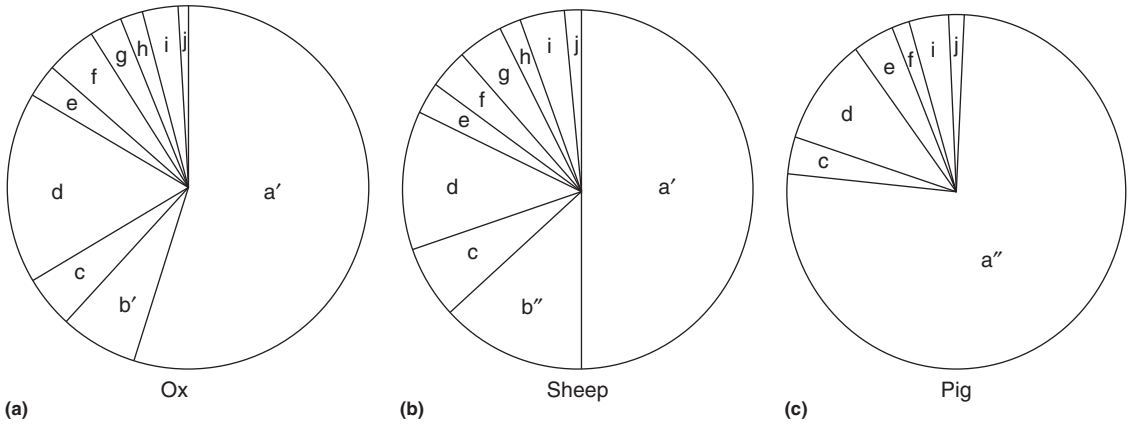

Figure 1 Composition of live animal: (a) ox, (b) sheep, and (c) pig. Components relevant to meat production are shown as a proportion of live body weight: a′, hot carcass, including cavity fat; a′′, hot carcass, including cavity fat, head, feet, and skin; b′, hide; b′′, pelt and fleece; c, empty gut; d, digesta; e, other viscera; f, visceral fat; g, head; h, feet; i, blood; j, remainder. Adapted with permission from Kempster, T., Cuthbertson, A., Harrington, G., 1982. Carcase Evaluation in Livestock Breeding, Production and Marketing. London: Granada.

Table 1 The composition of meat carcasses. Muscle, fat, and bone weights as percentages of total carcass weight for animals of 'lean' (L), 'average' (A), or 'fat' (F) condition. All carcasses are without head, feet, and skin. The carcasses of pigs have the highest muscle to bone ratio. Because muscle and fat content of pig carcasses is similar to that for cattle and sheep, the higher muscle to bone ratio appears to be due to a lower bone content

	Cattle			*Sheep*			*Pigs*		
	L	*A*	*F*	*L*	*A*	*F*	*L*	*A*	*F*
Muscle, %	66	59	50	64	57	48	67	59	53
Fat, %	16	25	37	14	24	38	22	31	38
Bone and remaining tissues, %	18	16	13	22	19	14	11	10	9
Muscle to bone ratio	3.7	3.7	3.8	2.9	3.0	3.4	6.1	5.9	5.9

Source: Adapted from Kempster, T., Cuthbertson, A., Harrington, G., 1982. Carcase Evaluation in Livestock Breeding, Production and Marketing. London: Granada.

Carcass Muscle

In living animals, muscles are essential for maintaining the shape of the body in a particular position and to provide physical movement. Even standing still requires muscular activity. Without normal muscle tone, such as during sleep or anesthesia, maintenance of posture is not possible. Muscles also enable the body of animals to bend and systematically move and change the support of their limbs, and thereby altering their relation to their environment. Such movement, when suitably coordinated, results in specific actions such as locomotion. Both posture and movement are basic to the survival of animals.

Carcass muscles are separated by loose connective-tissue sheets that might contain intermuscular fat. Each of these named muscles is free to act as a separate entity, contracting or resisting stretch between its unique attachments to the skeleton or other structures. Many muscles, especially those related to the vertebral column and ribs, act over many joints and have complex structures because of the number of attachments (Figures 2 and 3). The distribution of carcass muscle in cattle is shown in Figure 4: 55% of the muscle mass is involved in attaching the limbs to the trunk and moving the shoulder and hip joints (muscle groups 3, 4, and 9). The next largest group (14%) supports and moves the vertebral column (muscle groups 5 and 7). This accounts for the large masses of lean meat, relative to bone, obtained from the shoulder, loin, rump, and thigh regions.

Muscle Connective Tissue

Each muscle consists of a highly ordered arrangement of connective tissue in which muscle fibers are embedded. Muscle connective tissue shares similar features with the connective tissue of other organs.

Connective tissue contains fixed cells, free cells, and protein fibers, all embedded in a matrix. The fixed cells include the fibroblasts that produce both the fibrous component and the matrix of connective tissue. These remain subsequently in a less active secretory phase as fibrocytes. Also fixed within the tissue are the fat cells, which contain the major intramuscular fat of the carcass.

The two major types of protein fibers identifiable in the connective tissue of muscle are collagen and elastin. Collagen fibers contain a regularly oriented array of polypeptide chains, resulting in a highly fibrillar structure (Figure 5). Collagenous tendons can be stretched approximately 4% of their length

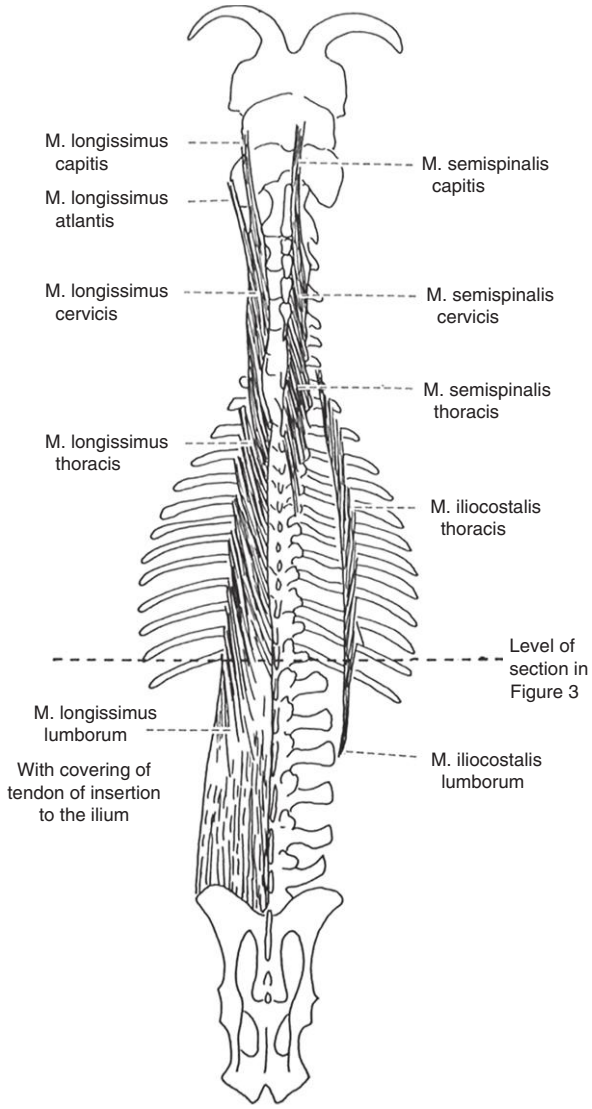

Figure 2 Some long and segmented muscles of meat carcasses. Selected muscles of the trunk of the goat are shown schematically, from a dorsal aspect. Although the longissimus muscle is a single segmented muscle reaching from the thorax to the hip bone, a series of names are given, depending on the region. The whole muscle was formerly called 'longissimus dorsi.' It is shown in transverse section for the sheep, at the level indicated by the dotted line in **Figure 3**. Adapted from Getty, R., 1975. Ruminant myology. In: Getty, R. (Ed.), Sisson and Grossman's The Anatomy of the Domestic Animals, fifth ed. Philadelphia, PA: WB Saunders, pp. 791–860.

elastically, i.e., they return to their original length without permanent deformation. Elastin in which polypeptide chains are arranged randomly provides the elasticity to the connective tissue. The fibers appear amorphous except for fine microfibrils embedded in them. Their elastic performance matches that of rubber, because they can be stretched 250% without permanent deformation. This can be demonstrated on the large elastic ligament in the neck of ruminants, easily seen on a half-carcass. This nuchal ligament, by stretching when the head is lowered, enables raising of the head again with little muscular effort. In tendons external and internal to muscles (Figure 6), collagen fibers are packed densely and have high tensile strength. The epimysium (Figure 7(b)), the connective tissue on the outside of a single muscle, is thick and strong when it forms part of the tendinous apparatus of the muscle. It is continuous with the grossly visible internal muscle connective tissue, the perimysium, which branches within the muscle to enclose bundles of muscle fibers (Figures 7(b), (c)). As for epimysium, perimysium can also, in some parts of a muscle, be thick and tendinous. In other locations within the muscle, the collagen fibers of the perimysium are arranged in a loose but well-ordered criss-cross lattice at an angle to the long axis of the muscle fibers. Elastin is also present in perimysium. Fat cells may accumulate. Vessels and nerves servicing the muscle are supported and protected within the perimysium. The 'grain' of a muscle, grossly visible, especially when cooked meat is broken apart, consists of perimysially enclosed bundles of muscle fibers. In the process of marinating or cooking, the integrity of the collagen fibers of the perimysium is partially lost and the muscle fiber bundles are easily separable.

The layer of connective tissue surrounding individual muscle fibers is endomysium (Figures 7(c), (d)). This contains only a light network of collagen fibrils, not bundled into fibers as in epimysium and perimysium. These collagen fibrils are the reticular fibers that form a continuous network around each muscle fiber. Reticular fibers are demonstrated by silver stains, apparently because of their thinness (0.5–2.0 μm) and their relationship to the interfibrillar matrix. The basal lamina is a relatively thin layer (1 nm), closely applied to the membrane of the muscle fiber. Endomysial collagen fibrils enter it. The basal lamina contains a nonfibrillar variant of collagen. Endomysium contains the capillary network from which muscle fibers obtain nutrition and respiratory gases, and to which waste products are passed. Also found here are the terminal ends of the nerves supplying each muscle fiber.

The matrix of muscle connective tissue is amorphous. It is composed predominantly of various types of large proteoglycan molecules, which have a protein core with multiple polysaccharide side chains.

The relevance of muscle collagen to meat production is due to the peculiar properties of the collagen molecule. To construct a tendon from polypeptide chains, these chains must first be woven together to form collagen molecules, then linked to form collagen fibrils, which in turn form collagen fibers, as shown in Figure 5. The binding of collagen molecules to form a collagen fibril that is visible electron microscopically is due to cross-linking (Figure 8). In newly formed collagen fibrils, these are solely intermolecular head-to-tail cross-links. However, the properties of collagen change with age. In mature animals, collagen fibers are much more stable owing to the cross-linking of several molecules, thereby linking fibrils in register (Figure 8(b)). Such fibers are more rigid, and less susceptible to enzyme degradation. Most significantly, their thermal properties are affected. At approximately 65 °C and above, collagen molecules are denatured. They lose their highly organized structure and become gelatin. The cross-linkages in mature collagen are heat stable, however, and some strength is progressively maintained with increasing animal age. The effect also results in more tension being generated under isometric conditions as the fibers shrink with

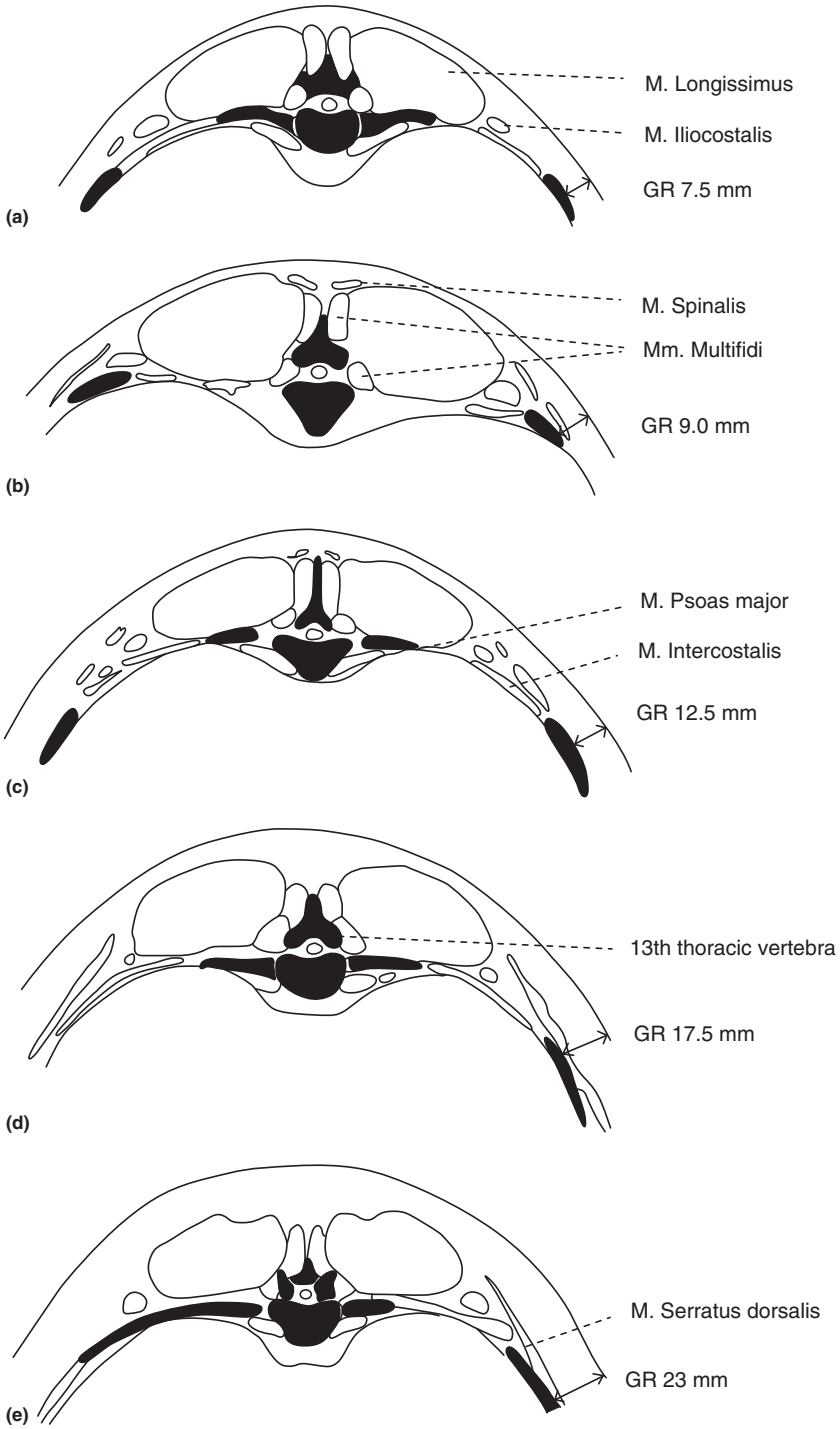

Figure 3 Anatomical features of a lamb chop. Transverse sections of the dorsal part at the level of the 13th thoracic vertebra of 5 lamb carcasses with increasing fatness (a, b, c, d, and e respectively), as indicated in **Figure 2**. The sections of the longissimus muscle ('eye muscle') have frequently been used to predict muscle mass in carcasses of cattle, sheep, and pigs. The series here shows sections from five lambs of the same carcass weight (22.7 kg), but increasing fatness as indicated by the increasing thickness of subcutaneous fat directly over the 13th rib ('GR' measurement). Adapted from photographs supplied by J.M. Thompson.

heat. The relevance of these properties to meat tenderness is discussed in another part of this encyclopedia.

Thus, even though muscle collagen content does not increase over the postnatal lifetime of animals, meat from older animals is tougher. It is the quality, not the quantity, of the collagen that is relevant. It is considered that collagen quality is a major determinant of texture in cooked meat.

The highest collagen content is found in muscles with much internal tendon, where strength is important rather than range of movement and where the muscle functions to some

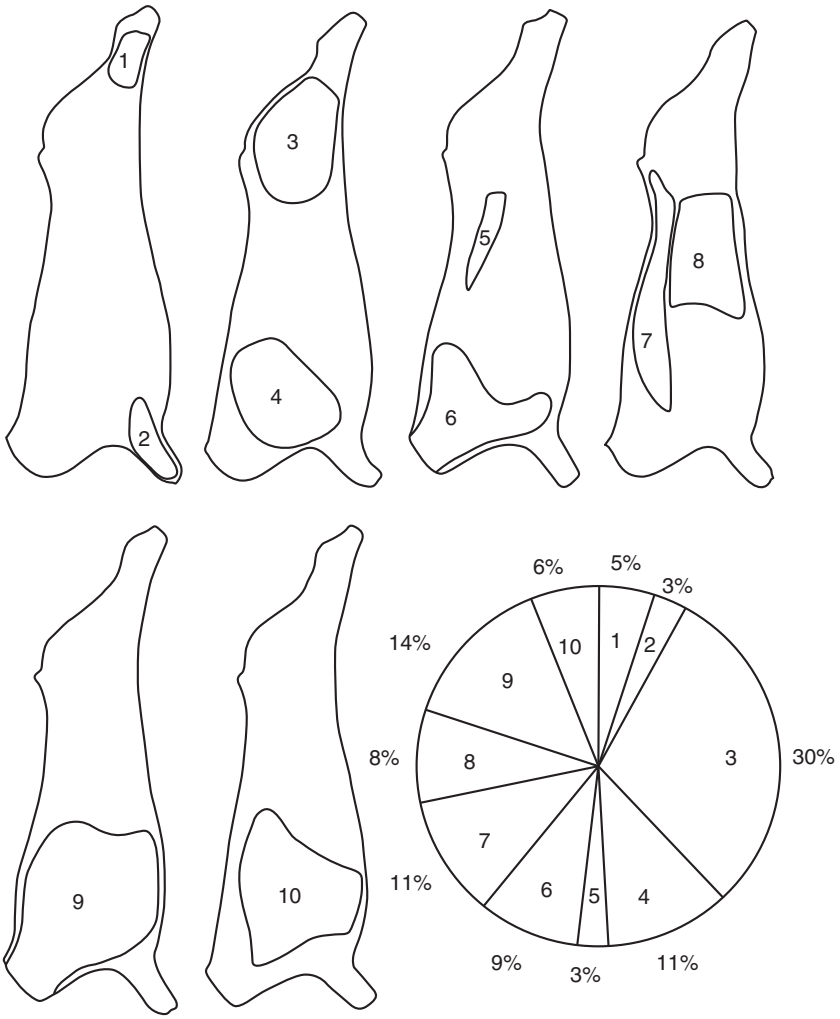

Figure 4 The distribution of muscles in carcasses. Muscle groups shown have weights appropriate to a Jersey cow of live weight 381 kg, carcass weight 178 kg, and total carcass muscle weight of 100 kg. The pie graph shows the weight of each muscle group as a proportion of total muscle. The muscle groups shown are: 1, muscles surrounding the tibia; 2, muscles surrounding the radius and ulna; 3, muscles surrounding the hip bone and femur; 4, muscles surrounding the scapula and humerus; 5, deep muscles ventral to the vertebral column; 6, deep muscles of the neck; 7, muscles dorsal to the vertebral column; 8, abdominal muscles, 9, muscles attaching the fore limb to the trunk; 10, deep muscles of the thorax. Data reproduced from Tan, G.Y., 1981. Carcass development and cellular growth of muscle and fat in male and female cattle. PhD Thesis, Massey University, Palmerston North, New Zealand.

extent as an adjustable ligament in the fixation of joints. A complete range of muscles, from those with a wide range of movement, to tendons, where only passive elastic movement occurs, is found in meat animals (Figure 6).

Muscles of the distal parts of limbs are especially high in collagen (Figure 9(a)). Collagen content is lowest in muscles of the back and abdomen and in muscles connecting limbs to the trunk (Figure 9(a)). These muscles have the widest range of movement.

The elastin content in the connective tissues of muscle varies considerably among individual muscles, but not in a pattern similar to that for collagen. It is especially high in the perimysium of the bovine semitendinosus and biceps femoris muscles, where it is approximately 20 times that for the surrounding thigh muscles. This may explain the strikingly mosaic appearance of the freshly cut transverse surface of these muscles.

The presence of intramuscular fat cells in the perimysium depends on overall carcass fatness. When this is high enough, the cut surface of a muscle has a 'marbled' appearance due to fat located between the muscle fiber bundles in the perimysium. Intramuscular fat also varies among muscles. In pigs, and presumably other species, the fattest muscles are those adjacent to the subcutaneous layer, especially in the neck, thorax, and abdomen (Figures 9(b) and 10). A certain amount of intramuscular fat is valuable for increasing palatability and adding flavor to meat.

Muscle Fiber Structure and Physiology

There are many millions of muscle fibers in a meat carcass. For a medium-sized hind limb muscle of a beef carcass, there

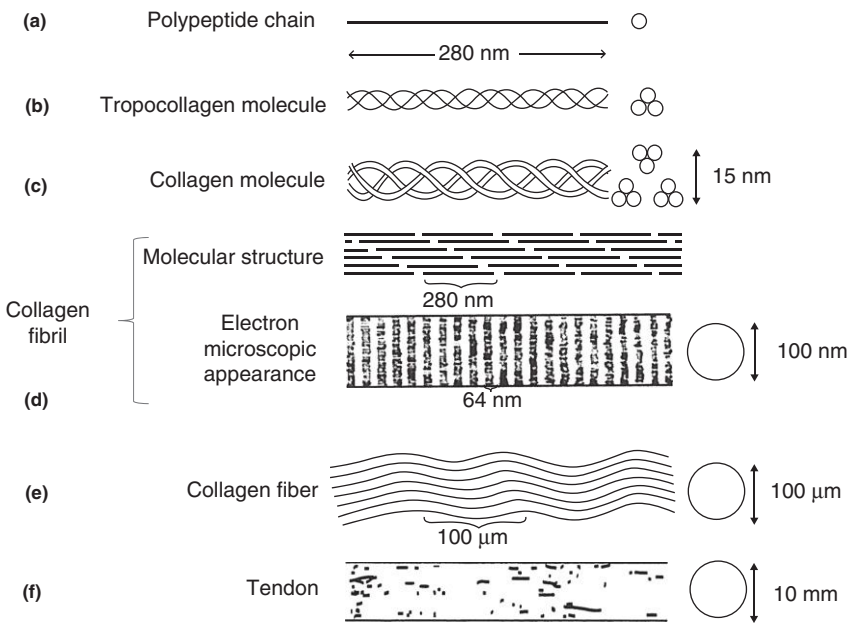

Figure 5 Molecular structure of collagen in a tendon. A diagrammatic representation of tendon structure as envisaged by (a) protein chemistry; (b), (c), and (d) X-ray diffraction confirmed by electron microscopy; (e) light microscopy; and (f) as seen grossly in, for example, the common calcanean tendon (Achilles) of cattle. Adapted from Evans, J.M., and Barbenal, J.C., 1975. Structural and mechanical properties of tendon in relation to function. Equine Veterinary Journal 7, 1–8.

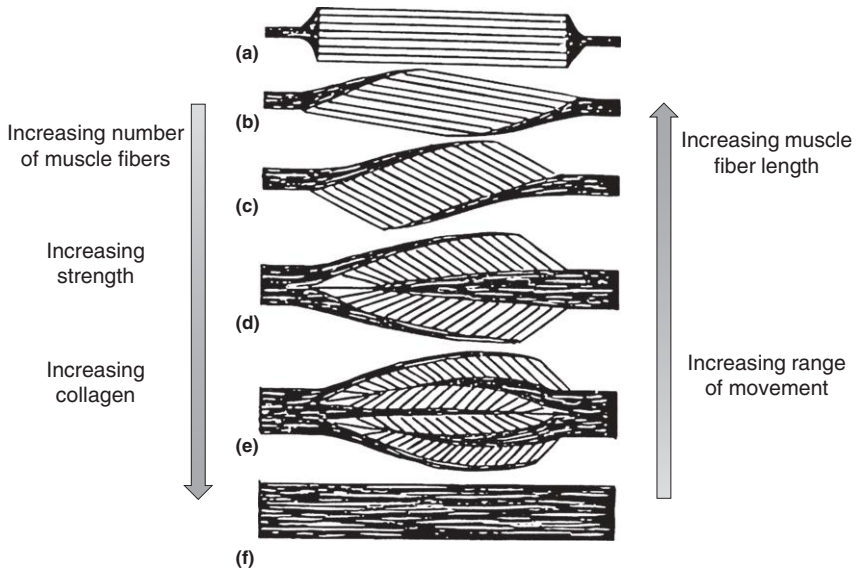

Figure 6 Fibrous architecture of muscle. Diagrammatic representation of possible arrangements of the muscle and collagen fibers within muscles. The extreme stages, from a strap muscle (a) to a ligament (f) are separated by a series of progressively more pennate muscles (b, c, d, e).

are approximately one million fibers in a transverse section (Table 2). Individual fibers are too small in diameter (50–100 µm) to be seen with the naked eye, but they span the length of small muscles, being several centimeters long.

The special property of muscle fibers is their ability to contract, or to resist stretching by an external force. Each fiber is an electrical unit, with its own triggering system. The apparatus involved in the electrical excitation of muscle fiber will be described first, followed by an account of the contractile apparatus itself.

Each muscle fiber of a carcass, together with from 5 to 200 other fibers that form a single motor unit, is linked to a large nerve cell in the spinal cord or the brain (Figure 11). Because the whole substance of the muscle fiber contracts simultaneously, there is an elaborate mechanism to ensure that the electrical impulse reaching the muscle is transmitted rapidly

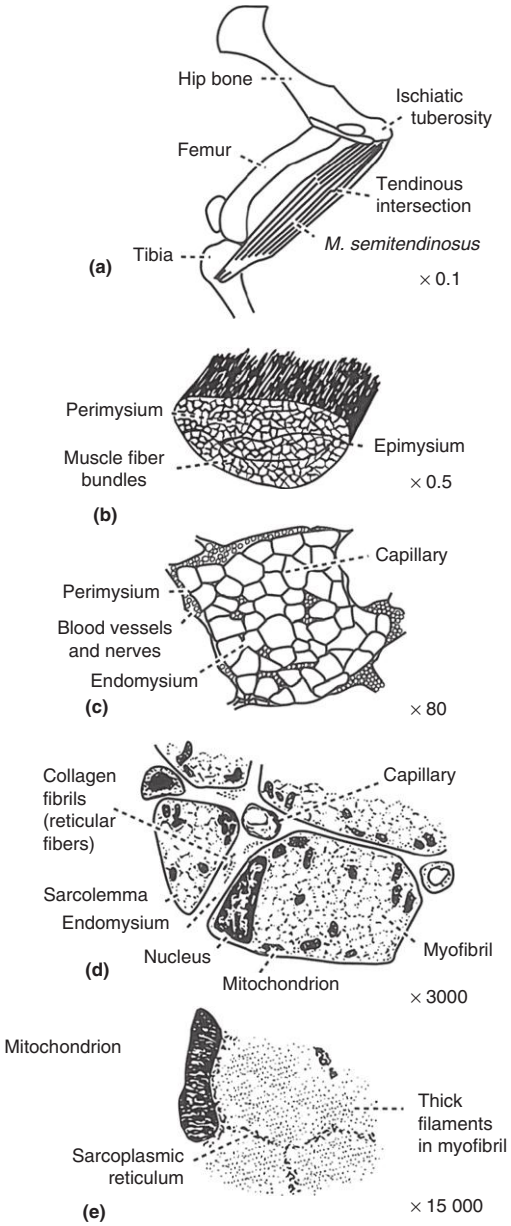

Figure 7 Gross and ultrastructural anatomy of the sheep semitendinosus muscle. The semitendinosus muscle lies caudally in the thigh region (a). Seen grossly in transverse section, perimysial strands course through the muscle and enclose muscle fiber bundles (b). Within a bundle, an endomysial connective tissue network is revealed by light microscopy to surround individual muscle fibers (c). Electron microscopic examination shows a network of sarcoplasmic reticulum enclosing myofibrils within the fibers (d). The two complete fibers drawn sectioned here are smaller than actual fibers in the sheep semitendinosus muscle (Table 2). At a still higher magnification, a pattern of thick filaments is visible within each myofibril (e).

throughout it, and that the contractile apparatus can react accordingly. The time taken for a muscle to produce peak tension is approximately 20 ms from stimulation. The components of the muscle fiber necessary to achieve this are summarized in Figure 11 and Box 1.

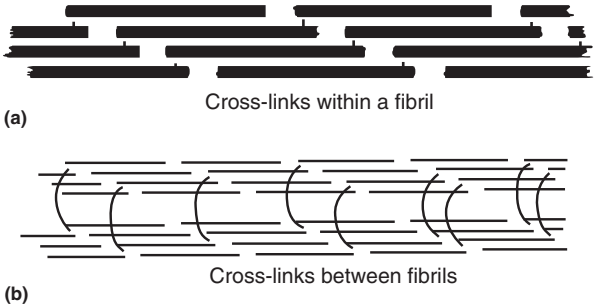

Figure 8 Cross-linking in collagen. The cross-links in immature connective tissue pass between collagen molecules within a fibril (a). The molecules are linked head to tail to form an infinite polymer. In a mature collagen fiber (b), the cross-links pass between the fibrils to link them in register. Adapted with permission from Davey, C.L., 1984. The structure of muscle and its properties as meat. In: Bailey, A.J. (Ed.) Recent Advances in the Chemistry of Meat. London: Royal Society of Chemistry, pp. 1–21.

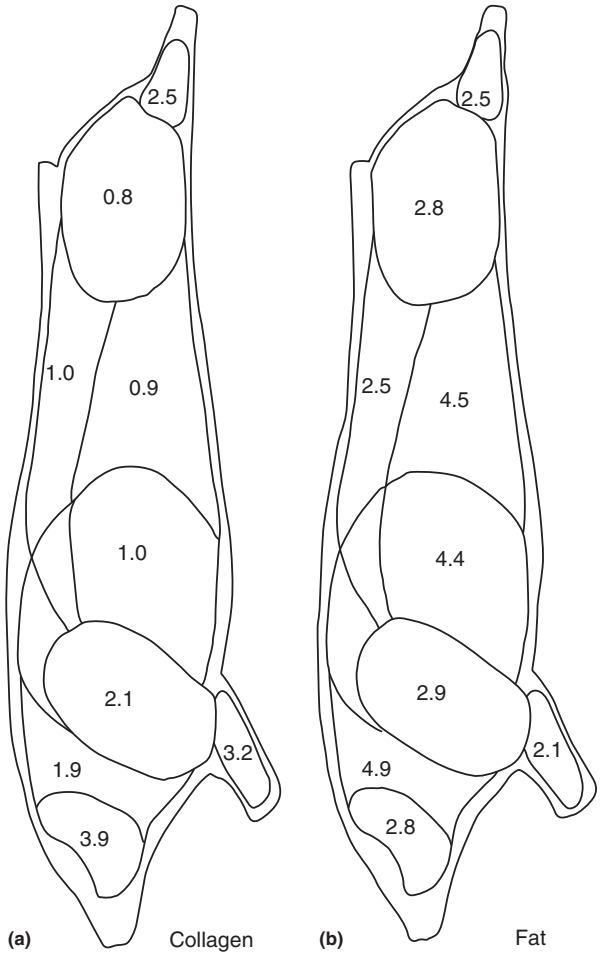

Figure 9 The collagen and fat content of muscles throughout the pig carcass. Estimations of collagen (determined by a colorimetric method for hydroxyproline) (a) and fat (determined by ether extraction) (b) in the carcass of a mature female German Landrace pig, expressed as a percentage of wet muscle weight. Data adapted from Davies, A.S., 1984. Wachstumsverlauf von Muskeln und Knochen bei Schweinen unterschiedlicher Endgroesse. Thesis, DrMed-Vet, Tieraerztliche Hochschule, Hannover.

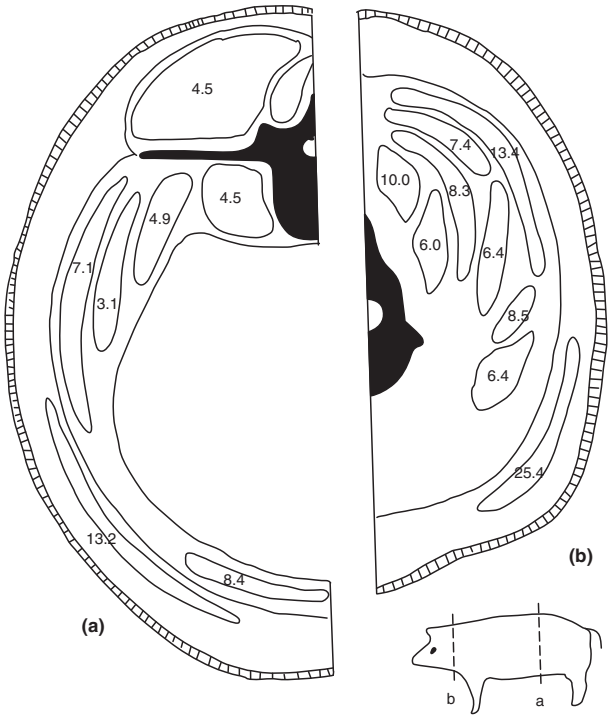

Figure 10 Intramuscular fat in individual muscles. Fat as a percentage of wet weight is shown for individual muscles in the abdominal and neck regions of a pig of 60 kg live weight. Data from Davies, A.S., Pryor, W.I., 1977. Growth changes in the distribution of dissectable and intramuscular fat in pigs. Agricultural Science 89, 257–266 (in Cambridge).

Table 2 The number and size of muscle fibers. Estimations of measurements for muscle fibers within the semitendinosus muscle of mature cattle and sheep

	Cattle	*Sheep*
Weight of semitendinosus muscle	3000 g	120 g
Mean transverse sectional area of single fibers	3500 μm^2	2500 μm^2
Number of fibers in a transverse section	1 000 000	300 000

Source: Data for cattle adapted from Tan, G.Y., 1981. Carcass development and cellular growth of muscle and fat in male and female cattle. PhD Thesis, Massey University, Palmerston North, New Zealand and Data for sheep from Sivachelvan, N., 1980. An anatomical study of adaptive processes in muscle. PhD Thesis, Massey University, Palmerston North, New Zealand.

Following the harvest of an animal for meat, muscle remains excitable for some time. The chemical changes necessary to convert muscle into meat are associated closely with the excitation apparatus.

Within the muscle fiber, and parallel to its length, are cylindrical myofibrils 0.5–1.0 μm in diameter (Figures 7(d), (e), and 12). Each myofibril consists of two types of protein filaments called 'thick' filaments and 'thin' filaments (Figure 13 and Box 3). Thick filaments are formed from myosin molecules that are grouped in bundles. Thin filaments are formed of actin, troponin, and tropomyosin molecules. More details about the myofibrils structure are available in another part of this encyclopedia. Myofibrils are isolated from each other mainly by sarcoplasmic reticulum but also by other structures in the nonmyofibrillar part of the fiber (sarcoplasm). These are listed in Box 2. Because contraction depends on the diffusion of calcium ions into and out of the myofibrils and the sarcoplasmic reticulum, myofibrils are the same diameter regardless of the size or the stage of development of the muscle fiber.

The filaments within a myofibril are aligned to the T-tubular system (Box 1). A T-tubule is associated with each A-I junction in mammalian muscles (Figure 12). The filaments in all myofibrils are, therefore, in register, right across the fiber. It is for this reason that a fiber in skeletal muscle appears striated. Early light microscopists recognized various elements of the striations that have been shown to be significant by electron microscopy and X-ray crystallography (Figure 13). The particular components of the contractile apparatus that are considered currently to be relevant to contraction in the living animal, as well as to processes occurring after slaughter, are listed in Box 3. The functional component of muscle is, therefore, intracellular. Muscle cells result from the fusion of many precursor cells that do not contain contractile proteins. The contractile apparatus is elaborated only after the fusion of myoblasts to form a multinucleated syncytium, the muscle fiber.

Muscle Fiber Metabolism

The energy for the interaction between the thick and the thin filaments is provided by the 'splitting' of the metabolic intermediary adenosine triphosphate (ATP). The site for the catalysis of this fundamental reaction is in the heads of the myosin molecule (Figure 13(d), Box 3). The enzyme is therefore known as myosin ATPase and is rate limiting for the speed of muscle contraction. The higher the level of activity, the faster is the speed of ATP splitting and the more energy that is available for mechanical work. Fast-contracting fibers, with a time to peak tension of approximately 10 ms, have a high level of myosin ATPase activity. Slow-contracting fibers (approximately 80 ms to peak tension) have a demonstrably lower myosin ATPase activity. This difference can be shown histochemically (Figure 14(a)).

Because all fibers within a motor unit are stimulated by the firing of their common motor neuron, they must contract together and have the same speed of contraction and the same level of myosin ATPase activity (Figure 15). In the living animal, fast-contracting fibers are used to accelerate parts of the body, whereas slow fibers are more economical for deceleration such as the maintenance of posture. In the latter case, chemical energy is not converted into mechanical energy but into heat. Muscles vary in their proportion of fast and slow fibers, according to their function in the body. There is also much variation in the proportion of these fiber types within and between muscles (Figure 16(b)).

The contraction speed is usually linked with the means by which ATP is regenerated after splitting. Figure 17 shows that when there is an adequate blood supply, this can be achieved aerobically. In this case, oxygen and nutrients diffuse into the fiber from surrounding capillaries, and waste products can diffuse back. Muscles must also often function without a

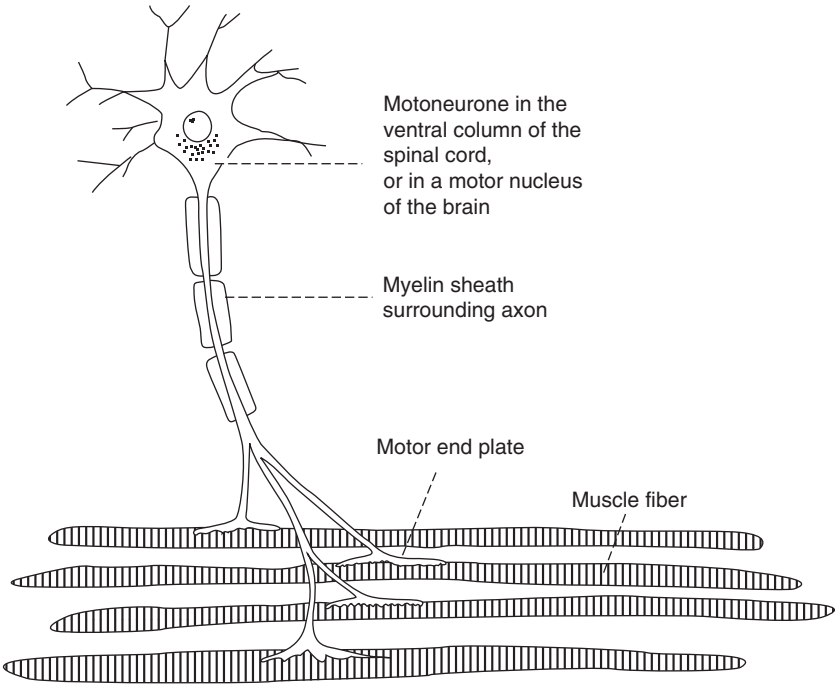

Figure 11 The motor unit. Several muscle fibers share the same motor neuron and are therefore stimulated simultaneously.

Box 1 The control of contraction in muscle

Motor end plate (**Figure 11**)
- Located at about midlength of the fiber.
- Transfers the electrical impulse from the nerve ending to the muscle fiber membrane.

Muscle fiber membrane (sarcolemma) (**Figure 7(d)**)
- 7 nm thick.
- Envelops the muscle fiber.
- Electrically excitable; on stimulation there is rapid depolarization over the whole fiber.

Transverse tubular (T-) system (**Figure 12**)
- Fine tubules with membranes continuous with the sarcolemma, 20 nm in diameter.
- By branching, the tubular system surrounds each myofibril at every A–I junction.
- Transfers the sarcolemma depolarization to the entire contractile apparatus within the fiber.

Sarcoplasmic reticulum (**Figure 12**)
- Forms a network of branching membranous sacs around each myofibril.
- Parts of it approach a T-tubule on each side to form a 'triad' in a section transverse to the T-tubule.
- On stimulation, releases and then actively reabsorbs calcium ions, which stimulate the chemical processes necessary for contraction within the myofibrils.

blood supply: this might be a temporary diversion or, if the muscle is seldom used, the capillary density around the fibers may be low. In this case, an intrinsic energy store (glycogen) must be used, and waste products cannot be removed from the muscle. The situation is essentially the same when muscles go on living after the death of animals. The anaerobic process is less efficient than the aerobic one, but wholly aerobic metabolism is possible only in continuously active muscles (such as the heart), which are very well irrigated. Slow fibers usually use aerobic metabolism because of their more continuous use, for which efficiency is desirable and, because in their deceleration function, they release heat that is only safely dissipated by a rich blood supply. Slow fibers are, therefore, found closest to the blood supply to a muscle (**Figure 16(b)**) and are surrounded by a dense network of capillaries. They contain more myoglobin in their cytoplasm for the transfer of oxygen and possess a high density of mitochondria with their enzymes of aerobic metabolism (**Box 2**, **Figures, 14**, **16(c)**, **17**).

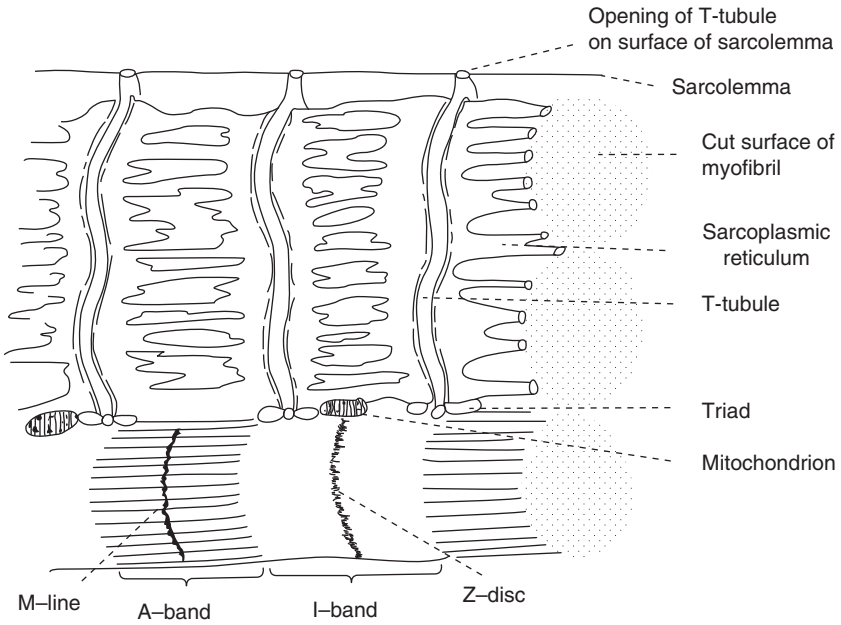

Figure 12 The sarcoplasmic reticulum and the T-system. This diagrammatic representation of three myofibrils extends **Figure 7(e)** three-dimensionally. The lower myofibril has been stripped of its surrounding T-tubular system and sarcoplasmic reticulum to show the relationship of these to the array of myofilaments within the myofibril. A 'triad' of one T-tubule and two parts of sarcoplasmic reticulum is sectioned at the level of each junction of A- and I-bands.

This association between slow fibers and aerobic metabolism does not imply, however, that slow fibers have no store of glycogen and cannot use anaerobic metabolism. Neither does it imply that fast fibers are exclusively anaerobic. Most muscles contain a high proportion of fast fibers with both aerobic and anaerobic metabolism. In fact, all metabolic combinations are possible and can be altered by a changing frequency of muscle use, such as prolonged aerobic training. **Box 4** shows some of the terms that have been used to describe the different fiber types in muscle. The supposed color distinction of 'red' and 'white' has largely been abandoned. It is especially inappropriate for the muscles of animals producing red meat. The terms 'type I' and 'type II' fibers, and their subsets, are still favored by pathologists.

Muscle Contraction

Muscle, a valuable nutritional source for humans, is nevertheless fundamentally a chemical machine driven by isothermal combustion. The contraction of voluntary muscles in all animals takes place by the mutual sliding of two sets of interdigitating filaments: thick, containing the protein myosin, and thin, containing the protein actin, organized in sarcomeres (**Figure 13**). The relative sliding of thick and thin filaments is brought about by the movement of cross-bridges, parts of the myosin molecules protruding from the thick filaments and acting cyclically with the thin filaments, transporting them by a kind of rowing action (**Figure 18**). It is the hydrolysis of ATP that provides the local energy for this linear motor. ATP is also a relaxing factor, in that it dissociates actin and myosin. Without ATP, the thick and thin filaments are bound together at the cross-bridges (**Figure 19**). The energy for the replenishment of ATP comes from the metabolic processes shown in **Figure 17**. A part of the ATP regeneration comes from phosphocreatine, which can donate a phosphate group to adenosine diphosphate (ADP) to form ATP. Moreover, adenylate kinase, also known as myokinase, catalyzes the formation of 1 molecule of ATP from 2 molecules of ADP.

The globular heads of myosin catalyze the hydrolysis of ATP. The rest of the myosin molecule forms a long helical coil. The myosin head is the structure forming a cross-bridge between filaments, as seen by electron microscopy. This cross-bridge binds to actin and changes its angle as one molecule of ATP is hydrolyzed in each cycle. Thin filaments are helical polymers of globular actin. Actin-binding sites have been identified in X-ray crystallographic models.

The chemical process by which animal movement takes place has long intrigued investigators. They have developed special technology to tackle the problem of three-dimensional chemical reactions at the atomic level taking place at high speed. Experiments using high-energy X-ray sources have confirmed a swinging cross-bridge hypothesis, although interpretations are ambiguous. Isomerism between an extended and a flexed state results in large changes of angle of the lever arm at the distal end of the myosin head. This accounts for a 12 nm power stroke (**Figure 19**). A burst of approximately 100 such cycles powers the sliding filaments so that peak tension in muscle can be reached in 10 ms.

Once an animal dies, the intrinsic metabolic processes that restore ATP eventually fail. The actin and myosin association becomes permanently fixed at the stage in **Figure 19(a)**. This rigor mortis state, an important consideration in how muscle becomes meat, is detailed in another part of this encyclopedia.

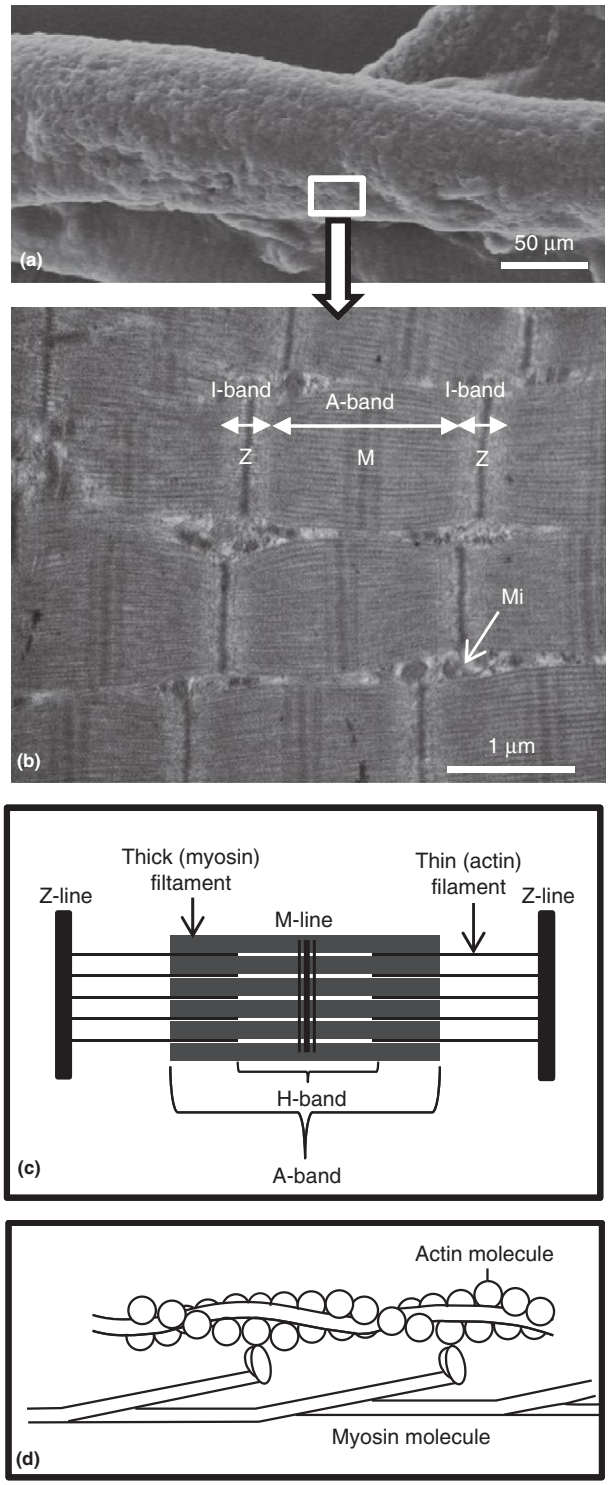

Figure 13 Fibrillar and molecular structure of the contractile elements of muscle. (a) Skeletal muscle fiber visualized by scanning electron microscopy. (b) Longitudinal section of a muscle fiber observed by transmission electron microscopy showing the myofibrils with a series of anisotrope (A-band) and Isotrope (I-band) areas, the sarcomere located between two Z lines with the M line in the middle part of the sarcomere. Mi, mitochondria. (c) Scheme of the sarcomere showing its organization. (d) Structure of thick (myosin) and thin (primarily composed of actin) myofilaments.

Carcass Fat

In living animals, fat insulates against cold and provides a store of energy. A large store is not essential for meat animals continuously supplied with enough food for optimal growth. The increased fat content that has almost invariably occurred with the domestication of meat animals must be contrary to natural selection and survival in the wild. Fat storage is,

Box 2 The contents of the sarcoplasm

Nuclei (**Figures 7(d)**)
- Located directly beneath the sarcolemma.
- Several thousand per fiber.
- Once a muscle fiber nucleus is involved in the synthesis of myofibrils, it is no longer capable of further division. A supply of nuclei in undifferentiated 'satellite cells', for growth and renewal, is located outside the muscle fiber.
- The function of the nuclei in mature muscle is to provide the DNA code for renewal protein following wear and tear of the myofibrils.

Lysosomes
- Small particles involved in intracellular digestion.
- May be significant in converting muscle to meat.

Mitochondria (**Figures 17**, **7(d)**, (e) and **13(b)**)
- Organelles containing the enzymes of aerobic metabolism.
- A high mitochondrial density results in dark, red meat. This is because some of these enzymes are pigments, and because aerobic metabolism in muscle is also associated with the red-pigmented iron-containing protein, myoglobin, in the sarcoplasm.

Glycogen
- These particles are usually numerous.
- They contain a carbohydrate nutritional store and associated enzymes.
- The preslaughter depletion of muscle glycogen is important in meat quality.

Box 3 Features of a myofibril

Bands (**Figures 12**, **13(b)**, **(c)**, and **18**)
- A-band: The region of thick (myosin) filaments.
- I-band: The region containing only thin filaments.
- Z-disk: A dark thin line in the middle of the I-band. It contains zig-zag elements that anchor the ends of the thin filaments.
- M-disk: A darker line in the middle of the A-band. Contains the protein myomesin. Connects the centers of the thick filaments together.

Sarcomere (**Figures 13(c)** and **18**)
- An assembly of thick and thin filaments between adjacent Z-disks forming the fundamental contractile units of muscle. The sarcomere length is greatest in stretched muscle (4.5 μ) and least in contracted muscle (less than 1.5 μ). These lengths are closely related to the toughness of meat. The length change is made without change in the length of the filaments. The fundamental interconversion of chemical to mechanical energy in muscle takes place between the thick and the thin filaments. This results in the sliding of interdigitating filaments (**Figure 19**).

Thick filaments (**Figure 13(c), (d)**)
- Each is an assembly of myosin molecules.
- Each filament is 12 nm in diameter and 1500 nm long.
- Each molecule has two heads, associated with the sliding of thick and thin filaments by forming cross-bridges between them. The enzyme catalyzing the splitting of ATP to achieve this is located in the heads.

Thin filaments (**Figure 13(c), (d)**)
- Each is an assembly of several proteins, predominantly globular actin, supported by troponin and tropomyosin.
- Each filament is 8 nm in diameter and 1000 nm long.

Cytoskeletal framework
- A distinct lateral component links adjacent myofibrils at the Z-disks. This remains after the removal of the thick and thin filaments. The most prevalent proteins present here are titin (connectin) and nebulin.
- T-filaments remain after the thick and thin filaments have been stretched beyond their overlap. Their diameter is 2 nm. They possibly contribute to the toughness of meat.

however, useful even under farm conditions in the case of red deer stags (**Table 3**), and in breeding females with pregnancy or lactation occurring at a time when grazing conditions are poor.

Joint fat and the epicardial fat surrounding the vessels in the grooves of the heart are present even in starved animals, and also presumably have a mechanical role. Because of their size or location, none of these fat depots are significant for meat production.

Fat is a particularly valuable component of meat because of its influence on the flavor of cooked meat and its ability to improve palatability.

Fat is distributed in meat carcasses in three anatomically distinct locations. The subcutaneous depot (panniculus adiposus) lies on both sides of the superficial fascia and the cutaneous muscle layer (panniculus carnosus) (**Figure 20**). The intermuscular depot lies especially thickly in certain regions. The fat immediately beneath the thin shiny membrane lining

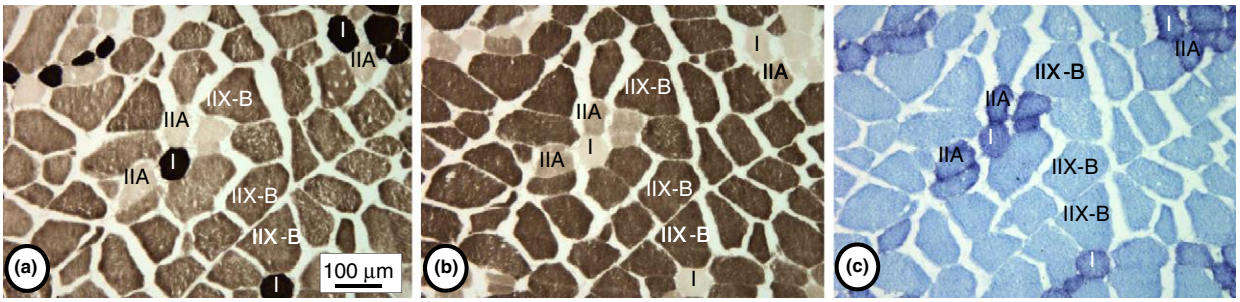

Figure 14 Intrinsic speed and metabolism in muscle fibers. Transverse serial sections of the pig longissimus dorsi muscle stained for myosin ATPase activity after section preincubation (a) at pH 4.45, (b) at pH 10.4, and (c) for succinate dehydrogenase. Type I fibers show a strong reaction after pH 4.45 preincubation (a), and a low reaction after pH 10.4 preincubation (b). IIX and IIB fibers not separable by this method and identified IIX–B are stained in dark grey while IIA fibers have an intermediate response (a and b). For succinate dehydrogenase staining (c), a dense reaction indicates a high mitochondrial density and therefore a high level of aerobic metabolism. Type I fibers are aerobic slow-twitch fibers, type IIA fibers are aerobic fast-twitch fibers, and IIX-B fibers are anaerobic fast-twitch fibers. Adapted from Davies, A.S., Gunn, H.M., 1972. Histochemical fibre types in the mammalian diaphragm. Journal of Anatomy 112, 41–60.

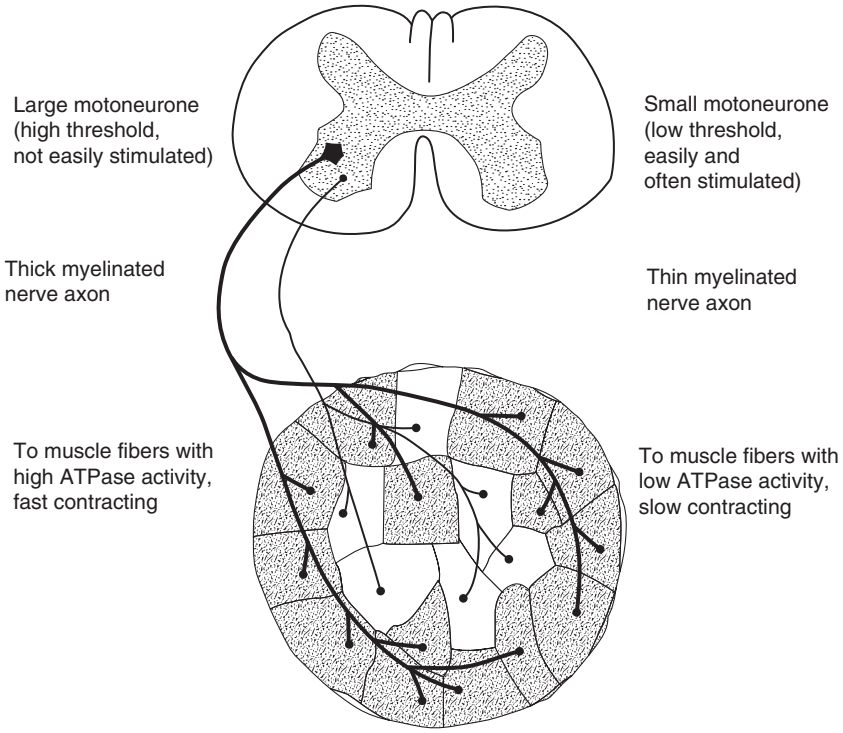

Figure 15 Innervation of fast and slow fibers. The speed of contraction of muscle fibers is related to motor neuron size. Small motor neurons are more readily, and hence more frequently, stimulated. They innervate slow fibers, which have a greater economy of energy use.

the body cavities (peritoneum and pleura) is called cavity fat and is especially thick around the kidneys and caudally into the pelvic cavity.

The subcutaneous fat depot is better developed in pigs than it is in ruminants (Table 4). In ruminants, it is possible to locate the sites in which it first develops perinatally, before they coalesce as the deposits grow (Figure 21). Each fat depot is composed predominantly of fat cells (adipocytes), which are spherical cells containing large amounts of lipid. Fat cell size varies between depots. When the cells are large, such as in the cavity fat surrounding the kidneys, the lipid content of the depot is higher (Table 5).

Subcutaneous, intermuscular, and cavity fat can be separated, with varying degrees of difficulty, from the other carcass tissues by dissection. Significant fat depots are also found within the perimysial connective tissue of muscles, as described above, as well as within the medullary cavity of bones. For this reason, and because fat has much less of a mechanical role than

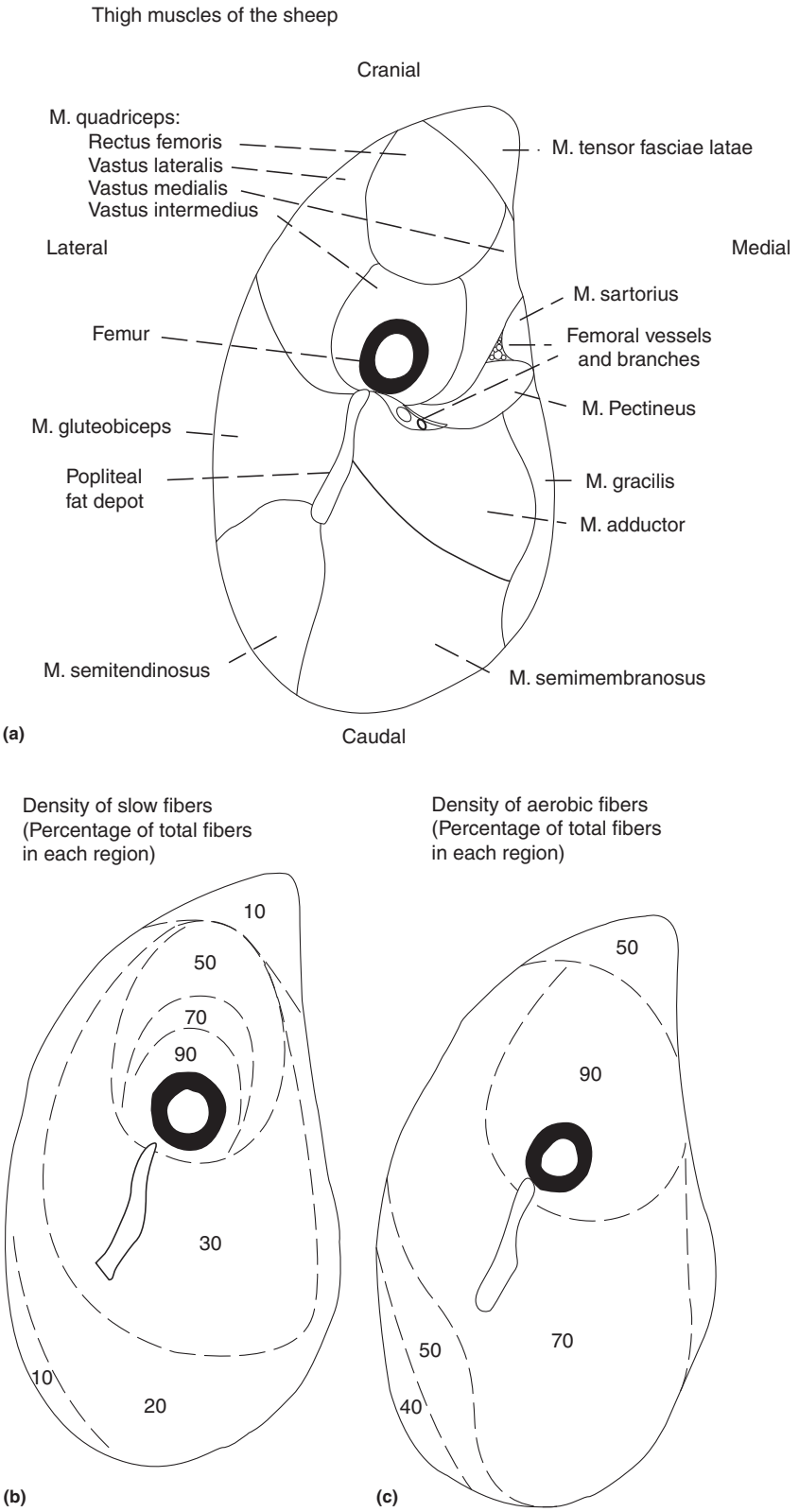

Figure 16 Heterogeneous distribution of fiber types in muscle. (a)Transverse section of the hind limb of the sheep. The density of slow-contracting fibers is shown in (b), and the density of fibers with a high level of aerobic enzyme activity is shown in (c). The muscles closest to the femur and the main blood supply to the limb have the highest density of slow, aerobic fibers. Adapted from Suzuki, A., Tamate, H., 1988. Distribution of myofiber types in the hip and thigh musculature of sheep. Anatomical Record 221, 494–502.

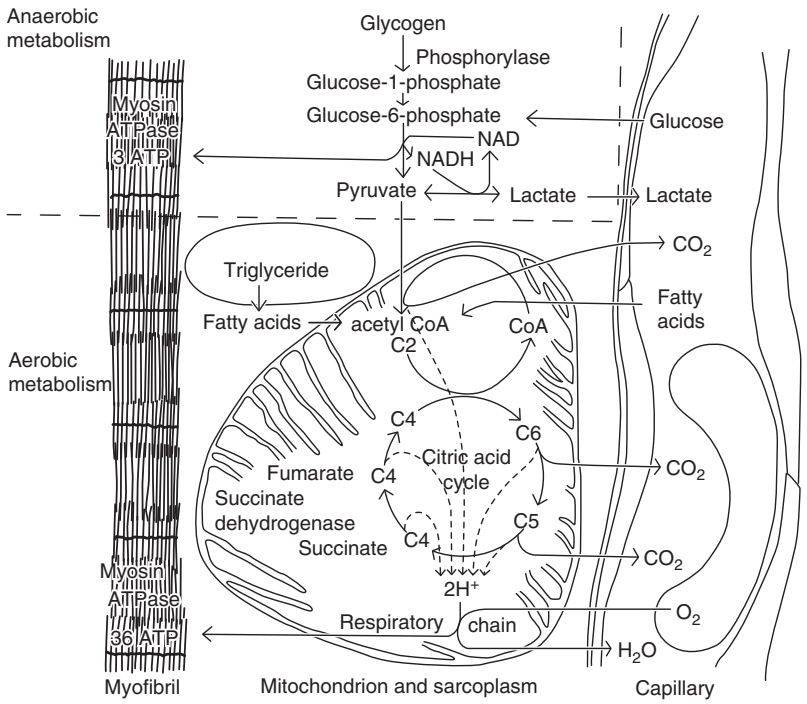

Figure 17 Aerobic and anaerobic metabolism in muscle. The metabolic processes enclosed by the dotted line in the upper left corner are those that required neither the availability of an extrinsic source of energy and oxygen nor the removal of metabolites from the muscle fiber. Under these circumstances, 3 mol of ATP are regenerated per mole of glucose-1-phosphate consumed. In contrast, 37 mol are regenerated per mole of glucose-1-phosphate metabolized through the citric acid cycle. The latter process is, however, effective only if an adequate blood supply is available. This cannot occur for all muscles during all periods of activity. This rationing of aerobic metabolism during life is curtailed following slaughter, when, without circulating blood, only anaerobic metabolism is possible. The length of carbon chains (between C2 and C6) is changed during the coenzyme A and citric acid cycles by the release of CO_2 as waste from aerobic metabolism. Lactate is the waste product from the anaerobic breakdown of glycogen.

Box 4 Mammalian muscle fiber types

An outline of chronological development of a classification system, showing the confused symbols used during the 1960s and the functional interpretation that became possible in the early 1970s. For most muscles of meat-producing animals, a functional classification of three types of fibers has been adequate in a variety of subsequent studies.

Date	*Species*	*Method*	*Classification*		
1873	Rabbit	Histology and physiology	Slow red		Fast white
1960	Rat, man	Histochemistry	I		II
1962	Rat	Mitochondrial distribution and histochemistry	B	C	A
1964	Rat	Histochemistry	III	II	I
1970	Pig	Histochemistry	Red	Intermediate	White
1970	Rat, man	Histochemistry	I	IIA	IIB
1970	Rat, cat	Histochemistry			
1971	Sheep	Histochemistry	C	A	B
1971	4 species (including ox, pig)	Histochemistry	β-red	α-red	α-white
1971	Cat	Physiology and histochemistry	Slow	Fast fatigue resistant	Fast fatiguable
1972	9 species (incl. ox, sheep, pig)	Histochemistry	Slow aerobic (fast aerobic and slow aerobic and anaerobic fibers were also identified)	Fast aerobic and anaerobic	Fast anaerobic
1972	Guinea pig, rabbit	Physiology, histochemistry, and biochemistry	Slow oxidative	Fast oxidative glycolytic	Fast glycolytic

muscle and bone and, hence, a less significant internal structure, chemical studies of total carcass fat are useful. However, these do not usually recognize the existence of anatomically definable fat depots and the morphological differences between them.

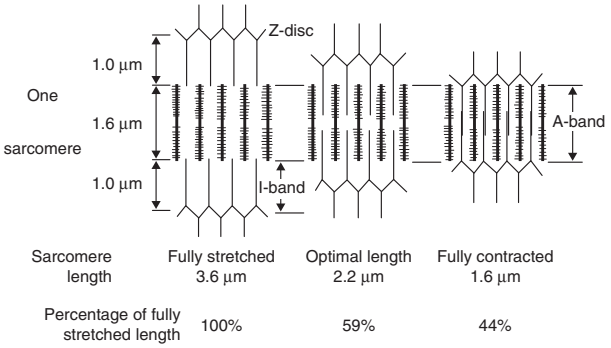

Figure 18 Sliding filaments in muscle. The sarcomere can normally contract to 44% of its fully stretched length. A sarcomere length of 2.2 μm is optimal for force generation, because the thin filaments are then maximally in apposition with the cross-bridges of the thick filaments. In some circumstances, if the fibers penetrate the Z-disk, sarcomere length can be as low as 1.3 μm.

The lipid within each fat depot is stored within the fat cells or adipocytes. Growth of a fat depot results from the degree of multiplication of adipocytes, depending on the depot (Table 5), but mainly in an accumulation of lipid droplets within the cytoplasm of cells.

Carcass Bone

Bone forms the passive part of the musculoskeletal system, in contrast to the active role of muscle. Whether functioning to support the animal or to make it move, muscles use energy. The main use of energy in bone is for growth and repair, processes that are remarkable in that they take place without loss of function. A bone is formed in a fetus as a cartilaginous template of its adult form and maintains an essential supporting role before and after birth while its tissues are replaced many times over (Figure 22).

Unlike muscle and fat, the structural components of bone tissue are formed outside the bone cells or osteocytes. Layered and sculpted formations of collagen and mineral remain in close contact with osteocytes so that they can direct growth and repair. Bones are remodeled extensively during growth,

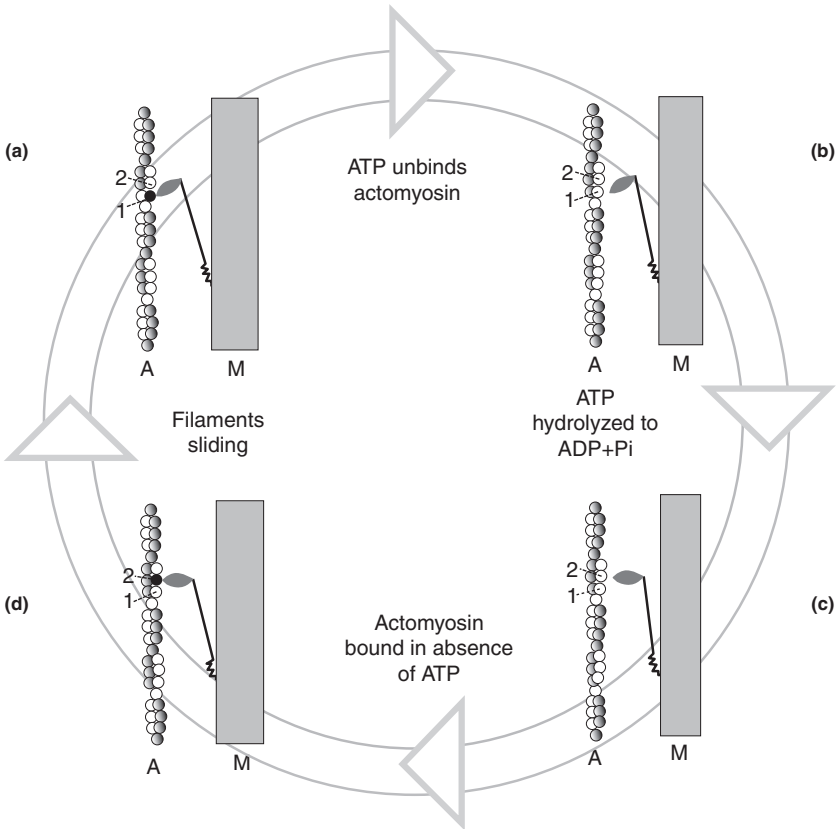

Figure 19 The actomyosin cycle. Reversible changes in myosin and actin association are due to the action of ATP and its products of hydrolysis. In the step (d) to (a), the actin filament A is 'rowed' past the thicker myosin filament M by an association of an adjacent globular actin molecule with the myosin cross-bridge. Adapted from Holmes, K.C., 1996. Muscle proteins – their actions and interactions. Current Opinion in Structural Biology 6, 781–789.

enabling regular complete replacement of collagen and mineral.

A bone is designed so that it performs a specific role with the lightest architectural structure possible. Such weight budgeting is essential because mineralized bones form the densest structures of the body and are therefore heavy to carry around. Of the tissues forming bone, fat is the least dense, followed by collagen and the blood-forming tissues, and then mineralized bone. The fat store within the bone is, therefore, located where there are no applied forces, whereas the mineralized cortex is thickest and densest where compression forces, in particular, are greatest.

Bones form a nutritional store, both of minerals and fat, but the bones of meat animals have no general value in human food production. The amount and distribution of carcass bone is, therefore, interesting mainly from a negative point of view because the amount of bone in any particular part of a carcass reflects how much of the part is to be considered as waste (Table 6). There are some breed differences in bone density that occur, and genetic gains in meat animal production can be made by selecting for lighter bone structure. Charolais cattle have distinctly less dense bone than Jerseys, for example, and the bone structures of wild and domesticated pigs appear quite different. The statement often made on mechanical grounds that larger animals must contain a higher

Table 3 Live weight and carcass changes in red deer stags. During the breeding season (rut), the single or combined effects of hormonal activity, physical activity, and/or the failure to graze result in a decrease in fat content but not apparently in the muscle and bone content of the carcass

Mean weight (kg)	*Prerut (n = 3)*	*Postrut (n = 3)*
Live weight	171	145
Carcass weight	100	85
Carcass muscle	70	68
Carcass bone	12.4	12.6
Carcass fat	19.4	4.6

Source: Adapted from Wallace, V., 1983. Pre- and postrut body composition of the red deer stag. B Phil Thesis, Massey University, Palmerston North, New Zealand.

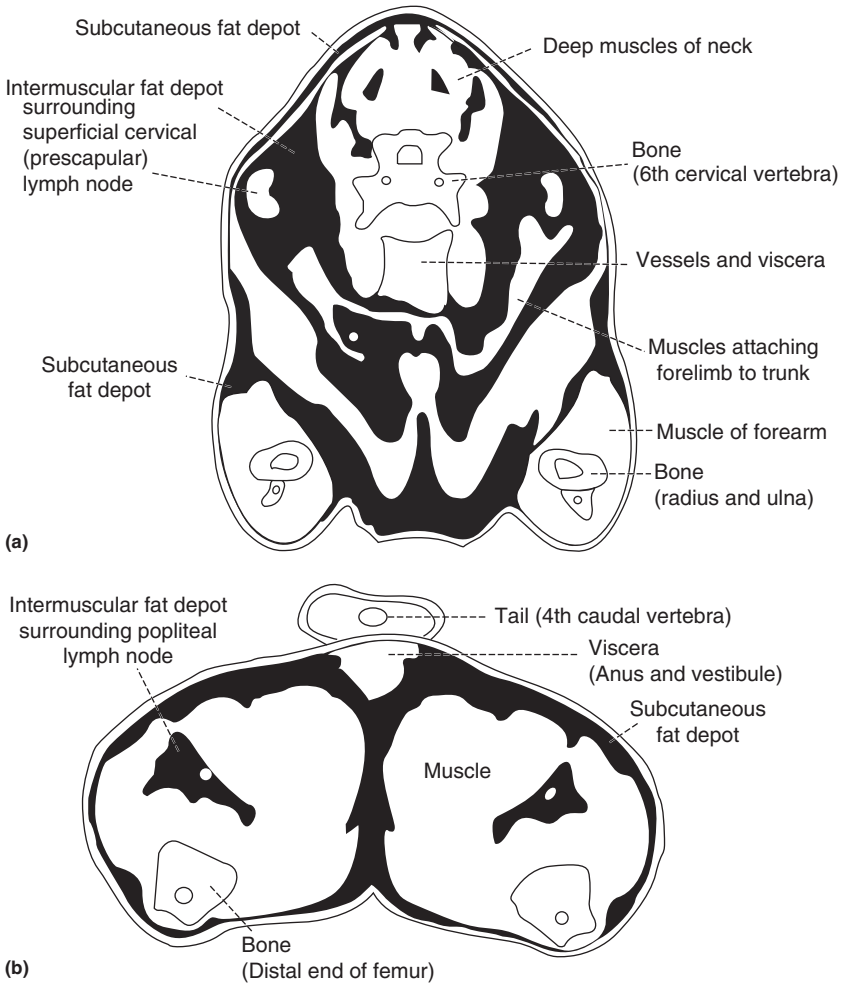

Figure 20 Distribution of fat in sheep carcasses. In these sections of the caudal neck region (a) and both thighs (b), the deposits of subcutaneous and intermuscular fat are shown in black. The drawings are adaptations of computed X-ray tomograms on a living sheep. Adapted with permission from Davies, A.S., Garden, K.L., Young, M.J., Reid, C.S.W., 1987. An Atlas of X-ray Tomo-graphical Anatomy of the Sheep. Wellington, New Zealand: DSIR-Science Information Publishing Centre.

proportion of bone is not shown in the tissue proportions of meat animals (Table 6). The ratio of muscle to bone in a carcass does, however, depend on the level of maturity of an animal. The proportion of the most desirable tissue, muscle, is higher in animals at a time when the fat proportion is higher than optimal and the collagen in their muscles breaks down less readily with cooking and the meat generally is, therefore, tougher. The age at which meat animals are slaughtered is decided by a trade-off of economic factors as well as the quality of the carcass based on muscle tenderness, fat development, and the muscle/bone ratio.

Table 4 The relative development of subcutaneous fat in lean, average, and fat pigs, sheep, and cattle. The values given are typical ranges for British livestock. With increasing fatness, the proportion of subcutaneous fat is higher, but the values for pigs are always much higher than in ruminants

Range of fatness	*Subcutaneous/intermuscular fat ratios*		
	Lean	*Average*	*Fat*
Pigs	3.0	3.7	4.0
Sheep	0.7	1.1	1.5
Cattle	0.3	0.6	0.9

Source: Adapted from Kempster, T., Cuthbertson, A., Harrington, G., 1982. Carcase Evaluation in Livestock Breeding, Production and Marketing. London: Granada.

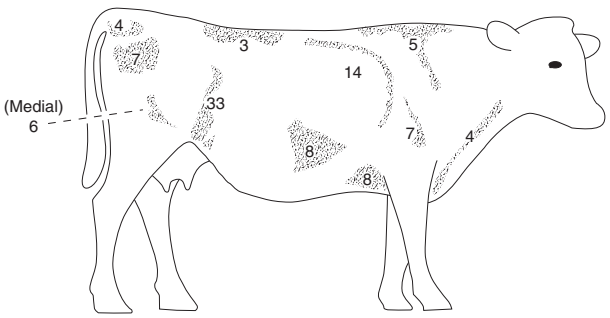

Figure 21 Subcutaneous fat distribution in cattle. The centers of subcutaneous fat development that become apparent around birth are shown here on an immature animal of 200 kg live weight. The proportions by weight of each area is shown as a percentage of the total subcutaneous fat weight of 1.5 kg. Data reproduced from Tan, G.Y., 1981. Carcass development and cellular growth of muscle and fat in male and female cattle. PhD Thesis, Massey University, Palmerston North, New Zealand.

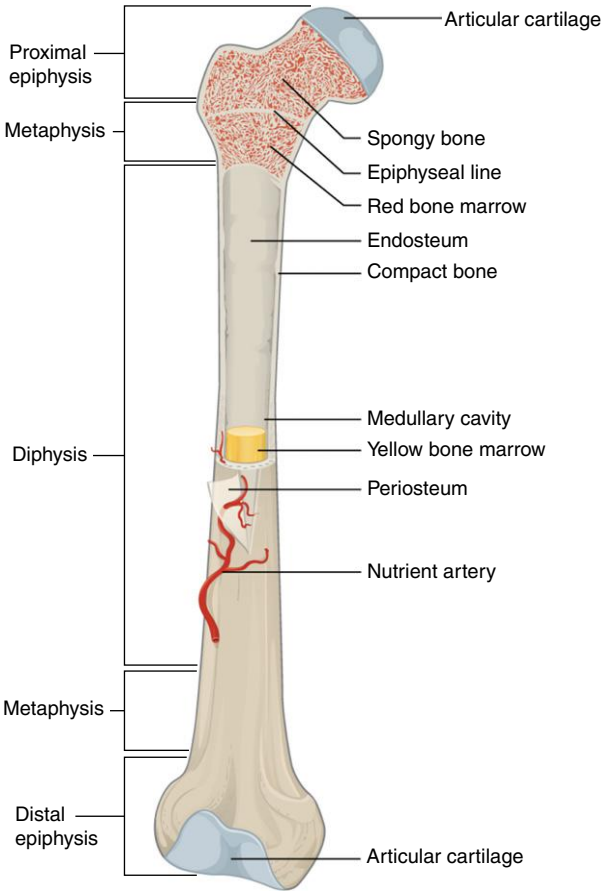

Figure 22 Tissues forming a bone. Reproduced from OpenStax College, 2013. Bone Structure. Connexions. Available at: http://cnx.org/content/m46281/1.3/ (accessed 24.09.13).

Table 5 The cellularity of fat deposits in cattle and sheep. The results are for the mature rams, and for bulls at 65% of mature live weight. Values for 'cavity fat' are for kidney fat in rams and for all the internal fat deposits in bulls. The lipid content of cavity fat (CAV) is higher than that for the subcutaneous (SC) and intermuscular (IM) depots. This corresponds to the larger fat cells of cavity fat and suggests that the better mechanically protected fat depots of the body can have larger fat cells with less supporting connective tissue between them

	Bulls			*Rams*		
	SC	*IM*	*CAV*	*SC*	*IM*	*CAV*
Lipid as a percentage of fresh depot weight	62.4	55.6	79.4	88.5	80.3	93.7
Fat cell volume ($\mu m^3 \times 10^5$)	0.40	0.67	1.03	0.55	0.63	1.18

Source: Data from Robelin, J., 1981. Cellularity of bovine adipose tissues: developmental changes from 15 to 65 percent mature weight. Journal of Lipid Research 22, 452–457 and Thompson, J.M., Butterfield, R.M., 1988. Changes in body composition relative to weight and maturity of Australian Dorset Horn rams and wethers. Adipocyte volume and number in dissected fat partitions. Animal Production 46, 387–393.

Table 6 Proportions of bone in carcass cuts. Muscle to bone ratios for several cuts and for the whole side of 300 kg bull carcasses

Carcass part	*Muscle to bone ratio*
Round	5.91
Loin	4.84
Fore rib	3.45
Fore shank	1.21
Total side	4.10

Source: Calculated from the data of Berg, R.T., Andersen, B.B., Liboriussen, T., 1978. Growth of bovine tissues. Genetic influences on muscle growth and distribution in young bulls. Animal Production 27, 51–61 and Genetic influences on patterns of bone growth and distribution in young bulls. Animal Production 27, 71–77.

See also: Chemical and Physical Characteristics of Meat: Adipose Tissue; Palatability. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Glycolysis. Growth of Meat Animals: Adipose Tissue Development; Endocrinology; Growth Patterns; Metabolic Modifiers; Muscle; Physiology. Modeling in Meat Science: Meat Quality

Further Reading

Bailey, A.J., Light, N.D., 1989. Connective Tissue in Meat and Meat Products. London: Elsevier Applied Science.

Bechtel, P.J., 1986. Muscle development and contractile proteins. In: Bechtel, P.J. (Ed.), Muscle as Food. Orlando, FL: Academic Press, pp. 1–35.

Davey, C.L., 1984. The structure of muscle and its properties as meat. In: Bailey, A. J. (Ed.), Recent Advances in the Chemistry of Meat. London: Royal Society of Chemistry, pp. 1–21.

Eisenberg, B.R., 1983. Quantitative ultrastructure of mammalian skeletal muscle. In: Peachey, L.D., Adrian, R.H., Geiger, S.R. (Eds.), Handbook of Physiology. Bethesda, MD: American Physiological Society, pp. 73–112. section 10, Skeletal muscle.

Kempster, T., Cuthbertson, A., Harrington, G., 1982. Carcase Evaluation in Livestock Breeding, Production and Marketing. London: Granada.

Leat, W.M.F., Cox, R.W., 1980. Fundamental aspects of adipose tissue growth. In: Lawrence, T.J.L. (Ed.), Growth in Animals. London: Butterworths, pp. 137–174.

Marcondes, M.I., Tedeschi, L.O., Valadares Filho, S.C., Chizzotti, M., 2012. Prediction of physical and chemical body compositions of purebred and crossbred Nellore cattle using the composition of a rib section. Journal of Animal Science 90, 1280–1290.

Marks, S.C., Popoff, S.N., 1988. Bone cell biology: The regulation, of development, structure and function of the skeleton. American Journal of Anatomy 183, 1–44.

Olsen, B.R., Reginato, A.M., Wang, W., 2000. Bone development. Annual Review of Cell and Developmental Biology 16, 191–220.

Relevant Websites

http://bovine.unl.edu/
Bovine Carcass Anatomy.

http://www.fao.org/docrep/010/ai407e/AI407E03.htm
Carcass Composition.

http://www.wisc-online.com/Objects/ViewObject.aspx?ID=AP14504
Carcass Composition.

http://www.fao.org/DOCREP/004/T0279E/T0279E05.htm
Carcasses Composition.

http://en.wikipedia.org/wiki/Muscle_contraction
Muscle Contraction.

http://www.youtube.com/watch?feature=endscreen&NR=1&v=e3Nq-P1ww5E
Muscle Contraction.

http://porcine.unl.edu/porcine2005/pages/index.jsp
Pig Carcass Anatomy.

CHEMICAL ANALYSIS

Contents

Analysis of Final Product Composition for Labeling

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Introduction

Labeling refers to the compilation of nutritional and nutrient content information printed on the packaging of retail-ready food products. Many countries have legislation that stipulates that nutritional labels must be printed on most, if not all, retail-ready foods. What nutritional or nutrient component qualifies to be included on a label varies to some degree from country to country. On a global scale, greater harmonization of food labeling standards is being developed through the Codex Alimentarius Commission. The overall purpose of labeling the nutritional content of food is to allow the purchaser of the food product to make an informed decision about the nutritional and nutrient value of the food, to protect the consumer from false and misleading claims, and to promote healthy diets. Nutritional and nutrient labeling requires product sampling and analytical methods that reliably measure nutritionally relevant processed food product components. Standard analytical methods, such as those of the International Organization for Standardization (ISO) and Association of Analytical Communities (formally Association of Official Agricultural Chemists) International, should be used, so that analyte values reported on food labels are consistent between countries. Values for analytes of many foods and food components already exist in numerous databases that can be sourced by food manufacturers to compile nutritional labels for final product composition without having to chemically test new formulations. However, chemical testing is still required to determine values of nutrients in new processed food product, when nutritional requirements change or for compliance verification.

Current Labeling Requirements

Most food manufacturing countries have legislation covering labeling requirements for retail foods. However, details may vary between countries. For example, in the US the Food and Drug Administration (FDA) Nutritional Labeling Education Act (NLEA) of 1990 amends the Food, Drug, and Cosmetic Act (FD&CA), and addresses serving size, health claims, and nutrient content labeling to help increase the consumer's ability to make healthy dietary choices. Currently, under the NLEA, full nutritional labeling is mandatory for 14 nutrients and voluntary for 36 additional nutrients. In 2003, the FDA amended the food labeling regulations to require *trans*-fatty acid declaration. The Food Allergen Labeling and Consumer Protection Act of 2004 further amends the FD&CA. It requires food labels to state the presence of the eight major food allergens identified by the act: milk, eggs, fish, shellfish, tree nuts, wheat, peanuts, and soybeans.

The European Union (EU) Regulation No 1169/2011 for Nutritional Labeling for Foodstuffs states that a nutrition declaration is mandatory. The nutrition label should include:

1. Energy value; or
2. Energy value and one or more of the following nutrients only:
   a. fat (unsaturates, monosaturates, and polysaturates),
   b. carbohydrate (sugars, polyols, and starch),
   c. salt,
   d. fiber,
   e. protein, and
   f. any of the vitamins or minerals listed in Annex XIII of the Regulation.

Figure 1 shows the Regulation's requirements for the expression and presentation of the nutritional declaration.

An increasing number of foods labeled and advertised bear nutrition and health claims. To ensure a high level of protection for consumers and to facilitate their choice, the European Community (EC) has issued a Regulation (No. 1924/2006) on Nutrition and Health Claims Made on Foods so that

Energy	kJ/kcal
Fat	g
of which	
– Saturate,	g
– Mono-saturates,	g
– Polyunsaturates,	g
Carbohydrate	
of which	
– Sugars,	g
– Polyols,	g
– Starch,	g
Fibre	g
Protein	g
Salt	g
Vitamins and minerals	The units specified in point 1 of Part A of Annex XIII

Figure 1 Expression and presentation of nutritional declaration according to Annex XV of EU No 1169/2011.

Table 1 Maximum fat and connective tissue content for ingredients designated by the term 'meat'

Species	*Fat content (%)*	*Collagen/meat protein ratio (%)*[a]
Mammals (other than rabbits and porcines) and mixtures of species with mammals predominating	25	25
Porcines	30	25
Birds and rabbits	15	10

[a]The collagen/meat protein ratio is expressed as the percentage of collagen in meat protein. The collagen contentis the hydroxyproline content multiplied by a factor of 8.

products put on the market are safe and adequately labeled. Article 4 of Regulation (No. 1924/2006) proposed the establishment of specific nutrient profiles that foods or certain groups of foods must respect to bear nutrition and health claims. The use of nutrient profiles aims to avoid a situation where nutrition or health claims could mislead consumers as to the overall nutritional quality of a food product when trying to make healthy choices in the context of a balanced diet. The Regulation requires that the setting of nutrient profiles should take into account the dietary role and importance of food groups and their contribution of nutrients to the overall diet of the population. Meat and meat products is one of the food groups with important dietary roles. However, to date the rule to require nutrient profiles has not been implemented because of disagreement with setting nutrient thresholds for some foods.

What constitutes a meat product? Excluding the obvious – meat cuts and anatomical parts that are sold without further processing – products that contain meat as an ingredient are usually defined in legislation. The Commission of the European Communities (Annex I to Directive 2000/13/EC) defines a meat product as containing skeletal muscle including naturally occurring attached fat and connective tissue subject to maximum limits (see Table 1). If these maximum limits are exceeded in formulations, the labeled 'meat' content must be downgraded accordingly and the list of ingredients must mention the presence of fat and/or connective tissue. Other animal parts, such as offal (including the heart, intestine, and liver) or fat, have to be labeled as such and not as 'meat.' Mechanically recovered meat (MRM) differs significantly from 'meat' as perceived by consumers and is excluded from the scope of the 'meat' definition. MRM, designated by the name of the species, has to be labeled separately and cannot form part of the meat content of any products in which it occurs. Mechanically recovered beef is banned entirely because of bovine spongiform encephalopathy (BSE). The BSE outbreak in Europe motivated the introduction of the compulsory (EC No. 1760/2000) beef labeling system requiring operators and organizations marketing beef to indicate its origin, in particular where the animal or animals from which the beef was derived were born, fattened, and slaughtered. A complimentary voluntary system covers labeling information on breed, type of production, and animal age, as well as information on the way the animals are reared and fed and their general welfare. This additional information is considered to help differentiate their products by identifying particular characteristics and thereby gain a commercial advantage, and to provide a more precise legislative framework than that created by the general principles of the labeling directive (Directive 2000/13/EC).

In addition to the 'meat' content defined by the Commission of the European Communities, the following particulars are compulsory in the labeling of foodstuffs:

- The name under which the foodstuff is sold.
- The list of ingredients, in descending order of weight. Important additions include compound ingredients, added water/concentrated foods, and cheese. The following ingredients require a specific statement on the label: genetically modified organisms, packaging gases, sweeteners, aspartame and polyols, quinine, and caffeine.

- The quantity of certain ingredients or categories of ingredients (Quantitative Ingredient Declaration).
- The net quantity of prepackaged foodstuffs expressed in metric units (liter, centiliter, milliliter, kilogram, or gram).
- The date of minimum durability in a specific format or the 'use by' date for highly perishable foodstuffs.
- Any special storage conditions or conditions of use.
- The name or business name and address of the manufacturer, packager, or vendor established within the Community.
- Particulars of the place of origin or provenance in cases where absence of such information might mislead the consumer.
- Instructions for use.
- The actual alcoholic strength for beverages containing more than 1.2% alcohol by volume.
- A mark to identify the lot to which a foodstuff belongs.
- Treatments undergone, with specific indications for irradiated foods or deep-frozen foods.

Global food trading requires greater harmonization of food labeling standards. This harmonization is being developed through the Codex Alimentarius Commission (CAC). The Codex Alimentarius (CAC) is a series of food standards and related texts that aim to provide a high level of consumer protection and fair practice in the international trade of food and agricultural products. The CAC, which administers the Codex standards and related texts, is an intergovernmental body jointly sponsored by the Food and Agriculture Organization and the World Health Organization.

Nutritional Databases

Compilations of nutrient data for various food components and products constitute nutritional labeling databases. Most of these databases are derived from published or proprietary chemical composition tables (e.g., United States Department of Agriculture (USDA), National Nutrient Database for Standard Reference). Some information contained within the databases is derived from ingredient databases that comprise components of a product's recipe. Software is used to assist food retailers and manufacturers in deriving average nutrient quantities for calculating information for a nutritional label. For example, the Food Standards Australia New Zealand website provides a Nutrition Panel Calculator to help food manufacturers calculate the average nutrient content of their food products and prepare a nutrition information panel. When nutritional information is unavailable in nutritional databases, when new food products are developed, or when food products require compliance testing, reliable and accurate analytical test methods are required to ensure that robust nutrient values are obtained for food products requiring labeling.

Chemical Analysis

Product Sampling

Meat and meat products are generally not as homogeneous as other animal-derived foods such as milk. The composition of meat can vary significantly within a carcass apart from the variation resulting from differences of species, breed, age, and nutritional status of the supply animals. This natural variation, along with other food components added during meat processing, warrants careful attention to a well-designed sampling plan to ensure that whatever nutritional claims are made on a food label are as representative as possible of the average composition of the final food product. Because most analytical methods are invasive and destroy part of the product tested, it is impractical to test every unit of a particular product. Instead, a sampling plan designed to take a representative sub-set of the total population of production units is needed. It is important to recognize that the samples being analyzed must be representative of the product that will be labeled with the results of the chemical analysis. If current production is not representative of future production, it is inappropriate to use current batches for sampling for representative testing.

The sampling plan must take into consideration any factors that will impact on the nutrient and composition content of the product. These could include changes in raw materials, processing procedures, storage conditions, and packaging and retail display conditions. The last three factors are important, and sometimes overlooked, when considering vitamin content of some food products, for example.

The FDA in the US recommends that at least 12 individual consumer units be selected randomly from a lot and then combined to make three composite samples of a minimum of four consumer units each. The three composite samples are then analyzed, and the mean value provides an estimate of the lot nutrient content.

Test Sample Preparation

Meat and meat products contain protein, fat, water, and ingredients from other food sources such as cereal-based material, stabilizers, and flavorings. Composite samples must be rendered homogenous, so that no biases in test results are encountered during analysis. Meat, fat, and connective tissue are particularly prone to causing problems during sample preparation. Excessive mixing or blending can result in the temperature of the sample rising excessively, causing fat to separate from the lean meat component and adhere to the surface of a mincer or food processor. Blunt mincing blades are ineffective in cutting connective tissue, resulting in the tissue collecting around the mincing auger.

An effective method of preparing homogeneous meat product samples is to soft-freeze the meat, dice it into chunks of approximately 2 cm with a knife, and pass it twice through a 3 mm mincing plate that has been precooled to approximately 4 °C. Alternatively, the diced chunks can be blended in a domestic or commercial food processor. Mincers or food processors should be of adequate size to accommodate all the composite samples to be mixed at one time. Once blended, samples are stored in an airtight container with minimum airspace to prevent the sample drying out. Samples stored in a refrigerator or freezer should be remixed thoroughly before analysis.

Test Procedures

Standard analytical methods, for example, those of the ISO and AOAC International, which have undergone peer review

Table 2 Association of Analytical Communities (formally Association of Official Agricultural Chemists, AOAC) methods[a] recommended for nutritional analysis

Component	*AOAC method numbers*
Protein	976.05, 981.10, 928.08, 977.14, 992.15, 960.39, 976.21, and 985.15
Fat	996.06 (Sum of fatty acids expressed as triglycerides)
Ash	920.153
Moisture	950.46 B (a) or (b)
Unsaturated/saturated fats	996.06
Trans-fatty acids	996.06 (Revised with 100 m column)
Cholesterol	994.10 and 976.26
Vitamins A	974.29
Vitamin C	984.26
Fiber	992.16, 985.29, 991.42, and 991.43
Sodium	968.08
Iron	985.01
Calcium	968.08 and 983.19
Sugars	Not available
Carbohydrate (mono- and disaccharides+starch+fiber+sugar alcohols +polydextrose)	By difference (100−%ash−%moisture−%protein−%fat)×(100/(100 $-CT_{limit}$))

[a]AOAC International (2000).

and collaborative laboratory comparison, should be used, so that analyte values reported on food labels are consistent between countries. The US Food Safety and Inspection Service specifies for nutritional analysis the methods of the USDA Analytical Chemistry Laboratory Guidebook; otherwise the Official Methods of Analysis of the AOAC International are to be used. Common methods of analysis for components listed on nutritional labels are given in Table 2.

Some vitamins and minerals in processed foods can undergo changes when exposed to heat, air, or light. Minerals such as copper, iron, and zinc can be affected by moisture, and may react with other food components such as proteins and carbohydrates. Vitamins and minerals can also be lost through leaching into cooking/processing water. It is important to take into account possible nutrient changes during processing and storage of meat products before consumption. One area that possibly needs more attention is the effect of storage on nutrients in meat products. Final product testing is usually done on freshly processed product, with little attention given to changes that might occur during cold chain transportation and storage.

Calculations

Meat Content

Many countries require statements of minimum meat content of meat products. Meat content can be calculated after the determination of the product's nitrogen, fat, carbohydrate, and collagen content. The Nitrogen Factor Method, adopted by the UK Food Standards Agency, is a method used for calculating the amount of meat, allowable fat and connective tissue, and excess fat and connective tissue in a meat product. The percentage of nitrogen, on a fat-free basis, is known as the nitrogen factor.

1. Meat protein% (P_M) (excluding contributions from non-meat nitrogenous sources) = meat nitrogen%×6.25.
2. Carbohydrate% (C) = 100−(water%+fat%+protein%+ash%).

Table 3 Some nitrogen factors for meat

Type of meat	*Nitrogen factor*	*References*
Beef	3.65	[a]
Pork	3.50	[b]
Lamb	3.50	[c]
Mutton	3.47	[d]
Chicken	3.70	[e]
Turkey	3.65	[f]

[a]Analytical Methods Committee, 1993. Nitrogen factors for beef: A reassessment. Analyst 118, 1217.
[b]Analytical Methods Committee, 1991. Nitrogen factors for pork: A reassessment. Analyst 116, 761.
[c]Analytical Methods Committee, 1996. Nitrogen factors for sheep-meat. Part 2; lamb meat. Analyst 121, 889.
[d]Analytical Methods Committee, 1995. Nitrogen factors for sheep. Part 1; mutton. Analyst 120, 1823.
[e]Analytical Methods Committee, 2000. Nitrogen factors for chicken meat. Analyst 125, 1359.
[f]Analytical Methods Committee, 2002. Nitrogen factors for turkey meat. Analyst 127, 859.

3. Connective tissue% (CT%) = (collagen%/P_M)×100 where collagen = (8×%hydroxyproline).

Added Water

Added water is subject to maximum limits and must be declared in the labeling of meat products. In general, for 'pure meat' the percentage of fat-free meat plus percentage of fat is taken as the meat content; if this does not add up to 100% then the discrepancy is considered 'added water.' Calculated apparent added water is dependent on the meat nitrogen factor (N_{ff}). Nitrogen factors (the percentage of nitrogen, on a fat-free basis) for a variety of meats are published by the Nitrogen Factors Subcommittee of The Royal Society of Chemistry (Table 3). In Europe, the three most common methods of calculating added water are the German Method, the Danish

Table 4 Some energy factors derived from Atwater tables

Class of food	Energy factor					
	Protein		Fat		Carbohydrate	
	(kJ g^{-1})	(kcal g^{-1})	(kJ g^{-1})	(kcal g^{-1})	(kJ g^{-1})	(kcal g^{-1})
Poultry	17.9	4.3	37.7	9.0	16.2	3.9
Beef	17.9	4.3	37.7	9.0	NA	NA
Beef, processed	17.9	4.3	37.7	9.0	15.4–16.9	3.7–4.0
Food of animal origin	17.9	4.3	37.5	9.0	16.2	3.9
Food of plant origin	15.0	3.6	36.6	8.7	16.7	4.0
Total food	16.7	4.0	37.3	8.9	16.6	4.0

Abbreviation: NA, not available.

Method, and a modification of the Stubbs and More Method. The Danish Method is probably the most used method for the calculation of added water and initially needs only the prior analysis of total water and nitrogen content of the meat product. If the resultant calculated 'apparent' added water value is excessive, then a more rigorous chemical analysis including fat, nitrogen, ash, and water is required for calculation of the added water content.

Danish Method I (initial)

%Added water = %total water − [(%nitrogen × 100/N_{ff}) − %protein]

Danish Method II (confirmatory calculation)

%Added water = 100 − [(%nitrogen × 100/N_{ff}) + %fat + (%Ash − 1)]

where (%nitrogen × 100/N_{ff}) is referred to as the %fat-free meat (%FFM).

Energy Values

The energy factor is the metabolizable energy (ME) of the food component calculated according to eqn [1], expressed in kilojoules (or kilocalories) per gram of food component, rounded to the nearest whole number.

$$ME = GE - FE - UE - GaE - SE \quad [1]$$

where ME, metabolizable energy; GE, gross energy (as measured by bomb calorimetry); FE, energy lost in feces; UE, energy lost in urine; GaE, energy lost in gases produced by fermentation in the large intestine; and SE, energy content of waste products lost from surface areas.

Energy factors have been derived by bomb calorimetry for a number of food types (see Table 4). Using the values in the table, the energy value of specific foods can be calculated. The number of grams of each of the macronutrients (protein, fat, alcohol, and carbohydrate) per 100 g of product is derived analytically. The energy value for each component is then calculated as in eqn [2] using an appropriate energy factor.

$$\begin{aligned}\text{Energy (kJ)} = {} & (\text{kJ g}^{-1}\ \text{protein} \times \text{g protein}) \\ & + (\text{kJ g}^{-1}\ \text{fat} \times \text{g fat}) \\ & + (\text{kJ g}^{-1}\ \text{carbohydrate} \times \text{g carbohydrate}) \\ & + (\text{kJ g}^{-1}\ \text{alcohol} \times \text{g alcohol}) \end{aligned} \quad [2]$$

The original work to determine energy factors for a range of foods was done by Atwater and others between 1899 and 1912 and was extensively reviewed in 1973, resulting in minor modifications. Generalized factors of 17/37/17 (kJ g^{-1}) or 4/9/4 (kcal g^{-1}) for carbohydrate, fat, and protein, respectively, are in common use. Average energy value for fiber should be 8 kJ g^{-1} (2 kcal g^{-1}).

Energy values can be expressed for nutritional labeling purposes either as kilocalories (kcal) or kilojoules (kJ) using the conversions factor in eqn [3]

$$1\ \text{kcal} = 4.184\ \text{kJ} \quad [3]$$

See also: Chemical Analysis: Raw Material Composition Analysis; Sampling and Statistical Requirements; Standard Methods. Chemical Analysis for Specific Components: Major Meat Components; Micronutrients and Other Minor Meat Components. Laboratory Accreditation. Microbiological Analysis: Standard Methods. Nutrient Claims on Packaging

Further Reading

AOAC International, 2011. Official Methods of Analysis, eighteenth ed. Gaithersburg, MD: AOAC International Reviews 4.

Bender, M., Rader, J., McClure, F., 1998. FDA Nutritional Labeling Manual – A Guide for Developing and Using Data Bases. Washington, DC: US Department of Health and Human Services, US Food and Drug Administration.

Codex Alimentarius Commission, Codex Alimentarius. Nutrition and Health Claims Made on Foods. Regulation (Ec) No 1924/2006 The European Parliament and the Council of the European Union. FAO, Rome.

FAO, 2003. Food energy – methods of analysis and conversion factors. FAO Food and Nutrition Paper 77, Food and Agriculture Organization of the United Nations, Rome.

Food Standards Agency, 2003. Labelling and Composition of Meat Products, Guidance Notes. London: Food Standards Agency.

FSANZ (Food Standards Australia and New Zealand). Available at: http://www.foodstandards.gov.au/foodstandards/nutritionpanelcalculator/ (accessed 11.10.13).

Körmendy, L., Zukál, E., Caspó, I., Török, N., 2007. Problems relating to the calculation of meat equivalent and 'meat content' with particular reference to the QUID (Quantitative Ingredient Declaration) regulation. Acta Alimentaria 36 (4), 439–453.

McLean, B., 1999. Meat and Meat Products: The Calculation of Meat Content, Added Water and Connective Tissue from Analytical Data. UK: Campden & Chorleywood Food Research Association Guideline No. 22.

Merrill, A.L., Watt, B.K. (Eds.), 1973. Energy Value of Foods – Basis and Derivation. Washington, DC: US Government Printing Office. USDA Handbook No. 74 (slightly revised).

Pennington, J.A.T., Stumbo, P.J. (Eds.), 2004. Papers from the Joint Meeting of the 5th International Food Data Conference and the 27th US National Nutrient Databank Conference, 2004. Journal of Food Composition and Analysis 17, 3–4, 247–580.

Schakel, S.F., Buzzard, M.I., Gebhardt, S.E., 1997. Procedures for estimating nutrient values for food composition databases. Journal of Food Composition and Analysis 10, 102–114.

Sullivan D.M., Carpenter D.E. (Eds.), 1993. Methods of Analysis for Nutritional Labeling. Arlington, VA: AOAC International.

Turner, A., 1995. Prepacked food labeling: Past, present and future. British Food Journal 97 (5), 22–31.

Physicochemical Analysis Methods

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Introduction

Analytical chemistry is concerned with identifying and determining a compound (the analyte) in a sample (the matrix). Occasionally, only the presence or absence of an analyte is determined; this is called qualitative analysis – in contrast to quantitative analysis where the actual concentration of the analyte is established. Qualitative analysis is seldom of use in meat science, and consequently this article is concerned solely with quantitative analysis, i.e., with verifying amounts (concentrations) of analytes. Concentrations can be estimated by a number of direct or indirect techniques, and here a number of examples of particular interest to meat science will be given.

Chemical analysis, or more correctly physicochemical analysis, is of interest in this context because it is intimately involved one way or another in almost all areas of meat science. Breeding programs, feed conversion studies, monitoring of residues of unwanted chemicals, nutritional studies, curing processes, fermentation processes, etc., all need chemical analysis data either as support or as a primary source of information in making decisions or achieving progress.

Consequently, the nature of the analytes and their abundances span the full spectrum: from elements to complicated organic molecules and concentrations expressed in percentage to parts per trillion. This, of course, impacts heavily on the number of methods needed and their complexities.

The most widely used instrumental techniques in current meat and meat product analysis are discussed. However, owing to space limitations, the treatment must be selective rather than comprehensive, and it is chosen to emphasize the traditional 'chemical' techniques. Enzymatic and immunological methods are, among others, not covered. For the same reason, the methods mentioned are not treated in any great detail; for some, though, more particulars are included when this appears justified.

Methods that are considered obsolete, although they are still being used in some laboratories, are omitted. Instead, their more modern counterparts are included.

Finally, the text is broken down into subsections in a natural manner, starting with pretreatment methods followed by classical methods such as gravimetry and volumetry and moving upward in complexity to end with sophisticated mass spectrometry (MS).

Sample Pretreatment

Samples taken from carcasses, i.e., different kind of tissue samples such as muscle, organs, fat, blood, etc., and samples of meat products are all rather complicated matrices. In contrast to some liquid samples, they need to be homogenized before analysis is attempted to secure representative samples and valid, meaningful results. The aspects of sampling are dealt with elsewhere, but it seems appropriate to list here some of the most common pretreatment methods currently being used.

Homogenization

Most samples encountered in a meat science laboratory are inhomogeneous and must therefore be homogenized to obtain representative samples. Mincing or blending of the samples usually achieves this. Connective tissue is sometimes an obstacle for adequate homogenization, and consequently several passages through the mincer are needed. In such circumstances, treatment in a blender appears to perform less satisfactorily.

Ashing/Mineralization/Hydrolysis

For elemental analysis, or the determination of ash content, a convenient way of separating the analyte from most of the matrix is by total oxidation of the organic constituents in the sample. This is referred to as ashing or mineralization, and it may be done in a wide variety of ways.

The simplest is probably complete combustion of the sample in a muffle furnace; this is used for determination of ash content, and as a first step in the analysis of many elements. However, the method must not be used uncritically in elemental analysis, as many elements, for example, mercury, arsenic, selenium, lead, or tin, will be (partly) lost owing to their high volatility.

Wet oxidation with oxidizing acids at elevated temperatures is often used, sometimes with the addition of a catalyst as in the Kjeldahl destruction. Often wet oxidation at elevated pressure as well as elevated temperature is called for, either in the so-called bombs (metal containers with quartz or Teflon inner beakers) or in heavy-walled Teflon or Kel-F containers. In the latter case microwave-assisted heating is often used.

For some analyses only partial breakdown is necessary, for example, when degradation of protein into individual amino acids is the target. This is the case in the predominant method for determination of collagen, which rests on a quantification of the amino acid hydroxyproline.

Extraction

Many analytical methods comprise one or more extraction steps in order to separate analyte from matrix.

Simple extraction

Simple extraction of a (finely divided) matrix with a (hot) solvent is in some cases sufficient, whereas multiple extractions in a Soxhlet apparatus under other circumstances are necessary. A variation on the theme of simple extractions is found in the accelerated solvent extraction method, which utilizes a solvent or mixture of solvents at elevated pressure and temperature. Supercritical fluid extraction works in a similar manner, but in this case a supercritical fluid is the extractant; CO_2 is the most commonly used compound for achieving the supercritical state and, as it evaporates rapidly under ambient conditions, the technique has the advantage of leaving the analyte solvent-free.

Liquid–liquid extraction

Liquid–liquid extraction (partitioning) between a polar and a nonpolar solvent is another versatile method. Often several extractions are carried out with pH changes in the polar solvent, facilitating a more complete separation of the analyte.

Solid-phase extraction

Solid-phase extraction (SPE) is a method similar to simple extraction; the main difference is that the extractant is a solid. The method is often used as a clean-up step after an initial simple or liquid–liquid extraction, where the analyte plus residual matrix elements are in a solution. The solution is treated with the solid extractant, which extracts either the analyte or matrix elements. In the former case, the analyte is subsequently liberated from the solid by another liquid extractant. Prefabricated cartridges filled with the solid extractant are commonly used. Immunoaffinity columns may be considered a subclass of SPEs.

Solid-phase microextraction

Solid-phase microextraction utilizes a thin coated fiber to extract analyte molecules from a gaseous or liquid phase. The fiber is subsequently introduced directly into a gas chromatographic column, where the extracted molecules are evaporated and separated.

Matrix solid-phase dispersion

Matrix solid-phase dispersion is a method that sometimes can be used successfully to extract residues, in particular residues of veterinary drugs, directly from a homogenized tissue. A solid extractant, for example, a C_{18} reversed-phase liquid chromatography (LC) column material, is mixed thoroughly (dispersed) directly with the tissue, using mortar and pestle. The large surface area of the column material and mechanical treatment of the mixture secure an efficient extraction.

Chromatographic Separation

Methods such as open-column- and thin-layer chromatography (TLC) are sometimes used as separation steps before the actual analysis takes place.

Derivatization

It often happens that the analyte has to be modified for it to be analyzed by a suitable method; in some instances because the analyte is 'difficult,' alternatively because the analyst would like to use a method to which the analyte is not suited per se.

Examples of the first case are encountered when the analyte does not have an 'analytical handle,' i.e., when it does not possess any highly absorbing chromophores, it does not fluoresce, it is neither oxidizable nor reductable, etc., and when the equipment at hand calls for such a 'handle.' As part of the pretreatment it is then obligatory to introduce such a suitable 'handle' by a chemical modification of the analyte.

The second situation often arises because there is a desire to use a certain method; then a modification of the analyte is necessary to make it amenable for analysis by this method. Trimethylsilylation of polar compounds to make them analyzable by gas chromatography (GC) is a well-known example.

Classical Methods

Gravimetric Analysis

In gravimetric analysis, the analyte is separated completely from the matrix and its concentration is subsequently determined by weighing. Gravimetric analyses are often used in meat science laboratories, for example, for the determination of ash content after a complete combustion of the sample in a muffle furnace, or for determination of water and dry matter content after drying to constant weight at 105 °C.

Volumetric Analysis

In volumetric analysis, a solution of the analyte is treated with a solution of known concentration of a reagent. The reagent solution is added to the analyte solution in small aliquots (titration), and a suitable detector monitors the subsequent immediate reaction between analyte and reagent to find the titration endpoint. The latter is the point where equivalent stoichiometric amounts of analyte and reagent are present. Suitable detectors depend on the chemical reaction taking place during the analysis; common examples are acid–base indicators that change color with changes in pH, or ion-selective electrodes that change potential as a function of analyte or reagent concentration.

Examples from meat and meat product analysis are the final back-titration step in the Kjeldahl determination of nitrogen (protein) content, where an acid–base indicator is used to find the endpoint, and the potentiometric determination of chloride in cured meat products utilizing an ion-selective chloride electrode for the same purpose.

It should be mentioned that at present most volumetric analyses are carried out by means of automated burets, and endpoints are registered automatically; this makes titrations less laborious than in the past.

Electrochemical Methods

Potentiometric Methods

Potentiometry is commonly used in the meat science laboratory, and its use may be divided into two major areas. In direct

potentiometry, the measurement of an electrode potential is used in finding the concentration (more correctly the activity) of an ionic species; in potentiometric titrations change in electrode potential on addition of a reagent is used to find the titration endpoint.

In both cases an ion-selective electrode and a reference electrode are used. The most common ion-selective electrode is the glass membrane electrode used in measurements of pH; however, solid-state electrodes and liquid–liquid membrane electrodes are also frequently used for measurements of activities of a wide variety of ions, most notably halides, sulfides, and some heavy-metal ions.

Voltammetric and Polarographic Methods

These are scarcely used in meat analysis. Both methods rely on the current–voltage relationships arising at a (micro) electrode when diffusion is the rate-determining step in the electrochemical reaction. The gross distinction between the two methods is that in voltammetry a stationary working electrode is used, for example, manufacture of glassy carbon, whereas in polarography the dropping mercury electrode does the job.

Some commercially available high-pressure (or high-performance) LC (HPLC) detectors use voltammetry as the working principle, and in some meat science laboratories voltammetry/polarography is used in the determination of heavy metals, for example, lead and cadmium. In such cases, the modified techniques of anodic stripping voltammetry or differential pulse polarography are normally employed.

Spectrophotometric Methods

Spectrophotometry is the name for a variety of instrumental methods that all have a common feature – the interaction of molecular species or elements with electromagnetic radiation (light). In the present context, the molecules or elements are analytes, and spectrophotometry is a method used in determining their concentration. Depending on the energy of the electromagnetic radiation, different processes take place in the analytes, but it is beyond the scope of this article to go into detail. A pragmatic approach without much theory will be followed and a look at spectrophotometry as a workhorse in the meat science laboratory will be presented.

UV–Vis Spectrometry

This method harnesses the properties of electromagnetic radiation at wavelengths from approximately 200–800 nm. As this embraces the ultraviolet (UV) and the visible (Vis) range in the electromagnetic spectrum, it is referred to as UV–Vis spectrometry. Measurements carried out in the 'Vis' range are sometimes referred to as colorimetry, i.e., the measurement of color. UV–Vis spectrometry measures absorption of radiation (energy).

Examples of meat and meat product analyses in which UV–Vis spectrometry has a role are numerous. It may be the single analytical principle apart from pretreatment procedures, as in the determinations of collagen/hydroxyproline and pigment/heme, or UV–Vis spectrometry may be used as a detector system, for example, in LC or flow injection analysis (FIA) in the form of diode array detectors (DADs).

Single- or variable-wavelength detectors are also used as endpoint monitors in pH titrations employing traditional color changing acid–base indicators as well as in LC and FIA.

Luminescence Spectrometry

Like UV–Vis spectrometry, luminescence spectrometry is mostly of interest in the wavelength range from approximately 200–800 nm; the distinction between the methods is that, whereas UV–Vis is concerned with absorption of radiation, luminescence has to do with emission of radiation. This emission can originate from two sources: either from excess energy imparted by radiation, fluorescence, or by a chemical reaction, phosphorescence.

Phosphorescence spectrometry is hardly ever used in meat analysis, whereas fluorescence spectrometry is more frequently of assistance, mostly as an LC detection principle.

Near Infrared Spectrometry

The wavelength area immediately neighboring the visible range is referred to as the near infrared (NIR). The total spectral area is normally considered to be from 800 to 2500 nm, but sometimes distinction is made between the near, NIR (N^2IR; 800–1050 nm), and the full range (NIR). In the N^2IR range, silicon photodiodes may be used as detectors; above 1050 nm, the more expensive or less sensitive detectors based on PbS, PbSe, InAs, InGaAs, etc., must be resorted to.

Another feature of the near, NIR is that transmission spectrometry is possible for most samples of interest, and commercially available spectrometers take advantage of this fact. At higher wavelengths, reflection spectrometry must be used owing to high absorbance from water in the samples.

NIR spectroscopy is an indirect technique measuring overtones and combination tones of fundamental absorptions taking place in the infrared region. This means that the technique must be calibrated with samples of known composition. Previously, this constituted a major task, involving many test samples with known composition and a good knowledge of statistics, a fact that was obstructive to the popularity of the technique for many years. This has changed recently, mostly as a result of new mathematical methods known as chemometrics, based on multivariate calibrations and artificial neural nets. This has made the calibration process less cumbersome and the resulting calibration models more robust. At present, some vendors of NIR instrumentation supply calibrations with purchase of their equipment.

NIR is currently used in many meat science laboratories for the simultaneous and nondestructive determination of protein, fat, and moisture in meat and meat products. Connective tissue content is sometimes determined in the same run, but so far it appears to be a less satisfactory method in terms of accuracy and precision.

Nuclear Magnetic Resonance Spectrometry

From 2500 nm and above, little interest to the meat science laboratory takes place until the megahertz (MHz) range is

reached. Here, nuclear magnetic resonance (NMR) spectrometry experiments are possible, in which interactions between nuclear spins in a magnetic field and high-frequency electromagnetic radiation may be harnessed for analytical purposes.

The only nucleus of immediate interest is the proton, ^{1}H, but as protons are highly abundant in meat and meat products, this does not constitute an obstacle (^{13}C, ^{19}F, and ^{31}P are other examples of NMR-active nuclei).

For a number of years total fat determinations in water-free matrices have been possible by 'low frequency' NMR, i.e., at frequencies in the range 10–30 MHz. The protons from water, if they were present, would be measured as well and would thus constitute a severe interference, hence drying of the samples is mandatory for this method. The actual measurement is carried out at 45–60 °C, where the fat is liquid and fat protons are mobile; only mobile protons give a response and the rest of the matrix protons, for example, from the protein part of the sample, are still in the 'solid state,' and are not measured.

Recently, a new generation of low-frequency NMR spectrometers has been introduced; they bring the possibility of applying strong pulses of electromagnetic radiation in discrete time domains, and such a pulse will completely swamp the signal from protons in that domain. As water and fat protons have slightly different properties, it is possible to pulse one kind out while measuring the other, and vice versa. With such an instrument it is thus within the realm of reality to measure both water and fat contents in the same (non-dried) sample.

Atomic Absorption and Emission Spectrometry

Elemental analysis is conveniently carried out by spectroscopic methods in which the elements are brought into a gaseous state before the interaction with electromagnetic radiation takes place. Two slightly different techniques are available.

The first technique vaporizes the analyte atoms without ionization, and subsequently the amount of energy at an element-specific wavelength absorbed by the analyte atoms to excite them to a higher energy state is measured. The more energy absorbed, the higher the concentration of analyte. Vaporization is facilitated by means of a flame or graphite furnace in this method, called atomic absorption spectrometry (AAS).

The second method also vaporizes the analyte atoms but ionization also takes place. The energy, again at an element-specific wavelength, that is emitted when the ionized atoms return to a lower energy state is measured. The more energy emitted, the higher the concentration of analyte. Vaporization is by means of a flame or an inductively coupled plasma (ICP) of argon; the method is known as atomic emission spectrometry (AES).

Both AAS and AES normally operate in the UV–Vis range, thus silicon photodiodes or photomultiplier tubes are common means of detection. However, when utilizing ICP as the energy source for the vaporization of the analyte atoms, it is also common practice to detect them using quadrupole MS, in which case the method is called ICP–MS.

Chromatographic Methods

It is a matter of temperament whether one regards chromatography a sample pretreatment technique or an analytical method in its own right. Strictly, because chromatography does not achieve the actual detection of analytes, but only their separation from interfering substances, it is a sample pretreatment. However, in common language and most contexts, chromatography is considered an analytical method, and it will be treated as such here.

In chromatography, two immiscible phases are brought into contact; one is the stationary (nonmoving) phase and the other is the mobile (moving) phase. A sample mixture is introduced into the mobile phase and interacts (partitions) with both mobile and stationary phase many times as it moves through the system carried by the mobile phase. Differences in chemical and physical properties in the sample components influence the rate of migration of each individual component to different degrees, i.e., some components move faster than others. The result is a separation of the components.

A column is an integral part of contemporary chromatography, and this may be packed or coated with stationary phases from a wide variety of different materials, solids or liquids. The mobile phase is a gas or liquid, again with many different choices of characteristics. The vast number of different combinations of stationary and mobile phases makes possible the separation of even closely related molecules.

Gas Chromatography

Analysis of contaminants in meat and meat products probably constitutes most of the work carried out by GC. Examples of analytes are trace amounts of organophosphorus and organochlorine pesticides, polychlorinated biphenyls, and residues of certain veterinary drugs, the last in rare cases in their native state but often after derivatization to make the drug residues more volatile.

Another use of GC analysis has more to do with meat and meat products themselves. Good examples are fatty acid composition analysis after saponification and derivatization of the individual fatty acids (the fatty acid methyl ester method) and headspace analysis in monitoring fermentation processes of meat products; the latter sometimes referred to as aroma analysis.

Capillary columns are invariably used in the modern laboratory, and a great variety of stationary phases is commercially available, both liquids (gas liquid chromatography) and solids (gas solid chromatography). The mobile phases normally encountered are nitrogen or helium, and sometimes also hydrogen.

A number of different detectors are available depending on the nature of the analyte. The most versatile and frequently used is likely to be the flame ionization detector, which although versatile is also very indiscriminate. Anything that will burn in a mixed atmosphere of oxygen and hydrogen will be detected with greater or lesser sensitivity.

For chlorinated or fluorinated species, and for molecules containing other highly electron-attracting substituents, the electron capture detector is often used because of high

sensitivity for such compounds while being blind to many matrix interferents.

Other detectors are suited particularly for molecules containing nitrogen and phosphorous (thermoionic emission detector) and sulfur and phosphorus (flame photometric detector).

In recent years the mass-specific detector (MSD) has become increasingly popular because of its versatility, high sensitivity, specificity, and its ability to identify unknown components. The MSD is a low-resolution mass spectrometer, either with a quadrupole or an ion-trap mass filter. When full electron impact (EI) mass spectra of unknowns can be obtained, comparisons with known spectra from a database will often be able to identify the unknown (EI is the most common ionization method for GC–MS experiments. The ionization is caused by collision with 70 eV electrons, and in most cases this leads to substantial fragmentation of the analyte molecular ion. EI is called a 'hard' ionization technique because of the surplus of energy transferred to the eluting components; hard implies substantial fragmentation).

Liquid Chromatography

A number of related methods come under this head. These include open-column chromatography, paper chromatography, TLC, and HPLC; but only the last will be included here. The reason is that open-column and paper chromatographic methods are almost totally absent from contemporary analytical science, and although TLC is sometimes used in screening for particular analytes, its use is limited, and confirmatory analyses of suspected analytes are always necessary.

However, HPLC is widely used for a variety of different analyses; examples include the determination of nitrite and nitrate in meat products by ion-exchange HPLC, and the numerous analyses harnessing reversed-phase HPLC such as vitamin determinations, boar taint component determination, and determination of veterinary drug residues.

In ion-exchange HPLC, the stationary phase is an ion exchanger and the mobile phase is an aqueous buffer; reverse-phase HPLC utilizes a hydrophobic stationary phase and polar solvent (mixture) as the mobile phase.

A number of detectors are used in HPLC; most common are spectrophotometric detectors in the UV–Vis range, either operating at single or variable wavelengths or as DADs covering a range of wavelengths. In addition, fluorescence detectors and electrochemical detectors are used; the former more often than the latter.

Recently, MS detection in combination with HPLC separation of analytes has come to a stage where routine analysis is possible. The predominant form is by single-stage MS, where soft ionization (see Section MS-MS and MS^n Methods) with little fragmentation takes place and only the ion originating from the analyte plus adduct ions between buffer constituents from the mobile phase and the analyte are detected. More sophisticated MS detection principles are mentioned in Section MS-MS and MS^n Methods.

Finally, it should be mentioned that other types of detectors are available, but they are not often used for analysis of meat and meat products.

Electronic Sensing; 'Noses' and 'Tongues'

Single chemical components or mixtures of components may be assessed by these methods; gaseous compounds by the electronic nose ('odor/flavor analysis') and compounds in solution by the electronic tongue ('taste analysis'). Electronic sensing is an often used term for attempting to mimic human sensory perception by such noses or tongues.

The principle of the measurements is normally adsorption of analyte molecules to an array of related, but not identical, sensors, thereby causing a physical change of the sensor. In quartz crystal microbalance sensors, for example, a measurable change of the crystal resonating frequency will occur upon adsorption (other sensor principles are also common). Many different signals from the sensor array are then treated mathematically (e.g., by chemometric methods) to yield outputs, which after calibration may be used in assessing intensity and/or identity of odors or tastes.

Known, but not very successful, uses of electronic sensing in meat science have been in boar taint intensity determination and monitoring progress of fermentation processes.

Flow Injection Analysis

FIA is a comparatively new form of automated analysis that is becoming increasingly popular in meat science laboratories. Previously, automation of analyses was carried out with the help of laboratory robots able to perform a wide variety of steps in an analytical method such as addition of reagents, mixing, timing, etc. However, this form of automation involves many moving parts that are likely to become worn and quite often involve a lot of cumbersome programing.

FIA, in contrast, is a continuous flow analysis method where all such manipulations take place in a continuous, nonsegmented stream of liquid. Samples pass through the same length of tubing, one after the other, with reagents being added at appropriate positions and other handling steps being performed while the sample is moving toward a flow-through detector (the auto-analyzer system, used for decades in many clinical laboratories, also utilizes a flow system that in many respects is like the FIA; however, the auto analyzer has bubbles of air segmenting the flow. The advantages of FIA are that the method has a higher sample throughput, requires smaller samples, and consequently uses less reagent).

The determination of nitrite and nitrate in meat products and brines by the Cd-column reduction method is an example of a newly introduced commercially available FIA method in meat analysis.

Mass Spectrometric Techniques

MS–MS and MS^n Methods

In the preceding sections, MS detection was mentioned in conjunction with chromatographic separation. In most cases the mass spectrometers are comparatively simple devices with low resolution (unit resolution meaning that mass X is distinguishable from mass $X+1$ over the available mass range)

based on single quadrupole or ion trap mass filters. In GC–MS, full mass spectra consisting of the molecular ion and fragments thereof may often be obtained as explained in the section 'Gas Chromatography', whereas only molecular ions and adduct ions normally are recorded from LC–MS experiments.

Such data are not always sufficient to give valid analytical results, and consequently more refined techniques such as MS–MS or MS^n can be helpful. The principle in both is the addition of more, normally low-resolution, mass filters; two in total for MS–MS and several for MS^n. The mass filters may be real or virtual as described in the last paragraph of this section.

Between the actual mass filtrations, further fragmentation of individual ions is often desirable; this is often the case in LC–MS–MS. As 'soft' ionization (soft implying no or little fragmentation) techniques such as electrospray or atmospheric pressure ionization are normally employed, only a few ions are generated in the ion source. Hence one of the ions can be selected with the first mass filter, and be fragmented by collision with an inert gas before being analyzed by the second mass filter. In this fashion a mass spectrum of the selected ion giving additional information of its identity may be achieved.

A similar procedure may, of course, be carried out on one or more of the multitude of ions normally generated by EI ionization.

More mass filters, and more fragmentation steps (MS^n), are sometimes needed, but very seldom for analytical reasons. This may be achieved either by a series of mass filters or by running a sequential series of experiments on an ion trap mass filter.

High-Resolution Methods

Most mass spectrometers used for routine analytical purposes have low-resolution mass filter with little or no focusing of the molecular beam. As there is always a spread in ionic energies between otherwise identical ions depending on where in the ion source the ion is formed, the result is peak broadening and hence low resolution.

For certain applications, high-resolution MS is necessary, and then double focusing mass spectrometers are employed. They have two different mass filters, a magnetic analyzer and an electrostatic analyzer, the combination of which may result in very high resolutions. For dioxin analysis, for example, the resolution must be better than 10 000 to distinguish the different dioxin congeners from overlapping interferents.

Miscellaneous Techniques

The pretreatments and instrumental techniques mentioned in this article are not restricted to meat science laboratories; they may be found in any analytical chemistry laboratory. There are, however, a number of methods that are more or less restricted in use to meat science.

Sarcomere length determinations by laser diffraction, meat tenderness determinations according to Warner–Bratzler or Volodkevich, and meat color determinations by reflection spectrometry (e.g., in Hunter Lab or Commission Internationale de l'Eclairage values) are examples of the methods. Such special methods are mentioned only for the sake of completeness; more information about them may be found in the literature.

Quality Assurance

Finally, some words concerning quality assurance and control of the analytical methods seem to be appropriate. It is, of course, of utmost importance that analytical results are valid in terms of accuracy and precision. Even the most expensive equipment will not guarantee valid results. Results obtained from the methods may be used for documentation of product composition and compliance with legal requirements. Consequently, analytical results are normally only trusted if they are produced in laboratories with implemented quality systems according to international standards for example, ISO/IEC 17025. The laboratories should ideally be accredited by an approved accreditation body, which is a member of the International Laboratory Accreditation Cooperation, an international cooperation of laboratory and inspection accreditation bodies formed more than 30 years ago to help remove technical barriers to trade.

Before analytical methods can be accredited, they must be validated or at least verified. A number of standards exist that describe how to validate analytical methods. Validation should always be done on matrices relevant for the use of the method. Validation should include a variety of matrices; the following properties of a method should be included in the validation: specificity, range, linearity, sensitivity, trueness, precision, and robustness.

Both for validation and for periodic control of a method, the laboratories can use certified reference materials (CRM's). A CRM is a material produced and certified by acknowledged institutes (e.g., National Institute of Standards and Technology (NIST) (USA) or Institute for Reference Materials and Measurements (IRMM) (EU)) and companies. A CRM contains certified amounts of compounds and substances. Comparing results from the analysis of CRM with the certified values can determine a bias (=systematic error) of the method, thereby making adjustments possible.

Participation in proficiency testing (PT) is also a must for laboratories. A number of providers of PT exist; as their quality varies, accredited suppliers should be preferred. When participating, a laboratory can compare its own results with those of other laboratories. Normally a z-score is calculated and this parameter should be > -2 and < 2. Interpretation of results may be difficult if participants use different methods and if the number of participants is too low for proper statistics.

On a daily basis methods should be controlled using stable and homogenous control materials. A material representative for the matrices analyzed should be used. Preferably two samples should be employed with low and high content compared to the measuring range. Some labs use CRM for daily control, however, this is expensive and not necessary. The results of the control analysis is followed on a Shewhart Control Chart, a system introduced for statistical process control introduced by Walter A. Shewhart in 1924. A system can easily be prepared in Excel. A number of commercial systems are also available.

See also: Chemical Analysis: Sampling and Statistical Requirements; Standard Methods. Chemical Analysis for Specific Components: Veterinary Drug Residue Analysis. Environmental Contaminants. Laboratory Accreditation. Microbiological Analysis: Standard Methods. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

AOAC, 2005. Official Methods of Analysis, 18th edn. Gaithersburg, MA: AOAC International revision 4 (ISBN 0-935584-82-X). Available as "continuous edition" at: www.eoma.aoac.org (accessed 15.10.13).

Garfield, F.M., Klesta, E., Hirsch, J., 2000. Quality Assurance Principles for Analytical Laboratories, 3rd edn. Gaithersburg, MA: AOAC International ISBN 0-935584-70-6.

ISO/IEC 17025, 2005. General Requirements for the Competence of Calibration and Testing Laboratories. ISO/IEC (see also ISO/IEC 17025:2005/Cor 1:2006). Available at: http://www.iso.org/iso/catalogue_detail.htm?csnumber=39883 (accessed 15.10.13).

NMKL (Nordic Committee on Food Analysis), 2010. Guidelines for Quality Assurance for Food Chemical Laboratories, NMKL General Secretariat, Oslo, Norway. Available at: www.nmkl.org (accessed 15.10.13).

NMKL (Nordic Committee on Food Analysis), 2011. Compiled Methods Collection, NMKL General Secretariat, Oslo, Norway. Available at: www.nmkl.org

Shewhart W.A. Available at: http://en.wikipedia.org/wiki/Shewhart_individuals_control_chart (accessed 15.10.13).

Willard, H.H., Merritt Jr, L.L., Dean, J.A., Settle, F.A., 1988. Instrumental Methods of Analysis, 7th edn. Belmont, CA: Wadsworth ISBN 0-5340814-28.

Relevant Websites

http://irmm.jrc.ec.europa.eu/reference_material_catalogue/
IRMM.

http://www.nist.gov/srd/
NIST.

Raw Material Composition Analysis

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Glossary

Artificial neural networks A nonlinear statistical data modeling tool that is used to find patterns in the data presented.

Dielectric constant The relative permittivity of a material to an electric field that is a measure of the polarity of a substance. Water, for example, has a dielectric constant of 80.1 whereas air has a dielectric constant of slightly more than 1.0.

Guided microwave spectrometry Measurement of the selective absorption and emission of microwaves of various frequencies.

Near infrared Spectroscopic method that uses light from the near infrared region (800–2500 nm) to determine selective absorption of infrared light by different substances.

Nuclear magnetic resonance Spectroscopic method that utilizes magnetic properties of atomic nuclei to determine properties of the molecules in which the atoms are contained.

Proximate composition The determination by prescribed methods of the composition of food in terms of the principal components. For meat, these components consist of moisture, fat, protein, and ash.

Introduction

Meat is composed of water, protein, fat, and ash, the total of which is commonly referred to as proximate composition. However, meat as a raw material is, for all practical purposes, a three-component mixture of water, protein, and fat. Consequently, composition analysis in the meat industry focuses on these three components in studies of proximate composition as it relates to fresh meat quality attributes or when attempting to achieve formulation targets during manufacturing of processed meat products. In the case of processed meats, precise control of product composition is essential to satisfying regulatory and labeling requirements, and to maximizing eating quality, shelf-life, and appearance. Further, control of composition is essential for the production of consistent finished products that have the same properties regardless of the raw meat materials used. Many different analytical methods have evolved over the years and have been generally categorized as 'official' or 'rapid' methods. The official methods have been critically evaluated for performance by an analytical methods organization such as AOAC International (AOAC), and are considered to be the most dependable. The rapid methods have been developed to be fast and easy but may be less accurate and/or less precise. More recently, new technology has resulted in methods that are both fast and reliable. In fact, some of these formerly 'rapid' methods have demonstrated a degree of accuracy and precision that is comparable with the official methods, and have been reclassified as official methods by the methods organizations.

Composition of Meat as a Raw Material

The average composition of muscle without external fat cover is, in general, composed of approximately 70% water, 20% protein, and 9% fat. The remaining 1% is ash or mineral content. The total water, protein, and fat contents will vary in different muscles and different animal species but the above values represent a typical average composition for raw muscle with the external fat cover removed. However, it is important to remember that meat as a raw material for further processed products includes much more than well-trimmed muscle. Subcutaneous and intermuscular fat deposits are usually included with trimmings from carcass fabrication and will dramatically alter the overall composition of raw materials used for processed meats. In addition, differences between species such as beef and poultry, and the effects of animal age will add even more variation to meat composition. Finally, some processed meat products may utilize hearts, livers, skin, or other nonmuscle animal protein sources, all of which add additional variation to product formulations. It should be no surprise that composition analysis of raw meat materials is critical to success in the processed meat industry.

Fat is the single most variable component in meat, and in raw materials it is likely to range from as low as 1–2% to as high as 80–90% or even more. As water and protein make up the remainder, the combination of water and protein together is inversely related to the fat content, and this is a consistent relationship. Water and protein in meat are also closely related to each other, and in raw meat will most often exist in a 3.6:1–3.8:1 ratio of water to protein. The relatively constant water: protein ratio is sometimes utilized for a quick approximation of composition when only one of the two components is actually measured. As fat is closely correlated with protein and water but in an inverse relationship, fat content of meat can also be estimated if either the water or protein content is known.

The extreme range in composition of meat raw materials means that an accurate and precise analysis is absolutely essential to the manufacture of consistent finished products that provide the eating quality desired by consumers. In the past, the official methods used to analyze raw materials were often too slow to provide useful information for high-speed, high-volume processing operations. Consequently, a large number of rapid methods have been developed for day-to-day

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compositional analysis of meat raw materials. The rapid methods can vary widely in performance capabilities and processors must evaluate methods carefully to choose those best suited to their needs. Rapid methods can provide practical, useful results without the same degree of performance as the official methods. Some of the methods first developed as rapid methods have since been improved to a level of performance that has resulted in their qualifying as official methods.

Performance of Analytical Methods

Regardless of the methods chosen for analysis, method performance should be measured on a routine basis to assure that the method used is performing as expected. Performance can be monitored by calculating variance such as the standard deviation of the mean of a series of measurements. Periodic analysis of a known standard for comparison of results with another laboratory provides additional measures of performance.

Performance of a method is usually evaluated as repeatability, reproducibility, and bias. Repeatability is defined as the standard deviation of repeated measures by the same laboratory; reproducibility is the standard deviation of repeated measures by different laboratories; and bias is the consistently high or low mean values produced by a method relative to known, authenticated values.

One example of performance criteria has been those used by the US Department of Agriculture (USDA) – Food Safety Inspection Service for USDA-accredited laboratories. These criteria include repeatability and reproducibility measures of 0.63% and 0.66%, respectively, for fat; 0.46% and 0.65%, respectively, for water; and 0.24% and 0.32%, respectively, for protein. These performance criteria provide a good basis for comparison of method performance. Rapid methods may result in larger performance variation values and, even so, can be very useful but, clearly, values close to the standards for performance are desirable. It should be noted that reproducibility will usually be larger than repeatability for a given method because it includes both intralaboratory and interlaboratory variation whereas repeatability includes only intralaboratory variation. It is possible, in rare instances, for these two measures to have the same value but, by the same token, repeatability can never be larger than reproducibility.

The standards for comparison of all methods are those methods approved as official methods and published by one or more of the several methods organizations. AOAC International, the American Oil Chemists Society, and the American Society for Testing and Materials are the organizations most widely recognized in the US, whereas the International Organization for Standardization, the Comité European de Normalization, and the Nordic Committee on Food Analysis are examples of international and European organizations that also publish standard methods for food analyses. A critical prerequisite for official or standardized methods is a performance evaluation, normally done by a collaborative study. AOAC International, for example, utilizes collaborative studies involving several laboratories (usually eight or more) to evaluate analytical methods in terms of repeatability and reproducibility. This evaluation, and subsequent publication of the method as 'official.' provides analysts with standardized methods that can be expected to provide accurate, precise, and dependable measurements.

After performance is evaluated, a second concern for analytical methods is often the disposal of waste reagents and byproducts of chemical reactions involved in the method. Many high-performance methods generate toxic and hazardous wastes that create significant disposal problems for laboratories. A considerable effort has been devoted to the development of analytical methods that minimize or eliminate laboratory waste disposal problems. Traditional wet chemistry is being replaced by new technologies made possible by the computer and electronics industries.

Another frequent question concerning methods is cost. The initial capital investment for an analytical method can range from less than US$1 000 to much more than US$100 000. Each laboratory needs to calculate a per-sample cost to determine which method will best serve its needs. The methods most commonly used in the US meat industry for the analyses of raw meat materials' composition are listed in Table 1.

Methods of Fat Analysis

Official Methods

As the fat content of meat raw materials is so variable and because fat is critical to finished product palatability and production economics, there have probably been more methods developed for fat measurement than for any other proximate component. The method of choice for 'official' fat analyses has long been a solvent-based extraction. AOAC International currently lists three solvent-based methods as approved methods for the measurement of total fat content of meat. These methods (Table 1) include ether extraction followed by gravimetric measurement (AOAC Method 991.36), which has been widely used by many laboratories; tetrachloroethylene extraction followed by specific gravity measurement (AOAC Method 976.21); and methylene chloride extraction followed by gravimetric measurement (AOAC Method 985.15). Conventional extraction with ether typically requires several hours whereas tetrachloroethylene and methylene chloride methods require only approximately 15 min. However, the latter two require specialized equipment that is no longer available because of concerns for the toxicity of the solvents used and because of the development of alternative methods such as near infrared (NIR) and nuclear magnetic resonance (NMR) spectroscopy (Table 1).

Additional Methods for Fat Analysis

The solvent extraction method with ether is commonly utilized to obtain a measure of total fat as triglycerides. However, other lipid components such as phospholipids and free fatty acids are not included in the extraction. These lipid components are relatively low in quantity in raw meat (1% or less), but in some cases, it may be important to include them for a true total fat measurement. To include phospholipids and other minor lipids in extraction procedures, a modified solvent

Table 1 Some of the more common methods available to the meat industry for the measurement of fat, moisture, and/or protein in meat raw materials used for further processing

Component	*Method*	*Method status/performance*
Fat	Ether extraction	Official (AOAC Method 991.36)
	Specific gravity	Official (AOAC Method 976.21)
	Microwave/solvent extraction	Official (AOAC Method 985.15)
	Nuclear magnetic resonance (NMR) (CEM SMART Trac II)	Official (AOAC Method 2008.06)
	Near infrared (FOSS Food Scan™)	Official (AOAC Method 2007.04)
	Caviezel (total fatty acids)	Correlation of 0.99 with ether extraction
	Accelerated solvent extraction	Rapid method; repeatability ±0.4–0.8%
	Supercritical fluid extraction	Rapid method; repeatability ±0.5–1.3%
	Modified Babcock	Rapid method; repeatability ±0.75%
	X-ray (KartridgPak Anyl-Ray; FOSS Meat Master™, EAGLE™ FA)	Rapid method; repeatability ±0.60–10%
	Database extrapolation (CEM SMART Profat)	Rapid method: fat determined from database following moisture measurement by microwave drying
Protein	Kjeldahl	Official (AOAC Method 928.08)
	Combustion	Official (AOAC Method 992.15)
	Protein tagging (CEM Sprint™)	Official (AOAC Method 2011.04) – direct protein measure
	NIR (FOSS Food Scan™)	Official (AOAC Method 2007.04)
Moisture	Oven drying	Official (AOAC Method 950.46)
	Microwave (CEM SMART Turbo)	Official (AOAC Method 2008.06)
	NIR (FOSS Food Scan™)	Official (AOAC Method 2007.04)
	Infrared, halogen lamp drying	Rapid method; can be variable
Multicomponent	NIR (FOSS Food Scan™) (fat, protein, moisture)	Official (AOAC Method 2007.04)
	Microwave, NMR (CEM SMART Trac II) (fat, moisture)	Official (AOAC Method 2008.06)
	Microwave, database extrapolation (CEM Profat Meat Analyzer) (fat, protein, moisture, ash)	Rapid method: fat, protein, and ash determined from database following moisture measurement by microwave drying
	NIR	Rapid method: repeatability ±0.42–0.51% (fat) ±0.32–0.36% (moisture) ±0.53–0.54% (protein)
	NMR	Rapid method: correlation of 0.967 with known fat values
	Guided microwave spectrometry	Rapid method: repeatability ±0.3% for all components (fat, moisture, protein)

(chloroform–methanol) or a pre-extraction acid hydrolysis treatment (Schmid–Bondzynski–Ratzlaff or Weibull–Stoldt method) must be used.

As the traditional extraction methods with ether measure primarily triglycerides, another extraction method, the Caviezel method (Table 1), has been developed for the measurement of total fatty acids in fat. This procedure is used to meet the US Food and Drug Administration (FDA) definition of total fat as lipids including all fatty acids for labeling of food products. The FDA definition includes fatty acids from triglycerides, phospholipids, and sterols as well as free fatty acids. The procedure uses *n*-butyl alcohol for fat extraction followed by saponification and isolation of fatty acids, which are then quantified by gas chromatography. A comparison of the Caviezel method with the conventional extraction methods have shown correlations of 0.99 or greater with values reported for the Caviezel method being slightly higher than that for conventional extractions because all fatty acids are included. Automated equipment is available that permits sample preparation and analysis in less than 2 h.

Accelerated solvent extraction (ASE) is another approach to solvent extraction that is based on the traditional solvents but uses elevated pressure and temperature to increase the rate and efficiency of the extraction process. Generally, less solvent is required for a quantitative extraction by ASE and the time required is considerably less, typically approximately 15 min. Equipment is available that can provide for continuous operation and recovery of extracts for further analysis. The ASE technique has been reported to be very effective for unbound lipids and is often used in environmental testing laboratories for recovery and analysis of lipid components.

Supercritical fluid extraction (SFE) is a method that uses carbon dioxide at elevated pressures and temperatures. Under the conditions used for SFE, carbon dioxide is a good nonpolar solvent and can quantitatively extract lipids. The procedure requires approximately 20 min and, most importantly, results in no waste solvent when samples are returned to normal pressure and temperature because carbon dioxide returns to its normal gaseous form. Performance has been reported to be close to that of traditional methods but the SFE method is easily affected by the presence of water. Water absorbers such as diatomaceous earth may have to be included with meat samples even after the samples have been dried.

One of the limitations of many of the solvent extraction methods is that they are matrix-dependent and require use of a specific solvent, depending on the objective of the extraction. Samples also usually require preparatory treatment such as drying and sometime use of water absorbers to prevent interference by water. Initial sample size should be relatively large for these methods because of the difficulties in collecting and preparing a representative sample of the original meat block.

Improved extraction procedures, which utilize microwave heating to accelerate the process and microsolvent extractors to reduce solvent volume, have been developed. However, the recent trends in methods for fat analysis have been away from extraction methods toward multicomponent analyses such as NIR and NMR that include fat as one of the components measured.

Rapid Methods for Fat Analysis

A simple and inexpensive method for fat measurement that is used by some laboratories in the meat industry is the Modified Babcock (Table 1). This approach utilizes an acid digestion to release fat from a sample, followed by centrifugation to bring all of the fat to the top of the fluid, digested mixture. A calibrated bottle is used to measure the percentage of fat present based on the height of the fat layer. Bottles are calibrated for sample weights of 9 g or 18 g, and for low (up to 10%) or high (up to 50%) fat content. Standard deviations for this method have been reported to be approximately 0.75%. Although performance can be quite good in some laboratories, this method is generally considered to be too variable for more than preliminary formulation decisions.

A more sophisticated approach to rapid methods for fat is the use of X-rays such as the Anyl-Ray instrument (KatridgPak, Tegetec, Frederikssune, Denmark), the MeatMaster™(FOSS, Hilleroed, Denmark), and the EAGLE™ FA (EAGLE Product Inspection, Tampa, Florida, USA). This approach utilizes an X-ray machine that measures the differential in X-ray absorption between fat and lean. The X-ray method is nondestructive so the sample is not damaged or lost. Calibration of the machine with a standard allows determination of the fat content in unknown samples. A unique aspect to the Anyl-Ray instrument is that it requires a 5.9 kg sample. The relatively large sample size is an advantage because it is easily representative of the larger amount being sampled. The MeatMaster™, on the contrary, is designed to scan and measure meat materials in-line at up to 20 t per hour. The measurement requires less than 1 min and standard deviations have been reported to be 0.60–0.80%. The EAGLE™ FA is designed to scan as many as 30 cartons (up to 28 kg) per minute or bulk products at up to 120 t per hour with results that are within 1.0% of actual fat content. This system includes a 45 s automatic calibration, is effective for fresh or frozen products, and will also detect contaminants such as metal, glass, and bone. All of these methods utilizing X-ray technology have been well accepted in the industry for formulation estimates but have not shown the performance required of official methods.

An indirect approach to fat measurement in meat developed by Least Cost Formulations Ltd (Virginia Beach, Virginia, USA) and called 'QC Assistant' is based on a software package coupled with a moisture measurement. This approach is dependent on a consistent moisture/protein/fat relationship in raw meat and draws from a database of values to estimate fat based on the measured moisture content. This system is combined with CEM Corporation's (Mathews, North Carolina, USA) microwave drying oven which has received official methods status (AOAC Method 2008.06) for moisture. The combined system is called SMART Profat and can provide moisture, protein, and fat values for a sample in approximately 3 min. It is important to keep in mind that the measurement of fat (and protein) by this method are indirect and dependent upon both the analysis for moisture and the assumption of a consistent moisture/fat relationship in the samples analyzed with the database of values. As moisture:protein, moisture:fat, and protein:fat relationships in raw meat are consistent, one component can be effectively estimated if one or two of the others are known.

Despite the development of several different rapid methods for fat analysis, some of which perform very well, the trend in methods for fat analysis has been toward the measurement of fat as part of multicomponent methods such as NIR and NMR.

Methods for Protein Analysis

Official Methods

The long-time standard for protein analysis has been the Kjeldahl method (AOAC Method 928.08) (Table 1). The longevity of this method is obvious when considering that the method was first published in 1883. In the past, the need to develop faster methods for protein analysis has probably not been perceived to be as important as the need for rapid fat and moisture methods. However, more recently, processed meat product definitions and labeling requirements have placed increased emphasis on protein content and the need for rapid, dependable protein measurements is now as great as for methods for fat and moisture. In addition, the heavy metal catalysts used in the Kjeldahl method plus the concentrated acid and alkali waste generated by conventional Kjeldahl procedures have been a disposal concern for laboratories. Some of the past method developments for protein have included approaches such as dye-binding procedures but none of these early efforts achieved major advances in methodology. Improvements in instruments that provide automated, relatively rapid Kjeldahl analysis have included accelerated acid digestion units and automated rapid distillation units to achieve an easier, more rapid analysis than the traditional Kjeldahl method.

A significant step forward in protein methods occurred in the 1990's when the combustion method received AOAC approval (AOAC Method 992.15). The combustion method is based on sample combustion (~850 °C) followed by quantitation of the released nitrogen. The method concept itself is even older than the Kjeldahl method, and was first published by Dumas in 1831, but only since about 1980 have instruments that utilize thermal conductivity to achieve accurate measurement of the released nitrogen been available. A major advantage to the combustion method is that no toxic or hazardous wastes are produced. The procedure is also relatively

fast requiring approximately 5 min or less per sample. Repeatability has been reported to be 0.12–0.41%. One concern for the combustion method has been that a relatively small sample size is required for combustion efficiency, making sample preparation critical to method success. Recent instrument developments have increased the sample size but it is still quite limited compared with most other methods.

A significant potential limitation to keep in mind for both the Kjeldahl and the combustion methods is that both of these methods measure sample nitrogen content from which protein content is then calculated. In the case of meat products, the nitrogen content is multiplied by a conversion factor of 6.25 because meat protein typically contains 16% nitrogen. However, nonmeat proteins will have different nitrogen contents and different conversion factors must be used. Further, recent fraudulent cases of nonprotein nitrogen addition to animal feeds to falsely increase the apparent protein content by Kjeldahl analysis has resulted in the development of improved methods that provide a direct measurement of the protein content.

Rapid Methods for Protein

Rapid methods for protein alone have not been developed as extensively as those for moisture and fat in terms of available commercial instruments. However, the developments of multicomponent methods that include protein measurement have fulfilled the need for rapid protein analysis. These methods for protein, as for fat, will be described in the discussion on multicomponent measurement. In addition, because of the limitation of the Kjeldahl and combustion methods for direct protein measurement, methods that utilize direct dye-tagging of proteins have been refined and improved to allow direct measurement of proteins. CEM Corporation, for example, provides an instrument (Sprint) that uses protein tagging technology to provide for rapid (approximately 3 min) and direct protein measurement. This method has also very recently qualified as an AOAC official method (AOAC Method 2011.04) (Table 1).

Methods of Analysis for Moisture

Official Methods

The standard reference method (Table 1) for the measurement of moisture in meat has been oven drying (AOAC Method 950.46). Weighed samples may be dried in a conventional oven (air drying) at 100–102 °C for 16–18 h or in a convection oven at ~125 °C for 2–4 h, and reweighed to determine the amount of moisture lost during drying. Use of higher temperature ovens and/or use of vacuum ovens can serve to shorten drying time but may not be suitable for samples with a high fat content. In all cases, it is necessary after drying to hold the dried samples in desiccators during cooling to prevent water absorption from the air before reweighing. It is also sometimes necessary, even at lower oven temperatures, to take precautions with samples that are high in fat content. If high-fat samples are dried in cellulose thimbles for subsequent fat extraction, for example, some melted fat may soak through the thimble and be lost, resulting in erroneously high values for moisture. The use of aluminum weighing dishes alleviates this potential problem. To be consistent, these methods should follow prescribed conditions and procedures carefully in order to achieve the expected results. It is also useful to keep in mind that oven drying does not determine moisture exclusively but also includes a mixture of volatile compounds that are volatilized at the temperature used. The volatiles released in addition to moisture is an extremely small quantity in terms of weight and does not significantly alter the quantitative measure of moisture but represents a change in some of the chemical components of the sample.

As the air-drying methods are relatively time-consuming, the use of microwave ovens has been investigated as a means of rapid drying. The most successful approach has been that of CEM Corporation, who designed a microwave analyzer (SMART Turbo), specifically for the measurement of moisture and solids content of food products. The procedure requires less than 3 min. As noted earlier, this CEM method and an early version of the microwave instrument has received AOAC International approval as an official method for moisture measurement of meat (AOAC Method 2008.06).

Rapid Methods for Moisture

A wide variety of rapid methods for the measurement of moisture content have been studied. Most of these have involved some form of accelerated drying such as infrared or halogen lamp heating sources (Table 1). These approaches give relatively rapid results (usually 15–30 min) but are typically highly variable. Heating rates, sample dispersion, and sample moisture content can each contribute to case hardening of the sample if conditions are less than ideal, and will result in highly variable measurement of moisture content. An exception to these limitations of rapid drying methods is the specially designed microwave instrument described earlier, which is even faster than the instruments using infrared or halogen heat sources. Other methods for the measurement of moisture content of meat that have been studied with somewhat mixed results include electrical conductance, toluene distillation, and Karl Fischer titration. However, the advances of multicomponent methods that include moisture measurement have shown these alternatives to be very successful and provide a good means of quickly measuring the moisture content of meat products.

Multicomponent Methods

The development of methods that will measure fat, protein, and moisture in meat simultaneously and almost instantaneously has been a monumental advance in practical analytical methods. There are three multicomponent concepts currently in use (Table 1). NIR has been used for many years for a variety of applications but new instruments have made this technology available for meat applications in the laboratory and for in-line analysis of products on the production line. Guided microwave spectrometry (GMS) has also been developed as a multicomponent measurement and has been

available as a component of meat processing equipment. NMR is a very effective method for rapid measurement of lipids and protein but functions best with dried samples, consequently a combination of NMR with rapid drying procedures has resulted in effective multicomponent measurement systems.

Near-Infrared Method

NIR methods have been used for more than 40 years for a variety of products, most notably moisture measurement of harvested grain. However, improvements in the method and especially in instruments have opened a very broad range of applications including analysis of the composition of meat products. NIR scans are unique to the measured sample, and for pure chemical compounds, essentially provide a 'fingerprint' that can be used for compound identification. Meat, however, is a complex mixture of chemical compounds and the infrared response to a meat sample is also a mixture reflecting the compounds present. Consequently, quantitative measurement of meat components requires measurement of several known samples for instrument calibration. Unknown samples of similar type can then be scanned and the components determined by comparing the response data with the calibration data. Once calibration is complete, the method provides a simultaneous measure of fat, moisture, and protein that is extremely fast and nondestructive.

The availability of economical microprocessors and electronic systems that provide for easy calibration using artificial neural networks has made NIR instruments commonly available for meat analysis. Examples of available instruments include those from NDC Infrared Engineering, Inc. (Irwindale, California, USA) called InfraLab™, and from FOSS (Hilleroed, Denmark) called Food Scan™. Currently, infrared analysis is finding a wide variety of meat applications in addition to proximate composition measurements. These include estimates of fat softness, determination of prior temperature history of meat, and identification of species of meat origin among other things. It is important to remember that NIR analysis is highly dependent on proper calibration of instruments with samples similar to the unknowns to be measured. Recalibration is necessary for any change in sample material that is outside the range of properties of the samples used for calibration. The need for careful and proper calibration is viewed by some analysts as a disadvantage of this method.

In the case of meat samples, water and fat have particularly high NIR absorption, whereas protein absorption is somewhat weaker, but all three components can easily be measured with proper calibration. Several instruments are available as tabletop analyzers for laboratories or as in-line analyzers on meat processing equipment. Changes in method technology have resulted in changes of most instruments from NIR reflectance measurement to NIR transmission (NIT). The transmission measurements utilize greater sample volume, which improves consistency of results. Although correlations between NIR and AOAC methods for fat, moisture, and protein have been very good (0.93–0.99), correlations between NIT measurements and AOAC methods have been reported to be 0.984–0.995, 0.987–0.992, and 0.949–0.957 for fat, moisture, and protein, respectively, in meat. Repeatability ranges from 0.42% to 0.51%, from 0.32% to 0.36%, and from 0.53% to 0.54%, respectively, for fat, moisture, and protein in meat have also been reported.

In addition to simultaneous, nondestructive measurement, NIT is typically very fast, and frequently repeated measurements are an attractive option with this method. The high frequency of measurements has facilitated the design of NIT systems as part of meat processing equipments such as grinders and mixers to provide continuous, on-the-spot monitoring of fat, moisture, and protein during grinding, mixing, and blending. In most cases, the NIT systems are built into the meat processing equipment to provide continuous formulation information and control. Wolfking (GEA Food Solutions, Dusseldorf, Germany), for example, has offered an NIT system called the GEA MultiTrack™ for use on mixers and grinders. This system utilizes an 850–1050 nm wavelength range to continuously monitor fat moisture and protein content during mixing of meat batches, and composition can be adjusted on the spot. Standard deviations of 0.3% for the measurements have been reported.

In addition to fat, moisture, and protein, NIR measurements have been studied extensively for the determination of collagen content in meat. Although this would be a very useful measurement for meat processors, correlations between NIR and other methods for collagen have been relatively low.

Guided Microwave Spectrometry

GMS has not been studied as extensively as NIR and NIT systems but this technology has been developed to the point of being offered commercially as part of meat processing equipment, similar to NIR and NIT. The GMS measurement is based on microwave energy absorption which is used to measure differences in conductivity and dielectric constants of water. The conductivity and dielectric constants are then used for the determination of sample fat, moisture, and protein content. Protein and fat are indirect measures with this method. Calibration with known samples is necessary for GMS measurements.

Although there is less information available on GMS systems than for most other analytical methods, one of the limitations to this method appears to be matrix sensitivity. For example, the presence of air bubbles or ice crystals in a meat mixture has been reported to have a significant effect on results.

Nuclear Magnetic Resonance

One of the most recent developments in commercial instruments for fat analysis of meat has been use of NMR. NMR data can distinguish between protons from different molecular sources, and can provide sharp contrasts between meat components such as fat and lean. Correlation between NMR measurements and known fat content in meat samples has been reported to be 0.967.

Studies of NMR applications for food products have suggested many potential uses, including identification of contaminants, specific composition of fats and oils, and changes in lipids as an indicator of product freshness. Although NMR

has been studied as a means of measuring water and protein as well as fat, the method appears to be most effective when used for the measurement of lipid components.

For meat samples, NMR applications for fat measurements are often limited by interference from water protons. Interference from water can be overcome by utilizing a dried sample but drying is ordinarily a time-consuming preparation step which has, in the past, reduced the practical usefulness of NMR as a rapid method. However, CEM Corporation has combined NMR technology with the CEM microwave oven-drying procedure to create a multicomponent moisture/fat method for meat that can be completed in 4–5 min. The CEM instrument, called SMART Trac II, has been approved by AOAC International as an official method for determination of both moisture and fat in meat (AOAC Method 2008.06). This combined methods approach offers significant advantages in terms of speed, no organic solvents or waste are formed, and there is no need for calibration.

Summary

A wide variety of methods are available for measuring the proximate composition (fat, moisture, and protein) of meat raw materials used for the manufacture of processed meat products. These methods range from traditional wet chemistry methods that have been in use for decades to extremely rapid, in-line multicomponent analyses that have evolved recently. A critical consideration for comparison of methods is performance measurement. Repeatability, reproducibility, and bias must be determined in order to permit selection of a method that will meet expectations. These performance measures must also be monitored while a method is in use to ensure continued acceptable method performance.

See also: Chemical Analysis: Sampling and Statistical Requirements. Chemical Analysis for Specific Components: Major Meat Components; Micronutrients and Other Minor Meat Components. Laboratory Accreditation

Further Reading

Horowitz, W., Latimer, G.W. (Eds.), 2005. Official Methods of Analysis, 18th ed. Gaithersburg, MD: AOAC International, Inc. Current through revision 4, 2011.
Jean, B.R., 2000. Guided microwave spectrometry for in-line analysis of flowable materials. Sensors Update 7 (1), 171–184.
Mahr, C., 2010. Technological analysis adding value for the food industry. Food Safety Magazine 16 (4), 59–62, 72.
Nielson, S.S., 2010. Food Analysis. New York, NY: Springer.
Nollet, L.M.L., Toldra, F. (Eds.), 2008. Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, Inc.
Perez, T., Ruiz, J., Martin, D., Muriel, E., Antequera, T., 2008. Comparison of different methods for total lipid quantification in meat and meat products. Food Chemistry 110, 1025–1029.
Prevolnik, M., Skrlep, M., Janes, L., *et al*., 2011. Accuracy of near infrared spectroscopy for prediction of chemical composition, salt content and free amino acids in dry-cured ham. Meat Science 88, 299–304.
Prieta, N., Roehe, R., Lauin, D., Batten, G., Andres, S., 2009. Application of near infrared spectroscopy to predict meat and meat products quality: a review. Meat Science 83, 175–186.
Shahidi, F., 2005. Extraction and measurement of total lipids. In: Wrolstad, R.E. (Ed.), Handbook of food analytical chemistry. Hoboken, NJ: John Wiley & Sons, Inc., pp. 425–435.
Thompson, M., Owen, L., Wilkinson, K., Wood, R., Damant, A., 2004. Testing for bias between the Kjeldahl and Dumas methods for determination of nitrogen in meat mixtures by using data from designated interlaboratory experiment. Meat Science 68, 631–634.
Wrolstad, R.E. (Ed.), 2005. Handbook of Food Analytical Chemistry. Hoboken, NJ: John Wiley and Sons, Inc.

Relevant Websites

http://cem.com/smart-profat.html
CEM Corporation.
www.eaglepi.com/fat_analysis.php
Eagle Product Inspection.
www.foss.dk/industry-solution/products/foodscan-meat-analyzer
FOSS.
www.Tegetec.com/EquipmentandSolutions/KatridgPak/tabid/20824/language/da-DK/Default.aspx
KatridgPak.
http://lcfltd.com
Least Cost Formulations.
www.ndc.com
NDC Infrared Engineering, Inc.
www.gea-foodsolutions.com/Wolfking.1305.aspx#.UFKUkm2QOOg
Wolfking.

Sampling and Statistical Requirements

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Glossary

Figures of merit Measurements, including limit of detection, sensitivity, R-square, and linear range that indicate how accurate and precise a standard curve will be at determining unknown concentrations.
Limit of detection The lowest value for an analytical assay that is reportable for an unknown sample.
Relative standard deviation This value is the standard deviation divided by the average and multiplied by 100.
Sensitivity The magnitude of change for a measuring device or analytical assay as compound concentration changes.
Significant figures The number of reportable digits for a result obtained in a chemical analysis.
Standard curve A linear regression analysis that is used to determine unknown concentrations. The regression analysis is based on known standard concentrations of a chemical analyte as the X-variable and an analytical parameter, such as absorbance units for ultraviolet-visible spectroscopy and peak area for gas or high performance liquid chromatography.
Standard deviation A measurement of analytical precision that indicates how close values are together. It is a measure of how much variability or spread is around the mean.

Introduction

Chemical analyses result in values that indicate compound concentration. However, even the most experienced person will not be able to repeat an analysis and obtain exactly the same value each time. For practical reasons, other people should also be able to do the analysis, at another time and perhaps in another place, and these factors will cause some deviation from the true (unknown) value and imply that the true value is present only within an observed interval.

Statistical analysis is used to determine confidence intervals (CIs) and account for variability. If the preconditions (environment, performance, etc.) have limited influence on the chemical analysis, only a few samples are necessary to obtain reliable results as compared with the use of techniques with low robustness or techniques with poor ability to give similar results under varying conditions. For example, a food processing plant might only take two measurements for protein, fat, and moisture percentage of a bologna meat batter because it is part of the daily production process and the variability in the process has been established. However, more than one measurement still needs to be taken to ensure repeatability and reliability. In research, >30 samples per diet may be necessary to determine the moisture, protein, and fat percentage of the resulting meat when feeding broilers diets with varying concentrations of lysine and methionine.

This article deals with the statistical methods related to the reliability of measurements and different aspects of the sampling problem. The subjects are discussed in relation to chemical analyses, but similar considerations should be made for all types of measurement.

Statistical Requirements of Analysis Techniques

Measurement of Central Tendency

A minimum of three measurements are commonly taken to determine the mean and standard deviation in a chemical analysis. Occasionally, two measurements can be taken if it is a robust measurement and a consistent industrial process. However, more than three measurements are commonly needed when there is a large standard deviation in the analysis, either due to the analytical measurement or due to heterogeneity of the process or product. This is often necessary in research and when setting specifications for a process. From these observations, a mean is calculated as an estimate of the true value. The equation for the mean is as follows:

$$\bar{x} = \frac{x_1 + x_2 + x_3 + x_4 + x_5 + \cdots + x_n}{n} = \frac{\sum x_i}{n}$$

Example: Three samples are randomly taken from a production batch to measure the protein percentage of a meat batter using near-infrared spectroscopy that is used to make frankfurters and values of 18.34, 18.73, and 18.92 are recorded. The calculated mean is 18.663 33. The value should be reported with 3 significant digits, 18.7, because it is clear that the first 2 values of the number are 18, but it is not clear that the value in the tenths place is 7. A measurement of the variability is needed so that the standard deviation and the relative standard deviation (RSD) (also called the coefficient of variability) are calculated. The standard deviation is a measure of how much variability or spread is around the mean and is calculated as s because the true mean is not known:

$$\text{Standard deviation} = s = \sqrt{\frac{\left(\sum(\bar{x}_i - x)^2\right)}{n}} \text{ if } n \geq 30 \text{ and}$$

$$s = \sqrt{\frac{\left(\sum(x_i - x)^2\right)}{n-1}} \text{ if } n < 30$$

For the example above, the standard deviation is the square root of $((18.34–18.66)^2 + (18.73 - 18.66)^2 + (18.92 - 18.66)^2)/(n-1) = \sqrt{0.0874} = 0.296$.

This mean should be reported as 18.7 ± 0.296 or 18.7 with a standard deviation of 0.296.

The RSD is calculated using the following equation:

$$\text{RSD} = \frac{s}{\bar{x}} \times 100$$

For the value 18.7, RSD = 0.296/18.7 × 100 = 1.58%.

An RSD ≤5% is generally considered acceptable for analytical measurements.

Confidence Intervals

The most CI used is the 95% CI, but 90% and 99% CIs are also commonly reported. If this is a repeated measurement in which >30 total measurements have been taken, and it has been proven that these values follow a normal distribution, then *z*-values can be used to determine the CI. The *z*-values for 90%, 95%, and 99% CIs are 1.645, 1.96, and 2.58, respectively. Therefore, a 90% CI for the example taken in this article would be $\text{x} \pm \text{Z value} \times s/n = \sqrt{18.66} \pm 1.645 \times (0.296/3) = \sqrt{18.66} \pm 0.28 = (18.38, 18.94)$. For a 95% and 99% CI, the values would be (18.32, 19.0) and (18.22, 19.10), respectively. Note here that even though one reports the mean as 18.7 due to significant digits, the mean is reported as 18.66 within the CI so that the CI is as accurate as possible.

If three measurements were taken and these samples were not part of a larger dataset with a normal distribution, the *t*-distribution would be used because $n < 30$, and a 95% CI with three measurements would yield $n - 1 = 2$ degrees of freedom. Table 1 contains the *t*-statistics that should be used for 90%, 95%, and 99% CIs. In this example, the 95% CI is $\text{x} \pm \text{t value}_{n-1} \times s/n - 1 = \sqrt{18.66} \pm 4.303 \times (0.296/2) = \sqrt{18.66} \pm 0.90 = (17.76, 19.56)$. The same mean and standard deviation with 10 measurements has a $\text{CI} = \sqrt{18.66} \pm 2.262 \times (0.296/9) = (18.43, 18.89)$. This demonstrates the effect of sample size on statistical confidence.

Example

Three protein measurements were taken for frankfurter batter as an example. However, the values for protein are 17.7, 18.7, and 19.7. The mean, standard deviation, and RSD are 18.7, 1.0, and 5.3%, respectively. Because these three measurements are taken for every batch on every day, the normal distribution is assumed and the 95% CI is $\sqrt{18.7} \pm 1.96 \times (1.0/3)$ (resulting in values of 17.56 and 19.84). It is assumed that a minimum of 18% protein is needed to emulsify the meat product and meet quality and sensory standards. This indicates that the lower number in 95% or 99% CI needs to be greater than 18% and, if it is not, an adjustment needs to be made to the formulation. This also demonstrates the importance of controlling variability in production, instrumental analysis, and sampling because a standard deviation such as this would not be acceptable in sausage production as the mean and lower CI need to be close to 18.0 to minimize costs.

Table 1 *t*-Statistics for two-sided 90%, 95%, and 99% confidence intervals (CI) and one-sided 95% CI within the *t*-distribution

Degrees of freedom (n − 1)	t-*statistic (two-sided 90% CI and one-sided 95% CI)*	t-*statistic (two-sided 95% CI)*	t-*statistic (two-sided 99% CI)*
1	6.314	12.701	63.66
2	2.920	4.303	9.925
3	2.353	3.182	5.841
4	2.132	2.776	4.604
5	2.015	2.571	4.032
6	1.943	2.447	3.707
7	1.895	2.365	3.499
8	1.860	2.306	3.355
9	1.833	2.262	3.250
10	1.812	2.228	3.169
30	1.697	2.042	2.750
∞	1.645	1.960	2.576

Standard Curves

Instrumental analyses, such as spectroscopy, gas chromatography (GC), and high-performance liquid chromatography (HPLC), require the use of a standard curve to determine unknown concentrations. Simple linear regression with concentration as the *x*-variable and either absorbance or peak area as the *y*-variable are used to determine $y = mx + b$. The slope, or sensitivity, is m, x is the concentration, and b is the *y*-intercept.

Example: A laboratory measures protein solubility as an indicator of protein functionality in processed meat products. The laboratory has been asked to determine total protein solubility for breast meat from broilers that are stressed before harvest by lengthy transport times in the summer (35–40 °C). A standard curve for the biuret assay is used to determine the concentration of solubilized protein with bovine serum albumin (BSA) as the standard. For this assay, the absorbance is determined using an ultraviolet–visible spectrometer at 540 nm.

The standard curve concentrations and absorbances are reported in Figures 1 and 2. The initial range of the protein standards was 0–14 mg ml^{-1} BSA. Simple linear regression was run on the samples with x as the concentration (explanatory variable) and y as the absorbance (response variable). Figures of merit were determined and include R^2 (coefficient of determination), limit of detection (LOD), linear range, and sensitivity. It is a general rule that a standard curve should have a minimum R^2 of 0.99. If $R^2 = 0.99$, this indicates that 99% of the variability in the absorbance (y) is explained by the concentration (x) and the remaining 1.0% is random variability. For the example in Figure 1, the excel output indicates an R^2 of 0.988. At first glance, this appears to be a sufficient standard curve because it is very close to the standard of 0.99. However, the line graph indicates that the data were only linear up to 12 mg ml^{-1} (Figure 2). Removal of the 14 mg ml^{-1} point improved the standard curve substantially and an $R^2 > 0.999$ was achieved. Therefore, the data from 0–12 mg ml^{-1} should be used because the R^2 is acceptable based on the requirement of 0.99 and the linearity of the curve. The LOD can be calculated for analytical instruments and an assay. For instruments, the LOD is 3 times the noise of the sample when a blank is run in the spectrometer, GC, HPLC, etc. The LOD for the assay is dependent on the random variability of the regression line and the slope and is calculated as either 3 × standard error (s.e.)/slope (m) or 3.3 × s.e./m. For the examples taken in this article, 3 × s.e./m is used to calculate LOD because this is what is done in a lab, but some literature reports using 3.3 × s.e./m to calculate LOD. Therefore, for the two

BSA (mg ml^{-1})	Absorbance (AU)
0	0
2	0.106
4	0.208
6	0.31
8	0.418
10	0.519
12	0.624
14	0.638

Summary output	
Regression statistics	
Multiple R	0.99404159
R Square	0.98811869
Adjusted R Square	0.98613847
Standard error	0.02796543
Observations	8
	Coefficients
Intercept	0.0155
X Variable 1	0.04819643

Summary output	
Regression statistics	
Multiple R	0.99997966
R Square	0.99995932
Adjusted R Square	0.99995119
Standard error	0.00156753
Observations	7
	Coefficients
Intercept	0.00057143
X Variable 1	0.05192857

Figure 1 Standard curve data and simple linear regression output from Microsoft Excel for the biuret analysis to determine protein solubility of turkey breast protein for use in further processed meat products. The output with eight observations is for 0–14 mg ml^{-1} BSA and with seven observations is for 0–12 mg ml^{-1} BSA.

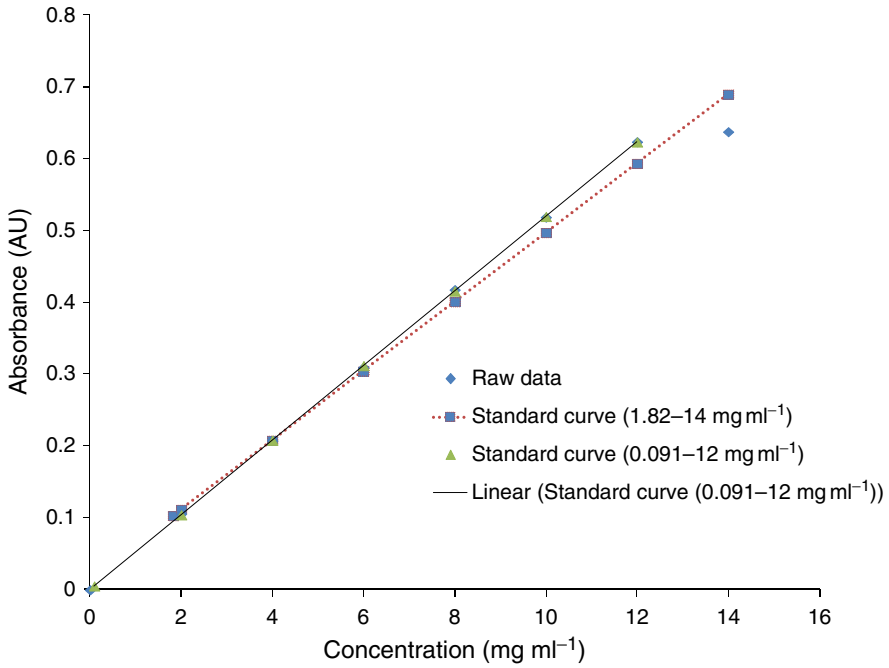

Figure 2 Standard curve graph for raw data and the standard curve lines 1.82–14 mg ml^{-1} BSA and 0.091–12 mg ml^{-1} BSA to determine protein solubility using the biuret assay.

regression assays, the LODs were calculated as $(3 \times .0280$ AU)/0.0482 AU (mg ml^{-1})$^{-1}$ and $(3 \times 0.00157$ AU)/0.0519 AU (mg ml^{-1})$^{-1}$, respectively (Figure 1). These LODs are 1.82 and 0.091 mg ml^{-1}. This large difference demonstrates the impact of a small increase in R^2 on the LOD when part of the curve is not linear. Any unknown sample that has a concentration below the LOD based on the standard curve should be reported as not detectable.

The limit of quantitation (LOQ) has been previously reported as either equal to the LOD or as $9 \times$ s.e./m. In addition, different organizations have varying definitions for LOD and LOQ. For clarification on LOD and LOQ, the Association of Official Analytical Chemists is the authority on analytical measurements of meat products. The third figure of merit is sensitivity or the slope and is defined as the change in response (absorbance) due to a 1-unit increase in concentration. A high-sensitivity assay should be used to determine small differences between samples, and a low-sensitivity assay, such as the biuret analysis, is sufficient for samples with higher concentrations or treatment samples that have large differences in concentration. The sensitivity for the two examples (Figure 1) are 0.0482 AU (mg ml^{-1})$^{-1}$ and 0.0519 AU (mg ml^{-1})$^{-1}$, respectively. This indicates that removing 14 mg ml^{-1} from the standard curve slightly increases the sensitivity of the test. The fourth and final figure of merit is the linear range. The linear range starts at either the LOD or LOQ and ends at the highest concentration that is used in the final regression analysis. The linear ranges of these two examples are 1.8–14 mg ml^{-1}

and 0.091–12 mg ml^{-1}. It is important to note that unknown samples can have concentrations only within the linear range for the assay to be valid for that sample. If a sample has a value outside of the linear range, it either needs to be concentrated or diluted for it to be readable using the assay.

Determining Unknowns

Example: Triplicate samples are run for each of the two treatments (stressed vs. nonstressed broilers) for the example above. To make sure that the values were representative of the treatments, eight whole breasts were ground and homogenized for each of the triplicate samples. The stressed broilers yielded breast meat with absorbance values of 0.431, 0.443, and 0.449 AU, and the nonstressed broilers yielded breast meat with absorbance values of 0.591, 0.587, and 0.573. To understand this data, the following protocol was followed: (1) verification that the concentration was within the linear range, (2) means, standard deviations, RSD, and CIs were calculated, and (3) data were reported as significant digits. To determine the unknown concentrations, the $y = mx + b$ equation was used to solve for x, in which $x = (y - b)/m$. For the regression with a linear range of 0.091–12 mg ml^{-1} $x = (0.431\ \text{AU} - 0.000\ 571\ \text{AU})/0.0519\ \text{AU}\ (\text{mg ml}^{-1})^{-1}$; $(0.443\ \text{AU} - 0.000\ 571\ \text{AU})/0.0519\ \text{AU}\ (\text{mg ml}^{-1})^{-1}$; and $(0.449\ \text{AU} - 0.000\ 571\ \text{AU})/0.0519\ \text{AU}\ (\text{mg ml}^{-1})^{-1}$, which are 8.29 mg ml^{-1}, 8.52 mg ml^{-1}, and 8.64 mg ml^{-1}, respectively. The mean, standard deviation, and RSD were determined for the concentrations and are 8.5 mg g^{-1}, 0.178, and 2.1%, respectively. The final concentration was reported as two significant digits because there was confidence that the eight was correct, slight confidence that the five was correct, but no confidence that the number in the hundredths place was correct. In addition, the concentration of the unknown samples was within the linear range, and the 95% CI was 8.28–8.69 for the normal distribution and 7.94–9.03 for the *t*-distribution. Even though two significant digits is correct based on the precision for the test, it is reported as three digits to make the CI as accurate as possible. Following the same calculations, the average, standard deviation, and RSD for the breast meat from nonstressed broilers was 11.2 mg ml^{-1}, 0.184 mg ml^{-1}, and 1.64%, respectively. The 95% CI was 11.02–11.43 g ml^{-1} and 10.68–11.77 mg ml^{-1} for the normal and *t*-distributions, respectively. Because the 95% CI for the nonstressed broilers does not overlap with the 95% CI for the stressed broilers, the breast meat from the nonstressed broilers had greater protein solubility, which indicates that it would have better water-holding capacity and texture than the breast meat from the stressed broilers in both fresh and processed meats.

Significant Figures

Determination of significant figures is reported in most freshman-level chemistry books. A few examples related to meat science are included to demonstrate the importance of accounting for both analytical and biological variability when determining how to report significant digits. For example, *CIE* L*(lightness/darkness) values are measured for porcine semimembranosus muscles from animals from two different genetic strains, in which one strain is more resistant to stress. In the summer time, 64 hogs were harvested from each genetic strain and the *CIE* L* values ranged from 48.00 to 54.00 for the resistant strain and from 54.00 to 60.00 for the nonresistant strain. The biological variability for the treatments is fairly large, even though the chroma meter is accurate to 0.01. In addition, the means are 50.432 13 and 56.243 12. There are three possibilities for reporting these data. Because the accuracy of the instrument is to 0.01, many researchers would report these values with four significant digits as 50.43 and 56.24. This would not be incorrect from an instrumental standpoint. However, if the biological variability in the sample is taken into account, you can assume that the average is being approached as sample size increases ($n > 30$), so there is confidence that 50 and 56 are correct digits for the averages, but it is unlikely that the 4 and 3 are correct in the tenths place. Therefore, it is logical to report the data as 50.4 and 56.2 because one can be confident of the numbers in the tens and ones place but not the tenths place. Even though there is a large biological variability that could infer that there should only be two significant digits, using two significant digits would make the results less accurate and therefore it would not be the best practice to report the data that way.

For the standard curve example above, averages of 8.5 and 11.2 mg ml^{-1} are used for the assay. However, 2.00 g of sample were initially homogenized to a total volume of 35.0 ml buffer. Therefore, the final concentrations are 8.5 mg ml^{-1} × (35.0 ml/2.00 g) and 11.2 mg ml^{-1} × (35.0 ml/2.00 g) = 148.75 and 196.00 mg g^{-1}, respectively. Because 8.5 has only two significant digits, the rule of thumb is to report this as 150 mg g^{-1}. However, 149 mg g^{-1} would also be appropriate because it is more accurate. The 196.00 mg g^{-1} sample should be written as 196 because 11.2, 35.0, and 2.00 all have three significant digits based on the standard curve, the scale, and the volumetric flask that was used. In a laboratory, these values would be reported as 149 and 196, even though 150 and 196 are more technically correct based on the following rule of thumb: A result cannot have more significant digits than the value in the multiplication, subtraction, or addition with the least number of significant digits. There are a few items to remember with respect to reporting data: (1) Be able to explain why you reported the data with the number of significant figures that were chosen. (2) Take into account the number of significant digits in all values going into a calculation. (3) Consider the biological variability in the meat tissue when research is conducted. (4) Consider the accuracy and precision of the analytical instrument. (5) Increase the number of figures by 1 if it increases the accuracy of the value. For example, if two significant figures are correct, but it decreases the accuracy of the value, use three significant figures. (6) Do not let significant figures be a stumbling block. There is usually more than one answer that is acceptable with respect to reporting the data. There are usually two and sometimes three values that are logical. However, there are many wrong ways to report the data.

Selecting the Test Samples

When analyzing meat, it is very important to know the objective of the sampling, i.e., what the test sample is supposed to represent. Should the test sample represent a muscle or the

meat ready for cooking? If a muscle, should it be a muscle in live animals, in the carcass just after the harvest, or after the postmortem process has ended? A muscle is not completely homogeneous because the muscle cells vary from the periphery to the middle and from one end to the other. From live animals, only biopsy samples will be possible; consequently, the test sample will represent only a very specific part of the body. After harvest or conversion to meat, representative test samples could be taken from the muscle after it has been homogenized in a mincing machine, but if only 50 g or less is needed for analysis, the experiment would be very expensive. In any case, the method might be intended not for minced meat but for a piece or slice of meat of a certain size. There is no single, and simple, solution to the sampling problem. An experiment has to be carried out to study the variation within the muscle. If the variation along the loin muscle, for instance, varies depending on different uncontrolled factors, several test samples from each muscle are needed to represent the whole muscle.

Survey Sampling

Representative Test Samples for Experiments

In meat science, exploratory sampling to gain information about the effect of different treatment of animals or subpopulations is the most common example of survey sampling. The first step will be to identify the objective of the problem and the next to clarify the experimental unit. Again, the lack of identical test units is a general problem. If examination of the effect on meat quality due to different treatments of the animals, such as feeding, stabling, or transport, is required, identical groups of animals treated differently are needed. If, say, pigs are studied, it is an obvious tactic to distribute pigs of the same sex from the same litter into groups. The pigs in a litter are not identical, but they will be more nearly identical than pigs from different litters. This is the best way to get replicates, but, because of the kinship, the observations (replicates) will be correlated. This is not a problem as long as the statistical model takes this fact into account and the sample size is large enough.

If one wished to obtain knowledge about a certain population, the first problem would be to choose pigs that represent the population in question. Ideally, pigs should be chosen at random. However, for this to happen, each pig should have the same probability of being selected for the sample; in practice, this could never be the case. There is no simple answer to this problem, but one must try to consider which factors could influence the results – the producer, the season, the time, or the place and so on. Once the sample is selected, investigations can be carried out if the sample is similar to the population with respect to certain key characteristics.

Sample Size

When determining the sample size, two problems must be addressed: How to compare treatments and the statistical power of the test to be carried out. Imagine an investigation for the effect of different treatments (e.g., different types of feed) on ordinary pigs with respect to the fat content in their meat. The statistical assumptions for making an analysis of variance (ANOVA) are present and the analysis shows that the fat content seems to vary because of the type of feed. This is probably not fully satisfactory as an additional question arises: Which type of feed implies the lowest or highest fat content? Consequently, comparison needs to be made of the results from the different treatments. When this is the objective, an ANOVA table should be used to determine overall significance of the model and a mean separation technique, such as Duncan's multiple range test or Tukey's honestly significant difference test, should be used to separate treatment means. When conducting these types of experiments, it is general practice to conduct at least three separate replications of treatments with enough animals/subsamples to have a sufficiently low alpha (0.05) and beta (<0.10). Beta is not often calculated when conducting analyses or reporting data, but it should always be considered when statistical analysis is utilized to determine differences between treatments.

To investigate the equality of two subpopulations, an experiment must be designed by selecting two representative samples of equal size, one from each population; the question is how large the sample size should be. In answering this question, small differences between the populations must be accepted and the size of the true difference must be detected with some probability. In the example above, a true difference of 0.5 units of fat content might be considered as not significant, whereas a difference of two units might be considered significant. It might be considered acceptable to have a probability of, say, 80% for detecting a difference of two units. Consequently, the experiment is designed to have the power of 80% for detecting two units of true difference. Finally, if the two (conceptually infinite) populations are expected to vary as normal (Gauss) distributions having the same variance and an estimate of the size of the variance, σ^2, is also available, the number of test samples in each sample can be estimated using the formula

$$Z_\beta = (-\sqrt{n})\frac{\delta}{\sqrt{2}\sigma} + Z_\alpha$$

where Z_β denotes the $(1-\beta)$ quantile and Z_α denotes the α quantile in the normal (Gauss) distribution. δ denotes the true difference to be detected at a power level $(1-\beta)$ (type II) and α denotes the risk (type I) of rejecting the null hypothesis wrongly. In the example above, $Z_\beta = -0.842$, $Z_\alpha = 1.96$, and $\delta = 2$; the only information missing before estimating n is an estimate of σ^2. The best estimate can often be obtained from the previous observations. Alternatively, a small pretest for this purpose can be performed. If the problem involves several comparisons or characteristics, the α level must be reduced accordingly. A best practice is to calculate β and power after each statistical analysis based on the data that are obtained.

To sum up, the following steps have to be considered before carrying out the sampling:

- Clarify the objective and the characteristics to be measured.
- Identify how test samples should be prepared.
- Estimate the level of type I and type II risks together with the number of replicates.

Acceptance Sampling

In the production of meat products, the process must be checked to insure that the requirements attached to the product can be met. On the one hand, the requirements can be set by the authorities to insure specific levels of compounds or microorganisms for health reasons. On the other hand, the producer is to ensure a defined level of quality. For this purpose, a Guide on Sampling for Analysis of Foods has been prepared by a working group established by the Nordic Committee on Food Analysis. The idea is to set up a sampling plan that describes how inspection should be carried out. Typically, the inspection consists of three levels: normal inspection, tightened, and reduced inspection. The inspections focus on the produced lots (or batches) of products; a 'lot' is a quantity of products produced under uniform conditions. From each lot, a number of sample items are selected, analyzed, and evaluated. The evaluation results in acceptance or rejection of the lot. If the inspection results in acceptance of consecutive lots, the inspection can be reduced, that is, fewer sample items need to be selected. However, if the inspection rejects consecutive lots, the inspection will be tightened.

A number of estimates are necessary to specify the sampling plan. First, the method for deciding on acceptance or rejection has to be determined; in principle, only two methods are included. In an attribute sampling plan, each selected test sample is classified according to the quality characteristics (i.e., acceptable or defective). In a variable sampling plan, the acceptance evaluation is based on the average and variability of the measurements, which must be considered as observations from a normal distribution. Besides this, the lot size has to be known and the acceptable quality level, which is an index corresponding to a maximum rate of defective items in the lots, has to be determined. With this information, it will be possible to determine a standard sampling plan (according to ISO 2859) that can be understood by all interested parties and that is known to be optimal as it includes an appropriate sample size.

Determination of Specifications

When determining processing capability or if a product meets specification, it is important to have statistical data during both the product development and the production phases to set specification limits and then have a sampling plan to verify that data for samples are within the specification limit. For example, a meat processor receives a request from a food service establishment for meatballs and will randomly sample 5 kg bags that are shipped to them for salt concentration and meatball diameter. Once the production process for the meatballs is set up and running correctly, sampling ($n > 30$) should be conducted on the initial production runs for 5–6 days. After setting the preliminary specifications, samples ($n > 30$) should be conducted for approximately 6 reps (processing days) to verify or change the specifications. Based on the data that are acquired, either a 95% or a 99% CI (if possible) should be used as the specification limits. Sampling should still be conducted for each lot of production (possibly a day's production, morning, afternoon, evening, etc.). If the sample fails to meet specifications, the lot should not be shipped without appropriate rework. These specification limits may need to be changed based on changes to the process or production process. This is not a five time and finished process. Data should continue to be added to the body of knowledge to adjust specifications and hopefully improve accuracy and precision.

See also: Chemical Analysis: Analysis of Final Product Composition for Labeling; Physicochemical Analysis Methods; Raw Material Composition Analysis; Standard Methods. Chemical Analysis for Specific Components: Major Meat Components; Micronutrients and Other Minor Meat Components; Veterinary Drug Residue Analysis. Chemical and Physical Characteristics of Meat: Chemical Composition; Color and Pigment; pH Measurement; Protein Functionality; Water-Holding Capacity. Environmental Contaminants. Microbiological Analysis: Standard Methods. Potential Chemical Hazards Associated with Meat. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

ISO 2859, 1995–2011. Sampling Procedures for Inspection by Attributes (Part 0–4. Geneva: ISO.

ISO 5725, 1994–2005. Accuracy (Trueness and Precision) of Measurement Methods and Results (Part 1–6). Geneva: ISO.

NMKL, 2002. Guide on Sampling for Analysis of Foods. Oslo: Nordic Committee on Food Analysis.

Smith, J.S., 2010. Evaluation of analytical data. In: Nielsen, S. (Ed.), Analysis of Food Products. New York, NY: Springer, pp. 55–67.

Relevant Website

http://www.sciencedirect.com/science/journal/08891575
Journal of Food Composition and Analysis.

Standard Methods

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Glossary

Applicability The method's ability to identify and measure the physical and chemical forms of the analyte that are likely to be present in the material, with regard to matrix effects.

Interferences The effects of any other components that are likely to be present at appreciable concentrations in matrices of interest and which may interfere in the determination.

Limit of detection The amount of an analyte corresponding to the lowest measurement signal which with a closely defined confidence may be interpreted as indicating that the analyte is present in the sample but without allowing exact quantification.

Precision The closeness of agreement between independent test results obtained under stipulated conditions.

Recovery The difference between the measuring results for a sample without the analyte and a sample to which a known amount of the analyte has been added, divided by the added amount.

Repeatability The precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility The precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

Standard developing organizations The organizations elaborating methods; in this context, within food analysis.

Trueness The degree of agreement between a sample's true content of a specific analyte and the result of the analysis. Trueness is not the same as accuracy. Accuracy is the sum of trueness and precision. Trueness is the systematic error, precision is the random error, and accuracy is the total error (systematic+random error).

Use of Standard Methods

Reliable analytical methods are required to achieve 'true' analytical results and compliance with national and international regulation. In some sectors, it is prescribed by law to use standard methods or fully validated methods. Most chemical standard methods are fully validated, i.e., the methods' performance characteristics have been studied in interlaboratory studies (also known as collaborative studies or collaborative trials). Conducting collaborative studies requires a lot of work, but if a method is required by a large number of laboratories, the cost of collaboratively validating a method is justified. When using a collaboratively studied method, the measures a laboratory needs to carry out before taking the method into routine use are reduced considerably. Using a collaboratively validated method gives an advantage when trying to get the method accredited, because the laboratories need only to demonstrate that they can achieve the performance characteristics stated in the method.

Standard Developing Organizations

There are several Standard Developing Organizations (SDOs), elaborating chemical methods for food analysis. Some of these SDOs provide standards for specific products, for example, the International Dairy Federation (IDF) elaborating standards in the field of dairy products and the American Oil Chemists' Society (AOCS) developing standards within the field of fats and oils. Other SDOs such as The Scientific Association Dedicated to Analytical Excellence® (AOAC International), the European Standardization Organization (CEN), International Standardization Organization (ISO), and Nordic Committee on Food Analysis (NMKL) are all developing methods for different food products. The structures of the different organizations vary. Some organizations consist of national committees or national standardization bodies. The SDOs depend on submission of method proposals from experts within these committees or national standardization bodies. Further, the national bodies forward method comments and give or withhold their approval of the method. The AFNOR (Association Francaise de Normalisation, France), DIN (Deutsches Institute für Normung, Germany), DS (Danish Standard, Denmark), BSI (British Standards Institution, UK), and NS (Norwegian Standard, Norway) are such national standardization bodies of ISO and CEN in Europe and the ANSI is the American National Standardization Institute.

The Food and Agriculture Organization of the United Nations and World Health Organization are both elaborating food standards (Codes) in the Codex Alimentarius – more than 180 countries representing 97% of the world's population are members. In these food standards, analytical methods are often referenced. These methods are primarily intended as international analytical methods for the verification of provisions in the Codex standards. They should be used for reference, in calibration of methods in the use, or introduced for routine examination and control purposes. To date, Codex has endorsed specific methods for analysis of the

provision of interest. Recently, Codex has adopted the Criteria Approach for the method performance. Instead of adopting specific methods, any methods fulfilling the given criteria might be used. In the Codex Alimentarius Commission Procedural Manual, twenty-first edition, Codex gives guideline for establishing numeric criteria for the method performance characteristics which corresponds to the characteristics listed below.

How a Method Becomes a Standard

Initially, the need for a standard is put forward by laboratories, government, or industry. A suitable method for the purpose has to be drafted and tested by experts within the specific topic. Most of the standardization work is voluntary; a number of experts and their institutions support standardization work by providing their valuable knowledge, effort, and time. Collaborative studies involve considerable effort and should, therefore, be conducted only on methods that have undergone prior testing in a laboratory. The collaborative studies should be conducted according to international acceptable rules. Several organizations have adopted the International Union of Pure and Applied Chemistry (IUPAC) protocol for the design, conduct, and interpretation of method performance studies of 1994. This protocol gives guidance on how to design, conduct, and interpret the results of a collaborative study.

Performance Characteristics Examined

In a collaborative study, the following characteristics are determined:

- Applicability: The method's ability to identify and measure the physical and chemical forms of the analyte that are likely to be present in the material, with regard to matrix effects.
- Interferences: The effects of any other components that are likely to be present at appreciable concentrations in matrices of interest and which may interfere in the determination.
- Limit of detection: The amount of an analyte corresponding to the lowest measurement signal which with a closely defined confidence may be interpreted as indicating that the analyte is present in the sample but without allowing exact quantification.
- Precision: The closeness of agreement between independent test results obtained under stipulated conditions.
- Repeatability: The precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.
- Reproducibility: The precision under conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.
- Trueness: The degree of agreement between a sample's true content of a specific analyte and the result of the analysis.
- Recovery: The difference between the measuring results for a sample without the analyte and a sample to which a known amount of the analyte has been added, divided by the added amount.

Determination of Performance Characteristics

Testing Materials Used in Collaborative Studies

A material is a given combination of an analyte (substance), its concentration, and a given matrix. For a single type of substance, at least five materials should be used. The number of materials used can be reduced to three if the method studied is applicable only for a single-level specification for a single matrix. The two or more test samples of blind or open replicates are considered as a single material. The concentrations of the analyte in the materials used should be of low, middle, and high level.

The main objective with the collaborative study is to generate data to form the basis of a reliable estimate of the repeatability and reproducibility. Repeated analysis of one single sample is not an acceptable basis. An estimate of the repeatability is best accomplished by using one of the following models (in prioritized order).

1. For each level, measurement is carried out on a single determination on two materials with the same or almost the same concentration of the parameter (split-level pair).
2. For each level, measurement is carried out on materials where some are split-level pair and some are disguised parallel test samples (combined uniform-level pairs).
3. For each level, measurement is carried out on disguised/hidden parallel test samples (uniform-level pair).

The Material Quality

Materials used in a study are subject to strict requirements. The samples should as far as possible be natural foodstuffs. It is of vital importance that the materials are properly homogenized, tested for homogeneity, and stored and shipped under the correct conditions. The conductor of the study must prepare enough samples to ensure homogeneity and stability and should also be prepared to provide additional samples to laboratories that for some reason might need them. It is important that the conductor keeps an open dialog with the participating laboratories about when the materials are to be analyzed.

The Number of Laboratories

In a collaborative study, results from at least eight laboratories should be obtained. To obtain this number of results, 12–15 laboratories should be appointed. If it is impossible to obtain this number of participating laboratories, for instance, if the study requires very expensive instrumentation or specialized laboratories, the study may be conducted with an absolute minimum of five laboratories. Usually, the laboratories do not receive any funding for their efforts in such study; they receive only honor of participating (in terms of good name and reputation).

Analysis of the Samples

The conductor of the study forwards the test samples to the participating laboratories. The laboratories must perform the analysis according to the method draft in question. Sometimes a prestudy is conducted in order to ensure that everyone has understood the procedure correctly and to eliminate any problems. If a laboratory modifies the method, the results from this laboratory must be excluded from the study.

Statistical Analysis of the Results

As an example, the 'statistical analysis' of the results conducted within the NMKL consists of the following six steps.

1. Recognition of valid data. Valid data are results that are not to be rejected. Results to be rejected are, for instance, results achieved from analyses in which the method was not followed precisely; a nonlinear calibration curve was used when a linear one was expected; the analytical equipment did not work satisfactorily and test samples were lost; the separation of peaks (in chromatography) was not satisfactory; or analyses in which unexpected reaction (chemical or microbiological) or any atypical phenomenon occurred. Quite often, the review of valid data is based on comments from the participating laboratories, which are attached to the study form distributed along with the study samples.
2. Variance analysis of the valid data. As described in the section "Testing Materials Used in Collaborative Studies" the samples in the studies are paired. The statistical analysis should be conducted on each pair. One-way analysis of variance is performed on all valid data for each sample pair and is the statistical procedure for obtaining the estimates of within-laboratory (intralaboratory) and between-laboratory (interlaboratory) variability on a material-by-material basis. Examples of calculations can be found in the NMKL Report No. 11 and ISO 5725–2, 1994.
3. Outlier tests by Cochran and Grubbs. The Cochran outlier test is used for testing the figures for significant differences in obtained results from a single laboratory compared with those obtained from other laboratories. The Grubbs outlier test is used for testing the figures for extreme mean values obtained at single laboratories compared with those obtained at the other laboratories. (For the testing, see IUPAC Harmonized Protocol.)
4. Recalculation of the variance, mean, the repeatability, and reproducibility. This is done after results have been removed in accordance with the outlier tests. According to the IUPAC Harmonized Protocol, no more than 22.2% (two of nine laboratories) should be removed.
5. Estimation of Horrat values. Based on several hundred method performance studies, including a huge number of analytes, materials, and techniques, Dr. William Horwitz established an equation describing the relation between the concentration ratio and the expected relative standard deviation. This is expressed as eqn [1], where C is the concentration ratio.

$$\text{Theoretical RSD} = (\text{PRSD}_R) = 2C^{(-0.1505)} \qquad (1)$$

C is the concentration ratio. For concentration of 10 mg kg^{-1}, C is $10\times10^{-6} = 10^{-5}$.

The Horrat value is the ratio of the estimated relative standard deviation (RSD_R) and the theoretical RSD_R ($PRSD_R$):

$$\text{Horrat value} = \text{RSD}_R/\text{PRSD}_R \qquad (2)$$

6. Evaluation of the method: A method can be approved as acceptable when:
   a. the study is performed according to the IUPAC-1994 recommendations.
   b. all the relevant (valid) results are examined for outliers, and fewer than one out of five samples contain less than 20% inexplicable outliers (no more than two out of ten laboratories or two out of ten samples can contain more than two outliers).
   c. the Horrat value is no more than 2 (in some occasions, less than 1.5 is recommended).

   The study reports are reviewed by experts within the relevant field of interest.

The collaboratively validated methods are reviewed after a given period. In the NMKL, the collaboratively validated methods are reviewed after 10 years, or sooner if necessary.

The Codex Alimentarius' Guidelines for Numeric Values for Method Performance Criteria

Only the analyte (provision) in the specific commodity of interest along with its maximum level, minimum level, normative level, or concentration range of interest (here noted as ML) is needed when establishing numeric values for method criteria. In the Codex, the numeric values are guidelines for the Codex purposes and hence relevant for the SDOs and laboratories when elaborating and selecting methods **Table 1**.

How Methods Come into Being

Establishing a collaboratively validated method is a demanding task. A lot of effort from different experts and laboratories is laid down in a method. Usually, it takes between 2 and 5 years to establish a method. Exactly how methods come into being in the different organizations varies quite a bit. However, to illustrate the process, the practice of the NMKL, is described in the flowchart in **Figure 1**. The NMKL is a Nordic organization consisting of microbiologists, chemists, and sensory analysts from Denmark, Finland, Iceland, Norway, and Sweden, with the primary objective of selecting, collaboratively validating, and publishing methods on analysis of foods.

Chemical Standards and Official Methods on Meat and Meat Products

In recent years, the organizations have been working toward making horizontal standards, methods applicable to all kind of foodstuffs, rather than vertical ones. References to Chemical standards and official methods applicable to meat and meat

Table 1 Guidelines for establishing numeric values for the criteria

Applicability:	The method has to be applicable for the specified provision, commodity, and level(s) (maximum and/or minimum) (ML). The minimum applicable range of the method depends on the specified level (ML) to be assessed and can be expressed either in terms of the reproducibility standard deviation (S_R) or in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).	
Minimum applicable range:	For ML≥0.1 mg kg^{-1}, [ML−3 S_R, ML+3 S_R]	
	For ML < 0.1 mg kg^{-1}, [ML−2 s_R, ML+2 s_R]	
	s_R = standard deviation of reproducibility	
LOD:	For ML≥0.1 mg kg^{-1}, LOD≤ML 1/10	
	For ML < 0.1 mg kg^{-1}, LOD≤ML 1/5	
LOQ:	For ML≥0.1 mg kg^{-1}, LOQ≤ML 1/5	
	For ML < 0.1 mg kg^{-1}, LOQ≤ML 2/5	
Precision:	For ML≥0.1 mg kg^{-1}, HorRat value≤2	
	For ML < 0.1 mg kg^{-1}, the RSD_{TR} < 22%	
	RSD_R = relative standard deviation of reproducibility	
Recovery (R):	Concentration (unit)	Recovery (%)
	≥10% (10 g 100 g^{-1})	98–102
	1% (1 g 100 g^{-1})	97–103
	0.1% (1 mg g^{-1})	95–105
	100 mg kg^{-1}	90–107
	10 mg kg^{-1}	80–110
	1 mg kg^{-1}	80–110

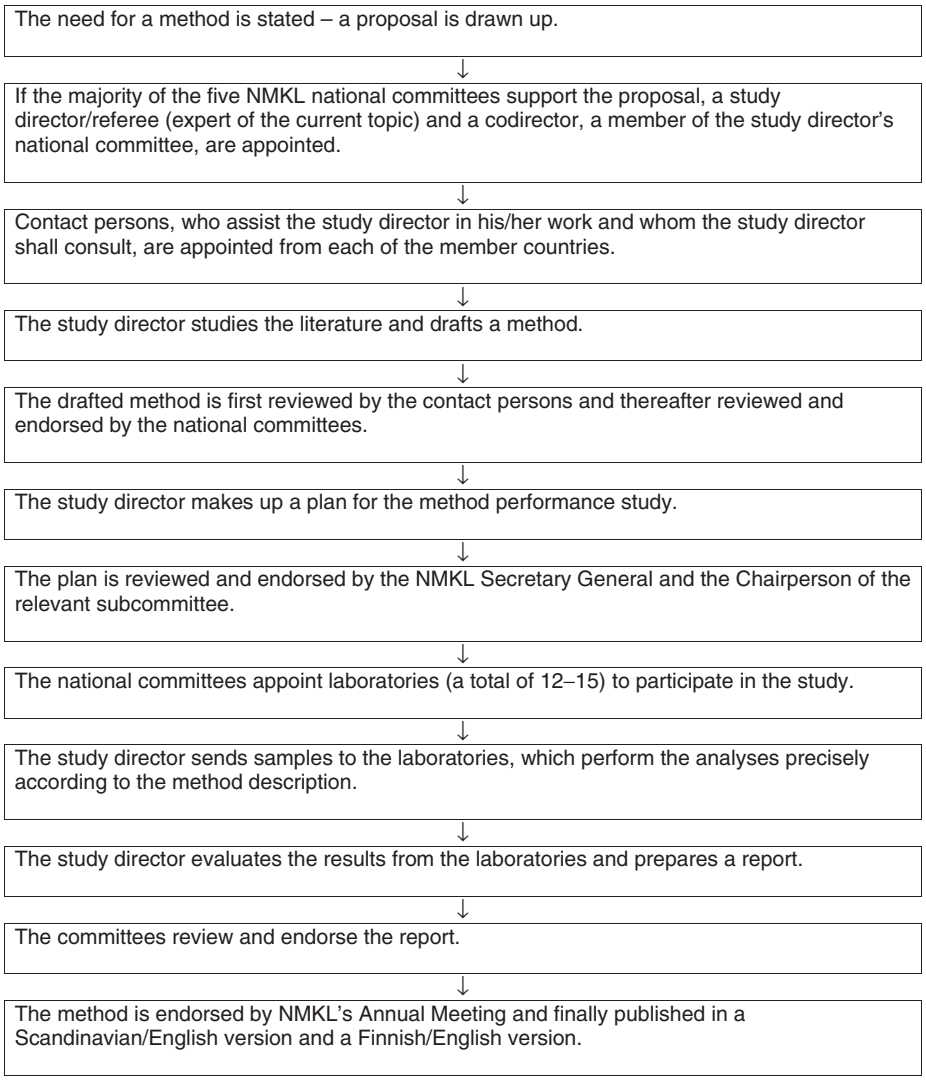

Figure 1 Flowchart of the NMKL method development process.

products collected from the organizations' web pages are listed below:

From ISO (www.iso.org):

- ISO 936:1998 Meat and Meat Products – Determination of Total Ash Content
- ISO 937:1978 Meat and Meat Products – Determination of Nitrogen Content (Reference method)
- ISO 1442:1997 Meat and Meat Products – Determination of Moisture Content (Reference method)
- ISO 1443:1973 Meat and Meat Products – Determination of Total Fat Content
- ISO 1444:1996 Meat and Meat Products – Determination of Free Fat Content
- ISO 1841–1:1996 Meat and Meat Products – Determination of Chloride Content – Part 1: Volhard method
- ISO 1841–2:1996 Meat and Meat Products – Determination of Chloride Content – Part 2: Potentiometric method
- ISO 2294:1974 Meat and Meat Products – Determination of Total Phosphorus Content
- ISO 2917:1999 Meat and Meat Products – Measurement of pH
- ISO 2918:1975 Meat and Meat Products – Determination of Nitrite Content
- ISO 3091:1975 Meat and Meat Products – Determination of Nitrate Content
- ISO 3100–1:1991 Meat and Meat Products – Sampling and Preparation of Test Samples – Part 1: Sampling
- ISO 3496:1994 Meat and Meat Products – Determination of Hydroxyproline Content
- ISO 4133:1979 Meat and Meat Products – Determination of Glucono-delta-lactone Content
- ISO 4134:1999 Meat and Meat Products – Determination of Glutamic Acid Content
- ISO 5553:1980 Meat and Meat Products – Detection of Polyphosphates
- ISO 5554:1978 Meat Products – Determination of Starch Content
- ISO 13493:1998 Meat and Meat products – Determination of Chloramphenicol Content – Method using Liquid Chromatography
- ISO 13496:2000 Meat and Meat Products – Detection of Colouring Agents – Method using Thin-layer Chromatography
- ISO 13730:1996 Meat and Meat Products – Determination of Total Phosphorus Content – Spectrometric method
- ISO 13965:1998 Meat and Meat Products – Determination of Starch and Glucose Contents – Enzymatic method

From the AOAC International (www.aoac.org)

- AOAC 2011.04: Protein in Raw and Processed Meats. Automated Dye-Binding Method
- AOAC 2008.06: Moisutre and Fat in Meats using Microwave and NMR
- AOAC 2007.04: Fat, Moisture, and Protein in Meat and Meat Products using NIR
- AOAC 993.03: Nitrate in Baby Foods
- AOAC 992.15: Crude Protein in Meat and Meat Products Including Pet Foods
- AOAC 991.36: Fat (Crude) in Meat and Meat Products
- AOAC 991.28: N-Nitrosamines in Minced Fish–Meat and Surimi-Meat Frankfurters
- AOAC 991.27: Phosphorus in Meat and Meat Products
- AOAC 991.19: Gliadin as a Measure of Gluten in Foods
- AOAC 990.26: Hydroxyproline in Meat and Meat Products
- AOAC 989.06: Aflatoxin B1 in Cottonseed Products and Mixed Feed
- AOAC 988.10: Soy Protein in Raw and Heat-Processed Meat Products
- AOAC 987.06: Beef and Poultry Adulteration of Meat Products
- AOAC 985.34: Niacin and Niacinamide (Nicotinic Acid and Nicotinamide) in Ready-to-Feed Milk-Based Infant Formula
- AOAC 985.16: Tin in Canned Foods
- AOAC 985.15: Fat (Crude) in Meat and Poultry Products
- AOAC 985.14: Moisture in Meat and Poultry Products
- AOAC 984.18: N-Nitrosopyrrolidine in Fried Bacon
- AOAC 983.19: Calcium in Mechanically Separated Poultry and Beef
- AOAC 983.18: Meat and Meat Products – Preparation of Test Samples
- AOAC 982.24: Aflatoxins B1 and M1 in Liver
- AOAC 982.22: N-Nitrosamines (Volatile) in Fried Bacon
- AOAC 981.10: Crude Protein in Meat
- AOAC 980.17: Preservatives in Ground Beef
- AOAC 977.14: Nitrogen in Meat (see 976.05)
- AOAC 976.21: Fat (Crude) in Meat
- AOAC 975.33: Arsenic in Meat (see 973.33)
- AOAC 974.45: Clopidol Residues in Animal Tissues
- AOAC 973.78: Arsenic (Total) Residues in Animal Tissues
- AOAC 973.60: Light Filth in Pork Sausage (Uncooked) and Ground Beef or Hamburger
- AOAC 973.31: Nitrites in Cured Meat
- AOAC 973.30: Polycyclic Aromatic Hydrocarbons and Benzo[a]pyrene in Food
- AOAC 969.31: Phosphorus (Total) in Meat
- AOAC 961.09: Sulfites in Meats
- AOAC 960.39: Fat (Crude) or Ether Extract in Meat
- AOAC 959.09: Boric Acid in Meat
- AOAC 958.06: Starch in Meat
- AOAC 950.46: Moisture in Meat
- AOAC 942.16: Milk (Nonfat and Dry) in Meat
- AOAC 935.50: Lead
- AOAC 935.49: Starchy Flour in Meat
- AOAC 935.47: Salt (Chlorine as Sodium Chloride) in Meat
- AOAC 928.08: Nitrogen in Meat
- AOAC 928.07: Water (Added) in Sausage
- AOAC 927.07: Lactose in Meat
- AOAC 913.01: Soybean Flour in Meat
- AOAC 892.02: Sulfurous Acid (Free) in Meats

From the NMKL (Nordic Committee on Food Analysis) (www.nmkl.org):

- NMKL 38, 2001, 4th Ed.: Acid Value/Free Fatty Acids. Determination in Fats
- NMKL 173, 2005, 2nd Ed.: Ash. Gravimetric Determination in Foods

- NMKL 103, 1984, corr 1991: Benzoic Acid and Sorbic Acid in Foods. Quantitative Determination by Gas Chromatography
- NMKL 2, 1979, 3rd Ed.: Benzoic Acid. Determination in Foods
- NMKL 124, 1997, 2nd Ed. 2007 Adm.: Benzoic Acid, Sorbic Acid and p-Hydroxybenzoic Acid Esters. Liquid Chromatographic Determination in Foods
- NMKL 178, 2004: Chloride (salt). Determination in Foods by Potentiometric Titration
- NMKL 32, 1959: Coloring Matters, Oil-Soluble, Synthetic. Isolation and Identification
- NMKL 114, 1985: Coloring Matters, Water-Soluble, Synthetic. Isolation and Identification
- NMKL 130, 1989: Colors, Synthetic, Water-Soluble. Liquid Chromatographic Determination in Foods
- NMKL 134, 1990: Colors, Synthetic, Water-Soluble, Semiquantitative. Determination by Chromatography and Spectrophotometry.
- NMKL 167, 2000: Cholecalciferol (Vitamin D_3) and Ergocalciferol (Vitamin D_2). Determination by HPLC in Foodstuffs
- NMKL 123, 1998, 2nd Ed.: Cyclamate. Spectrophotometric Determination in Foods
- NMKL 84, 1984, 2nd Ed.: n-Dodecyl Gallate (DG), n-Octyl Gallate (OG), n-Propyl Gallate (PG), and Nordihydroguaiaretic Acid (NDGA). Detection in Fat. TLC method
- NMKL 169, 2002: Dry Matter in Foodstuffs. The Vacuum method
- NMKL 131, 1989: Fat. Determination according to SBR in Meat and Meat Products
- NMKL 160, 1998: Fat. Determination in Foods
- NMKL 181, 2005: Fat. Determination in Meat and Meat Products using Butyrometer According to Gerber
- NMKL 129, 2003, 2nd Ed.: Fiber (Total Dietary). Gravimetric Determination after Enzymatic Degradation in Foods
- NMKL 162, 1998: Fiber (Total Dietary) Fiber. Gas Chromatographic, Colorimetric, and Gravimetric Determination in Foods – Uppsala method.
- NMKL 54, 1964: Formaldehyde. Determination in Foods
- NMKL 127, 2002, 2nd Ed.: Hydroxyproline. Colorimetric Determination as a Measure of Collagen in Meat and Meat Products
- NMKL 39, 2003, 3rd Ed.: Iodine value. Determination in Fats and Oils (Wij's method)
- NMKL 116, 1985: Iron. Photometric Determination in Foods
- NMKL 138, 1991: L-Glutamic Acid and Monosodium Glutamate. Enzymatic Determination in Meat and Fish and in Soups
- NMKL 155, 1996: Lactose and Galactose. Enzymatic Determination in Foods
- NMKL 153, 1996: Magnesium and Calcium. Determination by Atomic Absorption Spectrometry (AAS) after Wet Digestion in a Microwave Oven
- NMKL 161, 1998: Metals. Determination by Atomic Absorption Spectrophotometry after Wet Digestion in a Microwave Oven
- NMKL 139, 1991: Metals. Determination by Atomic Absorption Spectrophotometry in Foodstuffs
- NMKL 23, 1991, 3rd Ed.: Moisture and Ash. Gravimetric Determination in Meat and Meat Products
- NMKL 100, 2006, 2nd Ed.: Nitrite and Nitrate. Determination in Foods, Brines, and Salt Mixtures
- NMKL 165, 2000: Nitrite and Nitrate. Nitrite and/or Nitrate in Foodstuffs by Ion Chromatography
- NMKL 49, 1963: Nitrite: I Unspecific Titrimetric Routine method for the Determination of Nitrite in 'Nitrite Salt' and 'Nitrite Salpetre.' II Determination of Nitrite in 'Nitrite Salt' and 'Nitrite Salpetre,' and in Meat, Fish, and Brine
- NMKL 6, 2003, 4th Ed.: Nitrogen. Determination in Foods and Feeds According to Kjeldahl
- NMKL 63, 2000, 2nd Ed.: Oxalic Acid. Determination in Foods
- NMKL 158, 1997: Peroxide Value. Determination in Fats and Oils
- NMKL 57, 1994, 2nd Ed.: Phosphorus. Spectophotometric Determination after Dry Ashing in Food
- NMKL 179, 2005: pH: Determination in Foods
- NMKL 76, 1988, 2nd Ed.: Polyphosphates. Detection by Paper Chromatography in Meat Products, etc.
- NMKL 176, 2003: Proteins. Qualitative Method for Detection of Specific Proteins (Allergens) in Food by Immuno Diffusion
- NMKL 3, 1999, 3rd Ed.: Salicylic acid in Foods. Qualitative and Quantitative Analysis
- NMKL 180, 2005: Sodium. Determination in Foodstuffs by Flame AAS after Microwave Digestion
- NMKL 145, 1997, 2nd Ed. 2006, 2007 Adm.: Starch and Glucose. Enzymatic Determination in Foods
- NMKL 135, 1990: Sulfite. Enzymatic Determination in Foods
- NMKL 132, 1989: Sulfite. Spectrophotometric Determination in Foods
- NMKL 190, 2009: Tin (Sn). Determination in Foods by Flame or Graphite Furnace AAS
- NMKL 191, 2009: Tin (Sn). Determination in Foods by Inductively Coupled Plasma Mass Spectrometry (ICPMS)
- NMKL 186, 2007: Trace Elements – As, Cd, Hg, Pb, and Other Elements. Determination by ICP-MS after Pressure Digestion
- NMKL 104, 1984: Water Activity. Measuring with a Polyamide Filament Hygrometer

Collaboration

There is considerable collaboration between organizations. Organizations inform each other about ongoing activities in order to avoid duplicative efforts and to initiate cooperation in specific fields. Some of the organizations have agreements that allow them to adopt each other's methods.

See also: Chemical Analysis: Analysis of Final Product Composition for Labeling; Physicochemical Analysis Methods; Raw Material Composition Analysis; Sampling and Statistical

Requirements. Chemical Analysis for Specific Components: Major Meat Components. Laboratory Accreditation. Microbiological Analysis: Standard Methods

Further Reading

Fajgelj A., Amburs A., 2000. Principles and practices of Method Validation. The Royal Society of Chemistry 2000, ISBN 0-85404-783-2.

Horwitz, W., 1988. Protocol for the design, conduct and interpretation of method-performance studies. Pure and Applied Chemistry 60, 855–864.

Horwitz, W., 1995. Protocol for the design, conduct and interpretation of method-performance studies. Pure and Applied Chemistry 67, 331–343.

ISO (International Organization for Standardization). Accuracy (Trueness and Precision) of Measurement Methods and Results – Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method, ISO 5725-2:1994. ISO.

Latimer Jr., G.W. (Ed.), 2012. Official Methods of Analysis of AOAC International, nineteenth ed. USA: AOAC (aoac.org).

Mulholand, M., 1988. Ruggedness testing in analytical chemistry. Trends in Analytical Chemistry 7, 383–389.

NMKL, 1996. NMKL Procedure No 3: Control Charts and Control Materials in Internal Quality Control in Food Chemical Laboratories.

NMKL, 2000. Referentvejledning for det kemiske område. Udarbejdelse af analysemetoder indenfor NMKL (available in Danish only). NMKL Report No 11, second ed.

NMKL, 2005. NMKL Procedure No 5: Estimation and Expression of Measurement Uncertainty in Chemical Analysis, second ed.

NMKL, 2007. Procedure No 9: Evaluation of Method Bias using Certified Reference Materials, second ed.

NMKL, 2009. NMKL Procedure No 4: Validation of Chemical Analytical Methods, third ed.

NMKL, 2010. NMKL Procedure No 24: Guidelines in Quality Assurance for Food Chemical Laboratories.

Official Methods of Analysis of AOAC International, 19th Edition, 2012, Editor Latimer, G.W.Jr. (aoac.org)

Pocklington, W.D., 1990. Harmonized protocol for the adoption of standardized analytical methods for the presentation of their performance characteristics. Pure and Applied Chemistry 62, 149–162.

Thompson, M., Ellison, S.L.R., Wood, R., 2002. Harmonized guidelines for single-laboratory validation of methods of analysis. Pure and Applied Chemistry 74 (5), 835–855.

Youden W.J., Steiner E.H., 1975. Statistical Manual of the Association of Official Analytical Chemists, pp. 33–36, 70–71, 82–83. Arlington: The Association of Official Analytical Chemists.

Relevant Websites

www.aoac.org
AOAC International.

www.aocs.org
AOCS, American Oil Chemists' Society.

www.cen.eu
CEN, European Committee for Standardization.

www.codexalimentarius.org
Codex Alimentarius International Food Standards.

www.fil-idf.org
IDF, International Dairy Federation.

www.iso.org
ISO, International Organization for Standardization.

www.nmkl.org
Nordic Committee on Food Analysis, NMKL.

CHEMICAL ANALYSIS FOR SPECIFIC COMPONENTS

Contents

Curing Agents

KO Honikel[†]

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Glossary

Ingoing and residual amouts Nitrite and nitrate are toxic substances that react with meat batter constituents changing their concentration. Thus, for food safety reasons, some countries limit the ingoing (added) amounts at the time of production; other countries define the residual (remaining) amounts or both in the ready-to-eat meat products.

Introduction

This article describes the chemical reactions of nitrite and nitrate in meat products leading to their antimicrobial and antioxidative activity, as well as cured color and flavor. The formation of nitrosamines is also discussed. The analytical procedures for the determination of nitrate and nitrite are described.

Definitions

Curing of meat means the addition of nitrite and/or nitrate together with salt (NaCl) to meat in various degrees of comminution and at different processing steps.

The most basic method of curing is the covering of meat cuts (e.g., part of the hind legs of pigs) in a mixture of salt and curing agent. The salt and curing agents penetrate slowly into the muscular tissue. This dry-curing process lasts up to several weeks. The process can be accelerated by wet curing, in which the meat cuts are either inserted into brine and curing agents and/or brine is injected into the meat with needles.

In small pieces of meat, in minced or comminuted meat as in sausages, salt and the curing agent are mixed in or comminuted in a bowl chopper. This way of curing is fast and within a short time (less than 1 day), depending on the processing, leads to the characteristics and appearance of a cured product.

Legal Requirements

Various concentrations of curing agents are permitted to be added into the product or are required in the ready-for-sale product. In the European Union, the concentrations shown in Table 1 are permitted basically for addition and as maximum residual amounts in products for sale. But there exist quite a number of exemptions from the basic rules for defining not-heat treated meat products of the various EU member states.

Other countries differ somewhat in these legal requirements. In Russia, for example, nitrate is not allowed in meat products; in the USA, approximately 150 mg kg^{-1} nitrite is permitted as an ingoing amount in most products.

Reasons for Addition

The primary reason for the use of nitrate and nitrite is the preserving action of these compounds. They prevent growth of some microorganisms early in the processing and retard the oxidation of fats during storage.

Other effects are of secondary importance but are nevertheless regarded as positive actions of the curing agents. Cured

[†]Deceased.

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Table 1 Amounts of permitted curing agents in meat products in the European Union (Directive 2006/52/EC). There exist a number of traditionally produced mostly dry-cured nonheat treated (raw) ham products with other ingoing amounts and some with other residual amounts at the point of sale (residual amounts are presented in the range permitted for all meat products)

Additive	*EEC number*	*Foods*	*Ingoing amounts (mg kg^{-1})*	*Residual amounts[b] (m kg^{-1})*
Potassium nitrite	E 249	All meat products	150[a]	50–175
Sodium nitrite	E 250			
Potassium nitrate	E 251	Nonheat treated meat	150[a]	10–250
Sodium nitrate	E 252	Products		

[a]Expressed as $NaNO_2$.
[b]Residual amount at point of sale to the final consumer expressed as $NaNO_2$.

$$NaNO_2 \longrightarrow Na^+ + NO_2^- \quad [I]$$

$$4\ NO_2^- + 4\ H^+ \rightleftharpoons 4\ HNO_2 \quad pK_a = 3.35\ (K_a = 4.47 \times 10^{-4}\ mol\ l^{-1}) \quad [II]$$

$$4\ HNO_2 \rightleftharpoons 2\ N_2O_3 + 2\ H_2O \quad [III]$$

$$2\ N_2O_3 \rightleftharpoons 2\ NO + 2\ NO_2 \quad [IV]$$

$$2\ NO_2 + H_2O \longrightarrow HNO_3 + HNO_2 \quad [V]$$

$$\text{Sum [III] to [V]: } 3\ HNO_2 \longrightarrow 2\ NO + HNO_3\ \text{(oxidation/reduction)} + H_2O \quad [VI]$$

$$HNO_3 \longrightarrow H^+ + NO_3^-\ \text{(strong acid; completely dissociated)} \quad [VII]$$

$$NO + \text{Myoglobin} \longrightarrow \text{NO-Myoglobin (red colour)} \quad [VIII]$$

Oxygen sequestration:

$$2\ NO + O_2 \longrightarrow 2\ NO_2 \quad [IX]$$

Figure 1 Sequence of reactions of nitrite in aqueous solutions of pH approximately 5.5–6.0.

color, a heat-stable red color, results from the formation of nitrosyl myoglobin, and a so-called cured flavor, which can be detected by sensory assessment, is also produced by curing. However, it is not clear whether the flavor is caused by direct reactions of curing agents with meat constituents or whether oxidative processes like fat rancidity development, which result in a negative sensory reaction, are retarded.

Thus, curing agents are believed to have four effects within the permitted concentrations:

- Antimicrobial activity
- Antioxidative activity
- Formation of a red cured color
- Expression of cured flavor.

Chemistry of Curing Agents

Nitrate

Nitrate itself has no direct curing potential. Nitrate is only effective if it is reduced to nitrite. This is done primarily by microorganisms. Thus, nitrate as a curing agent is only relevant in raw meat products that are cured and fermented around ambient temperature for longer periods of time. In all meat products that are heated within hours after adding the curing agent, nitrate is useless and superfluous. Accordingly, nitrate is used mainly in raw hams and raw sausages that are fermented.

Nitrite

Nitrite is the basis for the real curing agent. Sodium or potassium nitrite is added to the meat with the salt. In many countries, nitrite must be premixed with common salt (NaCl) to prevent an overdose of nitrite, which is toxic in higher concentrations; 0.4–1.0% of nitrite in a salt mixture is common.

Nitrite salts dissociate almost completely in water to yield Na^+ (K^+) and NO_2^- ions (Figure 1, eqn [I]). The nitrite anion (NO_2^-) itself is not active as a curing agent. In a meat cut or meat batter, the pH value is approximately 5.5–6.0. In this slightly acidic environment a small proportion of NO_2^- anions react with H^+ ions to form nitrous acid (HNO_2); Figure 1, eqn [II]. HNO_2 is a medium-strong acid with a pK_a value of 3.35.

HNO_2 is in equilibrium with its anhydride N_2O_3 (Figure 1, eqn [III]). N_2O_3 breaks down into NO+NO_2 (Figure 1, eqn [IV]). NO is the effective curing agent that binds to myoglobin (Figure 1, eqn [VIII]).

The NO_2 reacts with water to yield HNO_2+HNO_3 (nitric acid) (Figure 1, eqn [V]). HNO_2 reenters the sequence of events (Figure 1, eqn [III]). HNO_3, as a strong acid, is completely dissociated into H^++NO_3^- (Figure 1, eqn [VII]). This means (Figure 1, eqn [VI]) that 1/3 of the HNO_2 (oxidation state +3) is oxidized to HNO_3 (oxidation state +5) and 2/3 are reduced to NO (oxidation status +2).

Table 2 Formation of HNO_2 in batters

Assumptions:

1. pH of raw batter 5.7 ($c_{H^+} = 2 \times 10^{-6}$ mol l^{-1})
2. 80 ppm of nitrite added $c_{NO_2^-} = 1.75 \times 10^{-3}$ mol l^{-1}

$HNO_2 \rightleftarrows H^+ + NO_2^-$ ($pK_a = 3.35$; $K_a = 4.47 \times 10^{-4}$ mol l^{-1})

$K_a = 4.47 \times 10^{-4}$ mol $l^{-1} = c_{H^+} \times c_{NO_2^-} / c_{HNO_2}$

Hence,

$c_{HNO_2} = (2 \times 10^{-6})(1.75 \times 10^{-3})/(4.47 \times 10^{-4})$

$c_{HNO_2} = 0.78 \times 10^{-5}$ mol l^{-1}

$c_{HNO_2} = 1.65 \times 10^{-4}$ mg kg^{-1}

$= 0.165$ ppm HNO_2

NO is easily oxidized by oxygen to NO_2 (Figure 1, eqn [IX]). In a comminuted meat batter, oxygen is present and reactions [IX] and [V] occur. This means that another part of the created NO is finally oxidized to HNO_3. This latter oxidation can be reduced by vacuum chopping or by antioxidants such as ascorbate, isoascorbate, or tocoferols. However, as a result of this series of reactions, a considerable part (1/3 or more) of the nitrite is oxidized to nitrate during curing of meat products. Thus, even if only nitrite has been added, nitrate will always be found in cured meat products.

As indicated above, the equilibrium of $HNO_2 \rightleftarrows NO_2^- + H^+$ is on the left side of the reaction. In a raw batter with a pH of 5.7 and a concentration of 80 ppm (80 mg kg^{-1}) nitrite added to the batter, a theoretical concentration of 0.165 ppm HNO_2 is formed (Table 2). This is approximately 1/500 of the nitrite concentration added.

In batters of higher pH (e.g., liver or blood sausages with pH 6.2–6.8) the concentration of HNO_2 is even lower (by as much as a factor of 10). Nevertheless nitrite is oxidized to nitrate as shown in Figures 2 and 3. In the more acid environment of a raw sausage or an emulsion-type batter with lower pH (e.g., due to the use of glucono-δ-lactone), the HNO_2 concentration is higher: more NO is formed and a more intense red color may be formed, as it will be in the presence of antioxidants that inhibit Figure 1, eqn [IX] and subsequently Figure 1, eqn [V] of Figure 1.

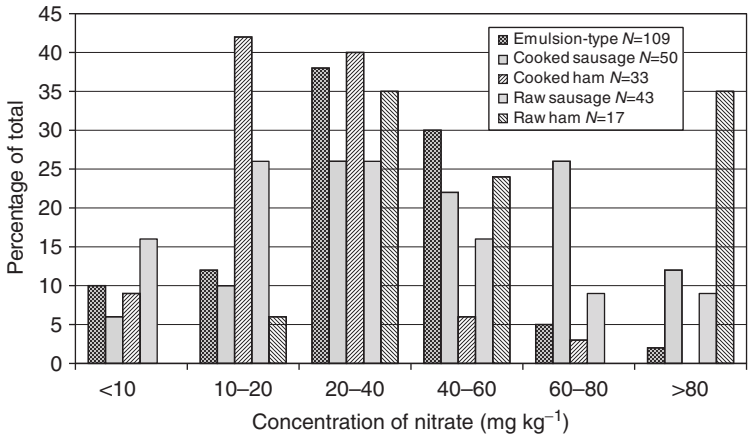

Figure 2 Nitrite concentrations in ready-to-eat meat products from Germany. Data from Dederer, I., 2001, personal communication.

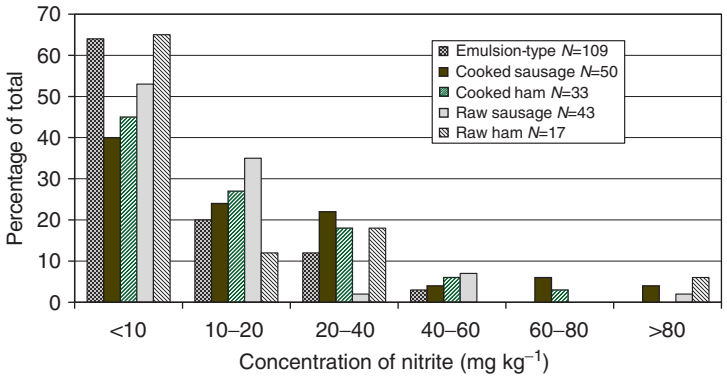

Figure 3 Nitrate concentration in ready-to-eat meat products from Germany. Data from Dederer, I., 2001, personal communication.

Concentrations of Nitrite and Nitrate in Meat Products

The concentration of nitrite in 252 samples of all groups of meat products is presented in Figure 2. For these German meat products it can be assumed that for emulsion-type, cooked sausages 80–100 ppm and for cooked hams 100–150 ppm of nitrite were added during preparation. The pointless use of nitrate can be discounted. Nitrate up to 150 ppm may have been used additionally in raw meat products.

Despite the addition of nitrite with 80–150 ppm, the concentration in the heated products ready for sale was below 20 ppm in more than 80% of all products at the time of measurement, which was 1–2 weeks after manufacturing.

In raw hams and raw sausages, the situation is similar. In these products nitrate (up to 250 ppm) has often been added as well as nitrite (up to 150 ppm). A total of 300 ppm, however, was not exceeded. Some products had concentrations of nitrite above 20 ppm on measurement, which took place 3 weeks to 3 months after manufacturing. The cooked sausages (liver and blood sausages) with pH > 6.2 cannot make much use of the nitrite, as the HNO_2 is formed only in very small concentrations. But nevertheless it is sufficient that a cured color is formed.

Figure 3 shows the nitrate concentrations for the same products as in Figure 2 for nitrite. The concentrations of nitrate are in general considerably higher than those of nitrite in Figure 2 with a maximum between 20 and 40 ppm.

Changes of Nitrite during Storage

The process of nitrite degradation happens not only during the manufacturing step, but it also occurs during storage. The results of Russian researchers are shown in Table 3. At concentrations of nitrite even beyond those permitted in Russia (75 ppm), the concentrations of nitrite are reduced to rather low concentrations during storage for 60 days. Thus, it is evident that reactions of nitrite occur even under storage conditions.

Analysis of Nitrite and Nitrate

There are a number of methods for determining the concentrations of curing agents. Nitrite and nitrate anions can be detected with high-performance liquid chromatography (HPLC) as an ion-exchange chromatography method and by isotachophoresis. Nitrite can also be determined titrimetrically with permanganate, but the most common method for determining the curing agents in meat products is to measure nitrite after a colorimetric reaction and nitrate after reduction to nitrite.

Table 3 Remaining nitrite (ppm; mg kg^{-1}) during storage at 2 °C of an emulsion-type sausage

Storage	*Concentration of nitrite added (ppm)*			
	75	*100*	*150*	*200*
After heating	21.9	30.5	59.5	53.7
20 days	7.5	9.3	10.2	15.4
40 days	3.6	6.4	7.6	7.7
60 days	0.5	0.9	4.0	5.8

Source: Data based on work of Kudryashow, L., 2003, Personal communication.

The colorimetric assay is based on the reaction of nitrite, sulfanilamide, and *N*-1-naphthylethylenediamine under acidic conditions to form a red azo dye that has an absorbance maximum at 540 nm. This method is described in many national standards (e.g., British Standard 1976; AOAC 1980; Germany L 08.0014 §64 LFBG (2006)).

The same method can be applied to nitrate after its reduction to nitrite. In older standards, nitrate is reduced with metallic cadmium, with the nitrate running through a column filled with cadmium particles. Cadmium is oxidized to Cd^{2+} and nitrate is reduced to nitrite. Newer standards use the enzyme nitrate reductase, which reduces nitrate to nitrite whereas the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) is oxidized to $NADP^+$. The reaction is carried out in one batch and followed by the color reaction.

Two colorimetric measurements must be made to determine nitrite and nitrate, one before and one after the reduction process. The result of the second analysis minus the first gives the nitrate concentration.

Reduction/colorimetry assays are potentially prone to interference from a number of sources, including incomplete reduction of nitrate to nitrite and also overreduction of nitrate, via nitrite, to lower oxides. High concentrations of inert salts may adversely affect the accuracy of the method, and the buffering effects of organic acids may affect the pH-dependent absorbance of the azo dye.

In principle, any component of the sample that is able to compete with the sulfanilamide reagent for nitrite during the color development reaction will cause a negative interference. The close comparability of the results obtained for the nitrite content of cured meats by colorimetry and by HPLC, where such an interference is not anticipated, suggests that this is not a widespread problem. Cured meats containing high levels of ascorbate are, however, known to give low results for nitrite owing to significant competition with the coupling reagent. In addition to chemical effects, the colorimetric assay may also be adversely affected by physical interferences arising from turbidity in the measuring solution. This is likely to occur if the postextraction precipitation and filtration steps are ineffective. Detection limits for nitrate and nitrite in cured meats are generally approximately 1–3 mg kg^{-1}. However, for samples that contain appreciable quantities of ascorbate or other interferents, the effective limit of detection may be an order of higher magnitude. The ascorbate interference is minimized or excluded by heating in alkaline solutions before the colorimetric reaction. Numerous standards recommend this step.

Effects of Nitrite in Meat Products

As nitrate is effective only after the reduction to nitrite, only the effects of nitrite in meat products are considered. Nitrite has antimicrobial effects in its form as HNO_2 or NO. Owing to its small concentrations in many meat products after manufacturing (pH range 5.0–6.8), the antimicrobial action of HNO_2 or NO is possible only in raw meat products and in the raw batter of emulsion-type sausages before heating.

Pathogenic microorganisms like *Salmonella* spp. and *Clostridium botulinum* are inhibited. Other microorganisms are not inhibited by nitrite or HNO_2. Spores, especially those of *C. botulinum*, are seen to be damaged or retarded by HNO_2/NO during the heating process. Nitrite alone does not protect against spoilage. Other microbiological hurdles such as salt, water activity (a_w), pH, redox potential, and temperature of heating are required to be crossed for stability.

Nitrite and its metabolites also act as antioxidants by sequestering oxygen and by binding to iron ions in the myoglobin cofactor heme. It is thus responsible for the heat-stable red cured color. Nitrite or its metabolites also play a part in the 'cured' flavor either be enhancing the meat flavor or by retarding its degradation. The latter may be an antioxidative effect.

Nitrosamine Formation

Some nitrosamines/amides are carcinogenic, and are formed by the reaction of amines or amides with nitrite under acidic conditions. Only secondary amines form stable nitrosamines (Figure 4). Furthermore, the pH must be low (<5.5). For these reasons, the formation of nitrosamines is small or negligible in emulsion-type and cooked sausages because of their pH values (>5.7) and the use of fresh meat (or early postmortem frozen meat) in which secondary amines are present only in very small amounts. The most convincing sequence of events is shown in Figure 4.

In low-pH raw sausages or raw hams (pH<5.5 for a longer period of time), with long fermentation and presence of secondary amines, the formation of nitrosamines is possible (Table 4). Nevertheless, the concentrations are mainly in the range 1–2 μg kg^{-1}. Reducing agents such as ascorbate prevent the formation of nitrosamines (see Table 4).

The problem of nitrosamines becomes larger with cooking, for example, in the frying of bacon, where the same conditions are present as in raw hams (low pH and possible formation of amines over the long storage time). In the frying of bacon, these conditions are combined with high temperatures (>130 °C), particularly in the case of frying to crispness.

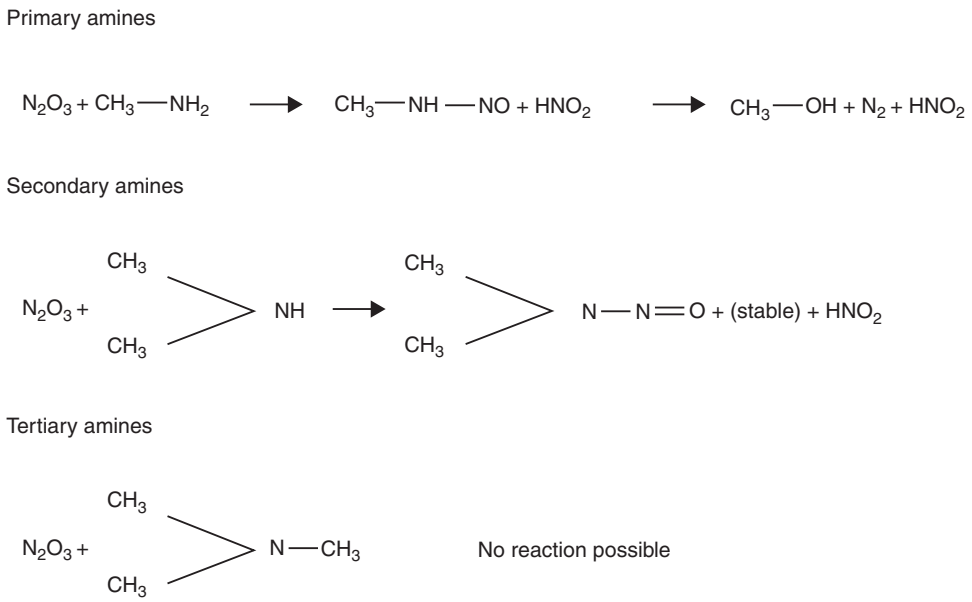

Figure 4 Nitrosamine formation.

Table 4 Frequency of various levels of nitrosamine concentration in raw sausages and ham

	Nitrosamine species[a]	*Number of samples with nitrosamine concentrations of μg kg^{-1}*					*Percentage of samples with <1 μg kg^{-1}*
		0–05	*<1*	*<2*	*<5*	*>5*	
Raw sausages (n=33)	NDMA	15	11	5	2	–	79
	NPIP	29	1	1	–	2	91
	NPYR	33	–	–	–	–	100
Raw hams (n=48)	NDMA	27	14	7	–	–	85
	NPIP	40	2	3	1	2	87
	NPYR	48	–	–	–	–	100

[a]NDMA, nitrosodimethylamine; NPIP, nitrosopiperidine; NPYR, nitrosopyrrolidine.
Source: Reproduced from Kuhne, D., 1995, Personal communication.

Table 5 Toxicity (lethal dose) of nitrate and nitrite

Nitrate	80~800 mg nitrate per kg bodyweight per day
Nitrite	33~250 mg nitrite per kg bodyweight per day

Source: Data from Schuddeboom, L.J., 1993. Nitrates and Nitrites in Foodstuffs. Strasbourg: Council of Europe Press.

Owing to the high temperatures, some of the volatile nitrosamines, which are the main carcinogenic ones, evaporate from the fried product, but fried bacon nevertheless has potential for higher concentrations of nitrosamines than any other meat products.

Toxicological Aspects

The acute toxicity of nitrite and nitrate is reported to cover a wide range (Table 5). A person of 60 kg who consumes 100 g of meat products with 20 mg nitrite per kg consumes 0.033 mg nitrite per day per kg body weight. This is 1/1000 of the lower limit of toxicity. With nitrate the intake is in a similar range. Thus, both curing salts *per se* can be regarded as posing no health hazard with normal use.

See also: Curing: Brine Curing of Meat; Dry; Production Procedures. Residues in Meat and Meat Products: Residues Associated with Meat Production

Further Reading

Bonell, A.E., 1995. Nitrate concentrations in vegetables. Epidemiological studies in humans. Proceedings of the International Workshop on Health Aspects of Nitrate and its Metabolites (Particularly Nitrite). Bilthoven 8–10 November 1994, Strasbourg: Council of Europe Press.

Cassens, R.G., 1994. Meat Preservation, Preventing Losses and Assuming Safety. Trumbach, CT: Food and Nutrition Press.

EU Directive 2006/52/EC of the European Parliament and Council, O.J. L204 of 27 July 2006.

Gow, A.J., Stauber, J.S., 1998. Reactions between nitric oxide and haemoglobin under physiological conditions. Nature 391, 169.

Hill, M. (Ed.), 1996. Nitrates and Nitrites in Food and Water. Cambridge: Woodhead Publishing.

Honikel, K.O., 2008. The use and control of nitrate and nitrite for the processing of meat products. Meat Science 78, 68–76.

Honikel, K.O., 2010. Curing. In: Toldra, F. (Ed.), Handbook of Meat Processing. Ames, Iowa: Wiley-Blackwell, pp. 125–142.

Hotchkiss, J.H., Parker, R.S., 1990. Toxic compounds produced during cooking and meat processing. In: Pearson, A.M., Dutson, T.R. (Eds.), Meat and Health, Advances in Meat Reserch, vol. 6. London: Elsevier Applied Science, pp. 105–134.

Lawrie, R.A., 1998. Lawrie's Meat Science. Cambridge, UK: Woodhead Publishing Ltd., pp. 188–195.

Möhler, K., 1980. Das Pökeln. Druckwerkstätten Alzey: Rheinhess.

Sofos, J.N., Raharjo, S., 1994. Curing agents. In: Maya, J.A., Maya, A., Tu, T. (Eds.), Food Additive Toxicology. New York: Marcel Dekker, pp. 235–267.

US Government 2005. Code of Federal Regulations, Title 21, vol. 3, Food and Drugs Part 170, Food Additives §§ 170.60/172.175/172/170. USA: US Food and Drug Administration.

Relevant Websites

http://www.aoac.org
AOAC Methods.

http://eur-lex.europa.eu/
EU-Rules.

http://www.bfr.bund.de/lfgb_sammlung-1137/html
German Methods.

http://www.gpoaccess.gov/cfr
USA Rules.

Major Meat Components

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Glossary

Amino acids Organic compounds, with relevant biological functions, made from amine and carboxylic acid groups. They have a side chain specific to each amino acid.
Carbohydrates Present in minor amounts in meat, mainly composed of glycogen and small amount of glucose.
Fat A major component in meat consisting of triacylglycerols and phospholipids as well as some free fatty acids and minor amounts of cholesterol and fat-soluble vitamins.
Fatty acid A carboxylic acid with a long aliphatic tail which is either saturated, monounsaturated, or polyunsaturated.
Gas chromatography (GC) Separation technique carried out in a column having gas as the mobile phase.
Internal Standard (IS) A substance not contained in the sample with physical–chemical properties as similar as possible to those of the analyte that has to be identified and which is added to each sample at a known concentration.
Liquid chromatography (HPLC) Separation technique carried out in a column packaged with small particles having liquid at a relatively high pressure as the mobile phase.
Meat proteins There are three major classes consisting of sarcoplasmic (soluble in water), myofibrillar (soluble in high ionic strength solutions), and connective tissue proteins (insoluble).

Introduction

Fat and protein are major components of meat. Fat, composed mainly of triacylglycerols and phospholipids, can make up as much as 50% of some meat products, whereas protein, composed of amino acids, can represent more than 20% of lean meat. The analysis of these major components is the subject of this article.

Carbohydrate, in the form of glycogen, is present in very low amounts in meat (approximately 0.5 g per 100 g in meat and 1.5–5 g per 100 g in liver (this higher value applies to beef liver; calf liver may go up to 10 g per 100 g liver) and is considered only briefly in this article.

The term fat is not clearly defined and can be subdivided in many ways. The analysis of the different lipid classes – such as tri-, di-, and monoacylglycerols, phospholipids, and free fatty acids – is very complex and falls outside the scope of this article. The analysis of specific proteins and peptides also falls outside the scope of this article.

Fat and protein can be analyzed using a wide range of methods, from traditional wet chemical analysis, which can be automated to some extent, to advanced gas chromatography (GC), high-pressure liquid chromatography (HPLC), and ion-exchange chromatography (IEC). Indirect methods such as nuclear magnetic resonance (NMR) and near-infrared transmission (NIT) and near-infrared reflectance (NIR) spectroscopy are also used.

Fat

The terms fat, total fat, crude fat, and total lipids are all terms covering more or less the same components. Triacylglycerol is the form in which fat chiefly occurs in the intermuscular and subcutaneous fat, or depot. Phospholipids are mainly contained in the structural fat. Fat also consists of minor amounts of other components, for example, free fatty acids, waxes, mono- and diacylglycerols, sterols, and fat-soluble vitamins. The extraction techniques can be classified into three different types, depending on the solubility of lipids in organic solvents or their physical characteristics (Figure 1).

The property of lipids, being soluble in organic solvents but insoluble in water, provides methods to separate lipid components in foods from water-soluble components, such as proteins, carbohydrates, and minerals. However, several new efficient methods have been developed during recent years. In solvent extraction methods, samples should be prepared by drying the samples to facilitate the organic solvent to penetrate. Particle size of samples should be reduced to produce a homogeneous sample and increase surface area. Before solvent extraction, it is necessary to submit the sample to acid hydrolysis in order to release bound lipids. Finally, the selection of the solvent depends on the polarity of lipids present in the samples. Polar lipids (e.g., phospholipids) are more soluble in polar solvents (alcohols) than in nonpolar solvents (hexane), whereas the opposite occurs for nonpolar lipids (triacylglycerols). Therefore, the total lipid content determined will be different depending on the nature of organic solvent used.

The first solvent extraction methods were discontinuous as they were based on the mixture of sample and solvent in a container, such as the Folch and Bligh and Dyer extraction methods, (Figure 1) where the lipid is extracted with a 3:1 mixture of chloroform:methanol. This very polar mixture of solvents extracts all the lipids, including structural lipids, and also extracts other components. Results for total lipid with this method, therefore, tend to be high. This method has fallen out

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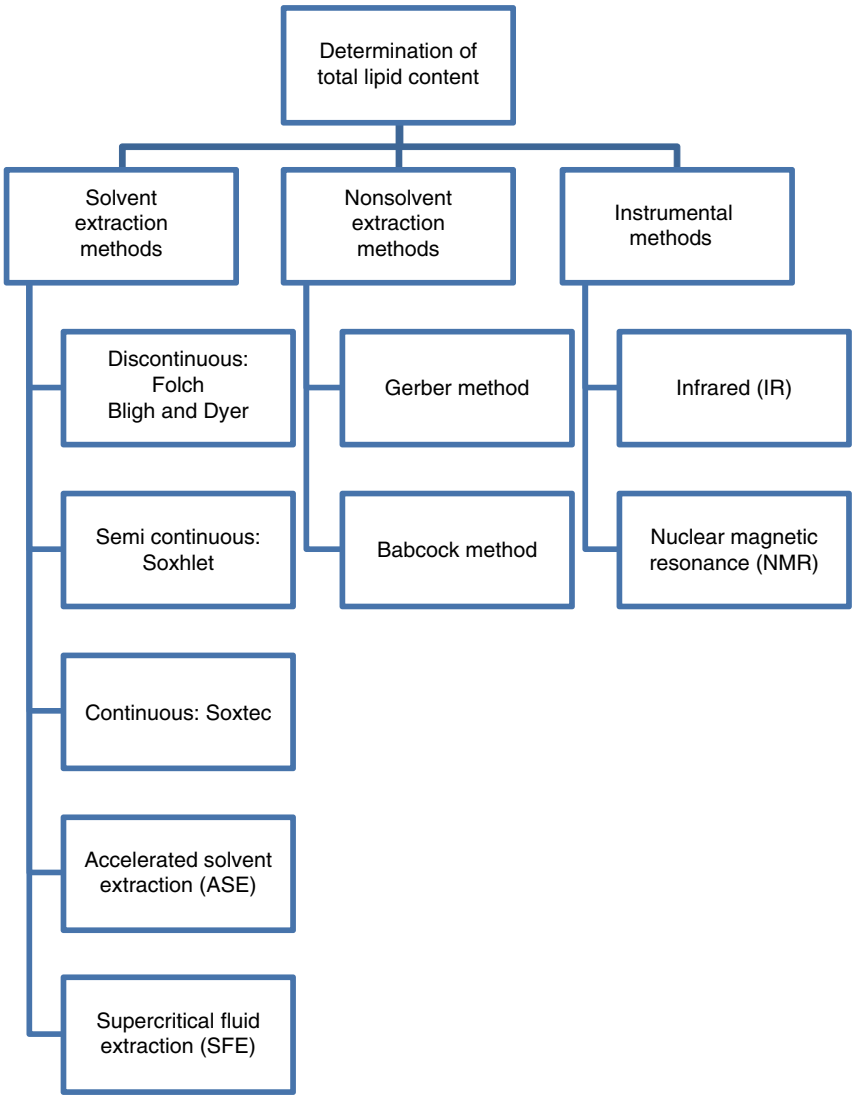

Figure 1 Scheme of methods to determine total lipid content.

of use for routine fat determination as chlorated solvents are banned for health reasons from many laboratories.

In semicontinuous classical Soxhlet extraction, the sample is extracted many times with a nonpolar solvent like hexane or petroleum ether, the solvent is evaporated, and the extracted fat is weighed. With this method, the major part of the triacylglycerol fraction and most of the cholesterol in meat will be extracted, but only a fraction of the phospholipids and lipoproteins. If extraction is carried out with diethylether, a more polar solvent, the yield will be higher. Modified versions of the Soxhlet method has been designed to reduce the use of solvents and time (e.g., Soxtec). The Soxhlet extraction method is the most used one, as it is the officially recognized method.

The Folch and Bligh and Dyer methods and Soxhlet method to determine fat content are tedious and time consuming, and high solvent consumption is a disadvantage from a health point of view. The newest extraction technique is the accelerated solvent extraction, which reduces extraction times down to minutes using very small amounts of solvent. The efficiency of extraction is increased by doing it at higher temperature and pressure than in normal process as well as by the availability of special instruments.

In addition, fat can be extracted using supercritical fluid extraction, with carbon dioxide as the solvent. Because this solvent is nonpolar like petroleum ether, only the triacylglycerols will be extracted, unless it is modified with other solvents, such as ethyl alcohol. The equipment is expensive, and it can be difficult to adjust the solvents to give comparable results to the classical reference methods; however, they are beneficial from a cost and environmental point of view by not using organic solvents.

Other liquid extraction methods do not use organic solvents but use other chemicals to separate lipids. The classical Gerber method, known for milk analysis, can also be used for meat. It is then known as the modified Babcock method. The sample is hydrolyzed with sulfuric acid in a special Gerber tube, a little amyl alcohol is added to aid the separation, and the mixture is centrifuged. The amount of fat can then be read

directly from the calibrated scale on the Gerber tube. It is necessary to adjust the temperature carefully at all times to secure correct calibration. The precision is not as high as for the other methods as it depends on the scale on the tubes, and a special centrifuge is necessary. However, this method is still in use in some laboratories.

There is a wide variety of instrumental methods for fat determination (Figure 1) and each one has its own advantages and disadvantages. In general, the advantages are nondestructive, require little or no sample preparation, and measurements are fast, precise, and simple, whereas the main disadvantage is the necessity to prepare a calibration curve specific to the type of food matrix used. NIT and NIR are instrumental methods based on absorption of light in the sample in the near-infrared range from 800 to 2500 nm.

NMR is another instrumental method based on the measurement of a spin echo of protons in a magnetic field. The samples must be heated to ensure that the entire fat phase is liquid. It is also necessary to dry the samples, as protons from water will give a signal. A linear regression with results from a reference method must be used for calibration, so the NMR technique has the same limitations as mentioned above for NIR/NIT. A newer NMR technique with pulsed field gradients allows measurement on nondried samples, and it is possible to measure both water and fat. However, a higher precision can be obtained from dried samples. Equipment needed for instrumental methods is expensive but allows very rapid measurements of fat on a large number of samples. In addition, the measurements are nondestructive, which makes it possible to use such techniques in process control.

Thus, the choice of method for fat determination depends on how much of the lipids should be included in 'total fat,' and also on both the sample load, which decides the degree of automation and the sample mix. Many different matrices are well suited to automated classical methods, but only a few are suited to instrumental methods. For meat and meat products, it is necessary to use acid hydrolysis to release all the fatty acids. A simple Soxhlet extraction is only sufficient for determination of added fat as triacylglycerols.

Fatty Acids

Methods for fatty acid analysis are much simpler to review as almost all rely on GC on capillary columns of fatty acid methyl esters (FAME), although there are many variations. The fatty acids are isolated either as total fat or as free fatty acids and are then converted into FAMEs, which are extracted with a nonpolar solvent, and a small proportion is injected into the GC. Results are calculated either per 100 g sample or per 100 g fat or fatty acids.

Isolation of Fat or Free Fatty Acids

Fat obtained from Soxhlet extraction or fatty acids extracted after acid hydrolysis can be used to determine fatty acids. Instead of evaporating the solvent, the extract is diluted to a known volume, an aliquot corresponding to approximately 100 mg fat is taken, and then the solvent is evaporated.

Methylation

The quantification of fatty acids by GC requires previous methylation consisting of the transformation of the analytes to methyl esters in order to make them volatile enough for GC. This conversion of the fat or fatty acids to methyl esters can be achieved by boiling in methanol for 2 h with strong acids like hydrochloric acid or sulfuric acid. The free fatty acids are methylated rapidly but the transesterification of triacylglycerols takes a little longer. Basic catalyzed transesterification is even more rapid. When using sodium methoxide in methanol as catalyst, the methylation takes only a few minutes. The presence of water must be avoided; if water is present, then free fatty acids will be formed instead of the methyl esters. The most commonly used catalyst is boron trifluoride. The sample is boiled for a few minutes in methanol and potassium hydroxide, cooled, boron trifluoride in methanol is added, and the mixture is boiled again for a few minutes in a stoppered glass tube. Care must be taken that the tube is tightly stoppered, otherwise the unsaturated fatty acids might be destroyed and the more volatile short-chain fatty acids might escape. After cooling the mixture, the FAMEs are extracted with n-hexane or isooctane, and they are now ready for the GC.

Gas Chromatography of Fatty Acids

The FAMEs are injected on a capillary column, usually 50–100 m long, with an internal diameter of 0.25–0.32 μm. The capillary column is coated with a highly polar material to achieve the necessary separation of FAMEs (100% biscyanopropyl polysiloxane or 90% biscyanopropyl/10% cyanopropylphenyl polysiloxane). Initial separation is according to chain length and then according to the number of double bonds. For instance, oleic acid will elute later than stearic acid and linoleic acid will elute later than oleic acid. *Trans* fatty acids (TFA) will elute before their corresponding *cis* fatty acids. For positional isomers, the elution time will increase with the nearness of the double bond to the methyl end of the molecule. Oleic acid ($C_{18:1\ n-9}$) will thus elute before $C_{18:1\ n-7}$.

Recent technologies for fatty acid analysis are based on 100-m capillary columns coated with high polarity cyanosilicone stationary phases. The most widely reported are CP Sil 88 (100 m×0.25 mm×0.2 μm, Varian Inc.) and SP 2560 (100 m×0.25 mm×0.2 μm, Supelco) capillary columns, both containing similar makeups (biscyanopropyl polysiloxane), and HP 88 (100 m×0.25 mm×0.2 μm, Agilent Tech.) with a different stationary phase (88%-cyanopropyl-methyl aryl polysiloxane) that shows a slightly different FAME profile. The elution of the fatty acids depends on their own characteristics: chain length, double bonds, position, geometry, and branches. In addition, temperature program affects the elution order of fatty acids.

The most used official method for fatty acid analysis is Association of Official Analytical Chemists (AOAC) method 996.06, which determines total, saturated, and unsaturated lipid in food. The major limitation of this method is the incomplete separation of TFA and the absence of validation data for their quantification.

A flame ionization detector (FID) is usually used to detect the eluted peaks. An internal standard is often used for

quantification of the fatty acids. The quantification is based on the assumption that the FID detector gives a linear response for each fatty acid over the entire range of concentrations present in the sample. The odd-numbered fatty acids, most commonly C_{17} but also C_{13}, C_{21}, and C_{23}, can be used as an internal standard, because they occur in nature in very small amounts. By adding an amount of internal standard to the sample before the extraction of fat, the amount of fatty acids can be determined per 100 g sample or expressed as a percentage of total fatty acids.

Detection can also be achieved using mass spectrometry (MS) with electron impact ionization. For FAMEs, only the molecular weight can be ascertained. Other structural information cannot be obtained because the double bonds shift back and forth during ionization. However, if the fatty acids are esterified with nitrogen-containing heterocyclic compounds, like picolinyl esters or dimethyloxazoline (DMOX) esters, the double bonds will be stabilized, and their position in the molecule can be seen, although the difference between *cis* and *trans* mass spectra is not visible.

Special Procedures for *Trans* Fatty Acids

The new regulations on labeling and limitation of TFA has introduced new or updated methods to be able to improve the separation of TFA from other fatty acids. The main limitation is the limited availability of reference materials to confirm the identifications. The latest AOAC methods for the determination of *cis*-, *trans*-, saturated, monounsaturated, and polyunsaturated fatty acids (official method Ce 1h-05 and recommended practice Ce 1j-07) are based on the separation through CP Sil 88 and Supelco 2650 capillary columns and provide the identification of FAME based on available reference materials. However, other fatty acids may be present and not indicated in the reference materials. In those cases, the identification is achieved by available literature and GC/MS/MS analysis. In addition, the elution order of fatty acids with different unsaturation can be affected by column polarity, column age, or oven temperature.

To solve problems associated with the direct analysis of TFA by GC, the use of fatty acids fractionation, such as liquid chromatography (silver ion chromatography), solvent phase extraction cartridge (Ag-ion cartridge), reverse-phase HPLC, or high-speed counter current chromatography, has been proposed. The elution pattern of *trans* monounsaturated fatty acids has been characterized; however, the quantification of polyunsaturated TFA is limited due to the lack of reference materials and confirmed identifications. The identification of fatty acids has been based on GC/MS/MS of DMOX derivatives, but they do not provide information about *cis*–*trans* configuration, and the identification needs to be confirmed by other techniques. Recently, a new technique based on covalent adduct chemical ionization MS/MS (CACI-MS/MS) was able to identify position and geometry of FAME double bonds.

Protein

Total protein or crude protein is usually determined by measuring the content of nitrogen and multiplying by a conversion factor that depends on the specific proteins in the different foods. Thus, a value for the meat protein content is obtained by multiplying nitrogen content by the factor 6.25. Only two methods, the Kjeldahl and the combustion methods (based on Dumas method), are of interest. Another option is to determine and add all the amino acids after hydrolyzing the sample to derive a value for the protein content as will be described in the next section. Some indirect methods will also be mentioned. Figure 2 illustrates the main methodologies for protein determination.

In the Kjeldahl method, the material is digested in a Kjeldahl digestion flask with concentrated sulfuric acid and several catalysts. After cooling, the mixture is made alkaline with sodium hydroxide and the released ammonia is distilled and trapped in a boric acid solution, which is then titrated back with hydrochloric acid. The method determines protein-bound nitrogen from protein, peptides, or amino acid origin and from ammonium salts if present in the sample as well as determines nitrogen from a very large number of other organic nitrogen compounds. In addition, nitrogen in nitrate and nitrite will be determined to some extent, with a very large variation of approximately 60%. The distillation and titration step can be automated to obtain high throughput and safe handling of samples. Even so, the method takes approximately 2–3 h to complete an analysis.

In the combustion method, the sample is placed in a combustion chamber, having temperature between 950 and 1300 °C, where all covalently bound nitrogen is converted into nitrogen gas, which is quantified by passing through a conductivity cell. The method can be automated, but special and rather expensive equipment is necessary, demanding a high sample load to be profitable. The combustion method tends to give slightly higher results than the Kjeldahl method, as nitrogen-containing salts, like nitrate and nitrite, will also be converted into nitrogen with higher recoveries. For meat products, where nitrate and nitrite salts have been used to cure the meat, this could be of importance. The major benefit of this combustion method is the very rapid turnaround time, usually 5 min at a rate of approximately 100 samples per day, and that hazardous and toxic chemicals are not utilized.

The NIR/NIT indirect method described for fat determination can also be used to determine protein. Again a calibration is needed, which means that the indirect method will inherit the precision and all the limitations of the reference method.

When the sum of amino acids is determined, called the net protein value, and compared with total protein calculated from the content of nitrogen, the net protein value will be significantly lower than the crude protein value that might be due to the Kjeldahl, and combustion methods determine more nitrogen than that from amino acids. The difference for meat is that net protein values are approximately 20% lower than crude protein values.

There are two general ways to calculate the total amount of amino acids. The simplest consists in the analysis of the global amino acids amount without discriminating each other, which would include free amino acids and small peptides. These methods are based on the reaction of the α-amino group with reagents like *o*-phthaldialdehyde (OPA), fluorescamine, cadmium-ninhydrin, or trinitro-benzene-sulphonic acid.

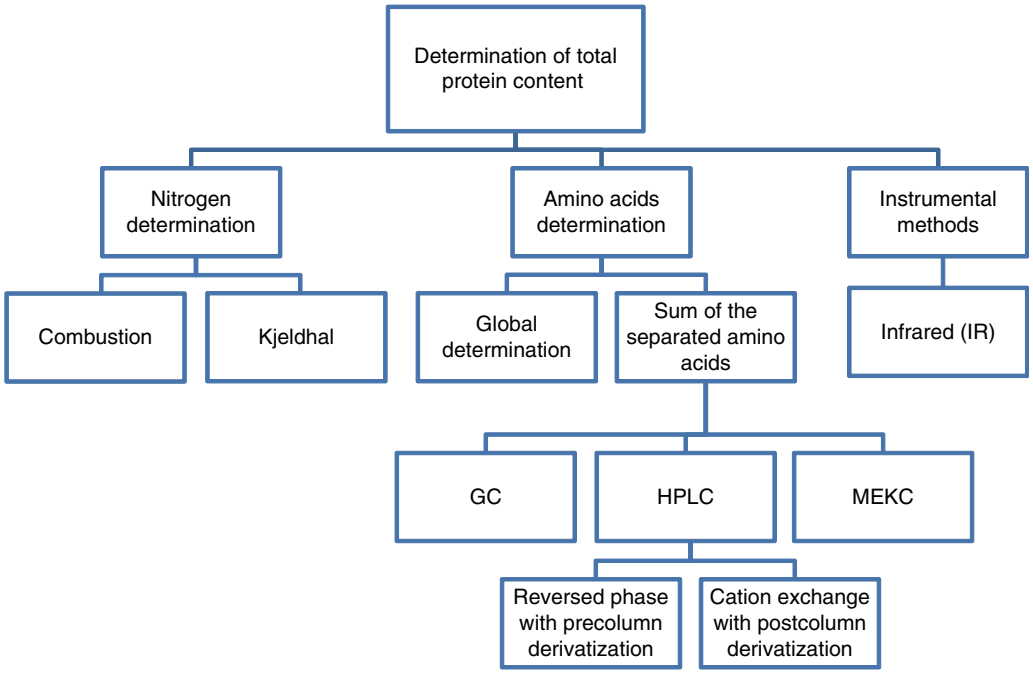

Figure 2 Scheme of methods to determine protein content.

Amino Acids

Amino acids constitute a very diverse group of chemical compounds that have in common an amine and carboxylic groups linked to the same carbon that support a wide variety of side chains, including sulfur-containing side chains like methionine and cysteine, benzene rings like phenylalanine and tyrosine, *N*-containing rings like tryptophan and histidine, or carbon chains like lysine and alanine that differentiate each other. This diversity gives them very different behavior in terms of oxidative stability, hydrolysis, reactiveness, charge as a function of the pH medium, and solubility in water. Thus, the simultaneous analysis of all naturally occurring amino acids is very complicated – far more so than the analysis of fatty acids. Here it is only possible to touch briefly on the most important factors in amino acid analysis.

Hydrolysis

Hydrolysis is the first step in the analysis of total amino acids. Hydrolysis is carried out by boiling samples in strong (6 mol l^{-1}) hydrochloric acid for approximately 24 h, either with reflux condenser or in sealed ampoules. Other possibilities are microwave systems with the use of special vessels, which can withstand high pressures. This allows the use of temperatures as high as 180 °C and reduces the hydrolysis time to 10 min. Not all the amino acids can be recovered after hydrolysis. Tryptophan is totally destroyed in the hydrolysis process, and so a special procedure with basic hydrolysis using sodium, barium, or lithium hydroxide and prolonged heating for approximately 16 h at 110 °C is used. However, tryptophan is also somewhat unstable by basic hydrolysis, which can be compensated by adding an internal standard – for instance, 5-methyltryptophan or α-methyltryptophan – before hydrolysis. Cysteine and methionine are not stable to the acid hydrolysis either and form diverse products of oxidation. This problem can be overcome by driving the oxidation to completion with phenol-containing performic acid to cysteic acid and methionine sulfone. However, this process destroys tyrosine, which then must be determined in an unoxidized sample. Bonds among serine, threonine, valine, leucine, and isoleucine amino acids are quite heat resistant and thus recoveries tend to be a bit low (85–95%), which is usually considered tolerable or can be rectified with the use of a correction factor. Glutamine and glutamic acid are determined together, as are asparagine and aspartic acid. Thus, the hydrolysis procedure is a series of compromises with many parameter sets described in the literature to obtain reasonable results for most of the amino acids. It is necessary to hydrolyze both an oxidized and an unoxidized sample and to carry out a special procedure for tryptophan, when analysis for total amino acids is the goal.

Chromatographic Separation

Separation of amino acids can be achieved by chromatographic techniques, like GC or HPLC, by either IEC or reversed-phase HPLC. Also, electrophoretic techniques like micellar electrokinetic capillary chromatography (MEKC) are used. Ion-exchange HPLC techniques are now gradually being replaced by reversed-phase HPLC carried out on standard equipment but with commercially available kits for the derivatization of the amino acids for a better detection.

Cation-exchange chromatography separates amino acids in their free form by gradient elution with a row of sodium-based buffers, giving a typical run time of approximately 1.5 h.

Detection of the amino acids is typically performed by light absorption after postcolumn reaction with ninhydrin, which forms a purple complex with all primary amino acids with a maximum at 570 nm. The secondary amino acid proline forms an intermediate reaction product with a maximum at 440 nm. Fluorescamine or OPA may also be used for the respective visible or fluorescent amino acid detection.

Reversed-phase HPLC is usually carried out on C_{18} columns with particle size of 5 μm or 3 μm, and gradient elution is common with a mixture of acetonitrile, methanol, and acetate or phosphate buffers. Precolumn derivatization to separate and detect the amino acids is necessary. Reagents like phenylisothiocyanate, OPA, 9-fluorenylmethylchloroformate (FMOC), 6aminoquinolyl-N-Hydroxysuccinimidyl carbamate, and dansyl and dabsyl chlorides are typically used, making ultraviolet or fluorimetric detection possible.

Hydroxyproline is an amino acid occurring predominantly in collagenous connective tissue, which contains 12.5% hydroxyproline. Therefore, a determination of hydroxyproline can be used to determine the amount of collagenous connective tissue in meat and meat products. Hydroxyproline can be determined in the analysis for total amino acids, but a much easier colorimetric procedure has been developed. The sample is hydrolyzed in sulfuric acid at 103 °C, filtered, and diluted. Hydroxyproline is oxidized with chloramine-T. After addition of 4-dimethylaminobenzaldehyde, a red-purple color is developed that is measured photometrically at 558 nm.

Because the free tryptophan is obtained after basic hydrolysis, it can be determined independently of the others amino acids, directly by reversed-phase HPLC and using its native fluorescence.

Amino acids to be analyzed by GC must be derivatized in order to increase their volatility and thermal stability. The application of *N*-methyl-*N*-tert-butyldimethylsilyl trifluoroacetamide as derivative reagent facilitates the GC analysis of amino acids. This reagent requires mild conditions to react, is stable, and has a very good behavior in MS detection together with the high efficiency of the GC technique, which makes this method a very good alternative to HPLC for free amino acid analysis.

The technique of capillary zone electrophoresis (CZE) is an extremely efficient technique for separations of charged solutes. Amino acids constitute a mix of basic, neutral, and acidic constituents and thus proper separation of all of them is not possible by this technique. MEKC is a modified version of CZE where surfactant-formed micelles are included in the running buffer to provide a two-phase chromatographic system for separating neutral compounds together with charged ones in a capillary electrophoresis system.

Glycogen

Glycogen is a starch-like molecule with a high molecular weight and is built of glucose units. The amount of glycogen depends on the conditions of the animal before harvest (resting, exercise, or fasting). Early postmortem, the reserve of glycogen is rapidly depleted and the generated glucose is rapidly converted into lactic acid. So, the amount of glycogen in meat is too small to be determined with the traditional method of subtracting fat, total protein, and ash from the dry matter. The glycogen can be broken down only partly with amylase but fully with amyloglucosidase or phosphorylases, releasing glucose phosphate. Glycogen can also be broken down by acid hydrolysis to glucose units, which then can be determined by an enzymatic method using commercially available kits or by reversed-phase or ion-exchange HPLC.

See also: Chemical Analysis: Raw Material Composition Analysis. Human Nutrition: Macronutrients in Meat

Further Reading

AOCS, 1989. Official Methods and Recommended Practices, fourth edn. Champaign, IL: The American Oil Chemists' Society.

Aristoy, M.-C., Toldrá, F., 2009. Amino acids. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, pp. 11–40.

Christie, W.W., 1998. Gas chromatography–mass spectrometry methods for structural analysis of fatty acids. Lipids 33, 343–353.

Delmonte, P., Kia, A.R.F., Hu, Q., Rader, J.I., 2009. Review of methods for preparation and gas chromatographic separation of *trans* and *cis* reference fatty acids. Journal of AOAC International 92, 1310–1326.

Delmonte, P., Rader, J.I., 2007. Evaluation of gas chromatographic methods for the determination of *trans* fat. Analytical Bioanalytical Chemistry 389, 77–85.

Etheridge, R.D., Pesti, G.M., Foster, E.H., 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. Animal Feed Science and Technology 73, 21–28.

Leffler, T.P., Moser, C.R., McManus, B.J., *et al*., 2008. Determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis: Collaborative study. Journal of AOAC International 91, 802–810.

Nollet, L.M.L., Toldrá, F. (Eds.), 2009. Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press.

Nollet, L.M.L., Toldrá, F. (Eds.), 2009. Handbook of Processed Meats and Poultry Analysis. Boca Raton, FL: CRC Press.

Official Methods of Analysis of AOAC International, 1999 or later. Method 988.05, 968.06, 990.03, Chapter 4. Arlington, VA: The Association of Official Analytical Chemists.

Official Methods of Analysis, 2000. Method 996.06, seventeenth ed. Gaithersburg, MD: AOAC International.

Official Methods and Recommended Practices, 2005. Official Method Ce 1h-05. Champaign, IL: AOCS Press.

Official Methods and Recommended Practices, 2006–2007. Methods Additions and Revisions. Recommended Practice Ce 1j-07. Champaign, IL: AOCS Press.

Pérez-Palacios, T., Ruiz, J., Martín, D., Muriel, E., Antequera, T., 2008. Comparison of different methods for total lipid quantification in meat and meat products. Food Chemistry 110, 1025–1029.

Terabe, S., Otsuka, K., Ando, T., 1985. Electrokinetic chromatography with micellar solution and open-tubular capillary. Analytical Chemistry 57, 834–841.

Ulberth, F., Buchgraber, M., 2000. Determination of total fat in foodstuffs. Lipid Technology 12, 135–137.

Relevant Websites

http://www.aoac.org/
AOAC International.

http://www.iata.csic.es/IATA/dcie/carn/
CSIC.

http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/default.htm
FDA.

Micronutrients and Other Minor Meat Components

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Glossary

Cholesterol Principal sterol synthesized by animals which is an essential structural component of cell membranes required to establish proper membrane permeability and fluidity, and for bile and steroid hormones synthesis.
Micronutrients Nutrients required throughout life in small quantities to orchestrate a range of physiological functions, including trace elements and vitamins.
Proteases Enzymes that catalyze the release of an amino acid from the amino terminus of a peptide (exopeptidases) or able to hydrolyze myofibrillar proteins to polypeptides (cathepsins and calpains).
Proteolysis Enzymatic breakdown of proteins with the formation of peptides and free amino acids.
Vitamins Essential organic compounds needed in low amounts for the control of metabolic processes which must be supplied through diet since the organism cannot synthesize them.

Introduction

The chemical analysis of the many micronutrients contained in meat and meat products will be addressed in this article. Vitamin content in meat derives from naturally available vitamins – meat being a good source of B vitamins: thiamine, riboflavin, and niacin are probably the most important in the group. Vitamins E and C are added to processed meat due to their reducing properties as antioxidants. Losses during processing can modify the vitamin content in processed meat. In many cases, the fortified levels of vitamins are high in comparison to the natural levels. Therefore, the availability of methodologies of use in monitoring the quality (technological indicators) of the product and for evaluating their nutritional value is of interest. Meat constitutes a major dietary source of iron, zinc, phosphorus, and magnesium. It is relatively poor in sodium and calcium. Nevertheless, the nutritional importance of foods of animal origin lies in their essential trace element contents. Of note in this sense is their iron content in the form of heme (hemoglobin and muscle myoglobin), offering high bioavailability in red meats. No other food contributes such high iron bioavailability. Moreover, heme iron contributes to the improvement of the organoleptic properties of meat as a result of its color. Meat contributes 50–70% of ingested zinc, and the latter (particularly in beef) is more bioavailable than zinc from vegetables. Despite the low selenium content of meat, its bioavailability is high – because the element is present in the form of selenomethionine and selenocysteine. Meat and meat products are also sources of cholesterol, considered by some relevant for health, which makes the analysis of cholesterol a part of this article.

Fat-Soluble Vitamins

Extraction and Purification

Analysis of fat-soluble vitamins in meat requires a complex sample preparation (extraction and purification) before vitamin determination via liquid chromatography (LC). Hot saponification with ethanolic potassium hydroxide solution containing an antioxidant is one of the most widely used procedures for extracting vitamins A, E, D, and carotenoids to disrupt the matrix, to release the free form from esters, hydrolyze triacylglycerols to glycerol, remove fats and other interfering substances, and produce soaps of free fatty acids. Vitamin K cannot be extracted by saponification because of its instability in alkaline media. The unsaponifiable fraction has been extracted with organic solvents such as hexane, isooctane, and petroleum-diisopropylether.

Liquid–liquid direct extraction using solvents such as hexane, ethanol, acetone, methanol, tetrahydrofurane, and petroleum ether is a more simple method of sample preparation. In meat products (liver, bacon, and sausage), extraction with 2-propanol (as protein denaturing solvent) and hexane as organic solvent to extract the vitamin was recommended for routine analysis of vitamin K in meat products. The main disadvantages are high consumption of organic solvents and the long time required for analysis.

As an alternative to these classical methods, saponification after supercritical fluid extraction (SFE) has been applied to fat-soluble vitamin determination in meat products (meat and liver paste). The advantages of SFE comprise minimal consumption of organic solvent (environmental protection), the exclusion of oxygen, reduction of thermal treatment, lesser economical cost, shorter extraction times, and less laborious processing, because these procedures can be automated.

In general, purification has only been applied to extracts of vitamins K and D from previously saponified samples to remove nonpolar components such as triacylglycerols. Samples with low fat contents ($<10\%$) are most effectively and easily purified with solid-phase extraction and the semipreparative high-performance liquid chromatography (HPLC).

Determination

HPLC became widely used for fat-soluble vitamin analysis, mainly because of its ability to effect rapid separation,

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nondestruction of the sample, and, more importantly, the good resolution achieved. Normal-phase HPLC on silica columns is by far the preferred technique for the analysis of vitamin A, which is eluted with small amounts of a polar organic solvent, often 2-propanol in *n*-heptane or isooctane, and often with gradient elution. Reverse-phase HPLC can also be used on C18 columns, but it is very difficult to achieve sufficient separation between all-*trans*-retinol and other vitamin A isomers, especially 13-*cis*-retinol. This is important, because other isomers do not have the same biological activity as all-*trans*-retinol. Fluorescence detection offers greater selectivity and sensitivity for all-*trans*-retinol and especially for tocopherols compared with ultraviolet (UV) detection, but usually the separation power of the column and the vitamin contents are high enough to permit UV detection to be used.

Capillary gas chromatography (GC)-flame ionization detection (FID) has been described for the determination of retinol (vitamin A) and α-tocopherol (vitamin E) in meat.

Water-Soluble Vitamins

Microbiological methods have been used for many years and are still employed for some of the water-soluble vitamins, including the determination of some B vitamins. This is based on the fact that certain microorganisms can only grow with a specific vitamin B in the growth medium. The vitamins are released by hydrolysis, growth medium is added, and the solution is inoculated with the microorganism. After incubation, the growth of the microorganism is measured using turbidimetry. The hydrolytic process is not always sufficient to release the vitamins in a form the microorganisms can use; enzymes therefore have to be added (papain, taka-diastase, and a phosphorylase) for thiamin and riboflavin. Folate is found in food in the form of polyglutamates, but the microorganisms can only use mono-, di-, or tri-glutamates; the bonds therefore have to be cleaved by enzymatic treatment. In vitamin B_{12} analysis, it is necessary to add potassium cyanide to transform naturally occurring hydroxycobalamin to more stable cyanocobalamin. In modernized versions of the microbiological assays, an enzyme-linked immunosorbent assay reader is used. This saves a lot of hard work for the laboratory staff and improves the precision of the assays.

Microbiological assays/methods are tedious and time-consuming; HPLC is therefore the most widely used technique for determining natural and fortified levels of water-soluble vitamins in meat products.

Thiamine (Vitamin B_1) and Riboflavin (Vitamin B_2)

Thiamine (the sum of thiamine, thiamine monophosphate, thiamine pyrophosphate, and thiamine triphosphate) and riboflavin (flavin mononucleotide-FMN and flavin adenine dinucleotide-FAD) are present in all animal tissues, and therefore in all natural unprocessed animal foods/meats.

HPLC is the method of choice for the simultaneous determination of B1 and B2, though excellent analytical data can be obtained using microbiological or fluorimetric procedures applied to thiamin (AOAC Official Method 942.23) and riboflavin (AOAC Official Method 970.65).

The extraction procedures generally applied in the determination of total thiamine and riboflavin by HPLC involve acid hydrolysis by boiling or autoclaving to release free thiamine and riboflavin and their phosphate esters from the food matrix (their association to proteins), followed by enzymatic hydrolysis (similar for the microbiological assay) of the phosphate esters to complete release. Generally, the acid hydrolysis step is carried out by heating the foodstuff with 0.1 M HCl, and using commercial enzymes (takadiastase, papain, pepsin, clara-diastase, acid phosphatase, and amylases).

Owing to the lability of flavins to light and to alkaline or extremely acidic pH values, special nonhydrolytic extraction conditions are required to quantify riboflavin and its coenzymes (FMN and FAD). This requires carrying out the extraction and analysis of the individual vitamers (compounds with similar molecular structure that show vitamin-activity in a vitamin-deficient biological system) between pH 5.0 and 7.0, and under subdued light in brown glassware.

The individual and simultaneous vitamin (B_1 and/or B_2) determinations are made by ion-pair reverse-phase HPLC using C18 columns. Elution with methanol or acetonitrile and phosphate buffer containing sodium heptane sulfonate, and fluorimetric detection, are the conditions normally used. Riboflavin has its own fluorescence, but the thiamine and phosphate esters must be oxidized to their fluorescent thiochromes. Simultaneous HPLC with UV detection determination of thiamine and riboflavin does not require the pre- or postcolumn derivatization needed in thiamine determination with fluorescence detection. However, higher levels of thiamine and riboflavin are needed (e.g., liver).

Capillary electrophoresis constitutes an interesting alternative to the HPLC method for determining thiamine, and is economical and ecologically sound.

Vitamin B_6

Vitamin B_6 comprises pyridoxine or pyridoxol (PN), pyridoxal (PL), and pyridoxamine (PM). Pyridoxamine phosphate and pyridoxal phosphate are the main vitamin B_6 vitamers in meat. HPLC techniques are the most widely used options for analyzing the various vitamers of B_6 (fluorescence detection). Determination of total B_6 requires hydrolysis: acid hydrolysis with HCl or H_2SO_4 and/or enzymatic hydrolysis (phosphatase or diastase, or an enzyme mixture of α-amylase, papain, and acid phosphatase). The official microbiological method (AOAC 961.15) allows the determination of PN, PL, and PM by using an anion-exchange resin and *Saccharomyces uvarum*.

Possible interconversion among vitamers due to the extraction and/or analytical procedure used, resulting in apparent losses and changes in B_6 vitamer composition, cannot be ruled out. This is the reason why identification of the vitamin B_6 vitamers by HPLC has been shown to be a source of error.

Vitamin B_{12} (Cyanocobalamin)

The predominant forms of cobalamin present in animal tissues include hydroxycobalamin and the two coenzyme forms, methylcobalamin and adenosylcobalamin.

The release of vitamin B_{12} from proteins is generally obtained by heat or protease treatment (pepsin). The addition of sodium cyanide during sample treatment before quantification converts the native vitamin forms into dicyanocobalamin. Vitamin B_{12} compounds are extracted in phosphate buffer containing a reducing agent (metabisulfite or ascorbic acid) to protect the cobalamins throughout extraction.

Generally, for the routine analysis of vitamin B_{12}, a microbiological method (AOAC Official method 952.20) has been used. HPLC methods for the determination of B_{12} have been reported but are less sensitive than the microbiological methods. Other techniques such as radioisotope dilution assay and chemiluminescence analysis have also been used.

Folates

HPLC methods for folate are not yet fully standardized. Poor stability of folates during extraction and the variability in deconjugation are the main problems. HPLC has been applied to the quantification of the main folate forms: tetrahydrofolate and 5-methyltetrahydroflate, using purification by ion-exchange chromatography before HPLC on a C18 column with gradient elution with acetonitrile–phosphate buffer and fluorimetric detection.

Minerals

Wet or dry decomposition procedures are the first step in the method of mineral analysis in meats. Wet digestion with concentrated acids (nitric, perchloric, and sulfuric acid alone or in combination) is the most common sample pretreatment technique, carried out at atmospheric pressure in open systems or at higher pressures in a closed vessel by conductive or microwave heating. Microwave energy has allowed a reduction of digestion time. The main limitations of these procedures are matrix interferences due to the use of concentrated acid in atomic absorption spectrometry (AAS); the formation of highly carcinogenic nitrous vapors; high blank values; the possibility of sample contamination; and long cooling times required before opening the low or high pressure pumps, etc. New sample pretreatment methods such as acid leaching assisted by microwave or ultrasonic bath have been developed. The procedure involves the solubilization of minerals in the leaching solvent (an acid and/or oxidant agent) without sample matrix decomposition. This type of procedure minimizes the main limitations of the wet digestion methods.

Other pretreatments such as enzymatic hydrolysis, consisting of the hydrolysis of proteins or lipids in the sample by using enzymes such as protease, trypsin, pronase, and lipase, and the determination of free mineral after a centrifugation step or the use of slurries represents an alternative to sample digestion before mineral determination. Slurry sampling offers the advantages of the direct solid and liquid sampling methods.

The methods for the determination of minerals are almost all atomic spectrometric techniques, AAS and inductively coupled plasma atomic emission (ICP-AES) or mass spectrometry (ICP-MS) – these being the most reliable options for multielement analyses. The extensive annual literature reviews in the section entitled Atomic Spectrometry Update-Clinical and Biological Materials, Food and Beverages, of the *Journal of Analytical Atomic Spectroscopy*, reflect the major role atomic spectroscopy has played in the development of the current databases on minerals in foods.

In general, iron and zinc may be determined by AAS using flame atomization and deuterium background correction with vaporization in an air-acetylene flame. Flame AAS and ICP-AES provide sufficient detection capability for the quantitation of elements of interest in meat (Fe, Zn, and Mg), and provide good precision over their entire calibration range. The dynamic range for ICP-AES is clearly larger, making the need for sample dilution less likely, and it is less prone to chemical matrix interference due to molecule formation than flame AAS – though there can be problems with spectral line overlaps. Selenium, present in much smaller quantities, is determined by the more sensitive graphite furnace AAS (GF-AAS) technique with Zeeman background correction, or by hydride-generation flameless AAS. ICP-MS is a fast and multielement technique with a wide dynamic range and excellent detection limits for element analysis in meats. The major drawback is that it suffers from spectral and nonspectral interferences.

The determination of phosphorous is the only gravimetric method proposed by the AOAC (969.31). Phosphorous can be precipitated as $Mg_2P_2O_7$. The precipitate is first dried and then ashed in a furnace at 550 °C. The spectrophotometric techniques remain valid for the determination of P and Fe. Fluorimetric methods for determining selenium involve wet digestion, where Se is converted to Se (IV) and determined by measuring the fluorescence of the piazselenol formed on reacting with 2,33-diaminonaphthalene or 3,3′-diaminobenzidine.

Other available techniques such as near infrared spectrometry, neutron activation analysis, and X-ray fluorescence are sufficiently sensitive (with detection limits of $1\,\mu g\,g^{-1}$ for Fe and Zn and $2\,\mu g\,g^{-1}$ for Se), and only very simple sample treatment is needed (drying and grinding to homogenize size, or dried and pelletized meat sample without dissolution). This simplifies the analysis and minimizes the risk of contamination.

Elements can be present in ionic form and/or complexed to various binding proteins. It is of special interest to be able to distinguish or speciate between heme and nonheme iron, because heme iron bound in myoglobin and hemoglobin is more bioavailable than nonheme iron. Several colorimetric methods are able to determine nonheme iron based on reaction with ferrozine or bathophenantroline. Heme iron can be determined by isolation of hematin with acetone-hydrochloric acid and colorimetric measurement. The hyphenated techniques such as size exclusion chromatography (SEC) coupled with ICP-MS have been successfully applied to Cu, Zn, Fe, Mg, among other elements, in liver. A magnetic sector mass spectrometer eliminates polyatomic ion interferences for Fe, S, and P, and provides high sensitivity. Sample preconcentration is not needed. This method generates information on the approximate molecular weight of proteins containing particular elements, without using a standard sample of the same protein. This is one of the advantages of SEC compared to RP-HPLC or ion-exchange chromatography. SEC and reverse phase (RP) chromatography coupled online to ICP-MS to investigate metallothioneins and superoxide

dismutase, Fe, Cu, Zn, and Mn species, has been applied to porcine liver and meats. Speciation of selenium (determination of selenomethionine and selenocysteine) in meats is feasible using SEC and/or ion exchange (IE) coupled to ICP-MS.

Cholesterol

Cholesterol in meat ranges from 50 to 70 mg per 100 g, partly independent of the fat content. Cholesterol can be analyzed routinely by GC-FID without derivatization. However, other methods, especially HPLC coupled to an UV/visible/photodiode array detector, can also be used when nondestructiveness is preferred, especially when cholesterol must be separated from other compounds such as tocopherols. More advanced methods, such as gas chromatography/HPLC-isotope dilution/mass spectrometry, are primarily used for quality control purposes.

Direct saponification with ethanolic KOH solutions at high temperatures has been preferred for hydrolyzing samples because of cost and time effectiveness, and has been shown to be effective in recovering cholesterol and in eliminating fatty acid interference. Nonsaponifiable matter is extracted with different solvents, but toluene seems to provide sufficient recovery in a single extraction – though the formation of an emulsion associated with this solvent is possible, and careful postsaponification manipulation is required. Derivatization with silylating reagents such as hexamethyldisilane has been used to form trimethylsilyl ethers (TMS), and has been applied by several methods, including the AOAC Official Method (994.10). TMS ethers, although allowing for greater sensitivity and a sharper peak shape, potentially cause deposits of silicon dioxide ion in FID, thereby affecting linearity of the FID response. In addition, methods involving derivatization are sensitive to moisture, which can facilitate hydrolysis of the ether derivative and reduce recovery. 5α-Cholestanol behaves like cholesterol during the analysis and is used as an internal standard, which can be added before saponification.

Methods involving direct saponification, smaller sample size, and lesser amounts of toluene for extraction compared with the AOAC method, and analysis of free cholesterol, without derivatization, have been applied. In this case, emulsification may occur during the purification process. The addition of ethanol, KOH, and water in subsequent steps of the cleanup is usually required.

Total sterols can be determined by an enzymatic method after saponification. Sterols are oxidized with cholesterol oxidase to stenones, with the release of hydrogen peroxide, which oxidizes methanol to formaldehyde in the presence of catalase. Formaldehyde reacts with acetyl acetone to form a yellow compound, which can be measured colorimetrically at a wavelength of 405 nm. The reagents can be acquired as a kit. The method works well if only cholesterol is present, as is the case for meat; however, if both plant sterols and cholesterol are present, the results will be difficult to interpret.

Enzymes

This section is only focused on muscle proteolytic enzymes, which constitute a group of enzymes involved in protein metabolism and thus affecting muscle growth processes in the living animals as well as the proteolysis phenomena during conversion of muscle to meat. These enzymes are also deeply involved in changes in quality during postmortem storage and further processing, especially in those related to fermented and dry-cured meat products. The most studied enzymes are the endopeptidases mainly consisting of the calpain system and cathepsins. The assays for their respective activities are briefly described below.

Calpain is an intracellular calcium-dependent cysteine endopeptidase. The two most commonly analyzed isoforms in meat are μ-calpain and m-calpain, which have been found in the range 10–100 μg g^{-1} of muscle. The calpain inhibitor calpastatin is also present in the muscle and extracted together with calpains making necessary its further purification previous to the assay. Usually either phenyl-Sepharose or butyl-Sepharose chromatography is used to remove calpastatin, followed by anion-exchange chromatography (diethylaminoethyl-Sepharose, or Mono Q fast protein LC) to separate μ- and m-calpain. A faster one-step procedure using only anion-exchange chromatography is also used, but incomplete separation of the μ-calpain fraction from calpastatin is often a problem. Casein is the classical substrate used for *in vitro* assays of calpain. Casein is cleaved by calpain during incubation at 25 °C and pH 7.5 in the presence of $CaCl_2$, and the remaining intact protein is precipitated by adding trichloroacetic acid. Subsequent to centrifugation, the absorbance of the supernatant containing the released peptides is measured at 278 nm. More sensitive assays are based on the use of fluorescent substrates like casein-fluorescein isothiocyanate at pH 7.5. The enzyme reaction is started by the addition of $CaCl_2$ and incubation at 25 °C for 20 min. Again, the remaining intact protein is precipitated by adding trichloroacetic acid and, subsequent to centrifugation, the fluorescence is measured at 485 nm and 538 nm of excitation and emission wavelengths, respectively. One unit of activity is defined as the amount of enzyme capable of hydrolyzing 1 mmol of substrate per hour at 25 °C.

Alternatively, calpain activity can be determined by casein zymography. Casein is incorporated in separating gels, and calpain extracts are loaded onto the gels that are run under denaturing conditions. After electrophoresis, calcium and a reducing agent are added to activate calpain, and the casein-gels are incubated for 16 h at 25 °C. After staining with Coomassie blue, the calpain activity will appear as a clear band in the dark blue gel corresponding to casein depletion (digested into peptides that have diffused out of the gel). Quantification can be done by expressing the density of the bands relative to the density of reference standards within each gel.

Muscle also contains other endopeptidases like cathepsins that are located in the lysosomes with a high hydrolytic potential. Main cathepsins are B, H, and L (cysteine) and D (aspartic) and are active in acid conditions. In meat, cathepsin activity is controlled by several factors including release from lysosomes, pH, extent of precursor activation, and specific endogenous inhibitors (cystatins). The activity of these enzymes can be assayed after a gentle homogenization procedure. Total lysosomal enzyme activities in meat extracts can be determined by extraction and homogenization at pH 5.0 in the presence of Triton X-100. The activity of cathepsin D can

be typically determined at pH 4.5 and 45 °C, using 10% w/v hemoglobin as substrate. The activity of cathepsins B, B+L, and H has been determined following isolation from their physiological inhibitors, cystatins, by affinity chromatography. The activity of cathepsins B, B+L, and H is usually determined at 37 °C and pH 6.0 for cathepsins B and B+L, and pH 6.8 for cathepsin H. Specific fluorescent substrates are used, *Z*-arginine–arginine-7-amido-4-methylcoumarin for cathepsin B, *Z*-phenylalanine–arginine-7-amido-4-methylcoumarin for cathepsin B+L, and *Z*-arginine-7-amido-4-methylcoumarin for cathepsin H. Once the reaction is stopped, the released fluorescence is measured at 380 nm and 440 nm of excitation and emission wavelengths, respectively. One unit of activity is defined as the amount of enzyme capable of hydrolyzing 1 μmol of substrate per minute at 37 °C.

The 20S proteasome is a muscle enzyme exerting endo- and exopeptidase activity and has been associated with the degradation of damaged oxidized proteins. This enzyme shows five main catalytic activities: trypsin-like, chymotrypsin-like, peptidyl–glutamyl peptide hydrolase, branched-chain amino acid-preferring, and small neutral amino acid-preferring, which hydrolyze synthetic peptides at the carboxyl group of alkaline, hydrophobic, acid, branched-chain, and neutral amino acids, respectively. The activity can be assayed with fluorescent substrates after extraction and homogenization at pH 8 in the presence of glycerol, ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), and inhibitors E64 and pepstatin A. The activity can be determined with leucine–serine–threonine–arginine-4-methyl-coumarin-7 amide, *N*-Suc-leucine–leucine–valine–tyrosine-4-methyl-coumarin-7 amide, or 100 mmol l^{-1} benzyloxycarbonyl-leusine–leusine–glutamic acid-2-naphthylamide to measure trypsin-like, chymotrypsin-like, and peptidyl–glutamyl peptide hydrolase activities, respectively.

See also: Chemical Analysis: Analysis of Final Product Composition for Labeling; Raw Material Composition Analysis. Chemical Analysis for Specific Components: Major Meat Components. Human Nutrition: Cardiovascular and Obesity Health Concerns; Macronutrients in Meat; Micronutrients in Meat

Further Reading

Alegría, A., Barberá, R., Lagarda, M.J., Farré, R., 2009. Vitamins. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Processed Meats and Poultry Analysis. Boca Raton, FL: CRC Press, pp. 291–326.

Alegría, A., Barberá, R., Lagarda, M.J., Farré, R., 2009. Minerals and trace elements. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, pp. 441–463.

Dinh, T.N., Thompson, L.D., Galvean, M.L., Brooks, J.C., 2012. Determination of total cholesterol in meat and poultry by gas chromatography: Single-laboratory validation. Food Composition and Addtives 95, 472–488.

Horwitz, W. (Ed.), 2002. Official Methods of Analysis, seventeenth ed. Gaithersburg: AOAC International.

Reig, M., Toldrá, F., 2012. Biochemistry of raw meat and poultry. In: Simpson, B.K., Nollet, L.M.L., Toldrá, F., Paliyath, G., Benjakul, S. (Eds.), Food Biochemistry and Food Processing, seconnd ed. Ames, Iowa: Wiley-Blackwell, pp. 287–302.

San Andrés, M.P., Vera, S., Torre, M. (Eds.), 2010. Recent Advances in Vitamin Analysis. Kerala: Research Signpost.

Toldrá, F., Flores, M., Sentandreu, M.A., 2009. Muscle enzymes: Exopeptidases and lipases. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, pp. 112–128.

Relevant Websites

http://www.aoac.org/
AOAC International.

http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/default.htm
FDA – Laboratory Methods.

Veterinary Drug Residue Analysis

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Glossary

Calibration standard A device for measurements that represents the quantity of substance of interest in a way that ties its value to a reference base.
Confirmatory method A method that provides full or complementary information enabling the substance to be unequivocally identified and quantified at the level of interest.
Decision limit (CCα) The limit at and above which it can be concluded with an error probability of α that a sample is non compliant.
Detection capability (CCβ) The smallest content of the substance that may be detected, identified, and/or quantified in a sample with an error probability of β. In the case of substances for which no permitted limit has been established, the detection capability is the lowest concentration at which a method is able to detect truly contaminated samples with a statistical certainty of $1 - \beta$. In the case of substances with an established permitted limit, this means that the detection capability is the concentration at which the method is able to detect permitted limit concentrations with a statistical certainty of $1 - \beta$.
Fortified sample material A sample enriched with a known amount of the analyte to be detected.
Interlaboratory study Evaluation of tests on the same sample by two or more laboratories in accordance with predetermined conditions to determine testing performance.
Internal Standard (IS) A substance not contained in the sample with physical-chemical properties as similar as possible to those of the analyte that has to be identified and which is added to each sample at a known concentration.
Laboratory sample A sample prepared for sending to a laboratory and intended for inspection or testing.
Level of interest The concentration of a substance or an analyte in a sample that is significant to determine its compliance with legislation.
Recovery True concentration of a substance recovered during the analytical procedure and expressed as a percentage.
Ruggedness Susceptibility of an analytical method to changes in experimental conditions.
Screening methods Methods that are used to detect the presence of a substance or class of substances at the level of interest. Generally are high throughput methods.
Specificity The ability of a method to distinguish between the analyte of interest being measured and other substances that may have similar physicochemical properties.

Introduction

There are many veterinary drugs that may be used in farm animals either for prophylactic purposes or to promote growth, to improve the distribution of fat and protein, and to increase feed-to-muscle conversion rate. After the administration of these veterinary drugs some residues may remain in the tissues at toxicologically unacceptable concentrations.

International and national legal bodies agreed for the so-called maximum residue limits (MRLs) for substances allowed for veterinary use. So, MRLs for specific veterinary drugs are derived from acceptable respective daily intakes from foods of animal origin. The MRLs should ensure that even in extreme consumption patterns, consumers are at an acceptable or nil health risk toward the intake of residues of certain veterinary drugs. There are no MRLs for banned drugs, including carcinogenic substances, because their residues are not tolerated at any concentration in food products. The national MRLs may be different from country to country probably due to differences in the level of risk that individual governments are prepared to accept and the methodologies for establishing MRLs.

Recent multiple outbreaks of residue-contaminated food products have scared consumers and increased the public's awareness of the use of veterinary drugs in the food chain. Furthermore, some of these residues may also exert carcinogenic, genotoxic, or other undesirable effects on health. So, the potential presence of residues in meat and poultry must be controlled to assure consumers' safety. This article is reporting the analytical procedure for the detection of residues in farm animals and meat.

Sample Preparation

Sampling

The concentrations of residues in foods of animal origin may be quite variable depending on the type of drug used, the type of administration, and the washout time left before slaughtering. Larger concentrations may be found near the injection site but also some substances may concentrate in certain organs. It is very important to select the appropriate target tissue or matrix where the analyte is expected to accumulate (e.g., liver, kidney, urine) because the concentrations are low and need very sensitive techniques for their detection. This is even worse when certain types of cocktails, prepared as a

mixture of low amounts of different growth-promoting substances with synergistic effect, are used. The same biological effect is obtained but making it rather difficult for the detection of the individual components because of their low individual concentrations. The sample must be chosen so that the result reflects the residue status of the laboratory sample and thus that of the sampled tissue or body fluid. Portioning of the laboratory sample, either at the sample collection site or in the laboratory, should always be considered to prevent unnecessary freeze–thaw cycles affecting residue integrity and allowing the possibility of further analysis in case of doubtful results or any other analytical problem.

Sample Storage and Sample Pretreatment

Sample storage is very important because sometimes the time elapsed between sample collection and analysis may be long. Depending on the postmortem processing of the sample and its storage, some reactions may convert the original residue into (unknown) derivatives. The residue may be object of physicochemical reactions like oxidation, proteolysis, or precipitation, even being covalently linked to proteins, making necessary the cleavage of such links for an adequate extraction. Residues may also be degraded by microbiological or enzymatic reactions. For instance, carbadox and chloramphenicol are susceptible to cytochrome P-450 degradation in liver and kidney samples, even during storage at −20 °C, but this degradation may be inhibited by adding piperonyl butoxide.

Sample Extraction

The target analyte is usually the parent residue molecule but its metabolites should also be considered. In all the cases, residues may be extracted with aqueous buffers or organic solvents. It must be taken into account that many veterinary drugs, including oestradiol, chloramphenicol, levamisole, and phenolic-type β-agonists like salbutamol, are present as conjugate forms (glucuronide or sulfate), as a way of detoxification, usually excreted through the urine. When analyzing such types of residues and before residue determination, it is necessary to hydrolyze the sample under mild conditions, usually achieved by incubating with *Helix pomatia* juice, which is a mixture of arylsulfatases and β-glucuronidases or any other hydrolysis procedure. Residues bound to proteins through weak interactions can be extracted after heat or acid treatments.

Solid matrices, including fat, kidney, liver, retina, and skin, are usually extracted by homogenization of the sample in the presence of solvents. Waring blenders, Ultra-Turrax, glass grinders, and stomachers can be used for homogenization; of course, heating of the sample must be avoided to preserve the integrity of the analyte. Sometimes, the sample can be grounded in liquid nitrogen. A cleanup of the sample is necessary to remove any matrix components that might be coextracted. The solid–phase extraction (SPE) is extensively used because the analyte can be concentrated in a single step with minimal sample and solvent consumption compared with the classic liquid–liquid extraction. There are many available stationary phases in SPE that facilitate the choice of the better phase for each particular analyte. SPE cartridges have evolved into miniaturized columns integrated in microwell assay plates and into disks consisting of membranes with high, reversed-, and ion exchange phase capacity.

An advanced extraction procedure can be obtained with immunoaffinity cartridges (IACs) that contain a support with linked antibodies for the specific target analyte that facilitates the affinity extraction with a high degree of specificity. An enormous concentration can be reached in a single step. The sensitivity is significantly increased even though these cartridges are expensive. IACs are commercially available for a number of residues, particularly of banned substances. Molecularly imprinted polymers (MIPs) are another alternative. MIPs can be synthesized from highly cross-linked polymers in the presence of the print molecule (a variant of the analyte). Removal of the print molecule leaves an imprinted sorbent for highly selective extraction of the analyte.

The so-called matrix solid-phase dispersion approach consists of simultaneous homogenization and extraction, which is accomplished by mixing the sorbent material in the homogenate and thus omitting liquid extraction of the homogenate as a separate step. The bonded reversed phase may act in this case as a surfactant and may help in unfolding and disrupting cellular structures to release contained residues. Columns prepared from the sample/sorbent mixture are eluted in a similar way to SPE cartridges.

Microwave-assisted extraction allows the rapid heating of the solvent, which accelerates the extraction by reducing the needed time and consuming less solvent. It also facilitates a high throughput. Supercritical fluid extraction has also been used as a suitable alternative to solvent extraction for lipophilic substances such as androgens, gestagens, and oestrogens, from a variety of matrices. Carbon dioxide is the most used fluid because it acquires physical properties intermediate between those of the liquid and gas phases at a certain combination of temperature and pressure. More recently, pressured liquid extraction is being used because a fast and efficient extraction of the analyte from the solid matrix is obtained. The solvent is kept in the liquid phase at temperatures above the boiling point by applying high pressure.

Following extraction, the analyte-containing solution is concentrated by removing the excess of solvent either by evaporation or sublimation. This can be achieved either by applying a vacuum or by using a flow of an inert gas (nitrogen), sometimes in combination with mild heating.

Analytical Methodologies

Analysis strategies are very important considering the wide variety of residues to be analyzed and the large number of samples, usually with results requested in a short period of time. Consequently, the initial phase consists of using low-cost screening methods with high throughput, acceptable false positive incidence and a very low false negative rate, are used. For those samples suspected to be noncompliant, the next phase consists of the use of confirmatory methods usually involving expensive instrumental techniques, almost exclusively based on mass spectrometry detection, and requiring highly skilled operators.

The samples that contain a residue at a concentration above the detection limit (CCα) (see section validation) within the EU, are considered as noncompliant (positive). A sample containing a substance with an MRL will be considered as noncompliant when the concentration of the target residue is found to be in excess of its MRL.

Screening Methods

There are different types of screening methods depending on the type of residues. The main types of screening techniques are listed in Table 1. So, residues of antimicrobial drugs can be screened with microbiological growth-inhibition assays like the European Union (EU) Four Plate Test, the New Dutch Kidney Test, and the Delvotest SP, which use antibiotic-sensitive bacterial reporter strains such as *Bacillus subtilis* and *Bacillus stearothermophilus* var. *calidolactis*. These bacteria are inoculated under optimal conditions with and without sample. After culturing, results are read from visible inhibition zones or color change of the bacterial suspension in agar gels.

The availability of relatively large amounts of immunoglobulins, for example, polyclonal antibodies or monoclonal antibodies, recombinant antibodies, or antiidiotype antibodies, of which the production is assured, has enabled the development of a range of immunoassays for many types of growth promoters and antimicrobials. These methods include radioimmunoassays, enzyme immunoassays, enzyme-linked immunosorbent assays, strip- and dip stick-based immunoassays. These methodologies have been expanded in the range of residues to detect and the sensitivity and have also developed for high throughput. Some commercial instruments include the CHARM Test II and Penzyme III.

Other types of screening methods are based on the detection of an interaction between biomolecule and analyte, not requiring any labeling. These biosensors allow realtime monitoring of the analyte immunoglobulin–receptor interaction. Commercial instruments include the surface plasmon resonance biosensors.

Finally, liquid chromatography (HPLC) and more recently ultra performance liquid chromatography (UPLC) are also used for the screening of multiple residues in a sample in a relatively short time, especially with the advent of new types of columns. Analytes that are not detected by light absorption or fluorescence at the level they are expected to occur in samples may require derivatization to render them active as fluorescents, chromophores, or UV-light absorbing compounds.

Confirmatory Methods

When samples are suspected to be noncompliant, the next phase consists of the use of confirmatory methods. It should be noted here that the use of an internal standard should be considered, to allow continuous assessment of the performance of the designed assay, and to facilitate calibration in quantitative determinations. Mass spectrometry is the most reliable for such confirmatory purposes especially when coupled to gas chromatography (GC) or liquid chromatography (LC). The main techniques are listed in Table 1 and major advantages and disadvantages of confirmatory methods are summarized in Table 2.

In GC analysis, the universality and discriminative power of the mass spectrometer (MS) in combination with electron-impact ionization have led to this being the method of choice for some residues like steroids.

In LC analysis, the evolution of mass selectors, ionization techniques and the available interfaces between them, have made mass spectrometry the standard equipment in a residue analysis laboratory. Mass selectors comprise triple quadropole,

Table 1 List of main analytical techniques available for screening and confirmatory purposes

Screening methods	*Confirmatory methods*
Microbiological plates bioassays	Gas chromatography coupled to MS, MS–MS, or MS^n
Dipsticks or enzyme-linked immunosorbent assays test kits	Liquid chromatography (HPLC) coupled to MS, MS–MS, or MS^n
Radioimmunoassay	Ultra performance liquid chromatography (UPLC) coupled to MS, MS–MS, or MS^n
Multiarray biosensors	HPLC or UPLC coupled to time-of-flight
High performance thin layer chromatography with full scan UV/Vis	HPLC or Gas chromatography coupled to Infrared (IR) spectrometric detector
Gas chromatography coupled to electron capture detector	
HPLC coupled to ultraviolet/visible (UV/Vis), diode array detector (DAD) or fluorescence detector	
UPLC coupled to UV/Vis, DAD or fluorescence detector	

Table 2 Major advantages and disadvantages of current confirmatory methods

Advantages	*Disadvantages*
High sensitivity	Need of sample preparation (extraction and purification)
High specificity	
Lot of information about the analyte	High initial investment (equipment)
Short time (few min/sample) to obtain the results	High maintenance costs
Screening and confirmation in one injection	Expertise required

Table 3 Some examples of the number of identification points earned for different analytical techniques

Technique	*Number of ions*	*Identification points*[a]
GC–MS Electron impact (EI) and Chemical ionization (CI)	2 EI and 2 CI	4
GC–MS EI or CI	N	n
LC–MS	N	n
GC–MS–MS	1 Precursor and 2 daughters	4
LC–MS–MS	1 Precursor and 2 daughters	4
GC–MS–MS	2 Precursor ions each with 1 daughter	5
LC–MS–MS–MS	1 Precursor, 1 daughter, and 2 granddaughters	5.5

[a]1 point per precursor and 1.5 points per daughter or granddaughter.

Source: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities of 17/8/2002, L221, 8–36.

ion trap, time-of-flight instruments and combinations thereof. In particular, the mild ionization techniques of atmospheric pressure chemical and electrospray ionization, combined with their suitability for interfacing with HPLC, have enabled MS analysis of both hydrophobic and hydrophilic molecules ranging from relatively small to large. In addition, MS-induced molecular fragmentation allows elucidation of the chemical structure of the residue. A major drawback is matrix-caused interference of the ionization process, which may reduce analyte signals dramatically.

Validation

Regulatory action can be taken only after unequivocal identification of the residue. The European Commission, for example, released the Decision 2002/657/EC, which describes the required quality performance criteria and guidelines for the interpretation of analytical results as well as validation of the methods. Other international organizations like International Organization for Standardization (ISO), International Union of Pure and Applied Chemistry (IUPAC), Association of Official Analytical Chemists (AOAC) International have issued similar criteria and recommendations. This Decision also includes the CCα, which is defined as the limit at and above which it can be concluded with an error probability of α that a sample is noncompliant and the detection capability (CCβ), which is defined as the smallest content of the substance that may be detected, identified, and/or quantified in a sample with an error probability of β.

Topics that have to be assessed are trueness and precision of the result, and specificity and selectivity of the method. Variables that may influence the measurement, such as minor changes in pH, evaporation conditions, temperatures, SPE flow rate, time intervals, and the use of different SPE batches or of different producers, have to be evaluated to establish the ruggedness of the method. The validation of analytical methods is largely based on the fortification of control samples with a known quantity of the analyte. Although fortification is a well-established procedure, it has to be recognized that the added analyte may be more available for extraction than its incurred counterparts. In addition, fortification and incubation time following the addition will most likely not imitate metabolic processes, including conjugation and formation of bound residues. Finally, the Decision 2002/657/EC also establishes the need to accomplish a number of the so-called identification points (IPs) for the correct identification of the residue substance. Examples of the number of IPs earned when using different techniques are shown in **Table 3**. At least three IPs should be obtained for a satisfactory detection of a substance with an MRL, whereas four IPs are needed for positive identification of banned substances.

Future Trends in Residue Analysis

Sensitivity and selectivity will continue to improve thanks to the rapid developments in analytical instrumentation, especially in computerization and miniaturization so that levels of detection will drop to very low ultratrace levels.

The time required per analysis will be reduced thanks to new columns, packing materials, elution conditions, and instrumental devices. The trends toward high throughput techniques will continue so that more multiresidue methods will be developed with more analytes per sample to be analyzed per unit of time.

Biosensor systems, with microchips containing antibodies against many different analytes in one single chip that requires only a few microliters of sample, are attracting particular attention for such applications.

See also: Chemical Analysis: Physicochemical Analysis Methods; Sampling and Statistical Requirements. Environmental Contaminants. Laboratory Accreditation. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Beta-Agonists; Bovine and Porcine Somatotropin. Residues in Meat and Meat Products: Feed and Drug Residues

Further Reading

Cháfer-Pericás, C., Maquieira, Á., Puchades, R., 2010. Fast screening methods to detect antibiotic residues in food samples. Trends in Analytical Chemistry 29, 1038–1049.

De Brabander, H.F., Noppe, H., Verheyden, K., *et al.*, 2009. Residue analysis: Future trends from a historical perspective. Journal of Chromatography A 1216, 7964–7976.

Dixon, S.N., 2001. Veterinary drug residues. In: Watson, D.H. (Ed.) Food Chemical Safety: Conaminants, Vol. 1. Cambridge, England: Woodhead Publishing Ltd., pp. 109–147.

EC 2002a. Commission Decision 2002/657/EC of 17 August 2002 implementing Council Directive 96/23/EC concerning the performance of the analytical methods

and the interpretation of results. Official Journal of the European Community L221, 8–36.
EC 2002b. Commission Directive 2002/32/EC of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Community L140, 10–21.
Gentili, A., Perret, D., Marchese, S., 2005. Liquid chromatography-tandem drugs in animal-food products. Trends in Analytical Chemistry 24, 704–733.
Kaufmann, A., 2009. Validation of multiresidue methods for veterinary drugs residues; related problems and possible solutions. Analytica Chimica Acta 637, 144–155.
Kaufmann, A., Butcher, P., Maden, K., Walker, S., Widmer, M., 2011. Development of an improved high resolution mass spectrometry based multiresidue method for veterinary drugs in various food matrices. Analytica Chimica Acta 700, 86–94.
Kinsella, B., O'Mahony, J., Malone, E., *et al*., 2009. Current trends in sample preparation for growth promoter and veterinary drug residue analysis. Journal of Chromatography A 1216, 7977–8015.
Le Bizec, B., Pinel, G., Antignac, J., 2009. Options for veterinary drug analysis using mass spectrometry. Journal of Chromatography A 1216, 8016–8034.
McGlinchey, T.A., Rafter, P.A., Regan, F., McMahon, G.P., 2008. A review of analytical methods for the determination of aminoglycoside and macrolide residues in food matrices. Analytica Chimica Acta 624, 1–15.
O'Keeffe, M., 2000. Residue Analysis in Food – Principles and Applications. Singapore: Harwood Academic Publishers, p. 307.
Reig, M., Toldrá, F., 2008a. Veterinary drug residues in meat: Concerns and rapid methods for detection. Meat Science 78, 60–67.
Reig, M., Toldrá, F., 2008b. Immunology-based techniques for the detection of veterinary drugs residues in foods. In: Toldrá, F. (Ed.), Meat Biotechnology. New York: Springer, pp. 361–373.
Reig, M., Toldrá, F., 2009. Veterinary drugs and growth promoters residues in meat and processed meats. In: Toldrá, F. (Ed.), Safety of Meat and Processed Meat. New York: Springer, pp. 365–390.
Reig, M., Toldrá, F., 2011. Growth promoters. In: Nollet, L.M.L., Toldrá, F. (Eds.), Safety Analysis of Foods of Animal Origin. Boca Raton, FL: CRC Press, pp. 229–247.
Stolker, A.A.M., Zuidema, T., Nielen, M.W.F., 2007. Residue analysis of veterinary drugs and growth-promoting agents. Trends in Analytical Chemistry 26, 967–979.
Toldrá, F., Reig, M., 2006. Methods for rapid detection of chemical and veterinary drug residues in animal foods. Trends in food science and technology 17, 482–489.
Verdon, E., 2008. Antibiotic residues in muscle tissues of edible animal products. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Meat Products Analysis. Boca Raton, Florida: CRC Press, pp. 856–947.

CHEMICAL AND PHYSICAL CHARACTERISTICS OF MEAT

Contents

Adipose Tissue

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Glossary

Conjugated linoleic acid (CLA) Fatty acids containing two double bonds, typically as 1,3-dienes with one double bond in the *cis*-configuration and one double bond in the *trans*-configuration, although some CLA isomers contain two *trans*-double bonds.

Monounsaturated fatty acids (MUFAs) Fatty acids, typically with the double bond in the delta-9 position in the *cis*-configuration.

Polyunsaturated fatty acids (PUFAs) Fatty acids containing at least two double bonds as 1,4-dienes with all double bonds in the *cis*-configuration.

Saturated fatty acids (SFAs) Fatty acids containing no double bonds, with relatively high melting points.

Stearoyl-CoA desaturase Delta-9 fatty acid desaturase that converts saturated fatty acids to monounsaturated fatty acids.

Thiazolidinediones A synthetic compounds that stimulate the differentiation and lipid filling of adipocytes.

Introduction

Fat (or lipid) contributes substantially to the caloric content of meat. This is especially true in the US, where total fat content can be as high as 30% in ground beef and 40% in some sausages. However, recent studies have indicated that ground beef produced from grain-fed cattle in the US is high in monounsaturated fatty acids (MUFAs) and actually may have important health benefits. It also has marked effects on mouthfeel and flavor of meat, which are primary components of palatability. For beef, the level of lipid in meat necessary to achieve consumer acceptance is between 3% and 7.5% (uncooked basis; Figure 1). The amount of fat and fatty acid composition both influence palatability. Additionally, the kinds of fatty acids present in meat and its cholesterol content influence the perceived healthfulness of meat. The location and composition of lipids within meat will be discussed, with particular emphasis on the adipose tissues associated with meat.

The composition of lipids in adipose tissues of meat varies in response to diet and the time at which the lipids are deposited. The fatty acid composition of pork is especially sensitive to dietary manipulation, whereas that of beef and lamb is affected primarily by the age of the animal and is modified by dietary means, particularly by feeding pastures as compared to grains. The cholesterol content of meat generally is resistant to dietary modification, except that any means of increasing the amount of intramuscular neutral lipids will ultimately cause small, incremental increases in cholesterol concentration.

Sources of Lipid in Meat

Although abdominal adipose tissues (kidney, pelvic, and heart fat; and omental fat) represent a large percentage of total body fat in livestock and poultry, they will not be included in this discussion because they are not associated with typical meat cuts. Furthermore, kidney, pelvic, and heart fat clearly originate as brown adipose tissue in sheep and cattle and, therefore, may represent a metabolically distinct adipose tissue depot.

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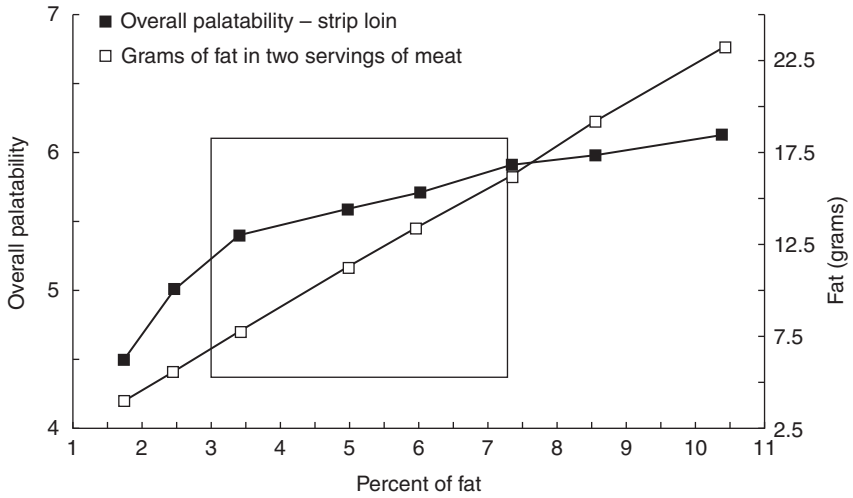


Figure 1 Window of acceptability for fat content of meat (palatability vs. grams of fat, two servings. The window is based on a fat content range of 3.0–7.5%, which is equivalent to the range for beef from the M. longissimus dorsi (12th–13th rib) that grades in the lower range of United States Department of Agriculture (USDA). Select (3.0–4.27% fat content) and that which grades in the high range of USDA Choice (4.28–8.0% fat content). Reproduced from Savell, J.W., Cross, H.R., 1988. The role of fat in the palatability of beef, pork, and lamb. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Research Council, pp. 345–355.

There are thus four sources of lipid in meat: the muscle fibers, subcutaneous adipose tissue, intermuscular (seam) adipose tissue between muscle groups, and intramuscular (interfascicular, marbling, and i.m.) adipose tissue (Figure 2; intermuscular adipose tissue is not indicated). Most whole cuts of beef in the marketplace now are closely trimmed of subcutaneous and intermuscular adipose tissue, but processed meat products such as ground beef and sausage can contain significant amounts of fat trim. Several countries, such as Japan and Korea, place a premium on soft beef fat, as do countries like Australia that export to these countries. In these countries, softness of the fat has a substantial impact on the value of beef. Fat softness in beef produced in Japan and Korea is achieved by an unusually high enrichment with MUFAs, whereas fat hardness in pasture-fed beef is the result of elevated levels of saturated fatty acids (SFAs). These issues will be addressed later.

Once subcutaneous and intermuscular fat have been removed, the primary contributor to the lipid content of meat is i.m. adipose tissue. Very lean beef, pork, or lamb, in which all subcutaneous and intermuscular adipose tissues have been removed, contain approximately 1% extractable lipid. At the other extreme, closely trimmed beef from Japanese Black (or Wagyu) cattle can contain more than 35% extractable lipid. This extraordinary concentration of lipid can be attributed solely to lipid in i.m. adipose tissue.

Intramuscular adipose tissue has several metabolic features that distinguish it from other depots. Intramuscular adipocytes typically display rates of fatty acid biosynthesis that are 5–10% of the rates observed in subcutaneous (s.c.) adipose tissue, and i.m. adipocytes are smaller (25–50 μm diameter) than s.c. adipocytes (100 μm to as much as 500 μm diameter) in beef and pork. Triacylglycerol biosynthesis from most fatty acids also occurs at lower rates in i.m. than in s.c. adipose tissue. However,

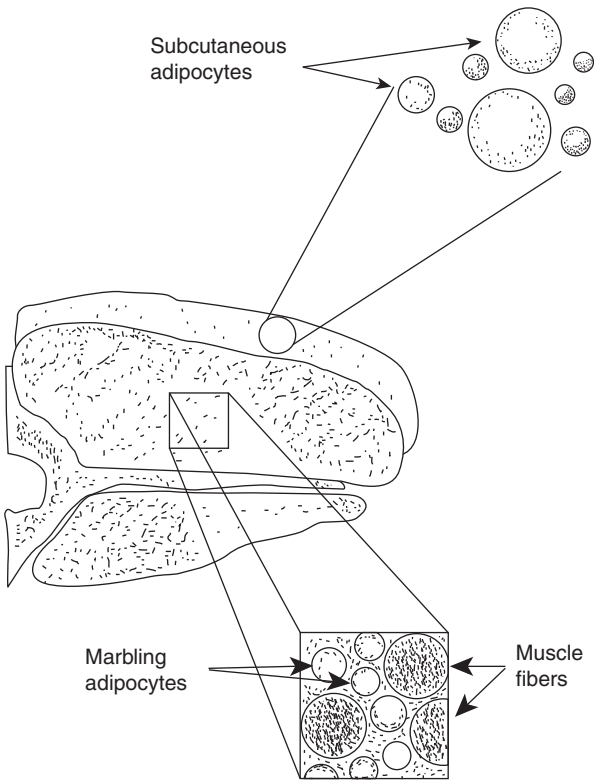


Figure 2 Sources of lipid in meat. Subcutaneous adipose tissue and intermuscular (seam) adipose tissue (not shown) contribute as much as 30% to the total lipid content of meat. Lipid associated with muscle fibers contributes approximately 1%, whereas intramuscular adipose tissue (marbling) can contribute from less than 1% to more than 35% of the lipid in meat in certain breeds of cattle.

i.m. adipose tissue incorporates palmitic acid into triacylglycerols at rates that exceed those in s.c. adipose tissue. Finally, glucose contributes a greater proportion of carbon to *de novo* fatty acid biosynthesis in intramuscular than in subcutaneous adipose tissue. Thus, the metabolic evidence indicates that intramuscular and subcutaneous adipose tissues are distinct.

Lipid Accumulation in Adipose Tissue

The accumulation of specific fatty acids in adipose tissue is regulated by the stage of differentiation of adipocytes. Preadipocytes first proliferate, followed by induction of the genes responsible for lipid filling. Thus, both the apparent number of adipocytes and the size of adipocytes increase with animal growth. In the case of intramuscular adipose tissue, adipocytes initially are visible as linear strings of small, lipid-filled cells embedded in perimyseal adipose tissue (Figure 3(a)). Adipocytes from cattle after 4 months of grazing on pasture do not enlarge measurably, nor does their apparent number increase (Figure 3(b)). In cattle after being fed a grain-based finishing diet, both the size and apparent number of adipocytes increase substantially (Figures 3(c) and (d)).

Initial lipid filling is characterized by the appearance of small lipid droplets (Figure 4). Although quite apparent in preadipocyte cell culture, this multilocular stage is difficult to detect *in situ* in growing animals. Lipid droplets coalesce as adipocytes enlarge, leading to the unilocular adipocytes characteristic of mature adipose tissue. There is evidence to indicate that preadipocytes within tissue beds of livestock species can proliferate even after accumulation of some lipid. The result is that in meat-bearing animals both processes – proliferation and lipid filling – are occurring concurrently.

Fatty Acid Composition of Subcutaneous, Intramuscular, and Muscle Lipids

Differentiation of preadipocytes is characterized by the expression of genes, such as those encoding stearoyl-coenzyme A desaturase (SCD, a delta 9 desaturase), and enzymes that support *de novo* fatty acid biosynthesis. Early in their differentiation, lipid-filling preadipocytes typically contain high concentrations of SFAs such as palmitic acid (16:0) and stearic acid (18:0), and concomitantly low concentrations of their delta 9 desaturase products, palmitoleic acid (16:1n-7) and oleic acid (18:1n-9). Treatment of bovine preadipocytes with insulin and thiazolidenediones, such as pioglitizone, markedly stimulate SCD differentiation (Figure 5(a)), which promotes lipid filling and increases the concentration of MUFAs in the cells. This can be depressed by concurrently treating the preadipocytes with *trans*-10, *cis*-12 CLA, which strongly inhibits SCD gene expression (Figure 5(b)).

Palmitic acid is produced by the combined activities of acetyl-coenzyme A carboxylase and fatty acid synthase, whereas stearic acid is produced by the addition of two carbons to palmitic acid via fatty acid elongase. Palmitoleic and

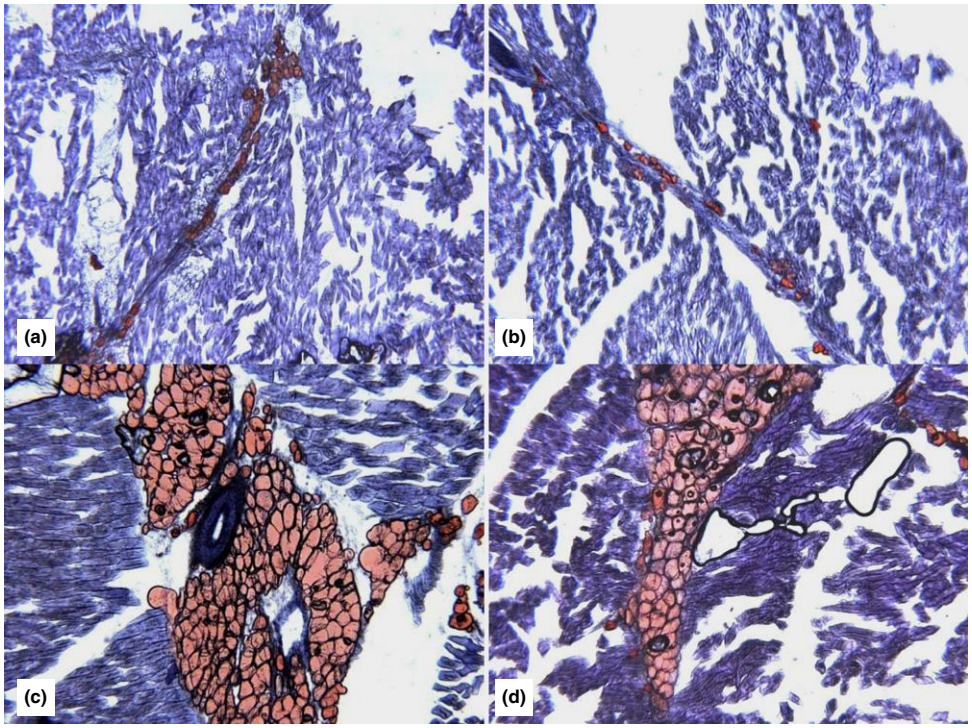


Figure 3 Intramuscular adipocyte development in M. longissimus thoracis of Angus steers. Cells were stained with Oil Red O to stain adipocytes and hematoxylin solution to stain for nuclei. (a) Sample taken at weaning (8 mo of age). (b) Sample taken after 4 mo on native Texas pasture (12 mo of age). (c) Sample taken in steers fed a corn-based, grain finishing diet for 4 mo after weaning (12 mo of age). (d) Sample taken in steers fed a corn-based, grain finishing diet for 4 mo after grazing native pasture for 4 mo (16 mo of age). Intramuscular adipocytes are visible as thin threads of adipocytes (a and b) or as clusters of roughly spherical adipocytes (c and d). Magnification approximately ×100.

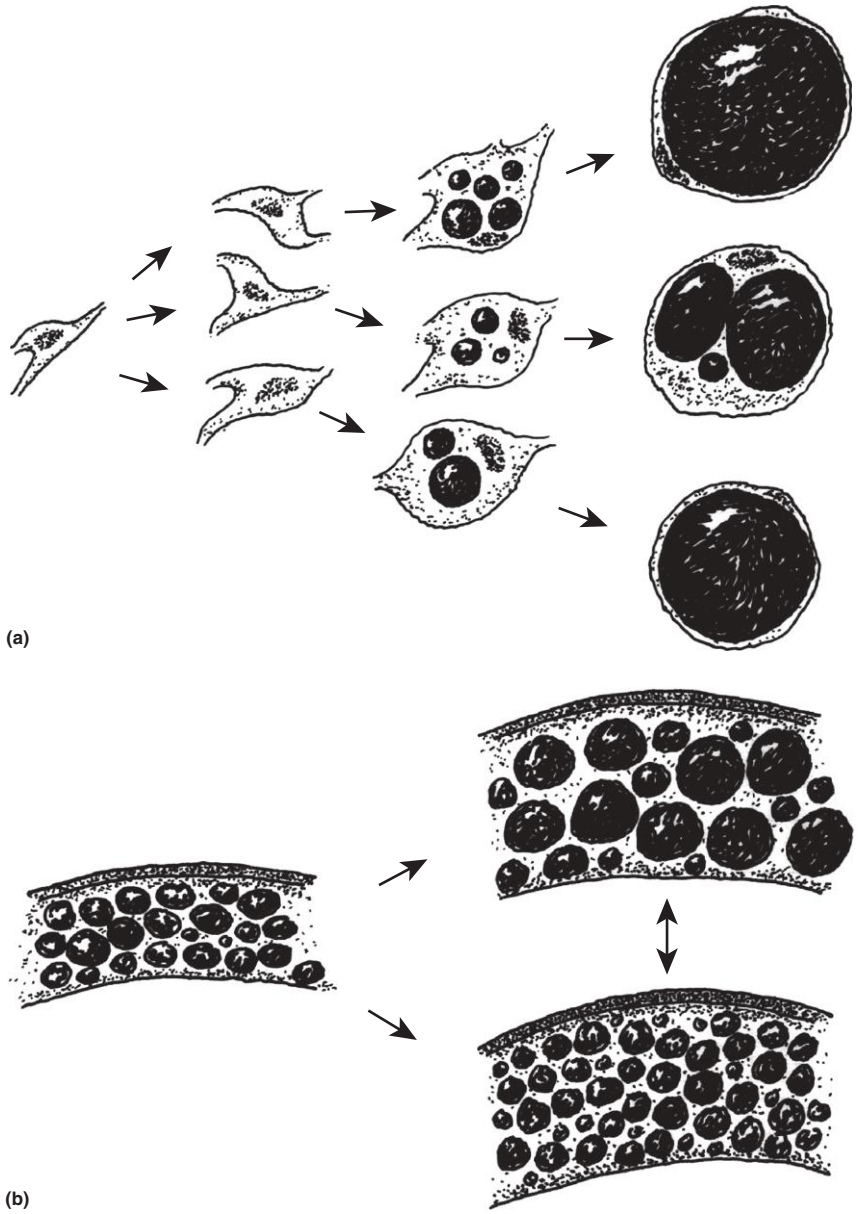


Figure 4 (a) Accumulation of lipid during preadipocyte differentiation and postnatal growth. After a series of proliferative divisions, preadipocytes begin to accumulate lipid (i.e., differentiate). In most species, this lipid arises primarily from *de novo* fatty acid biosynthesis, so it is composed primarily of palmitic, palmitoleic, stearic, and oleic fatty acids. (b) Postnatally, depots such as subcutaneous adipose tissue accumulate lipid in response to hypertrophy of adipocytes (upper right portion of figure), or by preadipocyte proliferation (lower right portion of figure), or by a combination of both processes (indicated by the double-headed arrow). The fatty acid composition of these adipocytes will be determined by *de novo* synthesis and by the composition of dietary lipids. This is especially true for monogastrics such as pigs.

oleic acid are the products of the delta 9 desaturation of palmitic acid and stearic acid, respectively, catalyzed by SCD. The activities of acetyl-coenzyme A carboxylase, fatty acid synthase, fatty acid elongase, and SCD persist at varying levels in adipose tissues throughout the life of the animal. For this reason, palmitic, palmitoleic, stearic, and oleic acids are always present in adipose tissues of animals. Relative concentrations of each are dictated primarily by the activity of SCD and availability of unsaturated fatty acids in the diet. The unsaturated fatty acids typically consumed by livestock species include oleic acid and the polyunsaturated fatty acids linoleic acid (18:2n-6) and α-linolenic acid (18:2n-3).

The MUFA:SFA ratio increases with time on a grain-based finishing diet in subcutaneous adipose tissue of postweaning calves (Figure 6). The MUFA:SFA ratio does not increase in calves grazing pasture, although there is a sharp increase in the concentration of MUFA in subcutaneous adipose tissue once the cattle are adapted to a grain-based diet. The net

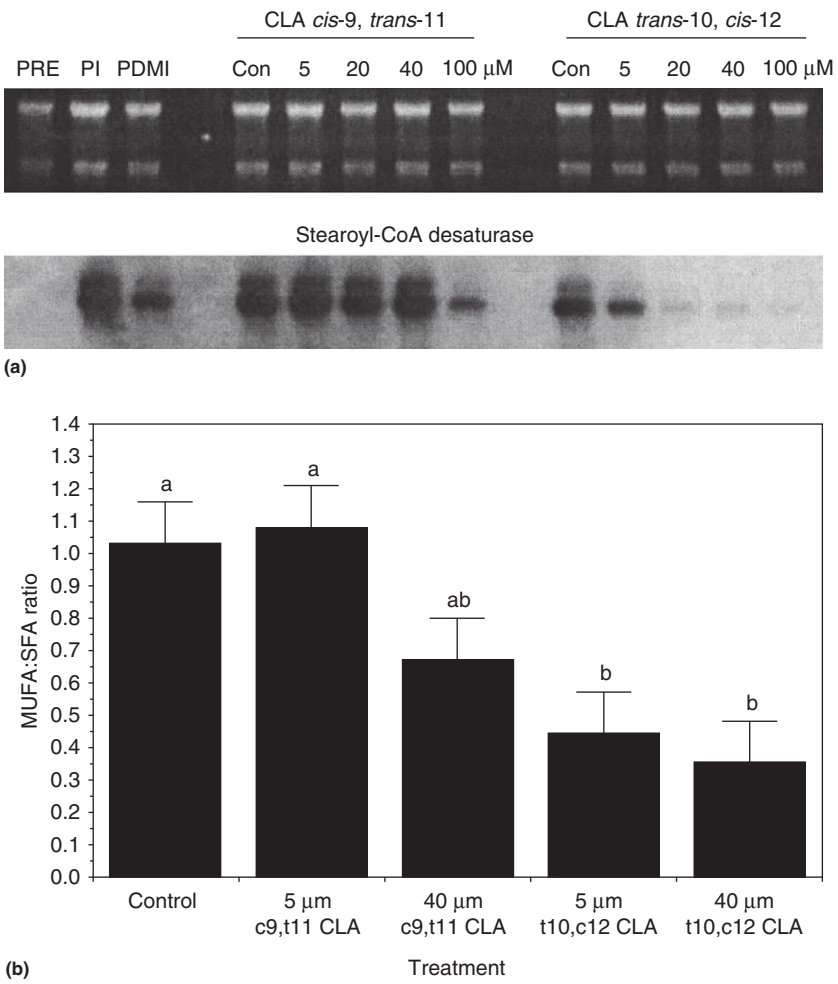


Figure 5 SCD gene expression and MUFA in bovine preadipocytes. (a) SCD gene expression in preconfluent bovine preadipocytes (PRE); in preadipocytes incubated with pioglitizone, insulin and holo-transferrin (PIM); in preadipocytes incubated with PIM plus dexamethasone (PDMI). Preadipocytes were cultured for 7 days PIM or PDMI, followed by 3 days of treatment with *cis*-9, *trans*-11 CLA or with *trans*-10, *cis*-12 CLA. (b) MUFA:SFA ratio of lipids from control preadipocytes and preadipocytes treated with 5 or 40 μM *cis*-9, *trans*-11 CLA or 5 or 40 μM *trans*-10, *cis*-12 CLA, MUFA = 16:1+18:1n-9+*cis*-9, *trans*-11 CLA; SFA = 14:0+16:0+18:0+18:1*trans*-11. Lipids were extracted from 7-day differentiated preadipocytes, followed by 3 days of treatment with the CLA isomers. [ab]Means with common superscripts are not different ($P > .05$).

accumulation of MUFAs apparently persists throughout the lifetime of livestock species in the US.

As seen for i.m. adipose tissue development, the MUFA:SFA ratio does not increase in pasture-fed cattle (Figure 6) and may actually be depressed by pasture feeding. Saturated fatty acids, especially stearic acid, accumulate in adipose tissues of pasture-fed cattle. Once the cattle begin consuming a grain-based diet, the MUFA:SFA ratio increases, although i.m. adipose tissue remains relatively higher in saturated fatty acids than s.c. adipose tissue. This may indicate that i.m. adipose tissue remains less differentiated than s.c. adipose tissue throughout production.

Lipid Accumulation in Meat

Fatty acids in adipose tissue are stored primarily as triacylglycerols (Figure 7), which coalesce in the large lipid vacuoles that are the central feature of adipocytes. The longissimus muscle of cattle produced in the US can accumulate at most approximately 11% i.m. lipid, primarily as triacylglycerols. For the US grading system, this represents carcasses that grade Prime, with Moderately Abundant marbling scores (Figure 8). Thus, i.m. adipocytes would provide the greatest amount of fatty acids in meat.

The most abundant fatty acids in i.m. adipose tissue of beef and pork are palmitic, stearic, oleic, and linoleic acids (Figures 9 and 10). As indicated previously, the first three of these fatty acids are derived from endogenous synthesis of fatty acids, whereas linoleic acid is derived from plant materials included in the diet.

The contribution of both *de novo* fatty acid synthesis and fatty acid desaturation to the fatty acid composition of adipose tissues is especially apparent in bovine adipose tissues (Figure 9). Stearic acid constitutes more than 80% of the fatty acids available for absorption from the duodenum in

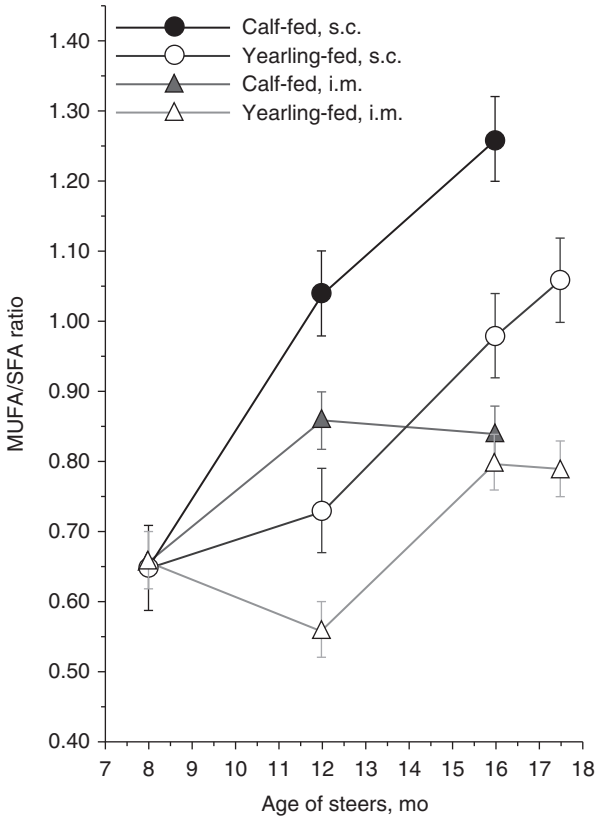


Figure 6 Changes in the MUFA:SFA ratio over time in calf-fed (closed symbols) and yearling-fed (open symbols) Angus steers. Steers were weaned at 8 mo of age. The CF steers were fed a corn-based, grain finishing diet for 4- or 8 mo after weaning. The YF steers grazed pasture until 12 mo of age, and then were fed the same corn-based, grain finishing diet for 4- or 5.5 mo of age, until they reached the same body weight as the calf-fed steers. At each sampling time (including 8 mo of age, immediately postweaning), fatty acids were measured in samples of intramuscular (i.m., triangles) and subcutaneous (s.c., circles) adipose tissue, at taken at the 5th–8th thoracic rib. Reproduced from Smith, S.B., Kawachi, H., Choi, C.B., *et al.*, 2009. Cellular regulation of bovine intramuscular adipose tissue development and composition. Journal of Animal Science 87(E. supplement), E72–E82, doi:10.2537/jas2008-1340.

beef cattle, yet the most abundant fatty acids in bovine adipose tissues are palmitic acid (the product of *de novo* synthesis) and oleic acid (from desaturation of stearic acid).

Typically, abdominal adipose tissue is more highly saturated than s.c. or i.m. adipose tissue. Perirenal adipose tissue develops very early in fetal life, and by the time cattle reach slaughter weight the perirenal adipose tissue is quite low in metabolic activity. In beef, i.m. lipid is also higher in stearic acid than is s.c. adipose tissue (Figure 9). In this respect, the smaller, less mature adipocytes of i.m. adipose tissue resemble preadipocytes of secondary cell lines. Unlike the situation for beef, i.m. lipid from pork contains less stearic acid than does subcutaneous adipose tissue (Figure 10).

As monogastrics, swine have adipose tissues with fatty acid composition that more closely resembles that of dietary fatty acids. Thus, diets enriched with oleic or linoleic acid will cause

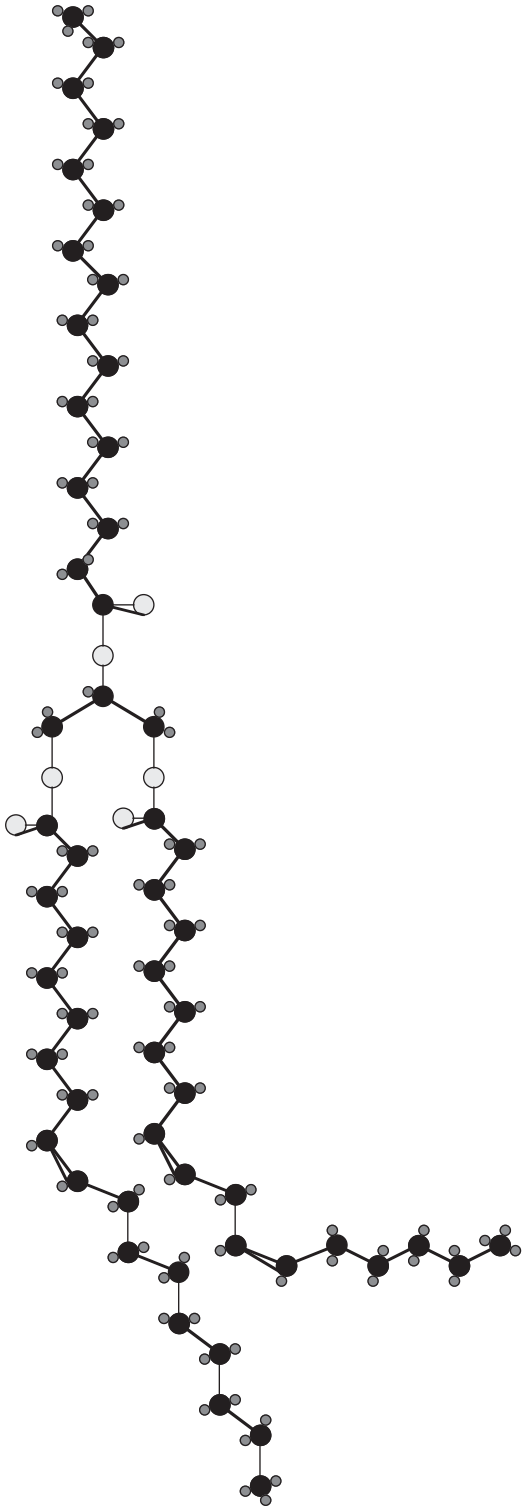


Figure 7 Typical structure of a triacylglycerol: *sn*-1 fatty acid (on left), oleic acid; *sn*-2 fatty acid, palmitic acid; *sn*-3 fatty acid, linoleic acid. This triacylglycerol would be common in porcine adipose tissue. In bovine and ovine adipose tissue, palmitic acid would occupy the *sn*-1 position and stearic or oleic acid would occupy the *sn*-2 position. Large filled circles represent carbon; large shaded circles represent oxygen; small shaded circles represent hydrogen. Reproduced from Savell, J.W., Cross, H.R., 1988. The role of fat in the palatability of beef, pork, and lamb. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Research Council, pp. 345–355.

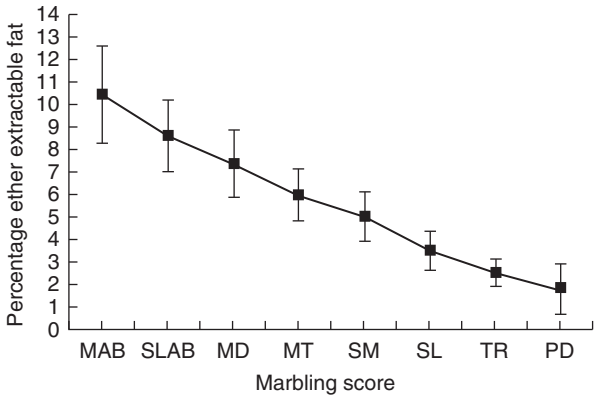


Figure 8 Marbling score and ether extractable fat. MAB, moderately abundant; MD, moderate; MT, modest; PD, practically devoid; SL, slight; SLAB, slightly abundant; SM, small; TR, traces. Reproduced with permission from Savell, J.W., Cross, H.R., 1988. The role of fat in the palatability of beef, pork, and lamb. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Research Council, pp. 345–355.

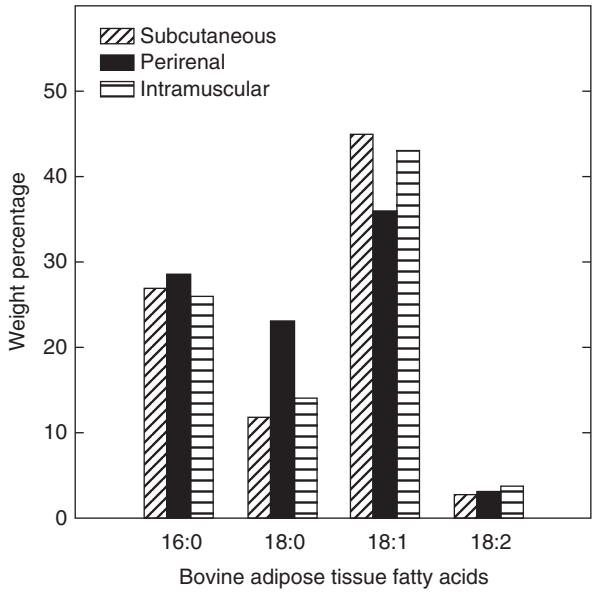


Figure 9 Fatty acid composition of bovine adipose tissues. Reproduced from Rule, D.C., Smith, S.B., Romans, J.R., 1995. Fatty acid composition of muscle and adipose tissue of meat animals. In: Smith, S.B., Smith, D.R. (Eds.), Biology of Fat in Meat Animals: Current Advances. Savoy, IL: American Society of Animal Science, pp. 144–165.

the lipids associated with pork to become similarly enriched with these fatty acids. Elevated unsaturated fatty acids do not have a major effect on the sensory properties of fresh pork. However, polyunsaturated fatty acids are especially susceptible to oxidation during long-term storage of pork or pork products. This results in the formation of undesirable aldehydes and ketones, rendering the meat less acceptable. Furthermore, feeding pigs diets enriched with polyunsaturated fatty acids causes the carcasses to be oily and may lead to production problems such as soft bellies. Enrichment of carcass lean and

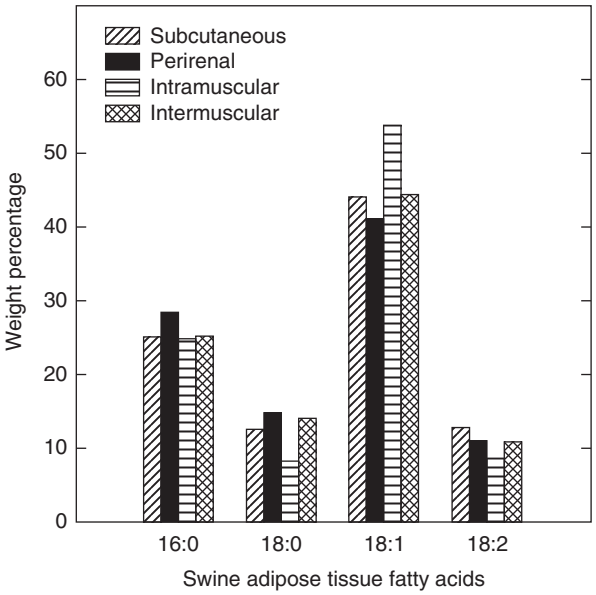


Figure 10 Fatty acid composition of porcine adipose tissues. Reproduced from Rule, D.C., Smith, S.B., Romans, J.R., 1995. Fatty acid composition of muscle and adipose tissue of meat animals. In: Smith, S.B., Smith, D.R. (Eds.), Biology of Fat in Meat Animals: Current Advances. Savoy, IL: American Society of Animal Science, pp. 144–165.

adipose tissues with oleic acid, which is a solid at typical freezer temperatures, does not appear to accelerate lipid oxidation or cause excessively soft bellies.

In contrast to pork, the fatty acid composition of beef is relatively resistant to dietary modification. Small increases in oleic acid have been demonstrated for cattle fed high-oleic acid sunflower seed. Similarly, adipose tissues and muscles of grass-fed cattle typically contain elevated concentrations of linoleic and α-linolenic acid. These increases are modest; even in grass-fed cattle, linoleic and α-linolenic acid concentrations rarely exceed 4% and 2%, respectively, of total fatty acids. Interestingly, the meat of range-fed bison has been reported to contain nearly 8% linoleic acid and nearly 3% α-linolenic acid. Also, beef from cattle fed in Australia can contain unusually high concentrations of stearic acid, although the biochemical basis for this is unknown.

Conjugated Linoleic Acid

Tissues of ruminants fed pasture grasses also are relatively enriched in by-products of ruminal biohydrogenation of linoleic and α-linolenic acid, vaccenic acid (*trans*-11), elaidic acid (*trans*-9), and a group of conjugated dienes collectively known as CLA. The most abundant of these are *cis*-9, *trans*-11 CLA (18:2*c*9,*t*11) and *trans*-10, *cis*-12 CLA (18:2*t*10,*c*12).

In addition to ruminal synthesis, 18:2*c*9,*t*11 may also be derived from desaturation of vaccenic acid in intestinal mucosa or within the muscle fibers or adipocytes. Similar to the synthesis of oleic acid from stearic acid, a *cis* double bond is inserted between carbons 9 and 10 of vaccenic acid by SCD. This

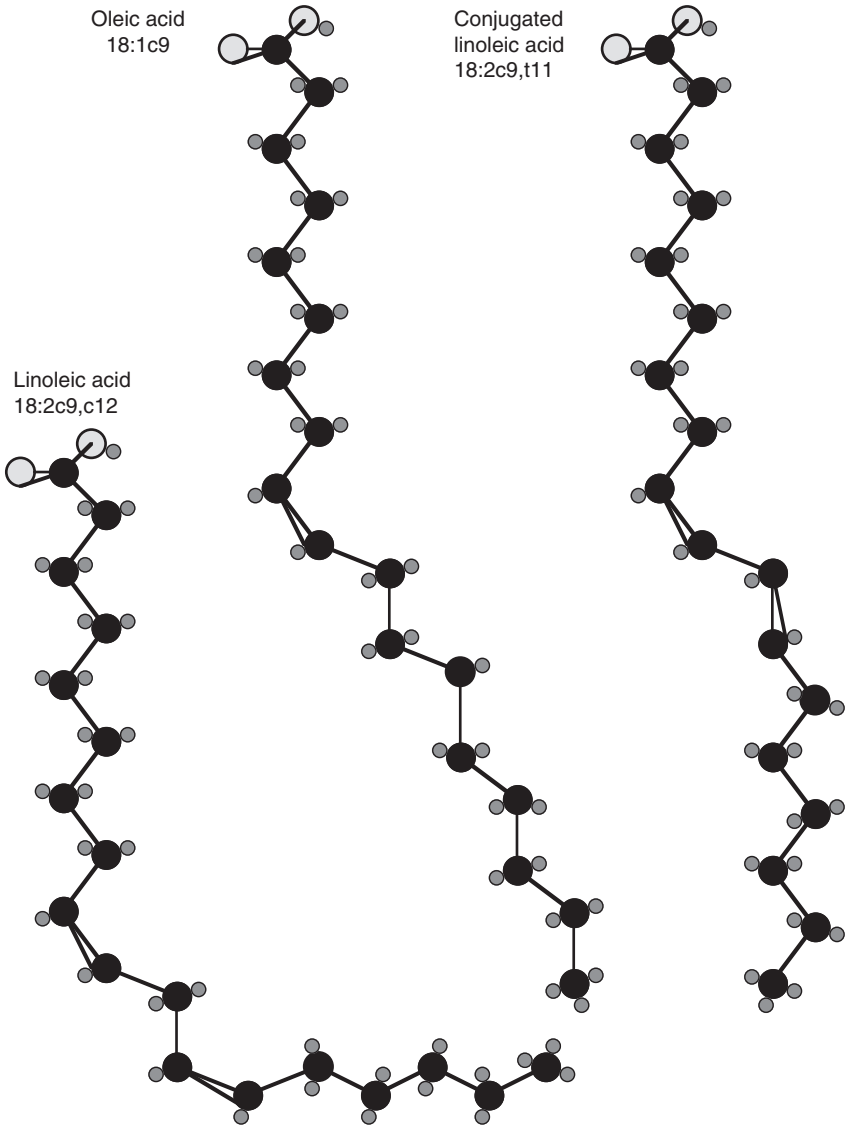


Figure 11 Structures of linoleic acid, oleic acid, and the *cis*-9, *trans*-11 isomer of CLA. Large filled circles represent carbon; large shaded circles represent oxygen; small shaded circles represent hydrogen.

results in a conjugated diene (Figure 11). Because of the *trans*-double bond between carbons 11 and 12, 18:2*c*9,*t*11 has a more linear configuration than linoleic acid. For this reason, free fatty acids or triacylglycerols of CLA have melting points that are similar to that of oleic acid and well above that of linoleic acid.

Although ruminant species produce and deposit CLA isomers in their tissues, the concentration of total CLA isomers rarely exceeds 2% of the total lipid. Attempts to increase CLA by dietary means (by feeding pasture grasses or flax seed) have caused the accumulation of vaccenic acid (produced from linoleic acid), with very little effect on the concentration of 18:2*c*9,*t*11. This indicates that SCD activity, either in the intestinal mucosal cells or muscle or adipose tissue, limits the rate of conversion of vaccenic acid to 18:2*c*9,*t*11.

Several investigators have demonstrated that porcine tissues can be enriched substantially by feeding mixtures of CLA isomers. Total concentrations of CLA isomers in pigs fed diets containing up to 3% CLA (as free fatty acids) are approximately 6% of the total lipid in the tissues. There appears to be no preference for the CLA isomers in porcine muscle or adipose tissue. Thus, 18:2*c*9,*t*11 and 18:2*t*10,*c*12 are enriched to the same degree in neutral lipids and cell membranes. However, porcine muscle and adipose tissue endoplasmic reticulum are enriched to less than 50% of the levels seen in other subcellular compartments. This indicates that there is greater specificity for the fatty acids that make up the endoplasmic reticulum, or that the fatty acids in this compartment turn over more slowly and thus are more difficult to modify by dietary means.

A primary effect of feeding CLA to pigs is to raise the melting point of the lipids of pork muscle and adipose tissue. This effect appears to be caused by the higher concentration of stearic acid typically observed in tissues of CLA-fed pigs. The amount of SCD mRNA is depressed in preadipocyte cell lines treated with CLA, and SCD enzyme activity is reduced in adipose tissue of pigs fed CLA. This has the beneficial effect of

producing pig carcasses with relatively hard bellies, which should improve ease of fabrication. However, the reduction in SCD activity caused by CLA concomitantly decreases the concentration of oleic acid, which is considered a healthful fatty acid.

Desaturation of Fatty Acids

Although ruminal hydrogenation of unsaturated fatty acids makes it difficult to enrich beef with unsaturated fatty acids, desaturation of fatty acids available for digestion in the duodenum ensures that plasma, muscle, and adipose tissue are not overly enriched with saturated fatty acids, especially stearic acid. The activity of SCD has been demonstrated in liver, muscle, s.c. adipose tissue, and intestinal mucosa of pigs and cattle (Figure 12). By far, the greatest SCD activity is located within adipose tissue, indicating its importance in the overall process of determining the fatty acid composition (and hence fluidity) of adipose tissue. However, substantial activity is also observed in intestinal mucosal cells, where it may function to modify the composition of chylomicrons and very-low-density lipoproteins produced within the mucosal cells. In this manner, SCD activity within mucosal cells would largely influence the composition of fatty acids available for deposition in other tissues.

Contribution of Intramuscular Adipose Tissue to the Concentration of Cholesterol in Meat

Cholesterol in meat exists in two forms: as free cholesterol and cholesterol ester. Free cholesterol is associated primarily with cellular and subcellular membranes of muscle and i.m. adipocytes. Because i.m. adipocytes are essentially lipid-filled spheres with very little membrane content (Figure 13), the amount of cholesterol associated with membranes is small (approximately 25% of total cholesterol in i.m. adipose tissue). Cholesterol ester, located within the triacylglycerol-rich central lipid vacuole, comprises approximately 75% of the total cholesterol in adipose tissue. Muscle fibers, which are rich in membranes but contain comparatively little lipid, have approximately 75% of their total cholesterol associated with membranes and the other 25% associated with their neutral lipids.

The concentration of cholesterol in lean, closely trimmed beef has been reported to be 45–65 mg per 100 g of muscle (wet weight). The variation may be due to methodology but is probably caused by varying amounts of neutral lipids contained within the muscle fibers themselves. Intramuscular adipose tissues contain approximately 115 mg of cholesterol per 100 g of i.m. adipose tissue or 1.15 mg cholesterol for every 1 g increase in total i.m. lipid content of meat. Several investigators have reported that increasing the amount of i.m. adipose tissue had no significant effect on the cholesterol content of meat. One study indicated that USDA Prime steaks (11% total lipid) contained 64 mg cholesterol per serving (100 g; approximately 3.5 ounces of uncooked beef). USDA Select steaks (2% total lipid) contained 61 mg cholesterol per serving. These values were not significantly different.

It seems inconsistent that increasing the amount of a relatively cholesterol-enriched source (i.m. adipose tissue) fails to increase total cholesterol in meat. There are two reasons for the minimal effect of i.m. adipose tissue on the concentration of cholesterol in beef. (1) Each gram of i.m.

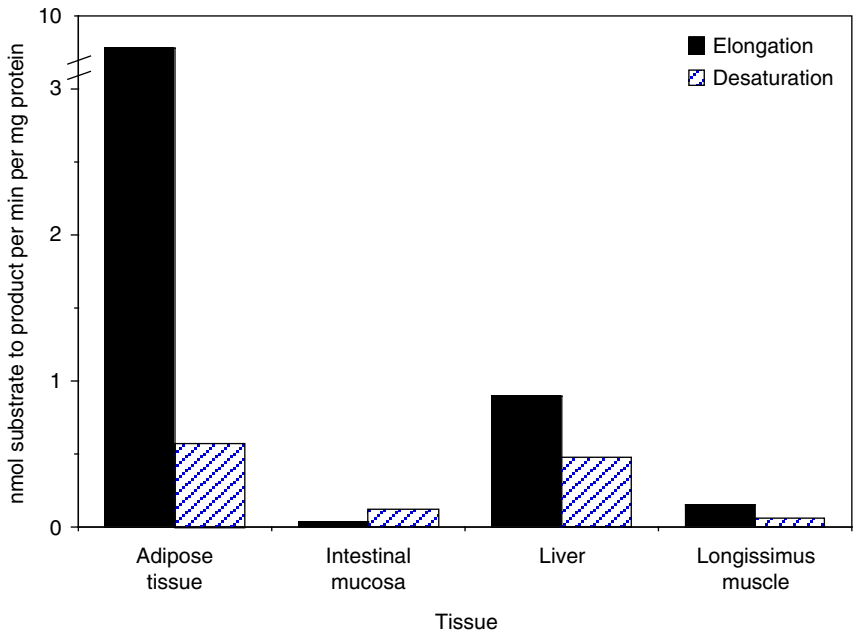


Figure 12 Fatty acid elongation and desaturation (SCD) enzyme activities in bovine tissues. Similar activities for SCD in adipose tissue, intestinal mucosa, liver and longissimus muscle have been demonstrated in porcine tissues. Reproduced from Smith, S.B., 1995. Substrate utilization in ruminant adipose tissues. In: Smith, S.B., Smith, D.R. (Eds.), Biology of Fat in Meat Animals: Current Advances. Savoy, IL: American Society of Animal Science, pp. 166–188.

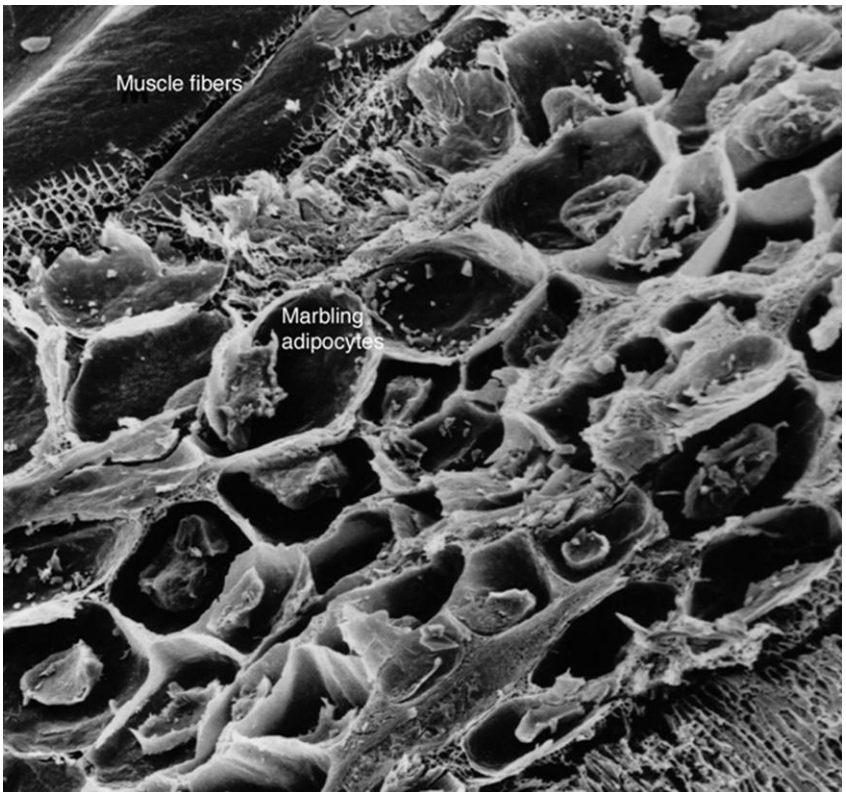


Figure 13 Distribution of marbling adipocytes within bovine longissimus muscle. Muscle fibers are oriented diagonally in this scanning electron micrograph. Reticular connective-tissue fibers connect the muscle fibers to the seam of marbling adipose tissue. Marbling adipocytes were dilapidated during the fixation process, and their upper surfaces were removed during sectioning. Diameters of the adipocytes are 10–50 μm.

adipose tissue contributes only 1.15 mg of cholesterol, which is below the level of sensitivity for some methods of cholesterol analysis. (2) Any increase in i.m. adipose tissue replaces an equal volume of muscle, which contributes approximately 0.65 mg of cholesterol per gram. Thus, any contribution of i.m. adipose tissue to total cholesterol is diluted by the amount of muscle it displaces. For this reason, Prime and Select cuts of meat may vary substantially in the number of calories from fat but will differ only slightly in their cholesterol content.

Fatty Acid Composition and Melting Point of Lipids

Several studies have described differences in fatty acid composition of adipose tissue and muscle across breed types of pigs, sheep, and cattle. In general, those breed types with larger adipocytes (s.c. or i.m.) have more saturated fatty acids in their tissues than those with smaller adipocytes. For example, s.c. adipose tissue from mature Hereford cows contained more lipid and saturated fatty acids than that from mature Brahman cows.

Another example of the relationship between adipocyte size and saturated fatty acid content is the Japanese Black. Japanese Black cattle were derived from Korean Hanwoo cattle but, unlike the Korean Hanwoo cattle, Japanese Black cattle were crossbred with Continental Europe and British dairy and beef breeds during the nineteenth century. Japanese Black cattle and the closely related American Wagyu cattle exhibit an unusual ability to accumulate i.m. adipose tissue. On the Japanese grading system, cattle raised in the US cannot achieve a Beef Marbling Standard (BMS) score greater than 5, whereas the best Japanese Black cattle achieve BMS scores of 12. The

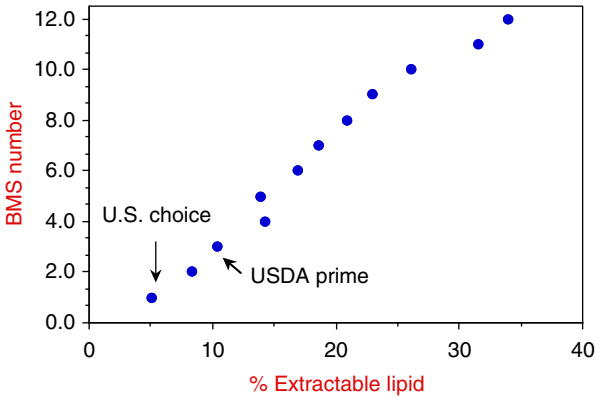
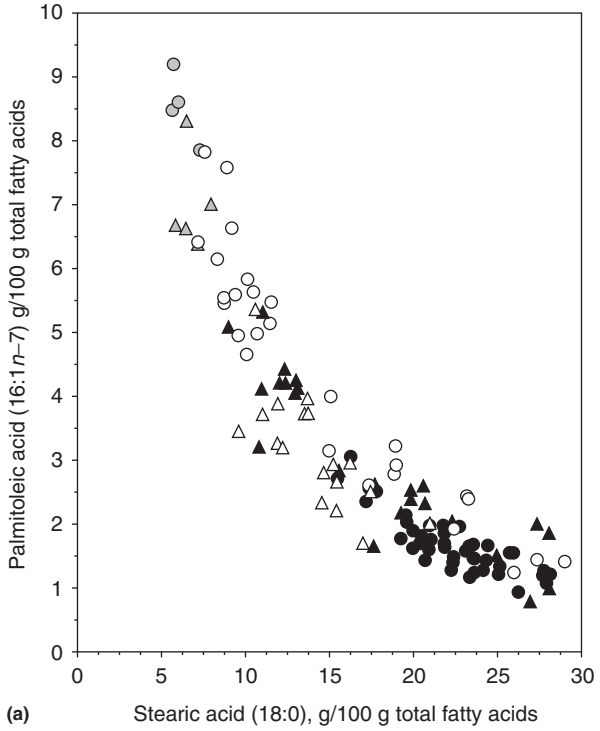
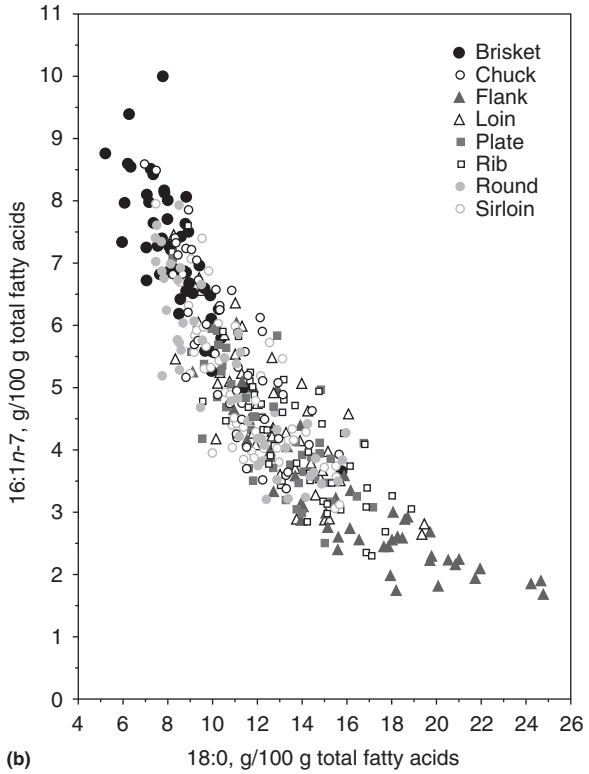


Figure 14 Japanese BMS as a function of extractable lipid in the 6th–7th ribs of the M. longissimus thoracis of purebred Japanese Black and American Wagyu cattle. Relative locations are indicated for USDA Choice and USDA Prime, adjusted for lipid concentrations at the 6th rib. Adapted from Cameron, P.J., Zembayashi, M., Lunt, D.K., *et al.*, 1994. Relationship between Japanese BMS and intramuscular lipid in the M. longissimus thoracis of Japanese Black and American Wagyu Cattle. Meat Science 38, 361–364. © Elsevier Science.

relationship of BMS number to extractable (primarily i.m.) lipid is illustrated in Figure 14. USDA Choice cattle occupy the very lowest portion of this curve, whereas the highest-grading Japanese Black cattle (with A5 carcasses) occupy the upper portion.





In spite of the great amount of i.m. lipid in beef from Japanese Black cattle, the i.m. adipocytes of Japanese Black steers are smaller (53 μm) than Angus i.m. adipocytes (61 μm). Intramuscular adipose tissue from Japanese Black cattle also contains more MUFA (57% of total fatty acids) than i.m. adipose tissue of Angus steers (50% of total fatty acids), even when raised under identical conditions. There is greater SCD activity in adipose tissues from Japanese Black cattle than in other breed types raised in the US, which would account for the greater concentration of MUFA in adipose tissues of Japanese Black cattle.

Studies that have included direct comparisons of American Wagyu cattle and other breed types have provided strong evidence for the genetic regulation of i.m. development and composition. Even when raised to the same physiological maturity under identical production conditions, American Wagyu steers accumulate more i.m. adipose tissue that is higher in MUFA than even those breed types that marble well (such as Angus cattle). Additionally, certain strains of Japanese Black cattle, such as those from the Hyogo Prefecture in Japan, can achieve BMS scores of 12 and their i.m. adipose tissue can contain more than 70% MUFA.

The contrast in fatty acid desaturation between Japanese cattle and cattle produced in other developed countries is best illustrated by comparing the concentrations of palmitoleic and stearic acid, as first proposed by Dr. Ron Tume, Commonwealth Scientific and Industrial Research Organization (CSIRO) Australia. Subcutaneous adipose tissue lipids from Japanese Black or American Wagyu cattle typically contain more than 6% palmitoleic acid and less than 7% stearic acid, indicating elevated SCD activity (Figure 15(a)). Conversely, s.c. adipose tissue lipids from young, Angus steers contain 1–2% palmitoleic acid and 20–30% stearic acid.

Interestingly, similar differences are observed across adipose tissues within the same carcasses (Figure 15(b)). Thus, brisket adipose tissue contains more than 6% palmitoleic acid and less than 10% stearic acid, whereas s.c. adipose tissue overlying the flank contains less than 3% palmitoleic acid and more than 15% stearic acid. The highly consistent, negative relationship between palmitoleic acid and stearic acid demonstrated in several studies indicates that the concentrations of these fatty acids are coordinately controlled by SCD.

Figure 15 Palmitoleic acid (16:1*n*-7) plotted as a function of stearic acid (18:0) in subcutaneous adipose tissue lipids. (a) Lipids from American Wagyu steers (shaded circles), Japanese Black steers (shaded triangles), mature Angus steers (open circles), young Angus steers (filled circles), Brangus steers (open triangles), and cattle raised in Australia (filled triangles). Subcutaneous adipose tissue lipids from American Wagyu and Japanese Black cattle contained the most palmitoleic acid and least stearic acid. Reproduced from Smith, S.B., Lunt, D.K., Chung, K.Y., *et al.*, 2006. Adiposity, fatty acid composition, and delta 9 desaturase activity during growth in beef cattle. Animal Science Journal 77, 478–486. (b) Lipids from eight subcutaneous adipose tissue depots, taken from 50 carcasses of unknown background. Lipids from brisket adipose tissue contained the greatest concentration of palmitoleic acid and least stearic acid, whereas lipids from the flank contained the least palmitoleic acid and the most stearic acid. Reproduced from Turk, S. N., Smith, S.B., 2009 Carcass fatty acid mapping. Meat Science 81, 658–663.

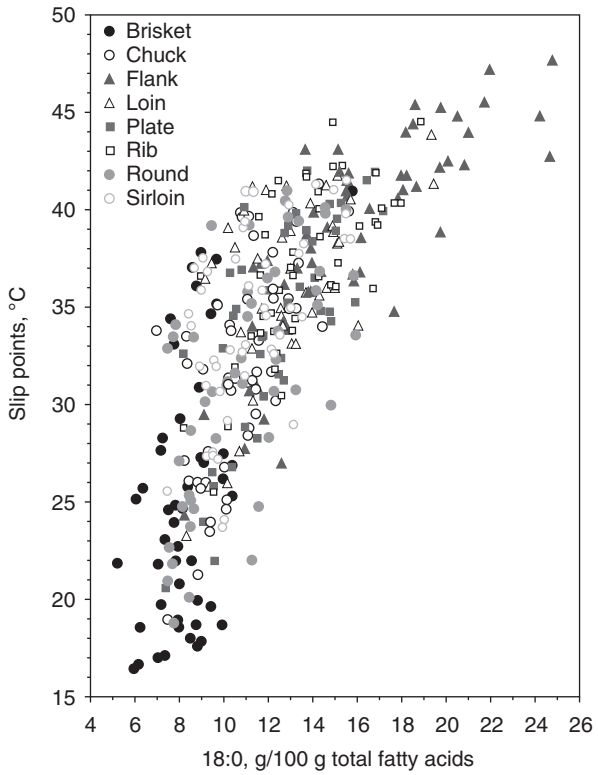


Figure 16 Lipid melting point (slip point) plotted as a function of stearic acid (18:0) in lipids from eight subcutaneous adipose tissue depots. Lipids from brisket adipose tissue had melting points less than 17 °C, whereas lipids from the flank had melting points greater than 45 °C. Reproduced from Turk, S.N., Smith, S.B., 2009. Carcass fatty acid mapping. Meat Science 81, 658–663.

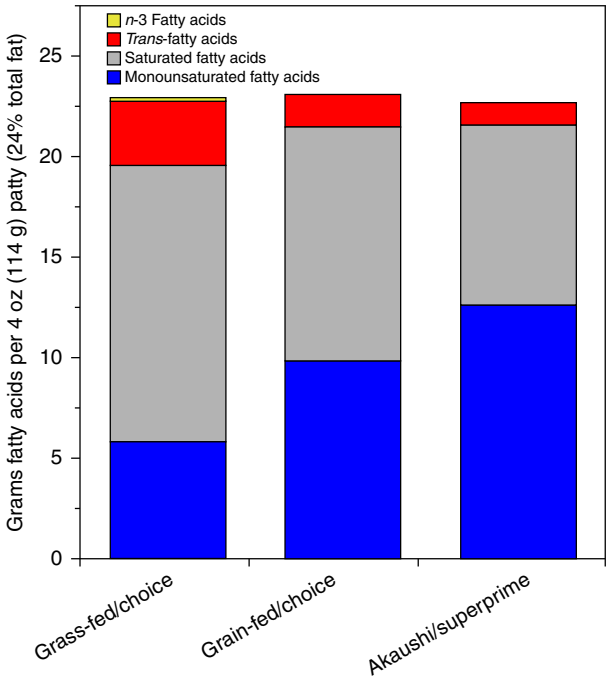


Figure 17 Total grams of fatty acids in one 4 oz (114 g) ground beef patty containing 24% total fat. Grass-fed/Choice, ground beef from Angus steers fed to USDA Choice on native Texas pastures, with no access to grains; Grain-fed/Choice, conventional ground beef purchased from a local retail outlet; Akaushi/Superprime, ground beef from Akaushi (red Japanese cattle) raised by Japanese production methods. *trans*-fatty acids include all 18:1 *trans*-isomers; saturated fatty acids include 14:0, 16:0, 18:0 and 20:0; monounsaturated fatty acids include 14:1n-5, 16:1n-7, 18:1n-7 and 18:1n-9. The only n-3 fatty acid that was quantifiable in the ground beef was α-linolenic acid.

The concentration of stearic acid in adipose tissue lipids is the primary determinant of lipid melting points (Figure 16), and even lipids extracted from adipose tissues of the same carcasses can exhibit a remarkable range of melting points. These differences in lipid melting point can have major practical importance. Pork carcasses enriched with unsaturated fatty acids may suffer from undue oiliness and belly softness, because the melting point of their tissue lipids is below 30 °C. Conversely, for those countries where carcasses are boned by hand, excessive fat hardness increases time and effort required for carcass fabrication. Perception of juiciness and mouthfeel also may be influenced by the melting point of lipids within meat, and depressing the concentration of MUFA in meat may reduce its healthfulness.

It is clear that breed type, production diet, stage of adipose tissue development, and adipose tissue depot all profoundly affect adipose tissue composition. Each one of these factors could have a significant impact on the healthfulness of beef, particularly ground beef. At least 40% of the 30 kg per year per capita beef consumption in the US is consumed as ground beef, and more than 31% of ground beef consumed in the US contains 22–30% fat, whereas the next 35% consumed contains 16–22% fat. Approximately 100 g of fat is consumed daily in the US, and ground beef, therefore, makes a significant contribution to total fat intake. Because pasture feeding of cattle depresses adipose tissue development and, concomitantly, SCD activity, a 4 oz (114 g, 24% total fat) ground beef patty from pasture-fed cattle can contain 2 g more *trans*-fatty acids and 2 g more saturated fatty acids, but only 60 mg more n-3 fatty acids, than conventionally produced ground beef (typically from grain-fed cattle in the US; Figure 17). The melting point of s.c. adipose tissue lipids of pasture-fed cattle can exceed 45 °C. Ground beef from cattle with a high genetic capacity to accumulate i.m. adipose tissue that is high in MUFA has 0.5-g less *trans*-fatty acids and 2.5-g less saturated fatty acids than conventional ground beef. These differences in fatty acid composition, due to differences in adipose tissue development among pasture-fed, conventional grain-fed, and highly marbled cattle, would have profound impacts on the eating quality and nutritional value of beef.

See also: Chemical Analysis for Specific Components: Curing Agents. Chemical and Physical Characteristics of Meat: Palatability. Classification of Carcasses: Beef Carcass Classification and Grading. Growth of Meat Animals: Adipose Tissue Development. Meat Marketing: Market Requirements and Specifications. Microbiological Safety of Meat: Thermotolerant *Campylobacter*. Nutrition of Meat Animals: Ruminants

Further Reading

Chung, K.Y., Lunt, D.K., Choi, C.B., *et al*., 2006. Lipid characteristics of subcutaneous adipose tissue and *M. longissimus thoracis* of Angus and Wagyu steers fed to US and Japanese endpoints. Meat Science 73, 432–441.

Cryer, A., Van, R.L.R. (Eds.), 1985. New Perspectives in Adipose Tissue: Structure, Function and Development. Butterworths: Boston, MA.

Gilmore, L.A., Walzem, R.L., Crouse, S.F., *et al*., 2011. Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. Journal of Nutrition 141, 1188–1194.

Haberstroh, C., Morris, C.E. (Eds.), 1991. Fat and Cholesterol Reduced Foods, Technologies and Strategies. Houston, TX: Gulf Publishing Company.

NRC, 1988. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Research Council.

Smith, S.B., Kawachi, H., Choi, C.B., *et al*., 2009. Cellular regulation of bovine intramuscular adipose tissue development and composition. Journal of Animal Science 87E (E supplement), E72–E82. doi:10.2527/jas.2008-1340.

Smith, S.B., Lunt, D.K., Chung, K.Y., *et al*., 2006. Review article: Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. Animal Science Journal 77, 478–486.

Smith, S.B., Smith, D.R. (Eds.), 1995. Biology of Fat in Meat Animals: Current Advances. Savoy, IL: American Society of Animal Science.

Chemical Composition

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Glossary

***Bos indicus* (or *Bos taurus indicus*)** Also called Zebu, are typified as herbivorous, humped domestic cattle and adapted to hot climates.
***Bos taurus* (or *Bos taurus taurus*)** Typical herbivorous domestic cattle descended from Europe, north-eastern Asia, and parts of Africa. They are referred to as 'taurine' cattle and many are adapted to cooler climates.
Bovine A diverse group of 10 genera of medium- to large-sized ungulates, including domestic cattle, bison, African buffalo, the water buffalo, the yak, and the four-horned and spiral-horned antelopes. Breeds of cattle fall into two main types *Bos taurus* and *Bos indicus*.
Ovine (*Ovis aries*) A genus of ruminant even-toed ungulate mammals that are herbivorous and typified as domestic sheep.
Porcine (*Sus domesticus*) A genus of domestic swine or pork that are omnivorous and classified as even-toed ungulates.
Poultry (*Gallus domesticus*) A domesticated fowl commonly referred to as poultry or chicken that has been adapted to commercial meat and egg production.
Turkey Fowl (*Meleagris gallopavo*) A domesticated version of wild turkey fowl adapted to commercial meat production.

Introduction

The material in this article includes the major and minor chemical constituents of meat tissues of bovine (*Bos taurus* or *Bos indicus*, humped cattle), ovine (*Ovis aries*), porcine (*Sus domesticus*), turkey fowl (*Meleagris gallopavo*), poultry (*Gallus domesticus*), and aquatic species. The primary components of meat tissues are moisture, proteins, lipids (fats), carbohydrates, and inorganic matter (ash or minerals). Alterations in the composition of meat tissues as they affect meat quality are briefly discussed.

Major Chemical Components of Meat Tissues

Edible animal tissues from carcasses are designated as 'meat' and consist of variable amounts of muscle, adipose tissue, connective tissue, blood, blood vessels, lymphatic tissues, nerve tissue, tendons, cartilage, and bone (the last three are typically removed before consumption). Meat tissues are composed of five primary chemical constituents: moisture (water), proteins, lipids (fat), carbohydrates, and inorganic matter (ash or minerals). Other components include nonprotein nitrogen compounds (e.g., nucleotides, peptides, creatine, creatine phosphate, urea, inosine monophosphate, nicotinamide-adenine dinucleotide and nonnitrogenous substances (e.g., vitamins, glycolytic intermediates, organic acids). Skeletal muscle tissue is composed of approximately 75% water, 19% protein, 2.5% lipid, 1.5% nonprotein nitrogenous compounds, 1% carbohydrate and nonnitrogenous components, and 1% inorganic matter.

The primary chemical components (water, protein, and fat) vary in meat tissues and meat products with species, maturity, anatomical location, amount of skin and bone, and the inclusion of added nonmeat ingredients such as salt, alkaline phosphates, sodium nitrite/nitrate, sugars, spices, or seasonings. In tissues, the percentages of water, protein, and ash are inversely related to the percentage of fat; in other words, the percentages of moisture, protein, and ash decrease with increasing amounts of fat (Figure 1). The percentage of carbohydrate, however, remains rather constant as the fat content of meat increases.

Eleven primary chemical elements make up over 99% of an animal's body composition whereas 25 essential and nonessential microelements are also present in the tissues. The major elements by weight include oxygen (65%), carbon (18%), hydrogen (10%), nitrogen (3%), calcium (1.5%), phosphorus (1.0%), potassium (0.35%), sulfur (0.25%), sodium (0.15%), chlorine (0.15%), and magnesium (0.05%). Essential elements that are required for normal metabolical function are cobalt, copper, iodine, iron, manganese, molybdenum, selenium, and zinc, whereas barium, bromine, cadmium, chromium, fluorine, and strontium are considered nonessential. For a more detailed listing of specific nutrient components of meat, the reader is referred to the United States Department of Agriculture National Nutrient Database for Standard Reference, Release 25 (2012).

Factors Influencing the Chemical Composition of Meat

Meat tissue composition varies according to differences in species, chronological and physiological maturity at harvest, plane of nutrition, genetic predisposition (e.g., pale, soft, and exudative (PSE) tissue vs. dark, firm, and dry tissue), and anatomical location of cuts within a carcass. Primarily, the composition of an animal carcass and the corresponding meat

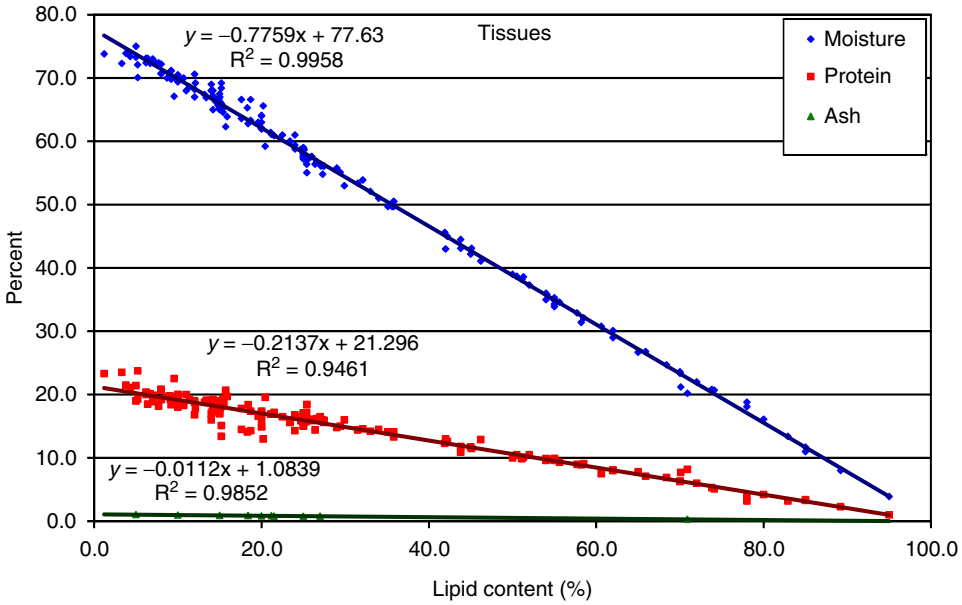


Figure 1 The inverse relationship of moisture, protein, and ash levels with increasing percentages of fat in boneless meat tissues.

tissues varies most depending on the stage of growth, the plane of nutrition, and the maturity level at which the animal is harvested.

Muscle, fat, and bone are the major carcass components that change in their proportions from birth to maturity. At birth, muscle (~67%) is the predominant component of a beef carcass, followed by bone (~25%), and lastly fat (~8%). At maturity, muscle may represent ~55% of a carcass whereas fat amounts to ~28% and bone ~15%. Thus, with growth and maturity, the percentages of separable muscle and bone decrease whereas the percentage of fat increases. The percentage of bone decreases only slightly once an animal's body or carcass weight is near that of an adult. Table 1 illustrates the decline in the percentage of moisture, crude protein, and ash with a corresponding increase in the fat content from birth to maturity. As noted in Table 1, the chemical composition changes when components are compared on the basis of a whole animal or a carcass (bone-in or boneless).

Chemical differences are also evident among species when carcass composites and selected skeletal muscles are compared (Table 2). Carcasses are chemically more diverse, whereas individual muscles from each species are more similar in gross composition (moisture, protein, and fat). However, muscles vary in the proportions of specific chemical components (e.g., collagen content, myoglobin concentration, and sarcoplasmic proteins) or nutrients (e.g., saturated, monounsaturated, and polyunsaturated fatty acids; iron).

Table 1 Changes in chemical composition (%) of animal carcasses from birth to maturity

Component	*Birth*			*Market Maturity*			
	Beef[a]	*Pork*[b]	*Turkey*[c]	*Beef*[d]	*Pork*[e]	*Turkey*[f]	*Chicken*[g]
Moisture	73.5	81.7	73.0	52.7	47.3	60.0	64.4
Crude protein	19.2	9.8	23.6	23.7	15.3	21.2	17.7
Fat	2.5	1.8	2.8	19.1	36.0	17.6	15.9
Ash	4.8	3.1	0.6	4.8	2.2	1.3	2.0

[a]Buckley, B.A., Baker, J.F., Dickerson, G.E., Jenkins, T.G., 1990. Body composition and tissue distribution from birth to 14 months for three biological types of beef heifers. Journal of Animal Science 68, 3109–3123.
[b]Shields Jr., R.G., Mahan, D.C., Graham, P.L., 1983. Changes in swine body composition from birth to 145 kg. Journal of Animal Science 57, 43–54.
[c]Ferket, P.R., Chen, F., Thomas, L.N., 1998. Amino acid profile of turkeys. In: Proceedings of the Turkey Nutrition Workshop, pp. 15–20. Raleigh, NC: North Carolina State University.
[d]Average composition values over 12–15 months of age. Reproduced from Buckley, B.A., Baker, J.F., Dickerson, G.E., Jenkins, T.G., 1990. Body composition and tissue distribution from birth to 14 months for three biological types of beef heifers. Journal of Animal Science 68, 3109–3123 and Early, R.J., McBride, B.W., Ball, R. O., 1990. Growth and metabolism in somatotropin treated steers: II. Carcass and noncarcass tissue components and chemical composition. Journal of Animal Science 68, 4144–4152. Garrett, W.N., Hinman, N., 1969. Reevaluation of the relationship between carcass density and body composition of beef steers. Journal of Animal Science 28, 1–5.
[e]Average composition values over 5–6 months of age. Reproduced from Swensen, K., Ellis, M., Brewer, M.S., Novakofski, J., McKeith, F.K., 1998. Pork carcass composition: I. Interrelationships of compositional end points. Journal of Animal Science 76, 2399–2404.
[f]Average composition values at 24 weeks of age. Reproduced from Ferket, P.R., Chen, F., Thomas, L.N., 1998. Amino acid profile of turkeys. In: Proceedings of the Turkey Nutrition Workshop, pp. 15–20. Raleigh, NC: North Carolina State University.
[g]Average composition values at 8 weeks of age contributed by Ferket, P.R., 2003. Personal Communication.

Specific Chemical Components

Moisture (Water)

In living muscle tissues, water may range from 65% to 80% of the total mass and serve as a basic component of cellular and

Table 2 Carcass composites and skeletal muscle tissue (raw) composition of various meat species

Analyses (%)	*Carcass composite (Boneless) (%)*											
	Beef[a]	*Pork*[b]	*Chicken*[c]	*Turkey*[d]	*Lamb*[e]	*Veal*[f]	*Salmon*[g]	*Mackerel*[h]	*Catfish*[i]	*Cod*[j]	*Shrimp*[k]	*Tuna*[l]
Moisture	58.21	49.83	65.99	70.40	59.80	72.84	75.52	71.67	79.06	81.22	83.01	70.58
Protein	17.48	13.91	18.60	20.42	16.74	19.35	20.50	19.29	15.23	17.81	13.61	22.00
Fat	22.55	35.07	15.06	8.02	22.74	6.77	4.40	6.30	5.94	0.67	1.01	1.01
Ash	0.82	0.72	0.79	0.88	0.92	1.04	1.52	1.27	1.05	1.16	1.86	1.30
	Skeletal muscle (%)											
	Beef[m]		*Pork*[n]		*Chicken*[o]		*Turkey*[p]		*Lamb*[q]		*Veal*[r]	
Moisture	69.29		73.21		75.46		74.16		76.13		74.80	
Protein	22.18		21.20		21.39		21.77		20.82		20.98	
Fat	7.68		4.82		3.08		2.86		3.28		3.08	
Ash	1.08		1.01		0.96		0.97		1.22		1.12	

Source: Data from USDA Nutrient Database Numbers: [a]13002, [b]10001, [c]05006, [d]05165, [e]17062, [f]17088, [g]15083, [h]15051, [i]15234, [j]15015, [k]15149, [l]15123, [m]13898 (Top Round, Select), [n]10002 (Leg, Loin, and Shoulder), [o]05011, [p]05167, [q]17070, [r]17094 USDA-NDSR (2012). United States Department of Agriculture Nutrient Database for Standard Reference (25): Beef, Pork, Lamb, Chicken, Turkey, Finfish and Shellfish Products.

organ metabolism, as a transport medium for metabolites and waste products, as a thermoregulator, as a solvent, and as a lubricant. In postmortem muscle tissue, water is the primary component of individual cells and comprises 75–80% of the cell mass. Thus, water comprises a major part of the sarcoplasm of muscle as well as surrounding the myofibrillar proteins. The myofibrils make up 75–92% of the volume of lean muscle and play a dominant role in the water-holding capacity (WHC) of the tissue. For the meat industry, the WHC of fresh meat is known to affect its economic value, such as processing yield and product quality. Poor WHC may reduce water retention, alter color, reduce sensory quality, and decrease acceptable appearance due to the presence of 'purge' ('drip') in a package.

The ionic environment (pH), availability of specific cations and anions, and the degree of contraction of myofibrillar proteins are the primary factors affecting the retention or loss of moisture from muscle tissues. As the postmortem pH is reduced by the accumulation of lactic acid, acidification of the muscle tissue reduces the pH from 5.6 to 5.8. The myofibrillar protein's ability to retain water is diminished and this may result in a corresponding increase in the percentage of protein. Table 3 illustrates the compositional changes and loss of WHC in porcine Longissimus muscle due to a decline in pH. The ionic environment of contractile protein side-groups and capillary entrapment of water within the myofibrillar matrices affect a muscle's WHC. At ~pH 5.1, myofibrillar proteins of postmortem tissue are near their isoelectric point (pI) and WHC is lowest at that point.

Moisture sorption isotherms of proteins estimate that $<0.1\%$ of the total water surrounding the protein is tightly bound or absorbed into the accessible polar sites of a protein's native structure. This water, designated as constitutional (bound or monolayer) water, is tightly bound within a protein and does not freeze at −40 °C. Additional 'layers' of water (~5%) interact with charged and polar protein surface groups to form a hydration shell around the protein. These water molecules participate in hydrogen-bonding, are slightly mobile, remain mostly unfrozen at −40 °C, and begin to have a plasticizing effect on solutes. When the hydration shell reaches a concentration of 0.38 g water per gram dry protein, a monolayer of water covers the surface of the protein. Additional water outside a protein's hydration shell is known as bulk-phase water, which constitutes ~95% of the water in a cellular system. Bulk-phase water consists of entrapped and free water, portions of which are susceptible to loss as 'purge' ('drip') or loss during thermal processing. This water is freezable and allows for a large degree of molecular mobility.

Table 3 Compositional differences in porcine *Longissimus* muscle exhibiting pale, soft, and exudative (PSE) characteristics

Component (%)	*Normal pork loin*	*PSE pork loin*
Moisture	68.7	70.0
Protein	20.9	21.7
Lipid	9.8	7.6
Ash	1.0	1.0
Expressible juice[a]	2.5	3.6
pH (no unit)	5.7	5.3
Reflectance	16.6	27.8

[a]Expressible juice is the ratio of moisture area to meat area when 300 g of meat is pressed between two pieces of filter paper using a Carver Press set at 136 kg.
Source: Reproduced from Ewan, R.C., Topel, D.G., Ono, K., 1979. Chemical composition of chops from pale, soft, exudative (pse) and normal pork loins. Journal of Food Science 44, 678–680.

Proteins and Nonprotein Nitrogen Compounds

Proteins constitute 16–22% of skeletal muscle tissue and are composed of 20+ amino acids connected via a peptide linkage as shown in Figure 2. Proteins are generally categorized according to function: myofibrillar (contractile), sarcoplasmic (metabolic), or stromal (connective or support). The metabolic turn-over or replacement rates for each of these tissues are intermediate, rapid, and very slow, respectively. Meat proteins as a whole contain ~16% nitrogen (including nonprotein nitrogen compounds) that can be converted into

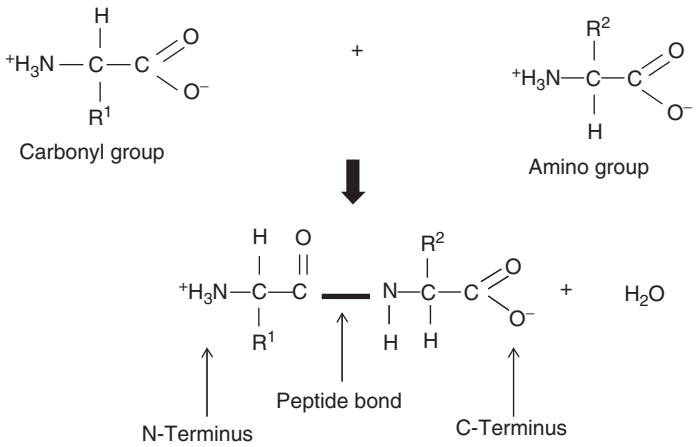


Figure 2 The peptide linkage between amino acids is formed by the condensation reaction of the carboxyl group of an amino acid reacting with an amino group of another amino acid.

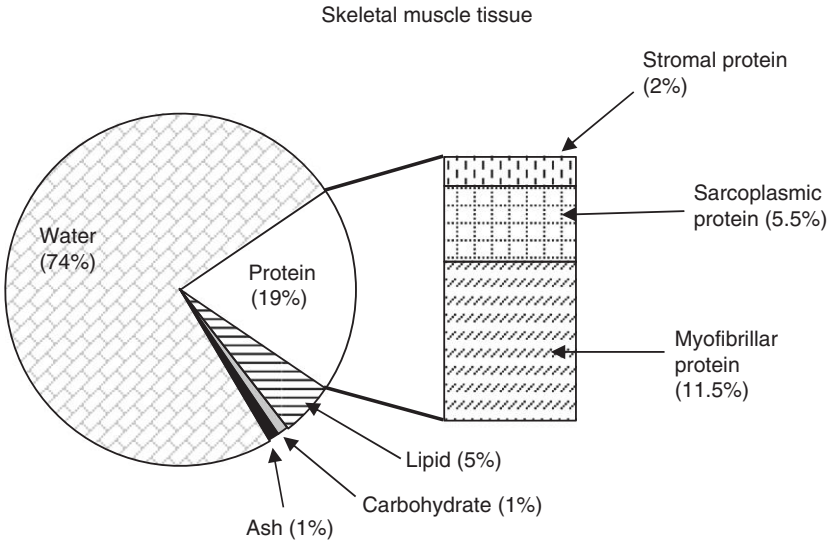


Figure 3 Composition of skeletal muscle tissue.

percent crude protein by multiplying percent nitrogen by 6.25 (100/16 = 6.25).

Myofibrillar, or salt-soluble, proteins comprise approximately 11.5% of the 19% total (Figure 3) and are extracted with a KCl solution of >0.3 mol l^{-1}. Myosin (43%), actin (22%), titin (8%), tropomyosin (5%), troponin (%), nebulin (3%), C-protein (2%), α-actinin (2%), M protein (2%), and desmin (<1%) account for ~93% of the 20+ different contractile proteins making up the myofibril. Actin and myosin constitute approximately 22% and 43%, respectively, or 65% of the total amount of myofibrillar proteins. Myosin (520 kDa; 2 subunits of 220 kDa, 4 of 20 kDa) and actin (42 kDa) complex during rigor mortis to form actomyosin and are the most important proteins influencing WHC (swelling) of muscle tissue, intermolecular binding in a meat gel matrix (gelation), and mechanical stability of a meat emulsion (encapsulation of fat globules).

Sarcoplasmic, or water-soluble, proteins account for approximately 5.5% of the 19% protein total and are extracted with low-ionic strength KCl solutions of ~0.06 mol l^{-1}. These proteins are found in the sarcoplasm or fluid surrounding the myofibrils and are made up of predominantly oxidative enzymes (cytochromes, the flavin nucleotides), various heme pigments (myoglobin), the mitochrondrial oxidative enzymes, lysosomal enzymes, and nucleoproteins. The glycolytic enzymes that make up most of the sarcoplasmic proteins are also bound to the myofibrillar protein actin. The concentration of sarcoplasmic proteins is ~55 mg ml^{-1}, with the most abundant proteins (mg g^{-1} tissue) in decreasing order of abundance being glyceraldehyde phosphate dehydrogenase (12), aldolase (6), enolase (5), creatine kinase (5), lactate dehydrogenase (4), pyruvate kinase (3), phosphorylase (2.5), and myoglobin (0.1–20+). Concentrations of these enzymes/proteins vary with species, muscle fiber type, maturity, and sex of the animal. Sarcoplasmic proteins are more effective emulsifiers in sausages or restructured meat products than are stromal proteins (collagen), but not as effective as myofibrillar proteins.

Myoglobin (Mb, 16 kDa) is the primary pigment giving color to muscle tissues, which is dependent on the concentration of myoglobin, the oxidation state of the iron atom in the planar porphyrin ring, and the molecule attached to the sixth site on the iron molecule. Examples of the iron oxidation state, the pigment form, the attached molecule, and the color of the tissue are as follows: Fe^{2+}, myoglobin–H_2O (purplish red); Fe^{2+}, oxymyoglobin–O_2 (bright red); Fe^{3+}, metmyoglobin–H_2O (brown); and Fe^{2+}, nitrosylhemeochromogen–NO (pink). Some uncertainty exists with regard to the specific molecule attached to some pigment forms. Concentrations of myoglobin in raw muscle tissue are shown in Table 4.

Stromal or connective tissue proteins normally comprise approximately 2% of the 19% total protein in skeletal muscle and are rather insoluble. Connective tissue consists of a viscous solution of soluble glycoproteins (proteoglycans) with extracellular fibers of collagen and elastin embedded in the glycoprotein matrix. Collagen is a unique triple-helical molecule and is the most abundant single protein in an animal. It can comprise 20–25% of the total body protein with the inclusion of skin, ligaments, tendons, cartilage, and bone. Collagen is extracted only with strong acid or alkaline solutions, or it can be digested with pepsin and collagenase. It has an unusual amino acid composition of approximately 33% glycine, 11% alanine, 9–10% proline, and 13–14% hydroxyproline. Because hydroxyproline is unique to collagen (and to a lesser extent elastin) and is present at a consistent concentration in collagen, chemical determination of the collagen content of meat tissues is performed via hydroxyproline analysis.

When heated (60–70 °C) slowly under moist conditions, collagen will unfold owing to the breakage of noncovalent bonds, some covalent (disulfide) intermolecular and intramolecular bonds, and a few peptide bonds, resulting in the collapse of the triple-helical polypeptide units. This conversion of helical collagen to an amorphous form (gelatin) is known as gelatinization. When it is chilled to refrigeration temperatures (~4 °C), partial renaturation occurs, resulting in a solidified, jelly-like gel or solidified gelatin. Reheating above 43–49 °C reliquefies the gelatin.

Elastin is a rubbery protein in a β-pleated sheet arrangement that is present in ligaments, in arterial walls and in the support structure for organs. The cervical ligament (*ligamentum nuchae*) in ruminants is primarily composed of elastin, which contains 1–2% hydroxyproline and two unique amino acids, desmosine and isodesmosine. It is very resistant to solubilization, cooking, or enzymatic digestion because of its high content (~90%) of nonpolar amino acids and desmosine cross-links.

Table 4 Myoglobin concentration in muscle tissues of various species

Species/maturity/color	*Myoglobin concentration (mg g^{-1} tissue)*
Mature beef (dark, red)	16–20
Young beef (bright, cherry red)	4–10
Veal (brownish pink)	1–3
Pork (grayish pink)	0.3–3
Mature pork (red)	8–12
Lamb (light red)	2.5–8
Mutton (brick red)	12–18
Horse (dark red)	7–8
Turkey (dull red)	0.4–1.5
Poultry (gray–white, dull red)	0.5–1.5
Fish (white to red)	0.3–1
Fish (dark red)	5–25
Whale (very dark red to black)	>25

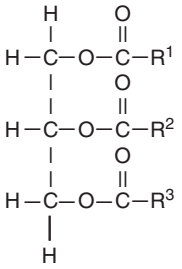
Source: Reproduced from Pearson, A.M., Gillett, T.A., 1996. Composition and nutritive value of raw materials and processed meats; least cost formulation and preblending of sausage. Processed Meats, third ed. New York: Chapman and Hall, pp. 53–78; Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., Jones, K.W., 2001. Sausages; meat as a food. The Meat We Eat, fourteenth ed. Danville, IL: Interstate Publishers, pp. 731–778; and Miller, R.K., 1994. Quality characteristics. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods Meat, Poultry and Seafood Technology. New York: Kluwer Academic/Plenum Publishers, pp. 296–332.

Lipids/Fat/Triacylglycerols

Animal adipose tissue (fat) is composed primarily of neutral lipids known as triacylglycerols and phospholipids that collectively range from 1.5% to 13% in muscle tissue. Lipids also exist as sterols and sterol esters (cholesterol and cholesterol components) and cerebrosides. Various lipid forms serve as an energy source for the cell, as a structural and functional component of the cell wall, as insulation or protection for vital organs, and as solubilizing agents for certain hormones and vitamins (A, D, E, and K). Fats can be metabolized to yield 2.25 times more energy than carbohydrates or proteins and thus are an energy-dense nutrient.

Neutral lipids (triacylglycerols) are glycerol esters composed of one glycerol molecule and three even-numbered long-chain fatty acids. Except in milk, the presence of fatty acids having a chain length of 10 or fewer carbon atoms in animals is rare. Triacylglycerols may be simple (all attached fatty acids are identical) or mixed (two or more attached fatty acids are different). A generalized structure of a triacylglycerol is shown in Figure 4.

Most fatty acids in animal fats contain an even number of carbon atoms, but a few odd-numbered carbon fatty acids are also known. Fatty acids are characterized as saturated (containing no double bonds between carbon atoms); monounsaturated (containing one double bond between two carbon atoms); and polyunsaturated (containing two or more double bonds in the carbon chain). The combined chemical



{Glycerol} + {Fatty acid} = Triacylglycerol

Figure 4 General structure of a triacylglycerol; R^1, R^2, and R^3 represent fatty acid chains.

Table 5 Melting point characteristics of fatty acids and triacylglycerols commonly found in animal tissues

Fatty acid	*Chemical designation*	*Melting point (°C)*
Saturated		
Butyric	C_4	−8.0
Caproic	C_6	−3.4
Caprylic	C_8	16.7
Capric	C_{10}	31.6
Lauric	C_{12}	44.2
Myristic	C_{14}	52.0
Palmitic	C_{16}	63.1
Stearic	C_{18}	69.6
Arachidic	C_{20}	75.4
Monounsaturated		
Myristoleic	$C_{14:1,\ 9C}$	−1
Palmitoleic	$C_{16:1,\ 9C}$	13
Oleic	$C_{18:1,\ 9C}$	39
Vaccenic	$C_{18:1,\ 11C}$	
Polyunsaturated		
Linoleic	$C_{18:2,\ 9C,\ 12C}$	−5.1
Linolenic	$C_{18:3,\ 9C,\ 12C,\ 15C}$	−11.2
Arachidonic	$C_{20:4,\ 5C,\ 8C,\ 11C,\ 14C}$	−49.5
Triacyglycerols		
Palmitodiolein		Solids at body temperature
Oleopalmitostearin		
Oleodipalmitin		
Sterodiolein		
Dipalmitostearin		
Palmitodistearin		
Tripalmitin		
Triolein		

Source: Reproduced from Dugan Jr., L.R., 1987. Lipids. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products. Westport, CT: Food and Nutrition Press, pp. 103–113 and Aberle, E.D., Forrest, J.C., Gerrard, D.E., *et al.*, 2001. Structure and composition of animal tissues; growth and development of carcass tissues; principles of meat processing; nutritive value of meat. Principles of Meat Science, fourth ed. Dubuque, IA: Kendall/Hunt, pp. 9–43.

characteristics (carbon chain length, number of double bonds, melting point, fluidity, hardness, and susceptibility to lipid oxidation) of the fatty acids attached to a triacylglycerol give a fat its own particular set of physical characteristics. Melting point characteristics of fatty acids and triacylglycerols commonly found in animal tissues are presented in Tables 5 and 6. Palmitodiolein and oleopalmitostearin are the two most prevalent triacylglycerols in beef, lamb, and pork.

The melting point of fats is determined by the carbon chain length and the number of double bonds (degree of saturation) within the fatty acid carbon chain. As the carbon length of a fatty acid becomes longer, the melting point increases, whereas, as the number of double bonds increases, the melting point decreases. Table 7 illustrates the effect of chain length and double bonds on melting points of common fatty acids in animal tissues.

Internal fats surrounding the organs are generally more saturated and have higher melting points than external subcutaneous fats. Saturated fatty acids having more than 12 carbon atoms are solids at body temperature as shown in Table 5.

Table 6 Melting points of lipids in meat cuts and tissues commonly used in sausage processing

Source species	*Melting Point (°C)*
Lamb	32.2–46.1
Beef	31.7–43.3
Pork	30.0–40.0
Poultry	26.7–43.3

Source: Reproduced with permission from Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., Jones, K.W., 2001. Sausages; meat as a food. The Meat We Eat, fourteenth ed. Danville, IL: Interstate Publishers, pp. 913–918.

Table 7 Change in melting points due to carbon chain length and degree of saturation – examples of common fatty acids in animal tissues

Fatty acid characteristics	*Melting point (MP) change (°C)*
Incremental addition of two carbons	MP increase of
$C_{12:0}$ to $C_{14:0}$	7.8
$C_{14:0}$ to $C_{16:0}$	11.1
$C_{16:0}$ to $C_{18:0}$	6.5
$C_{18:0}$ to $C_{20:0}$	5.8
$C_{20:0}$ to $C_{22:0}$	5.6
$C_{22:0}$ to $C_{24:0}$	3.2
Incremental addition of one double bond	MP decrease of
$C_{18:0}$ to $C_{18:1}$	56.2
$C_{18:1}$ to $C_{18:2}$	22.4
$C_{18:2}$ to $C_{18:3}$	8.0

Source: Selected values from Voet, D., Voet, J.G., 1995. Lipids and membranes. Biochemistry, second ed. New York: John Wiley and Sons, Inc., p. 278.

Fatty acid nomenclature is derived from numbering the carbon atoms from the carboxyl (COOH) or methyl (CH_3) ends of the molecule. Using the carboxyl numbering method, oleic acid ($C_{18:1,\ 9C}$) is named *cis*-9-octadecenoic acid. In the methyl numbering scheme the double bond is located on the third carbon from the methyl end (*n*-3). Fatty acids designated in this manner are called 'omega-3' fatty acids and are primarily found in cold-water fish. Most unsaturated fatty acids in meat are of the *n*-6 variety (sixth carbon from the methyl end).

The degree of unsaturation of a fat is typically determined by measuring the number of grams of iodine reacting with 100 g of fat, to produce an iodine number. Unsaturated fatty acids may be made more saturated by hydrogenation of the unsaturated carbons with the use of a catalyst such as nickel or sodium methoxide. A *trans* fatty acid conformation can result from this process and is more stable having less mobility and fluidity. The *trans* fatty acids have been implicated in contributing to coronary heart disease and increasing low-density lipoprotein cholesterol while decreasing high density lipoprotein cholesterol. Most fatty acids in foods are in the *cis* conformation, which is less stable than the *trans* form, and are also more fluid. Isomers of linoleic acid that have double bonds on the C9 and C11 sites rather than the C9 and C12 sites are designated conjugated linolenic acid and may have

unique and beneficial properties for processing and dietary purposes.

The proportions of fatty acids making up a specific fat depot vary with species and diet. In general, oleic acid ($C_{18:1}$) (20–47%) is the most abundant fatty acid in the animal body of lamb, cattle, and pigs, whereas palmitic (C_{16}) (26%) is the most abundant in poultry. The fatty acid composition, ranking from most saturated (harder fat) to least saturated (more oily) by species is as follows: lamb > cattle > pigs > poultry > fish. For cattle, lamb, pigs, and poultry, palmitic (C_{16}) (25–30%) and stearic (C_{18}) (7–27%) saturated fatty acids predominate, with lesser quantities of lauric (C_{12}) (trace), myristic (C_{14}) (0.1–5%), and arachidic (C_{20}) (trace to 3%). Linoleic ($C_{18:2}$) (2–20%), palmitoleic ($C_{16:1}$) (1–7%), linolenic ($C_{18:3}$) (0.2–0.6%), and arachidonic ($C_{20:4}$) (0.2–2%) unsaturated fatty acids are also present in the amounts indicated. Linoleic and linolenic are considered essential fatty acids in humans.

Phospholipids are the structural and functional components of cell membranes comprising 0.5–1% of the lipid in skeletal muscle. The most common phospholipids in muscle tissue are: phosphatidylethanolamine (cephalin) (33%), phosphatidylserine (6%), phosphatidylcholine (lecithin) (58%), and sphingomyelin (3%). These lipids are more readily oxidized by oxygen than are triacylglycerols, resulting in the development of specific off-aromas and flavors in meat products known as warmed-over flavor. Phospholipids are very similar to triacylglycerols with a phosphoric acid inserted between the glycerol ester bond and the R^3 fatty acid shown in Figure 4. Saturated fatty acids are the least susceptible to lipid oxidation, followed by monounsaturated fatty acids, and lastly polyunsaturated fatty acids, which are the most susceptible to free radical lipid oxidation. Free-radical oxidation produces a variety of undesirable breakdown products, especially short chain acids, aldehydes, and ketones.

Sterols are minor constituents present in the fat of the human diet. Cholesterol is the main animal sterol and is the precursor of bile acids, provitamin B, and the steroid hormones. Cholesterol can be present in the free form or esterified at the hydroxyl group with fatty acids of various chain length and saturation. Cholesterol is a component of meat lipids, and high blood levels in humans have been shown to increase the risk of cardiovascular disease. Cholesterol intake has been recommended not to exceed 300 mg per day. The content of cholesterol in meat and meat products is influenced by diverse factors, such as type of meat, anatomical location, and preparation conditions. The average cholesterol content of domestic meat species and their by-products are summarized in Table 8.

Fat content varies with species, maturity (Tables 1 and 2), and diet. From birth to maturity, fat is deposited in the following order: around vital organs, subcutaneously, intermuscularly (between muscles), and lastly intramuscularly (between muscle bundles as 'marbling'). Adipose tissue is dynamic and is constantly being stored or mobilized. All meat animals synthesize fatty acids in the liver and/or in adipose tissue from carbohydrates and proteins. Fats in the diet of monogastric animals are broken down to their parent fatty acids and may be assimilated and deposited in relatively unchanged form; thus carcasses have characteristics related to the fatty acid composition of the dietary fat. Without careful management of the diet, problems can arise during processing or at the time of consumption. For example, the carcass fat of pigs fed a diet containing lower melting point fats such as flax seed, highly unsaturated vegetable oils, fish meal, or peanuts takes on the characteristics of the dietary fat. In this case, pigs would have more oily, softer carcasses resulting in meat cuts with poor appearance, poor slicing characteristics (bacon), painty/fishy off-flavors (especially when heated), or raw oyster-like fat in the center of a ham slice. Ruminants, however, deposit more saturated fats that have higher melting points (harder fat) due to the degradation and resynthesis of dietary fats by rumen bacteria before assimilation and deposition in the tissues.

Limiting fat and cholesterol intake are considered to be important measures to prevent obesity and hypercholesterolemia, conditions that are thought to predispose humans to various chronic diseases of the circulatory system. Epidemiological and clinical studies have suggested that high-fat diets, regardless of their fatty acid distribution, increase the concentration of cholesterol in the blood. Therefore, several strategies have been applied to reduce the fat content of carcasses due to consumer demand for leaner meats. These strategies to reduce fat include selection of leaner genetic lines within breeds, and changes in animal rations to include feed supplements such as probiotics, antibiotics, β-agonists, and growth hormones. These practices have allowed for a substantial reduction of intramuscular, intermuscular, and subcutaneous fat. However, reduction of intramuscular fat ('marbling') should be carefully controlled to maintain meat quality attributes such as tenderness, juiciness, and flavor.

Table 8 Average cholesterol content of meat species and by-products

Type of meat/by-products	*Cholesterol (mg per 100 g edible portion)*
Type of meat	
Beef[a]	61
Pork[b]	68
Chicken[c]	64
Turkey[d]	65
Lamb[e]	64
By-products	
Beef liver[f]	275
Beef heart[g]	124
Pork liver[h]	301
Pork heart[i]	131
Chicken liver[j]	345
Chicken heart[k]	136
Turkey liver[l]	331
Turkey heart[m]	147
Lamb liver[n]	371
Lamb heart[o]	135

Source: Data from USDA Nutrient Database Numbers [a]23636 (top round, steak, separable lean only, trimmed to 1/8″ fat, select), [b]10010 (whole, leg, ham, separable lean only), [c]05062 (broilers or fryers, breast meat only), [d]05167 (all classes, meat only), [e]17013(leg, whole, separable lean only, trimmed to 1/4″ fat, choice), [f]13325, [g]13321, [h]10110, [i]10103, [j]05027, [k]05025, [l]05177 (all classes), [m]05175 (all classes), [n]17199, [o]17191 (USDA–NDSR, 2012). United States Department of Agriculture Nutrient Database for Standard Reference (25): Beef, Pork, Poultry, Lamb, Veal and Game Products.

Carbohydrates

Glycogen is a branched polysaccharide consisting of α-D-glucose units linked by α-1, 6 glucosidic and α-1, 4 glucosidic bonds. It is the most abundant carbohydrate in animal tissues and is present in the liver at 2–8%, but ranges from 0.5% to 1.5% in living skeletal muscle tissue. The initial amount of glycogen in the muscle tissues at slaughter affects ultimate muscle color, texture, firmness, WHC, emulsifying capacity, and shelf life.

Glycogen can have a molecular weight in the millions and is stored in the muscle cell for subsequent conversion to glucose. The amounts of initial pre-slaughter glycogen depend on preslaughter stress conditions and in particular the levels of adrenaline (epinephrine) and/or preslaughter exercise that play a significant part in its conversion to lactate with potentially adverse effects on meat quality. Postslaughter anaerobic glycolysis essentially results in the glucose moiety being metabolized to two moles of lactate. The final lactate concentration dictates the end-point muscle pH and is proportional to the initial glycogen concentration. Glucose in a living muscle cannot leave but will be used up by muscular exercise (glucose can be transported in but not out of the muscle). Lactate leaves muscle, is transported to the liver, and eventually over time (a long time in ruminants) recycles in living animals. Ordinarily, fatty acids are the low-level energy source and glycogen is used only in intensive bursts of muscle activity or is used only preferentially when adrenaline is available. Even then exercise (e.g., trembling, shivering) is needed to use up the glycogen over time. The glycogen replenishment rate depends on species and is higher in pork than ruminants.

Typical resting muscle glycogen and lactate levels vary depending on species (Table 9). At 24 h post mortem, the lactate concentration can range from 77 to 130 mmol kg^{-1} of tissue whereas the glycogen drops to less than 10 mmol kg^{-1} of tissue. Variations of postmortem glycogen and lactate concentrations among species are presented in Table 9. As a consequence of the build-up of lactate, the pH drops from 7.1–7.3 to 5.5–5.7. The pH decline of a muscle or carcass is temperature dependent, with the pH of warm muscle declining at a faster rate than that of muscles or carcasses that have been chilled quickly.

Other carbohydrates that are found in animal tissues include the glycosaminoglycans and proteoglycans that are associated with the extracellular matrix of connective tissues, as well as the glycoproteins found in plasma and blood, and some hormones, glycolytic intermediates, nucleotides, nucleosides, and the glycolipids. Of all these carbohydrates, D-glucose is the most abundant. Glycosaminoglycans are covalently linked to core proteins to form complex proteoglycans. Hyaluronic acid, the chondroitin sulfates, dermatan sulfate, keratan sulfate, and heparin-like polysaccharides are components of the glycosaminoglycans.

Some genetic strains of swine are susceptible to stress and as a consequence, exhibit a PSE condition in some major muscle groups due to a rapid decline in pH to an endpoint of 5.2–5.4 (in comparison to a 'normal' post mortem pH of 5.5–5.7). The accelerated pH decline results in denaturation of muscle protein with changes in muscle chemical composition and damaging consequences to the quality of the tissue. PSE muscle (ham and loin) typically exhibits poor WHC, pale color, soft texture, excessive cooking losses (lower processing yields), poor cured color, and a dry, 'mealy' mouthfeel.

Inorganic Matter (Minerals)

Approximately 3.5% of total body weight consists of inorganic matter (bones and teeth) that is typically analyzed as percent ash. Minerals in ash are in the form of oxides, sulfates, phosphates, nitrates, chlorides, and other halides. In meat tissues, the percent ash is an estimate of the total mineral content that makes up cellular constituents (myoglobin, hemoglobin, and enzymes), bone (bone fragments, mechanically separated tissue, and advanced meat recovery systems), or ingredients (sodium chloride, potassium chloride, alkaline phosphates, lactate salts, spices, seasonings, batters, and breading) used in processing. Bone is a major component of carcasses and contributes primarily calcium and phosphorous. Muscle tissue is low in calcium (3–6 mg g^{-1}), but abundant in potassium (250–400 mg g^{-1}), phosphorus (167–216 mg g^{-1}), sodium (55–94 mg g^{-1}), magnesium (22–29 mg g^{-1}), zinc (1–5 mg g^{-1}), iron (1–3 mg g^{-1}), and copper (0.5–0.13 mg g^{-1}). The heme iron from meat is more readily absorbed as a nutrient, and heme iron accounts for 40–60% of the total iron. Calcium, magnesium, sodium, and potassium are directly involved in contraction in living muscle, whereas magnesium and calcium contribute to muscle fiber contraction post mortem. Sulfur (2.5 mg g^{-1}) is present in sulfur-containing amino acids that compose proteins, whereas chlorine (0.65 mg g^{-1}) is principally an anion for salts in soft tissues and intracellular fluids.

Iron, copper, zinc, iodine, manganese, molybdenum, cobalt, and selenium are essential microelements in the diet, whereas barium, bromine, cadmium, chromium, and fluorine are microelements within meat tissues with defined functions. Aluminum, arsenic, boron, lead, lithium, nickel, rubidium, silicon, silver, strontium, titanium, and vanadium are present,

Table 9 Resting and postmortem muscle glycogen and lactate concentrations

Species	*Resting*		*Postmortem*	
	Glycogen (mmol g^{-1})	*Lactate (mmol g^{-1})*	*Glycogen (mmol g^{-1})*	*Lactate (mmol g^{-1})*
Bovine	60–100	10–16	16–37	72–100
Porcine (normal)	52–85	16–28	1–10	79–97
Avian	37–56	10–40	0–7	89–120

Source: Adapted with permission from Keeton, J.T., Benli, H., Claflin, A.E., 2009. Carbohydrates. In: Nollet, L.M., Toldrá, F. (Eds), Handbook of Muscle Food Analysis. Boca Raton, FL: CRC Press, pp. 263–279.

but may not have well-defined functions or may be environmental contaminants.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment. Conversion of Muscle to Meat: Glycolysis. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns; Macronutrients in Meat; Micronutrients in Meat; Vegetarianism

Further Reading

Aberle, E.D., Forrest, J.C., Gerrard, D.E., *et al*., 2001. Structure and composition of animal tissues; growth and development of carcass tissues; principles of meat processing; nutritive value of meat. In: Elton, D.A., John, C.F., David, E.G., Edward, W.M. (Eds.), Principles of Meat Science, fourth ed. Dubuque, IA: Kendall/Hunt, pp. 9–43, 67, 137, 247–260.

Buckley, B.A., Baker, J.F., Dickerson, G.E., Jenkins, T.G., 1990. Body composition and tissue distribution from birth to 14 months for three biological types of beef heifers. Journal of Animal Science 68, 3109–3123.

Cheng, Q., Su, D., 2008. Factors affecting the water holding capacity of red meat products. A review of recent research advances. Critical Reviews in Food Science and Nutrition 48, 137–159.

Chizzolini, R., Zanardi, E., Dorigoni, V., Ghidini, S., 1999. Calorific value and cholesterol content of normal and low-fat meat and meat products. Trends in Food Science and Technology 10, 119–128.

Dugan Jr., L.R., 1987. Lipids. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products. Westport, CT: Food and Nutrition Press, pp. 103–113.

Early, R.J., McBride, B.W., Ball, R.O., 1990. Growth and metabolism in somatotropin treated steers: II. Carcass and noncarcass tissue components and chemical composition. Journal of Animal Science 68, 4144–4152.

Ewan, R.C., Topel, D.G., Ono, K., 1979. Chemical composition of chops from pale, soft, exudative (pse) and normal pork loins. Journal of Food Science 44, 678–680.

Fennema, O.R., 1996. Water and ice. In: Fennema, O.R. (Ed.), Food Chemistry, third ed. New York: Marcel Dekker, pp. 17–94.

Ferket, P.R., Chen, F., Thomas, L.N., 1998. Amino acid profile of turkeys In: Proceedings of the Turkey Nutrition Workshop. Raleigh, NC: North Carolina State University, pp. 15–20.

Foegeding, E.A., Lanier, T.C., 1996. Characteristics of edible muscle tissues. In: Fennema, O.R. (Ed.), Food Chemistry, third ed. New York: Marcel Dekker, pp. 879–942.

Garrett, W.N., Hinman, N., 1969. Reevaluation of the relationship between carcass density and body composition of beef steers. Journal of Animal Science 28, 1–5.

Greaser, M.L., 1986. Conversion of muscle to meat. In: Bechtel, P.J. (Ed.), Muscle as Food. Orlando, FL: Academic Press, pp. 37–87.

Greaser, M.L., Wang, S.M., Lemanski, L.F., 1981. New myofibrillar proteins In: Proceedings of the 34th Annual Reciprocal Meat Conference. Savoy, IL: American Meat Science Association, pp. 12–16.

Immonen, K., Kauffman, R.G., Schaefer, D.M., Poulanne, E., 2000. Glycogen concentrations in bovine longissimusdorsi muscle. Meat Science 54, 163–167.

Keeton, J.T., Benli, H., Claflin, A.E., 2009. Carbohydrates. In: Nollet, L.M., Toldrá, F. (Eds.), Handbook of Muscle Food Analysis. Boca Raton, FL: CRC Press, pp. 266.

Lawrie, R.A., 1998. Chemical and biochemical constitution of muscle; meat and human nutrition. In: Lawrie, R.A., Ralston, A.L. (Eds.), Lawrie's Meat Science, sixth ed. Boca Raton, FL: CRC Press, pp. 58–95, 258–269.

Miller, R.K., 1994. Quality characteristics. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods Meat, Poultry, and Seafood Technology. New York: Kluwer Academic/Plenum Publishers, pp. 296–332.

Pearson, A.M., Gillett, T.A., 1996. Composition and nutritive value of raw materials and processed meats; least cost formulation and preblending of sausage. Processed Meats, third ed. New York: Chapman and Hall, pp. 53–78.

Pearson, A.M., Gillett, T.A., 1996. Composition and nutritive value of raw materials and processed meats; Least cost formulation and preblending of sausage. In: Pearson, A.M., Gillett, T.A. (Eds.), Processed Meats, third ed. New York: Chapman and Hall, pp. 23–52, 196–198.

Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., Jones, K.W., 2001. Sausages; meat as a food. The Meat We Eat, fourteenth ed. Danville, IL: Interstate Publishers, pp. 731–778, 913–918.

Shields Jr., R.G., Mahan, D.C., Graham, P.L., 1983. Changes in swine body composition from birth to 145 kg. Journal of Animal Science 57, 43–54.

Swensen, K., Ellis, M., Brewer, M.S., Novakofski, J., McKeith, F.K., 1998. Pork carcass composition: I. Interrelationships of compositional end points. Journal of Animal Science 76, 2399–2404.

U.S. Department of Agriculture, Agricultural Research Service, 2012. USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data Laboratory Home Page. Available at: http://ndb.nal.usda.gov/ndb/search/list (accessed 19.08.13).

Voet, D., Voet, J.G., 1995. Lipids and membranes Biochemistry, second ed. New York: John Wiley and Sons, Inc. p. 278.

Relevant Websites

http://ndb.nal.usda.gov/ndb/search/list
Nutrient Data Laboratory Home Page.

http://www.ars.usda.gov/ba/bhnrc/ndl
United States Department of Agriculture.

Color and Pigment

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Introduction

The color of meat is the most important attribute influencing consumers' purchase decisions at the point of sale. Consumers often correlate cherry-red color of fresh meats with wholesomeness. However, at the point of consumption, a dull-brown color of cooked meats is used as an indicator of doneness. Cured meat products have a characteristic pink color. Myoglobin is the sarcoplasmic heme protein primarily responsible for the color of meat harvested from well-bled carcasses. Nonetheless, hemoglobin, cytochrome, and other pigments may also be present in meats at low levels and contribute to color to a lesser extent. Although exsanguination of food animals removes significant amount of blood, residual blood/hemoglobin can be found in blood vessels within the skeletal muscles and can contribute to meat color. In general, pigments other than myoglobin are more relevant to color in poultry and game meats than in red meats.

Myoglobin

The pigment myoglobin is composed of a protein moiety (globin) and a heme prosthetic group. In live muscles, myoglobin is responsible for oxygen binding and delivery functions. The globin polypeptide chain consists of eight helical segments – A through H, which forms a coil around the heme moiety (Figure 1). The hydrophobic heme group is arranged such that the vinyl groups are oriented toward the hydrophobic interior and the propionic acid groups toward the outer surface of the molecule (Figure 2). The heme group of myoglobin contains an iron atom that can exist in a reduced (ferrous/Fe^{2+}) or oxidized (ferric/Fe^{3+}) form. The globin portion of the molecule confers water solubility upon the hydrophobic heme group and, more importantly, protects the heme iron from oxidation. The resonant nature of the conjugated double bonds of heme is responsible for the ability of myoglobin to absorb visible light.

The ferrous iron within the heme group can accept six electrons in its outer orbital and can thus form six coordinate bonds, four with pyrrole groups of the porphyrin ring of heme and one with histidine (F8 position in globin), which connects heme to the globin chain. The sixth position is available for binding oxygen or other small ligands such as carbon monoxide (CO) or nitric oxide (NO).

Amino Acid Sequence of Myoglobin

In the postgenomic era, the amino acid sequences of myoglobins from several hundred species are available in the protein databases. Mammalian and avian myoglobin molecules consist of a single polypeptide of 153 amino acids. The molecular masses of beef, water buffalo, pork, sheep, goat, and horse myoglobins are 16 946, 17 034, 16 953, 16 923, 16 896, and 16 952 Dalton (Da), respectively. However, avian myoglobins are approximately 300–400 Da heavier than their mammalian counterparts, and the molecular masses of myoglobins of turkey, chicken, ostrich, and emu are 17 291, 17 291, 17 297, and 17 380 Da, respectively. Several low-molecular weight amino acids in red-meat myoglobins are replaced by heavier ones in avian myoglobins leading to the mass increment. In addition, these substitutions provide improved protection to the heme group in avian myoglobins. Tuna myoglobin has only 146 amino acid residues and a lower molecular weight (15 529 Da) than avian and mammalian myoglobins. Livestock and poultry myoglobins are among the few proteins in biological systems that do not contain cysteine residues. In contrast, highly oxidizable cysteine residues are present in the myoglobins of humans, great apes, rodents, and tuna. The presence of cysteine contributes to the rapid oxidation in tuna myoglobin.

In mammalian and avian myoglobin molecules, the heme is attached to the globin moiety at histidine F8, which is

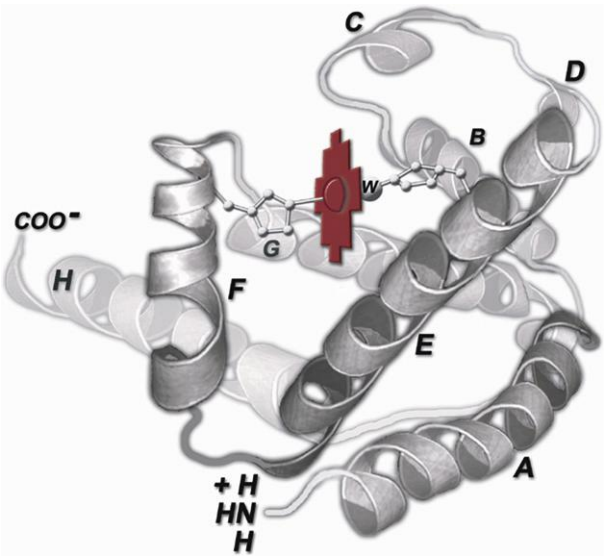

Figure 1 The myoglobin molecule consists of heme attached to globin. A to H indicate the eight helical segments of the globin moiety. The heme group is located in a hydrophobic cleft, where only small ligands such as oxygen and carbon monoxide have ready access. Owing to the hydrophobic environment, even water (W) has limited access to the heme group.

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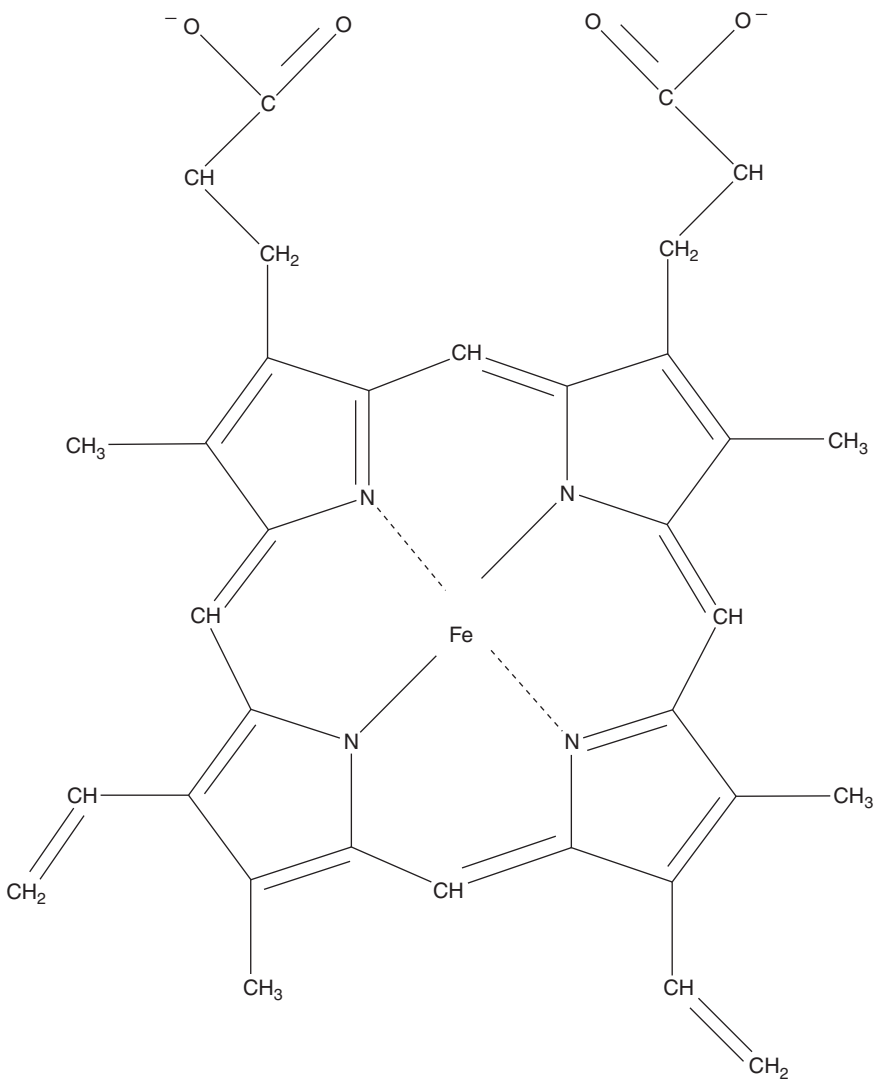

Figure 2 Planar view of the heme group of myoglobin. The iron can be in a ferrous (Fe^{2+}) or ferric (Fe^{3+}) form.

residue 93 from the amino terminal. Histidine 93 is termed the proximal histidine. The heme group is also stabilized in the heme pocket by coordination with histidine 64, the distal histidine, which is located in the E segment of globin. Whereas distal and proximal histidines are conserved in livestock myoglobins, in elephant myoglobin distal histidine is substituted by glutamine, and this substitution contributes to the absence of proton-catalyzed autoxidation. Despite the differences in amino acid sequences, several biological, structural, and functional properties of myoglobin are conserved across mammalian and avian species. Nevertheless, the primary structure of myoglobin influences biochemical attributes such as autoxidation, heme retention, structural stability, thermostability, and oxygen affinity, which are critical to meat color stability as well as the protein's function in live animals.

Several phylogenetically closely related mammalian and avian species have identical or nearly identical myoglobins. The myoglobins of European cattle, American bison, and yak have the same amino sequence. Zebra myoglobin is identical to horse myoglobin; myoglobin of European red deer is identical to that of the North American white-tailed deer. Among poultry species, chicken and turkey myoglobins share the same primary structure. Furthermore, myoglobins of several meat-producing ruminant livestock demonstrate high homology, albeit differences at key amino acid positions (e.g., European cattle and water buffalo; sheep, and goat). Histidine residues in myoglobin are susceptible to nucleophilic attack by reactive lipid oxidation products. The number of histidines in livestock myoglobins is 13 in beef and water buffalo; 12 each in horse, sheep, and goat; and 9 in pork (Figure 3). However, turkey and chicken myoglobins contain 9 histidines.

Myoglobins of meat-producing livestock share 85–100% homology in primary structure. The amino acid sequences of pig, cattle, sheep, goat, water buffalo, and horse myoglobins differ at 29 positions. Five mutations are required to obtain pig myoglobin from ancestral mammalian myoglobin, whereas 24, 19, and 13 mutations are necessary to obtain cattle, sheep, and horse myoglobin, respectively. Thus, pig myoglobin can be considered the most primitive among the domestic mammals.

Sequence No.	1	10	20	30	40	50
Beef	GLSDGEWQLV	LNAWGKVEAD	VAGHGQEVLI	RLFTGHPETL	EKFDKFKHLK	
Water-buffalo	GLSDGEWQLV	LNAWGKVETD	VAGHGQEVLI	RLFTGHPETL	EKFDKFKHLK	
Sheep	GLSDGEWQLV	LNAWGKVEAD	VAGHGQEVLI	RLFTGHPETL	EKFDKFKHLK	
Goat	GLSDGEWTLV	LNAWGKVEAD	VAGHGQEVLI	RLFTGHPETL	EKFDKFKHLK	
Pig	GLSDGEWQLV	LNVWGKVEAD	VAGHGQEVLI	RLFKGHPETL	EKFDKFKHLK	
Horse	GLSDGEWQQV	LNVWGKVEAD	IAGHGQEVLI	RLFTGHPETL	EKFDKFKHLK	

Sequence No.	60	70	80	90	100
Beef	TEAEMKASED	LKKHGNTVLT	ALGGILKKKG	HHEAEVKHLA	ESHANKHKIP
Water-buffalo	TEAEMKASED	LKKHGNTVLT	ALGGILKKKG	HHEAEVKHLA	ESHANKHKIP
Sheep	TEAEMKASED	LKKHGNTVLT	ALGGILKKKG	HHEAEVKHLA	ESHANKHKIP
Goat	TGAEMKASED	LKKHGNTVLT	ALGGILKKKG	HHEAEVKHLA	ESHANKHKIP
Pig	SEDEMKASED	LKKHGNTVLT	ALGGILKKKG	HHEAELTPLA	QSHATKHKIP
Horse	TEAEMKASED	LKKHGTVVLT	ALGGILKKKG	HHEAELKPLA	QSHATKHKIP

Sequence No.	110	120	130	140	150
Beef	VKYLEFISDA	IIHVLHAKHP	SDFGADAQAA	MSKALELFRN	DMAAQYKVLG FHG
Water-buffalo	VKYLEFISDA	IIHVLHDKHP	SDFGADAQAA	MSKALELFRN	EMAAQYKVLG FHG
Sheep	VKYLEFISDA	IIHVLHAKHP	SDFGADAQGA	MSKALELFRN	DMAAQYKVLG FQG
Goat	VKYLEFISDA	IIHVLHAKHP	SDFGADAQGA	MSKALELFRN	DMAAQYKVLG FQG
Pig	VKYLEFISEA	IIQVLQSKHP	GDFGADAQGA	MSKALELFRN	DMAAKYKELG FQG
Horse	IKYLEFISDA	IIHVLHSKHP	GDFGADAQGA	MTKALELFRN	DIAAKYKELG FQG

Figure 3 Comparison of amino acid sequences of livestock myoglobins. Differences in the amino acid sequence are indicated by arrows. Adapted from Joseph, P., Suman, S.P., Li, S., *et al.*, 2010. Characterization of bison (*Bison bison*) myoglobin. Meat Science 84, 71–78.

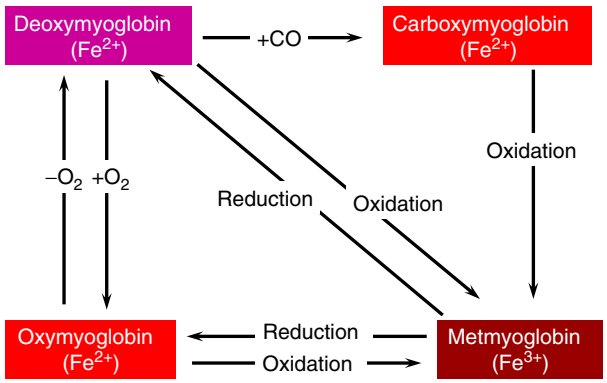

Figure 4 Interconversions of myoglobin redox forms in packaged fresh meats. Adapted from Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Science 71, 100–121 and Rousseaux, J., Dautrevaux, M., Han, K., 1976. Comparison of the amino acid sequence of pig heart myoglobin with other ungulate myoglobin. Biochimica et Biophysica Acta 439, 55–62.

Pigments in Fresh Meats

Myoglobin Redox Forms in Packaged Fresh Meats

In packaged fresh meats, myoglobin can exist in any of the four redox states – deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin (Figure 4). Deoxymyoglobin, oxymyoglobin, and carboxymyoglobin are in the ferrous state. Deoxymyoglobin is purplish-red, whereas oxymyoglobin and carboxymyoglobin provide bright cherry-red color critical to consumer acceptance. Deoxymyoglobin does not have any ligand bound with the heme iron, whereas the sixth coordinate of heme iron is occupied by oxygen in oxymyoglobin and CO in carboxymyoglobin. Formation of brown metmyoglobin results from oxidation of the ferrous forms to a ferric state and is associated with meat discoloration. Metmyoglobin has a water molecule bound at the sixth coordinate of the ferric heme. It is incapable of binding oxygen and thus is physiologically inactive. All the four forms of myoglobin are soluble in cold water, and the best extraction can be achieved by 0.04 M phosphate buffer at pH 6.8. The visible light absorption maxima and chemistry of fresh meat pigments are presented in Table 1.

The porphyrin ring of heme pigments absorbs visible light in the Soret region (400–440 nm). The interactions between heme iron and ligands are responsible for the alpha and beta absorption peaks. Whereas deoxymyoglobin exhibits a strong absorption maximum at 555 nm, oxymyoglobin absorbs at 420 nm in the Soret region and has large twin maxima at 544 and 582 nm. Very similar to oxymyoglobin, carboxymyoglobin also has two absorbance maxima at 543 and 581 nm. Metmyoglobin exhibits peaks at 409 and 500 nm, with a small peak at 630 nm. The absorption spectra of the four redox forms intersect (isobestic point) at 525 nm. Thus, spectrophotometric absorbance at 525 nm is used to estimate total myoglobin concentration in fresh meats. Treatment of meat samples with potassium ferricyanide converts all myoglobin forms into cyanometmyoglobin, allowing quantitation by spectrophotometric measurement of absorbance at 540 nm.

Carbon monoxide has a greater affinity to hemoglobin than oxygen, leading to the displacement of oxygen from blood resulting in carbon monoxide poisoning. Similarly,

Table 1 Chemistry of major pigments in fresh meats

Pigment	*Source species*	*Color*	*Formation*	*Oxidation state of heme iron*	*Status of globin*	*Absorption maximum (nm)*			*Reference*
						Soret	*Alpha*	*Beta*	
Deoxymyoglobin	Horse	Purplish-red	Deoxygenation of oxymyoglobin; reduction of metmyoglobin	Fe^{2+}	Native	439	555		Broumand *et al.* (1958)
Oxymyoglobin	Horse	Cherry-red	Oxygenation of deoxymyoglobin	Fe^{2+}	Native	420	582	544	Bowen (1949)
Metmyoglobin	Horse	Brown	Oxidation of oxymyoglobin and deoxymyoglobin	Fe^{3+}	Native	409	630	500	Bowen (1949)
Carboxymyoglobin	Horse	Cherry-red	Binding of CO with deoxymyoglobin	Fe^{2+}	Native		581	543	Suman *et al.* (2006)
Cyano-metmyoglobin	Pig	Brown	Addition of cyanide to myoglobin	Fe^{3+}	Native			540	Warriss (1979)
Cytochrome c	Horse	Red		Fe^{2+}	Native	415	550	521	Girard *et al.* (1990)
Sulfmyoglobin		Green	Reaction of hydrogen sulfide with myoglobin	Fe^{2+}	Native	420	617		Nicol *et al.* (1970)
Metsulfmyoglobin		Red	Oxidation of sulfmyoglobin	Fe^{3+}	Native	405	715	595	Nicholls (1961)
Acid ferrimyoglobin peroxide		Green	Reaction of hydrogen peroxide with metmyoglobin under acidic conditions (pH 4.5); distal histidine is oxidized	Fe^{3+}	Native			589	Fox *et al.* (1974)
Ferrimyoglobin peroxide		Red	Reaction of hydrogen peroxide with metmyoglobin under alkaline conditions (pH 8)	Fe^{3+}	Native			547	Fox *et al.* (1974)
Ferrocholem yoglobin		Green	Irreversible oxidation of heme in myoglobin with ring opened	Fe^{3+}	Native		635		Nicol *et al.* (1970)

Source: Reproduced with permission from Bowen, W.J., 1949. The absorption spectra and extinction coefficients of myoglobin. Journal of Biological Chemistry 179, 235–245; Broumand, H., Ball, C.O., Stier, E.F., 1958. Factors affecting the quality of prepackaged meat II. E. Determining the proportions of heme derivatives in fresh meat. Food Technology 12, 65–77; Fox, J.B., Nicholas, R.A., Ackerman, S.A., Swift, C.E., 1974. A multiple wavelength analysis of the reaction between hydrogen peroxide and metmyoglobin. Biochemistry 13, 5178–5186; Girard, B., Vanderstoep, J., Richards, J.F., 1990. Characterization of the residual pink color in cooked turkey breast and pork loin. Journal of Food Science 55, 1249–1254; Nicholls, P., 1961. The formation and properties of sulphmyoglobin and sulphcatalase. Biochemistry Journal 81, 374–383; Nicol, D.J., Shaw, M.K., Ledward, D.A., 1970. Hydrogen sulfide production by bacteria and sulfmyoglobin formation in prepacked chilled beef. Applied Microbiology 19, 937–939; Suman, S.P., Mancini, R.A., Faustman, C., 2006. Lipid-oxidation-induced carboxymyoglobin oxidation. Journal of Agricultural and Food Chemistry 54, 9248–9253; and Warriss, P.D., 1979. The extraction of haem pigments from fresh meat. Journal of Food Technology 14, 75–80.

myoglobin also has a high affinity for CO, forming bright cherry-red carboxymyoglobin. The visible light absorption spectra of carboxymyoglobin and oxymyoglobin are nearly identical, and the red color of these two proteins is similar and indistinguishable to the naked eye. Nevertheless, there are distinguishable differences between the absorbance spectra of these two cherry-red redox forms in the alpha and beta peaks. The differences in the relative intensities of the two peaks can be used to differentiate these redox forms in aqueous solutions.

Carboxymyoglobin is more resistant to oxidation than is oxymyoglobin, owing to the strong binding of CO to the iron-porphyrin site on the myoglobin molecule. Therefore, low levels of carbon monoxide are beneficial in modified atmosphere packaging (MAP) of fresh meat to maintain a stable cherry-red color. Between 1985 and 2004, the Norwegian meat industry had safely and effectively used low levels of carbon monoxide in fresh meat MAP. The MAP system consisted of 0.4% CO and variable combinations of nitrogen and carbon dioxide. In 2004, Norway discontinued the use of CO MAP in compliance with the regulations in European Economic Area. A similar MAP system was approved for retailing fresh red meats in the US in 2004. Although myoglobin of fresh meat strongly binds CO, the CO is released

upon exposure to light and heat. Interestingly, carboxymyoglobin is one of the pigments responsible for the pinking of irradiated chicken breast meat, as a result of CO production during the irradiation process.

Cytochrome c

Cytochrome c is a low-molecular weight (13 000 Da; 104 amino acids) hemoprotein, which participates in the redox reactions involving the synthesis of ATP (adenosine tri phosphate) in the mitochondria. Cytochrome c, associated with electron transport chain and inner mitochondrial membrane, can be spectrophotometrically distinguished from myoglobin by its absorption peaks at 521 and 550 nm (Table 1). Relative to the myoglobin content, cytochrome c is found at low levels in red meats than in poultry. However, cytochrome c possesses greater heat stability than myoglobin. Thus it can contribute to the persistent pink color in cooked turkey rolls after other heme pigments have undergone denaturation-induced browning.

Sulfmyoglobin

Reaction of hydrogen sulfide with ferrous heme iron of myoglobin yields the green pigment sulfmyoglobin, with a characteristic absorption peak at 617 nm (Table 1). Greening is associated with the growth of sulfhydryl-producing bacteria *Pseudomonas mephitica*. This organism requires low oxygen tension (less than 1%) and a pH greater than 6.0 for the production of hydrogen sulfide from sulfur-containing amino acids. To avoid the green discoloration, meat with high ultimate pH (such as dark cutting beef) should not be packaged under low oxygen tensions (vacuum packaging or low oxygen-modified atmosphere packaging systems). Oxidation of sulfmyoglobin by oxygen or ferricyanide yields the red pigment metsulfmyoglobin (Table 1), leading to the disappearance of the absorption peak at 617 nm.

Acid Ferrimyoglobin Peroxide

This green pigment is the product of hydrogen peroxide-induced oxidation of myoglobin under acidic conditions, along with oxidation of the distal histidine. It is also known as hydroperoxymetmyoglobin. Lactic acid-producing microbes such as *Lactobacillus viridescens*, *Leuconostoc*, and *Pediococcus* generate hydrogen peroxide under aerobic conditions. These facultative bacteria are salt-tolerant, catalase-negative, and are capable of growing at low temperatures.

Ferrimyoglobin Peroxide

This red pigment is also a product of hydrogen peroxide-induced oxidation of myoglobin. The action of peroxide on myoglobin first generates the red intermediate ferrimyoglobin peroxide. The red pigment accumulates under alkaline conditions (pH 8.0) and is subsequently converted into the green pigment (acid ferrimyoglobin peroxide) under mild acidic conditions of meat.

Ferrocholemyoglobin

The green pigment ferrocholemyoglobin is formed when myoglobin oxidation proceeds extensively leading to cleavage of the porphyrin ring. The degree of myoglobin oxidation can be determined by the addition of a dilute solution of sodium dithionite (sodium hydrosulfite), which is a strong reducing agent. The green pigments sulfmyoglobin and hydroperoxymetmyoglobin are only mildly oxidized and therefore can be converted back to myoglobin by the addition of a reducing solution. However, the green ferrocholemyoglobin is almost completely oxidized and cannot be converted into myoglobin by dithionite.

Lipid Oxidation-Induced Myoglobin Oxidation

Lipid oxidation generates secondary reactive products, such as aldehydes and ketones, which are responsible for the off-odors. Aldehyde products of lipid oxidation covalently bind to myoglobin and accelerate heme oxidation and metmyoglobin formation, leading to meat discoloration. Lipid oxidation-induced meat discoloration has been studied using oxymyoglobin and 4-hydroxynonenal (a highly reactive aldehyde generated by the oxidation of lipids containing polyunsaturated fatty acids). Hydroxynonenal forms adducts with the histidine residues in myoglobin, including distal and proximal histidines, and compromises the protein's redox stability. Lipid oxidation-induced meat discoloration is species-specific; beef oxymyoglobin is more susceptible to a nucleophilic attack by lipid oxidation products than its pork counterpart. The differences in the primary structure of myoglobins, along with the number and location of histidines within the globin chain, are responsible for this observation. Greater oxidation rates are observed in oxymyoglobins with greater number of histidine residues, such as beef and sheep (12 histidines) than in pork and chicken (9 histidines). Comparative kinetic studies using mass spectrometry observed 4-hydroxynonenal adduction at distal histidine (93) in beef myoglobin, but not in pork myoglobin, indicating that lipid oxidation is more critical to beef color than to pork color. Furthermore, 4-hydroxynonenal preferentially adducted 2 histidines (at positions 88 and 81) in the close vicinity of heme pocket in beef myoglobin compared to only one histidine residue (at position 36) far away from the heme pocket in pork myoglobin.

Within a meat species, carboxymyoglobin shares same amino acid sequence with oxymyoglobin, and therefore can theoretically interact with lipid oxidation products in a way similar to oxymyoglobin. Although horse carboxymyoglobin demonstrated greater resistance to browning than horse oxymyoglobin on exposure to 4-hydroxynonenal, the amino acid residues adducted by 4-hydroxynonenal were the same (histidines at 24, 36, 48, 81, and 93) in both the redox forms suggesting possible similar mechanistic interactions.

Pigments in Cooked Meats

Exposure to heat during cooking of meat causes denaturation (unfolding) of the globin protein. The exposed heme is more

prone to oxidation than the one in its native state. Denaturation of globin in metmyoglobin results in the formation of denatured globin hemichrome (also called as ferrihemochrome), which is the pigment responsible for the dull-brown color of cooked meats. This dull-brown pigment is also formed when meat is cooked in the presence of air. Globin denaturation in ferrous myoglobin forms leads to the formation of pink/red ferrohemochrome (denatured globin hemochrome), which subsequently and readily is oxidized to brown ferrihemochrome. Cooking of meat under anaerobic conditions (canned meats and in vacuum bag in hot water) also generates the pink ferrohemochrome. Owing to heat denaturation of the globin protein, these pigments coagulate and therefore are insoluble in water or buffers. Thus, reflectance measurements are used to study cooked meat pigments. The pink denatured globin hemochromes are characterized by the reflectance minima at 530 and 558 nm (Table 2). The minimum at 558 nm is much more pronounced. However, brown hemichromes are characterized by the reflectance minima at 495 and 545 nm. Heat-induced denaturation of carboxymyoglobin (cooking of CO-treated meats) leads to the formation of pink-red denatured globin CO hemochrome.

A variety of pink hemochromes are possible if the heme iron is maintained in the reduced ferrous state (Table 2). All such hemochromes have reflectance minima near 530 and 560 nm. After heat-induced denaturation, nicotinamide or other nitrogen-containing ligands can bind with the heme group at the coordinate previously occupied by globin. Alternatively, the denatured globin may remain associated with heme, but nicotinamide or nitrogen-containing ligands (such as the histidine side chains of other denatured proteins) may associate with the sixth coordinate of heme. Nicotinamide hemochrome and related pyridine hemochromes have been investigated as a replacement for nitrite as additives to generate pink color in cooked meats.

Premature Browning

Premature browning (PMB) is the phenomenon observed in cooked ground beef wherein myoglobin denaturation occurs at a temperature lower than that is required to destroy foodborne pathogens. Because the dull-brown color of cooked beef is often considered as an indicator of doneness, PMB can lead to food safety concerns. The thermal denaturation temperatures are different for myoglobin redox forms, and therefore the incidence of PMB is influenced by the predominant redox form of myoglobin in beef before cooking. The resistance of myoglobin redox forms to thermal denaturation is in the order: deoxymyoglobin > oxymyoglobin > metmyoglobin. Processing strategies that increase the proportion of oxymyoglobin and metmyoglobin in beef, such as oxygen-rich packaging (high-oxygen MAP and aerobic packaging), thawing frozen beef, and bulk packaging, increase the incidence of PMB. In these cases, meat pigments are exposed to oxidative conditions before cooking, causing accelerated pigment denaturation during cooking. However, antioxidants and vacuum packaging increase the relative proportion of deoxymyoglobin and thus minimize PMB. Ground beef packaged in CO MAP demonstrates a low incidence of PMB, presumably due to the increased resistance of carboxymyoglobin pigment to oxidative environments and/or due to the pink-red denatured globin CO hemochrome resulting from the cooking of CO-treated beef.

Pink Color Defect

Pink color defect (PCD) is another color quality problem in cooked meats (primarily observed in poultry), which results in an uncooked pink appearance in fully cooked, uncured products. Consumers often correlate the pink appearance to an uncooked product, although the microbiological safety is

Table 2 Chemistry of major pigments in cooked meats

Pigment	*Source species*	*Color*	*Formation*	*Oxidation state of heme iron*	*Status of globin*	*Reflectance minimum (nm)*			*Reference*
						Soret	*Alpha*	*Beta*	
Denatured globin hemochrome	Cooked pork or beef	Pink or red	Heat-induced denaturation of ferrous myoglobin; reduction of globin hemichrome	Fe^{2+}	Denatured	424	530	558	Tapel (1957); Ghorpade and Cornforth (1993)
Denatured globin hemichrome	Cooked pork	Brown, tan, or gray	Heat-induced denaturation of metmyoglobin; oxidation of globin hemochrome	Fe^{3+}	Denatured	405	495	545	Tarladgis (1962)
Nicotinamide hemochrome	Turkey	Pink or red	Reaction of heat-denatured myoglobin with nicotinamide under reducing conditions	Fe^{2+}	Denatured	420	529	558	Tapel (1957); Cornforth *et al.* (1986)
Denatured globin CO hemochrome	Cooked beef	Pink or red	Heat-induced denaturation of carboxymyoglobin	Fe^{2+}	Denatured		542	571	Tapel (1957); John *et al.* (2004)

Source: Reproduced with permission from Cornforth, D.P., Vahabzadeh, F., Carpenter, C.E., Bartholomew, D.T., 1986. Role of reduced hemochromes in pink color defect of cooked turkey rolls. Journal of Food Science 51, 1132–1135; Ghorpade, V.M., Cornforth, D.P., 1993. Spectra of pigments responsible for pink color in pork roasts cooked to 65 or 82 °C. Journal of Food Science 58, 51–52, 89; John, L., Cornforth, D.P., Carpenter, C.E. *et al.,* 2004. Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. Journal of Food Science 69, 608–614; Tappel, A.L., 1957. Reflectance spectral studies of the hematin pigments of cooked beef. Food Research 22, 404–407; and Tarladgis, B.G., 1962. Interpretation of the spectra of meat pigments. I. Cooked meats. Journal of the Science of Food and Agriculture 13, 481–484.

Table 3 Chemistry of major pigments in cured meats

Pigment	*Source and species*	*Color*	*Formation*	*Oxidation state of heme iron*	*Status of globin*	*Absorption maximum (nm)*	*References*
Nitrosyl metmyoglobin	Pork	Brown	Reaction of nitric oxide with metmyoglobin	Fe^{3+}	Native		Killday *et al.* (1988)
Nitrosyl myoglobin	Pork	Red	Reduction of nitrosyl metmyoglobin	Fe^{2+}	Native		Killday *et al.* (1988)
Nitrosyl hemochrome	Cooked, cured pork	Pink	Head-induced denaturation of nitrosyl myoglobin	Fe^{2+}	Denatured	540	Hornsey (1956)
Nitrimetmyoglobin	Uncooked, cured meat	Green	Addition of excess nitrite	Fe^{3+}	Native		Fox (1987)

Source: Reproduced with permission from Fox, J.B., Jr. 1987. The pigments of meat. In Price, J.F., Schweigert, B.S. (Eds.) The Science of Meat and Meat Products, 3rd ed. Westport, CT, USA: Food and Nutrition Press, pp. 193–216; Hornsey, H.C., 1956. The color of cooked cured pork. I. Estimation of the nitric oxide-heme pigments. Journal of the Science of Food and Agriculture 7, 534–540; and Killday, K.B., Tempesta, M.S., Bailey, M.E., Metral, C.J., 1988. Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the curing reaction. Journal of Agricultural and Food Chemistry 36, 909–914.

assured, and reject such products. Nondenatured myoglobin can be present at significant levels in meats cooked to internal temperatures as high as 80 °C. Meat pH above 6.0 (typical in poultry) also stabilizes myoglobin against heat-induced denaturation, resulting in the pink color after cooking. Although various preharvest and postharvest factors contribute to the occurrence of PCD, the interactions of myoglobin with ligands and small biomolecules are considered as a major endogenous factor contributing to PCD. Pink color may result if meat is exposed to nitrite in ingredients and water used in the processing plants, or to nitrogen dioxide and carbon monoxide in combustion gases from gas-fired ovens. Furthermore, the interiors of large roasts may appear brown immediately after cooking but turn pink during refrigerated storage due to slow conversion of the brown cooked pigment (denatured globin hemichrome) to its reduced form (denatured globin hemochrome). Molecular studies indicated that the unique biochemistry of poultry myoglobins, along with the protective pH of poultry meat (pH 6.2), contributes significantly to the increased thermostability of poultry myoglobins leading to the incidence of PCD.

Pigments in Cured Meats

Cured meat products have a stable pink color, which is generated through the reaction of meat pigments with nitrates/nitrites in curing mixture or with nitrogen dioxide in smoke. Sodium nitrite, applied either onto the surface or injected into the meat, is the ingredient responsible for the pink color of conventionally cured meat. In Roman times and before, meat was preserved using salt. Sea salt contains sodium or potassium nitrates, which are reduced to nitrites by bacteria leading to pink color development. In 1925, the US Department of Agriculture approved the direct addition of sodium nitrite to cured meats, accelerating the curing process. Smoked meats also have a desirable surface pinking, and nitrogen dioxide is the compound in smoke responsible for the cure reaction.

In all three curing pathways (nitrate, nitrite, and nitrogen dioxide), it is nitrous acid that reacts with myoglobin. On contact with water, nitrite forms nitrous acid, which reacts with myoglobin. Myoglobin is oxidized to metmyoglobin by nitrous acid, and subsequently the nitrous acid is reduced to heme-bound NO. Nitric oxide metmyoglobin is brown in color and is reduced to nitrosyl myoglobin (red pigment) under anaerobic conditions (brine equilibration). When subjected to cooking, nitrosyl myoglobin is denatured and converted into nitrosyl hemochrome, which is the final cured pink pigment (Table 3).

Nitrosyl hemochrome levels in meats are determined after extraction in 80% acetone, and the absorbance of the extracted NO–hematin is measured at 540 nm. If the extraction solution is acidified with concentrated HCl, total heme (as hematin) can be determined by absorbance at 640 nm. Cured meats with desirable pink color have 60–80% total pigment existing in the nitrosylated form. Very low levels of nitrite are needed for pinking; as little as 4–6 ppm of sodium nitrite is sufficient for pink color development. Addition of excess nitrite (>1000 ppm) causes excessive myoglobin oxidation to green nitrimetmyoglobin (nitrite burn).

Although nitrosyl hemochrome is stable, it is sensitive to the presence of oxygen, temperature, and light. Exposure to oxygen and light leads to the fading of cured color. Under these conditions, the bound NO dissociates and the heme iron is oxidized to the ferric form. Pink color dissipates as the concentration of gray–tan cooked meat pigment (denatured globin hemichrome) increases. Therefore, cured products are vacuum packaged in opaque protective films for retailing.

See also: Conversion of Muscle to Meat: Color and Texture Deviations. Curing: Brine Curing of Meat; Dry; Physiology of Nitric Oxide; Production Procedures. Packaging: Modified and Controlled Atmosphere; Overwrapping; Vacuum

Further Reading

Bowen, W.J., 1949. The absorption spectra and extinction coefficients of myoglobin. Journal of Biological Chemistry 179, 235–245.

Brown, W.D., Mebine, L.B., 1969. Autoxidation of oxymyoglobin. Journal of Biological Chemistry 244, 6696–6701.

Cornforth, D.P., Rabovitser, J.K., Ahuja, S., *et al.*, 1998. Carbon monoxide, nitric oxide, and nitrogen dioxide levels in gas ovens related to surface pinking of cooked beef and turkey. Journal of Agricultural and Food Chemistry 46, 255–261.

Faustman, C., Phillips, A.L., 2001. Measurement of discoloration in fresh meat. Current Protocols in Food Analytical Chemistry. New York, USA: Wiley and Sons Chapter F3 Unit F3.3.
Faustman, C., Sun, Q., Mancini, R.A., Suman, S.P., 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science 86, 86–94.
Fox Jr, J.B., 1987. The pigments of meat. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products, 3rd ed. Westport, CT, USA: Food and Nutrition Press, pp. 193–216.
Hornsey, H.C., 1956. The color of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. Journal of the Science of Food and Agriculture 7, 534–540.
Joseph, P., Suman, S.P., Li, S., *et al*., 2010. Characterization of bison (*Bison bison*) myoglobin. Meat Science 84, 71–78.
King, N.J., Whyte, R., 2007. Does it look cooked? A review of factors that influence cooked meat color. Journal of Food Science 71, 31–40.
Livingston, D.J., Brown, W.D., 1981. The chemistry of myoglobin and its reactions. Food Technology 35, 244–252.
Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Science 71, 100–121.
Rousseaux, J., Dautrevaux, M., Han, K., 1976. Comparison of the amino acid sequence of pig heart myoglobin with other ungulate myoglobin. Biochimica et Biophysica Acta 439, 55–62.
Suman, S.P., Joseph, P., 2013. Myoglobin chemistry and meat color. Annual Review of Food Science and Technology 4, 79–99.
Suman, S.P., Mancini, R.A., Faustman, C., 2006. Lipid-oxidation-induced carboxymyoglobin oxidation. Journal of Agricultural and Food Chemistry 54, 9248–9253.
Tang, J., Faustman, C., Hoagland, T.A., 2004. Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. Journal of Food Science 69, C717–720.
Wittenberg, J.B., Wittenberg, B.A., 2003. Myoglobin function reassessed. Journal of Experimental Biology 206, 2011–2020.

Relevant Websites

http://www.pdb.org
Research Collaboratory for Structural Bioinformatics.
http://www.expasy.org
Swiss Institute of Bioinformatics.

Palatability

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Glossary

Cold shortening A physiological occurrence that results from rapid temperature decline during the onset of rigor mortis; this process results in muscle fibers that are contracted to a greater extent than muscle fibers from muscles that were not subjected to very cold temperatures during the onset of rigor mortis.

Endomysium The connective tissue that surrounds muscle fibers.

Myofibrillar component The portion of muscle cells that contains the muscle proteins responsible for muscle contraction.

Perimysium The connective tissue that surrounds muscle bundles.

Phospholipid A lipid that contains phosphoric acid and fatty acids esterified to glycerol; found in all living cells and in the bilayers of the plasma membrane.

Protease A protein that is involved in breaking down or degrading other proteins.

Protein denaturation The process of modifying the molecular structure of a protein; alteration of the protein from its original state.

Sarcomere length A measurement of the distance between the two Z lines (outer boundaries) of one sarcomere.

Triglycerides Esters of glycerol that contain three ester groups and either one, two, or three fatty acids attached to the ester group.

Volatiles Compounds that are vaporized from a product.

Introduction

Meat palatability is important as it relates to the eating quality and consumer acceptance of meat as a protein source. Meat palatability relates to how meat tastes and is defined as juiciness, tenderness, and flavor. These three attributes have been related to consumers' perception of overall acceptability and preference. Juiciness is the amount of perceived juices in the meat during chewing or mastication. Tenderness is how easily meat breaks down during chewing. Toughness would be the opposite of tenderness or it is the resistance of meat to breakdown during chewing. Flavor is a combination of smell from the olfactory senses; aromatics perceived during consumption of meat from the olfactory senses; the basic tastes of salt, sour, sweet, and bitter are perceived from the tongue; and feeling factors in the mouth during the consumption of meat. Also, flavor consists of aftertastes perceived after consuming the product. Although these three palatability components have been shown to individually impact consumer preferences, they are also interrelated and changes in one component might affect another component. For example, trained sensory panelists can measure whether a meat sample is dry or juicy and if the same sample is tough or tender. However, when serving the same sample to consumers, they might not differentiate between juiciness and tenderness. Additionally, consumers may perceive a dry product as tougher, even if the samples are similar in mechanical measurements of tenderness.

There are many factors, both antemortem and postmortem, that affect meat palatability. These factors influence meat palatability by affecting the underlying chemical and physical components within meat. Therefore, it is important to understand how chemical and physical components of meat impact meat palatability before understanding how meat palatability can be managed, altered, or impacted by live animal management, harvesting, or processing. Meat is comprised of lean tissue or muscle fiber cells (also called the myofibrillar component of meat), fat, and connective tissue. Fat or adipose cells are found intramuscularly as marbling, contained between muscles as seam fat, or fat that is deposited externally as subcutaneous fat. The three major components of meat – fat, lean or the myofibrillar component, and connective tissue – impact meat palatability in different ways.

Fat or Lipids: Effects on Meat Palatability

Intramuscular fat content or marbling affects flavor, juiciness, and tenderness of meat. The 'Window of Acceptability' presented in Figure 1 illustrates the role of increased intramuscular fat on pork, lamb, and beef palatability. In general, as fat content increases, palatability increases, but the rate of improvement in palatability with each incremental increase in fat is not constant. When meat contains less than 3% fat, meat palatability is lowest and is outside the acceptability window. As fat increases from less than 1% to 3%, palatability increases at the highest rate. In fact, this is the steepest slope on the curve or where the greatest improvements in meat palatability occur. As fat increases from 3% to approximately 6%, meat palatability improves, but not as dramatically as reported at the lower levels. As fat content exceeds 7.3%, meat fat content is again outside the Window of Acceptability as the fat is plainly visible and might be perceived by health-conscious consumers as too high in fat content. High fat content in foods has been related to increased incidence of coronary heart disease, obesity, or some forms of cancer in humans. Diet/health-conscious consumers may be willing to sacrifice palatability for lower fat content meat in order to reduce their risk of the aforementioned dietary-related diseases. Therefore, meat with fat content between 3% and 7.3% is generally considered acceptable.

As meat fat content increases, juiciness increases. During initial chewing of high fat content meat, fat is released. Humans

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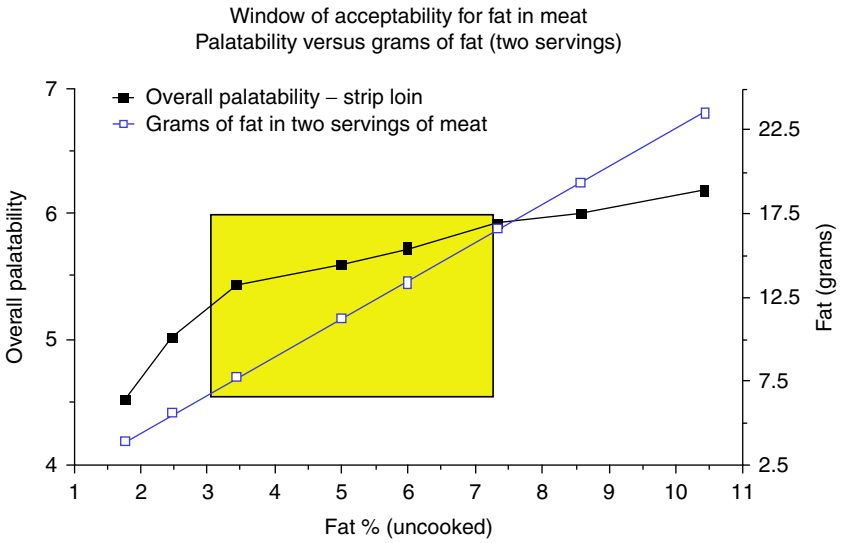

Figure 1 The window of acceptability to demonstrate the relationship between grams of fat and meat palatability. Adapted from Savell, J.W., Cross, H.R., 1988. The role of fat in the palatability of beef, pork, and lamb. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Academy Press.

perceive this fat as juice. Additionally, when fat is released early in the chewing process, the salivary glands are stimulated. With increased salivation, the meat is perceived as juicier. Meat with higher fat content will have longer sustained perception of juiciness. Also, fat affects juiciness by lubricating the muscle fibers during cooking and by increasing tenderness of meat.

Four Theories of Marbling or Lipid Contribution to Meat Tenderness

Intramuscular fat content affects meat tenderness, even though this relationship is not always strong. There is conflicting information on the relationship between meat fat content and tenderness. Four theories are proposed on why meat with higher fat content is tender. The 'bulk density theory' says that as fat is not as dense as heat-denatured meat proteins, meat with higher amounts of fat or soft tissue is more tender. Another way to express the relationship is that in high fat meat, a higher percentage of the bulk of meat is soft, the meat is more tender.

The second theory is the 'lubrication effect.' The majority of fat in meat is stored as triglycerides that are found in adipose cells. Adipose cells make up marbling or intramuscular fat and these cells are embedded in connective tissue surrounding muscle fiber bundles called perimysium. When meat is chewed, these lipids are released and they provide lubrication between muscle fibers. With higher lubrication, muscle fibers can give more during chewing or provide less resistance. If there is less resistance during chewing and the meat is perceived as more tender.

The third theory is the 'insurance theory.' This theory proposes that fat slows or provides insurance against severe heat-induced toughening of muscle fibers during cooking. Muscle cells are approximately 17% protein and approximately 75% water. Meat proteins help to hold water within the muscle. When meat proteins are heated, they denature and lose some of their abilities to hold water. The more severe the denaturation or the higher proportion of muscle fibers that are severely denatured, the greater the amount of water that is released from meat during cooking. This phenomena is called cooking loss. The weight of meat lost during cooking is mainly water, even though small amounts of water-soluble proteins and lipid also are lost. The goal is to limit the amount of water lost during cooking so that the muscle fiber proteins are not concentrated; the more concentrated the muscle fiber proteins are, the tougher the meat is. As fat is an insulator, or heat does not easily transfer through fat, meat with a higher fat content will slow down heat transfer during cooking. When heat transfer is not as rapid or severe, meat proteins do not denature to a great extent and less moisture is lost during cooking and the resulting meat is more tender.

The last theory is called the 'strain theory.' Adipose cells or marbling are imbedded in connective tissue perimysium. As marbling increases, perimysial connective tissue is weakened and does not play a large role in meat toughness. Therefore, the connective tissue is strained and the meat is more tender.

The four theories are difficult to prove and they are most likely not independent. Each theory could be contributing to fat's relationship with meat tenderness. Although some studies show a low to no relationship between meat fat content and tenderness, in general, whether palatability is measured using trained sensory panels or by consumers, meat with a higher fat content tends to be more tender, juicier, and flavorful. Meat with higher marbling provides some insurance against toughening and drying when cooked to higher degrees of doneness.

Use of Marbling or Intramuscular Fat to Segment Meat for Expected Palatability Differences

An example of marbling as an indicator of meat palatability is found in the United States Department of Agriculture (USDA), Agricultural Marketing Service (AMS) Beef Quality Grading System. The purpose of the USDA, AMS Beef Quality Grading

System is to segment beef carcasses into classes based on expected meat palatability. Beef cuts that qualify for higher beef quality grades are expected to be more tender, juicier, and flavorful and the value of this beef is greater. For carcasses from young cattle, the most common USDA, AMS Beef quality grades are from highest to lowest: prime, choice, select, and standard. To classify for a quality grade, the beef carcass is assessed for physiological age and the marbling in the Longissimus muscle at the 12th and 13th rib interface is assessed. The greater the marbling score, the higher the quality grade assigned and the higher the expected meat palatability. Figure 2 shows the relationship between the four major USDA quality grades for young beef and overall palatability rated by a trained sensory panel for beef top loin steaks. The figure shows the sensory attribute overall palatability score that was a combination of juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness, and flavor intensity using eight-point descriptive scales. Marbling predicted beef palatability attributes with 30–38% accuracy and as quality grade increased, beef top loin steaks were more palatable. As the quality grade increased, the range of variation in palatability decreased as exemplified by the reduction in the size of the box for each higher quality grade. The size of boxes in Figure 2 represents the range of sensory scores for top loin steaks in that quality grade. Using a five on the bottom scale as the lowest level of acceptability (a five on this scale represents a verbal score slightly desirable for juiciness, tenderness, and flavor), the percentage of ratings less than five decreased as the quality grade increased. Note that the greatest improvements in reducing the number of steaks with ratings less than five occurred when quality grade changed from standard (traces and practically devoid marbling or less than 2% chemical lipid) to select (slight marbling or ~3% chemical lipid). As beef quality grade moved from select to choice, the percentage of samples with ratings below five decreased from 26.4% to 10.8%, respectively. Steaks from Prime carcasses only had 5.6% of the steaks with ratings less than five for overall palatability. This figure shows that marbling, or quality grades, are positively related to overall palatability. However, sensory ratings across quality grades overlap or there were standard steaks that were as palatable as prime steaks (Figure 3).

Consumers also can detect differences in meat palatability as marbling score changes. In the United States, consumers tended to rate top loin steaks with the highest amount of marbling (Top Choice that includes USDA, AMS marbling scores of modest and moderate) higher for palatability traits than those with low slight marbling (Table 1). Trained sensory panelists found that as marbling score increased, cooked beef top loin steaks from top choice were juicier, more tender, had more intense flavor, and they had higher levels of beef flavor and beef fat flavor than those with the lower marbling score of slight. Warner–Bratzler shear force values (a mechanical measure of meat tenderness) decreased, or steaks were more tender, for marbling scores of moderate, modest, and small (Top and Low Choice grades) than those with slight marbling (Select grade). The marbling to meat palatability relationship is not always strong for cuts from different muscles within an animal. When top round and top sirloin steaks were evaluated in the same study, marbling did not affect consumer ratings. Steaks derived from lower fat beef muscles tend not to be affected by the marbling to meat palatability relationship. As there is not as much fat and the fat content is less variable in other muscles, it would stand to reason that marbling would not impact palatability to the same extent. In muscles used for locomotion that have higher amounts of connective tissue, the higher amount of connective tissue plays a greater role in the palatability or tenderness than marbling or fat content.

The marbling or lipid to meat palatability relationship may not be as strong in meat from animals of other species as reported for beef. In a US pork consumer study, consumers rated pork loin chops the same regardless of lipid content (Table 2). pH of the meat had a greater impact on consumer

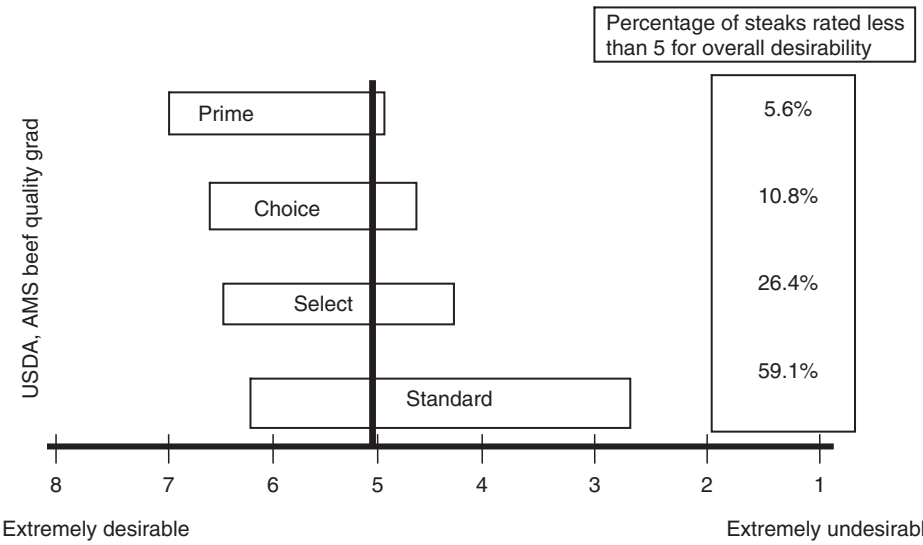

Figure 2 Percentage of loin steaks receiving desireable and undesirable overall palatability ratings, where 8 = extremely desirable palatability and 1 = extremely undesirable palatability. Adapted from Smith, G.C., Savell, J.W., Cross, H.R., *et al.*, 1987. Relationship of USDA quality grades to palatability of cooked beef. Journal of Food Quality 10, 269–286.

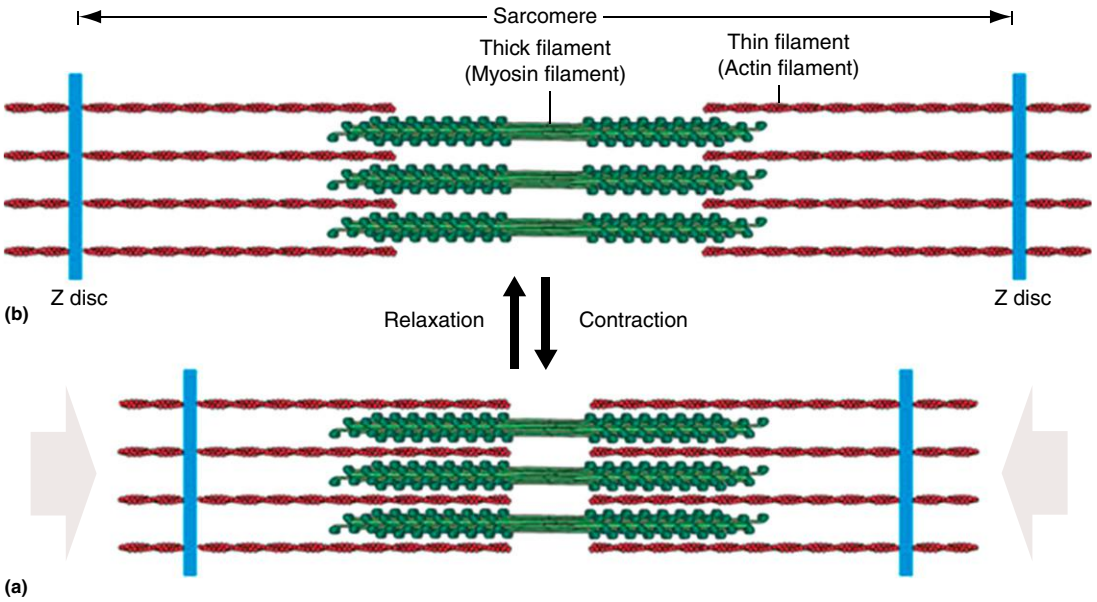

Figure 3 Illustrations of muscle fiber contractile state of postrigor muscle, where (a) shows a muscle fiber with a shorter sarcomere length than (b). The sarcomere length in (a) would be considered cold shortened and the sacromere length in (b) would be normal. The subsequent cooked meat from sample (a) would be tougher than the cooked meat from sample (b).

Table 1 Least squares means of top loin steaks from US Beef Customer Satisfaction Study for consumer sensory attributes,[a] trained meat descriptive sensory attributes and Warner–Bratzler shear force (kg) as effected by USDA quality grade

Sensory attribute	*USDA quality grade*				
	Top choice	*Low choice*	*High select*	*Low select*	P-*value*
Consumer sensory attributes[a]					
Overall like/dislike	19.3[e]	19.0[d]	18.9[c,d]	18.7[c]	.0004
Juiciness	18.6[e]	18.3[d]	18.2[d]	17.9[c]	.0006
Tenderness like/dislike	19.0[c,d]	19.2[d]	18.6[c,d]	18.6[c]	.0001
Flavor intensity	19.1[d]	19.2[d]	18.9[c,d]	18.9[c]	.0009
Flavor like/dislike	19.4[e]	19.2[e]	19.1[d]	18.9[c]	.0002
Trained meat descriptive sensory attributes[b]					
Juiciness	5.8[d]	5.6[c,d]	5.5[c,d]	5.4[c]	.0001
Muscle fiber tenderness	6.7[e]	6.6[e,d]	6.5[c,d]	6.5[c]	.01
Connective tissue amount	6.8	6.9	6.9	6.9	.55
Overall tenderness	6.6	6.6	6.5	6.5	.06
Flavor intensity	5.7[d]	5.7[c]	5.6[c]	5.6[c]	.002
Beef flavor intensity[f]	3.5[e]	3.5[d]	3.3[c]	3.3[c]	.0001
Beef fat flavor intensity	2.1[e]	2.0[d]	1.8[c]	1.8[c]	.0001
Mechanical tenderness measurement[b]					
Warner–Bratzler shear force, kg	2.70[d]	2.75[d]	3.00[c]	2.95[c]	.0002

[a]Values from Neely *et al.* (1998) and Lorenzen *et al.* (1999). Some values differ from citations because values were not reported and models differed slightly in order to generate these least squares means. Consumers sensory attributes were rated as 1 = dislike extremely, not at all juicy, not at all tender, dislike extremely, and no flavor at all, respectively and 23 = like extremely, extremely tender, extremely juicy, like extremely, and an extreme amount of flavor, respectively.

[b]Values are unpublished data, but they were derived from the same data set as published by Neely *et al.* (1998) and Lorenzen *et al.* (1999). Some values differ from citations because values were not reported and models differed slightly in order to generate these least squares means.

[c]Least squares means within a row and a cut lacking a common superscript differ ($P < .05$).

[d]Least squares means within a row and a cut lacking a common superscript differ ($P < .05$).

[e]Least squares means within a row and a cut lacking a common superscript differ ($P < .05$).

[f]Least squares means for top choice and low choice differ although numerically the same due to rounding to the nearest tenth.

palatability and acceptability than lipid content. However, Japanese consumers rated pork loin chops with greater lipid or marbling as juicier, more flavorful, better in taste with more desirable color. Moreover, Japanese customers tended to like the amount of fat and visual appearance when compared to pork chops with lower marbling (Table 3). Therefore, the

Table 2 Least squares means for pork consumer sensory traits[a] as effected by predetermined categories of lipid, Warner–Bratzer shear force, and pH from loin chops from the US Pork Consumer Sensory Study

Trait	*n*	*Juiciness*	*Tenderness*	*Flavor*	*Overall like*
pH category[b]		.04	.0165	.06	.03
Low	648	3.3[d]	3.3[d]	3.2	3.2[d]
Medium	620	3.3[d]	3.3[d]	3.2	3.2[d]
High	498	3.5[e]	3.4[e]	3.4	3.4[e]
RSD[c]		1.13	1.08	1.10	1.03
Lipid category[b]		.20	.19	.09	.18
Low	427	3.4	3.3	3.3	3.2
Medium	857	3.3	3.3	3.2	3.2
High	482	3.4	3.4	3.4	3.3
RSD[c]		1.3	1.08	1.05	1.03
Shear category[b]		.0004	.0001	.0004	.0001
High	379	3.2[d]	3.1[d]	3.1[d]	3.0[d]
Medium	844	3.4[d]	3.3[e]	3.3[e]	3.3[e]
Low	520	3.5[e]	3.5[f]	3.4[e]	3.4[e]
RSD[c]		1.12	1.07	1.05	1.03

[a]Consumer attributes were evaluated using a 5-point hedonic, end-anchored sensory scale where 1 = dislike extremely and 5 = like extremely.
[b]*P*-value from the Analysis of Variance table.
[c]RSD = Residual Standard Deviation from the Analysis of Variance table.
[d]Least squares means within a column and a trait lacking a common superscript differ ($P < .05$).
[e]Least squares means within a column and a trait lacking a common superscript differ ($P < .05$).
[f]Least squares means within a column and a trait lacking a common superscript differ ($P < .05$).
Source: Adapted from Miller, R.K., Moeller, S.J., Goodwin, R.N., Lorenzen, C.L., Savell, J.W., 2000. Consistency in meat quality. International Congress of Meat Science and Technology 46, 566–580.

marbling to meat palatability relationship is also affected by cultural and geographic influences on consumer preferences.

Marbling or Intramuscular Fat as an Indirect Measure of Meat Tenderness

Intramuscular fat also has an indirect relationship to meat tenderness. As animals grow and develop, fat is deposited sequentially into five different fat depots – mesenteric fat; kidney, pelvic, and heart fat; subcutaneous fat; seam fat; and marbling or intramuscular fat. As marbing is the last fat depot to be deposited, it can be used as an indication of growth and nutritional status of animals. If animals are fed high energy-based diets, they grow rapidly or they have high rates of protein and lipid accretion. Therefore, these animals are heavier with higher levels of subcutaneous, seam, and intramuscular fat and greater muscle mass. These heavier, fatter, and more muscular carcasses chill slower and are less susceptible to cold-induced toughening. Meat from early postmortem muscle subjected to cold shortening or cold-induced toughening has shorter muscle contractile state that results in tougher meat. Additionally, animals fed energy-based diets that grow rapidly have higher collagen solubility. Meat that has greater collagen solubility will be more tender because, during cooking, more of the collagen matrix (the main component of connective tissue) will melt. As more collagen melts, the connective tissue within the muscle will not contribute toward meat toughness or the meat is more tender.

Marbling or intramuscular fat positively affects meat flavor (Tables 1–3). As fat level increases, consumers tend to like the flavor of beef and pork. Fat has a characteristic flavor and is one of the major components of meat flavor. Many times it is not the predominant flavor in meat, but it does provide a balance with lean meat flavors. When meat contains very low levels of fat, the predominant flavors are associated with the

Table 3 Least squares means for consumer sensory scores of pork loin chops from the Japanese Pork Consumer Study that vary by National Pork Producers Council (NPPC) marbling scores determined at the 10th rib in the Longissimus muscle

Consumer attribute	*Marbling score*[b]						P-*value*
	1	*2*	*3*	*4*	*5*	*6*	
Aroma like/dislike[a]	3.20	3.11	3.16	3.27	3.87	3.00	.13
Juiciness like/dislike[a]	3.09[c,d]	3.00[c]	3.01[c,d]	3.13[c,d]	4.12[d]	3.36[c,d]	.048
Tenderness like/dislike[a]	3.34	3.29	3.25	3.39	4.25	3.82	.07
Flavor like/dislike[a]	3.15[c]	3.19[c]	3.14[c]	3.29[c]	4.12[d]	3.64[c,d]	.04
Overall taste like/dislike[a]	3.15[c]	3.16[c]	3.12[c]	3.34[c,d]	4.25[e]	3.82[d,e]	.006
Appearance like/dislike[a]	3.01[c]	3.11[c,d]	3.19[c,d]	3.32[c,d]	3.75[d]	2.82[c]	.02
Color like/dislike[a]	3.07[c]	3.17[c]	3.23[c,d]	3.28[c,d]	3.87[d]	2.82[c]	.04
Amount of fat like/dislike[a]	3.06[c]	3.19[c,d]	3.26[d]	3.36[d]	3.75[d]	3.09[c,d]	.02

[a]Consumer attributes were evaluated using a 5-point scale where 1 = dislike extremely and 5 = like extremely.
[b]National Pork Producers Council new fresh meat marbling scores where 1 ≤ 1% lipid, 2 = 2% lipid; 3 = 3% lipid, 4 = 4% lipid, 5 = 5% lipid, and 6 ≥ 6% lipid.
[c]Least squares means within a row lacking a common superscript differ ($P < .05$).
[d]Least squares means within a row lacking a common superscript differ ($P < .05$).
[e]Least squares means within a row lacking a common superscript differ ($P < .05$).
Source: Adapted from Miller, R.K., Moeller, S.J., Goodwin, R.N., Lorenzen, C.L., Savell, J.W., 2000. Consistency in meat quality. International Congress of Meat Science and Technology 46, 566–580.

lean, such as cooked beef lean, serumy, bloody, grainy, metallic, livery/organy, and brothy flavor aromatics. As the level of fat or marbling increases, the cooked fat aromatic or flavor increases in meat and this aromatic can assist in decreasing or masking flavor attributes associated with lean, thus providing a balance of meat flavors. The chemical basis of how adipose tissue and lipids contribute toward meat flavor will be discussed in the Section Lipids and Off-Flavor Development.

Muscle Fiber or Lean Components Contribution to Meat Palatability

Muscle fibers, or the cellular structure of meat, are the main components of lean meat. The unique aspect of muscle fibers is that they contain a very organized array of proteins that perform various functions. Muscle fiber proteins are classified either as contractile proteins, structural proteins, or regulatory proteins. The contractile proteins are the most abundant proteins and they perform work within the muscle fiber as well as function to bind water within the muscle cell. Structural proteins provide the lattice work or structural support for the muscle fiber and assist in organizing components of the fiber. The structural integrity, the ability of the muscle proteins to bind water, and the contractile state of the muscle fiber affects meat tenderness and juiciness.

In living tissue, muscle fibers are elastic and have the ability to contract and relax. During the conversion of muscle into meat, muscle undergoes a stiffening process called rigor mortis. In this process, muscle fibers lose their ability to relax. They stiffen and become inelastic. Sarcomere length is the distance between the two Z lines within a sarcomere. Z lines are rigid structures that compose the exterior of a sarcomere. They are very strong structures that have to withstand the forces applied during contraction. As Z line concentration increases within a given quantity of meat, meat is tougher as there is a higher concentration of rigid structures to bite or chew through. Conditions that result in shorter sarcomere lengths produce meat that is tougher; whereas conditions that result in muscles with longer sarcomere lengths produce more tender meat.

As sarcomere length shortens, there is less physical space for water to bind to the contractile proteins of actin and myosin. As a result, there is less physical water bound in the meat and juiciness decreases.

Strength of the structural components within the muscle fiber impacts meat tenderness. As the structural apparatus of muscle is degraded and weakened, meat tenderness improves. The structural apparatus of muscle fibers consist of Z lines and multiple structural proteins that hold the myofilaments in an organized array and help to strap the protein structures together and to the sarcolemma. With timely postmortem, degradative enzymes work to break apart the muscle fiber structural apparatus. As the structural component of the muscle fiber degrades, meat is more tender. This phenomena is called meat aging. Meat that is inherently tender immediately postrigor tends to not undergo substantial meat aging or tenderization with storage. Meat aging is also impacted by sarcomere length. Muscle fibers that are shorter do not degrade as rapidly postmortem as there is not sufficient room for degradative enzymes to work within the crowded sarcomere.

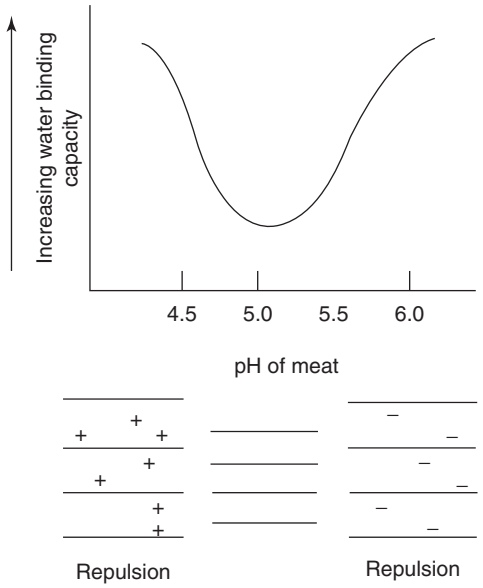

Figure 4 An illustration of the relationship between pH and water-holding capacity of muscle, where the isoelecric point is the pH where water-holding capacity is lowest. Adapted from Aberle, E.D., Forrest, J.C., Gerrard, D.E., Mills, E.W., 2001. Principles of Meat Science, fourth ed. Debuque, IA: Kendall/Hunt Publishing Company.

The ability of myofibrillar proteins to bind water within the muscle fiber also is related to meat tenderness and juiciness. Myofibrillar proteins have charged side-groups that contain ionic charges and these ionic charges bind water. Actin and myosin, the most abundant proteins in the muscle fiber, bind the majority of water within the muscle fiber. The charge on proteins can be either positive or negative. Charges on proteins can be altered by changing the pH. As pH increases, there is a net increase of negative charges and as pH decreases, protein side-groups become more positively charged (Figure 4). As the net charge of proteins become either more positively or negatively charged, ionic forces increase and water is bound or held more tightly to the proteins. An increase or decrease in meat pH will change the ratio of positive and negative charges on protein side-chains and will alter the ability of muscle proteins to bind water. The isoelectric point of a protein is the pH where there is a balance of positive and negative charges on the protein side-groups. The isoelectric point is where muscle proteins have the lowest ability to bind water. As meat pH reaches the isoelectric point, meat loses more water as drip loss during storage or water loss during cooking. As meat loses more water either during storage or during cooking, the meat becomes drier and tougher. Therefore, meat pH is an important component of meat quality as it relates to the ability of muscle proteins to bind water and the subsequent juiciness and tenderness of the meat.

Connective Tissue Influences on Meat Palatability

Muscles used for work or major movements have higher connective tissue content than muscles that provide structural support. Muscles with higher connective tissue are tougher. In

Table 4 The relationship between muscle, Warner–Bratzler shear force, collagen content (mg gm^{-1}), and collagen solubility (%) in beef

Muscle	*Warner–Bratzler shear force, kg*	*Total collagen content, mg gm^{-1}*
Adductor	4.5	12.31
Biceps brachii	3.3	13.14
Biceps femoris	4.5	12.36
Brachialis	4.8	11.81
Brachiocephalicus	7.0	11.28
Compexus	4.7	12.59
Cutaneous omobrachiallis	–	10.72
Deltoideous	5.3	13.57
Gluteus medius	6.0	11.80
Gracilis	4.1	15.20
Infraspinatus	3.5	8.72
Intertransversales	4.7	13.82
Latissimus dorsi	4.9	12.53
Levatores costarum	3.0	8.87
Longissimus capitis et altantis	4.0	11.87
Longissimus costarum	5.2	13.39
Longissimus dorsi	5.0	14.49
Multifidus/spinalis dorsi	3.4	16.20
Pectineus	3.7	12.97
Rectus femoris	3.6	11.06
Rhomboideus	6.1	12.27
Sartorius	4.5	10.49
Scalenus dorsalis	5.1	10.06
Semimembranosus	4.3	10.40
Semitendinosus	4.7	11.56
Serratus ventralis	3.8	8.78
Splenius	4.9	11.16
Subscapularis	3.4	10.64
Superficial pectoral	4.4	8.21
Supraspinatus	5.2	17.77
Tensor fasciae antibrachii	6.4	9.95
Teres major	3.7	11.33
Trapezius	–	8.85
Triceps brachii	4.2	9.97
Vastus intermedius	4.0	9.89
Vastus lateralis	5.3	12.71
Vastus medialis	3.7	14.92

Source: Adapted from the National Cattlemens' Beef Association Muscle Profiling Study from the University of Nebraska Lincoln Department of Animal. Available at: http://bovine.unl.edu/ (accessed 20.03.14).

general, muscles from the hindquarter of animals used for locomotion, such as the Biceps femoris and Semimebranosus are inherently tougher than structural support muscles, such as the Longissiumus lumborum. Table 4 shows the relationship between collagen content and tenderness as measured by Warner–Bratzler shear force for 37 different beef muscles. As collagen content increases, muscles tend to be tougher.

Connective tissue is composed of proteins bound in a lattice-type structure. The major protein in connective tissue is collagen, even though elastin, reticulin, proteoglycans, and glycoproteins are also components of the connective tissue matrix. Collagen crosslinks within the connective tissue matrix and the type of crosslinks impacts meat tenderness. Crosslinks are either heat soluble or heat insoluble. In raw meat, collagen is elastic; however, with cooking, collagen undergoes thermal shrinkage and it can shrink to one quarter of its length. If collagen is bound by heat-soluble crosslinks, the protein can solubilize from the meat and not contribute to meat tenderness. However, if the collagen is crosslinked into stable linkages, the collagen matrix will shrink and develop force. The end result is tougher meat. Therefore, solubilized crosslinks are more tender and, on solubilization, collagen does not contribute negatively to meat tenderness. As animals age, the percentage of heat-stable or insoluble crosslinks increases. As the percentage of heat-stable crosslinks increase, meat is tougher. Meat from older animals is usually tougher than meat from younger animals and this phenomena is mainly due to increased proportion or percentage of collagen crosslinks in the heat-stable form.

Chemical Development and Reactions of Meat Flavor

Cooked meat flavor is the result of chemical reactions that occur within and between the lipid and lean portions of meat during cooking. Raw meat contains very little aroma. Raw meat aroma can be described as bloodlike or one that has a serumy taste, but precursors to cooked meat flavor are contained within raw meat even though in the raw state these precursors are nonvolatile or nondetectable. In general, cooked meat flavor develops as a result of interactions between amino acids, peptides, reducing sugars, vitamins, and nucleotides or their breakdown products during cooking from the lean component. Lipids also play a role in meat flavor and much of the species-specific flavor of meat is derived from adipose tissue. Lipid degradation and oxidation both contribute to meat flavor, usually negatively by contributing off-flavors.

Sulfurous- and carbonyl-containing volatile compounds are thought to be mainly responsible for flavor aromatics in meat. These chemical reactions are complex and intermediate reaction products can interreact with multiple products.

From a sensory standpoint, meat flavor is segmented into aroma or smell before consumption by the olfactory senses, the flavor aromatic perceived by the olfactory senses during chewing, basic tastes of sweet, sour, bitter, and salty sensed from taste receptors on the tongue; mouthfeels identified from the trigeminal receptors in the mouth that provide astringent and metallic sensory attributes; and aftertastes that are perceived after swallowing that are almost always flavor attributes perceived by the olfactory senses. The underlying chemical components that contribute to these sensory attributes have been extensively studied.

Cooked Meat Flavor Development

Cooked meat aroma or flavor aromatics are mainly derived from volatile compounds that develop during heating. In beef, 880 volatile components have been identified, but only 25 have meaty odors. Poultry has more than 450 volatile components with 16 primary odor compounds having been identified. Most of these compounds are the result of the effect of heat on sugars or amino acids. These reactions are defined

as Strecker degradations and Maillard reactions, and the secondary products of these reactions also can react and contribute to cooked meat aroma and flavor aromatics. Strecker degradation is the reaction of α-amino acids that results in the formation of alkylpyrazines. These Strecker aldehydes have meat aroma. Maillard reactions are the interaction of a free amino group, such as an amino acid, amines, peptides, proteins, or ammonia with a carbonyl compound. Examples of carbonyl compounds are aldehydes, ketones, and reducing sugars. Numerous volatile compounds or products result from this reaction as a wide array of precursors and reactive substances are available for reaction.

Maillard reactions are a series of reactions involving the reaction of aldehydes with amines and the ultimate development of meat flavor and dark pigments. In meat, the first reactions involve the reaction of an α-amino with an aldose or ketose. This results in the formation of Amadori or Heyns nonvolatile compounds. These compounds are heat labile, and with heat they decompose in the second-stage reactions. The result of the second stage is the formation of highly reactive compounds that decompose and further interreact. Also dehydration of cyclic forms of Amadori compounds contribute to the formation of volatile compounds. Maillard reactions with cysteine or cystine, sulfur-containing amino acids, have been extensively studied as these reactions and the subsequent products are major contributors to meat flavor.

Thermal degradation of thiamine is also an important meat flavor reaction. During heating, thiamine decomposition can result in the formation of carbonyls, furanoids, thiophenoids, thiazoles, and aliphatic sulfur compounds. These compounds contribute toward flavor and can also react with Maillard reactions products to form meat volatile compounds.

Basic Tastes in Meat

Compounds in muscle foods that contribute to the basic tastes of sweet, sour, bitter, and salty have been identified. Sweet basic tastes in muscle foods are related to the presence of carbohydrate and L-amino acid compounds. Levels of carbohydrates, such as glucose, fructose, ribose, and many L-amino, such as glycine, alanine, serine, threonine, lysine, cysteine, methionine, asparagines, glutamine, proline, and hydroxyproline contribute toward the sweet taste in muscle foods. Sour basic taste is derived from acid components in meat, such as aspartic acid, glutamic acid, histidine, asparagines, succinic, lactic, inosinic, orthophosphoric, and pyrrolidone carboxylic acids. Inorganic salts and the sodium salts of glutamate and aspartate contribute toward the salty basic tastes in muscle foods. Bitter basic taste comes from the presence of hypoxanthine, anserine, carnosine and other peptides, and the L-amino acids of histidine, arginine, lysine, methionine, valine, leucine, isoleucine, phenylalanine, tryptophan, tyrosine, asparagines, and glutamine. Umami tastes or the savory characteristic of meat is mainly derived from glutamic acid, but the presence of monosodium glutamate, 5′-inosine monophosphate, 5′-guanosine monophosphate, and other specific peptides also can contribute to umami tastes in muscle foods.

Species-Specific Flavor of Meat

Lipids not only contribute fat flavor to meat, but species-specific flavors are also found in the fat component. Lipid derived from the phospholipid component in meat (phospholipids are mainly found in muscle fiber membranes and make up $\sim$1% of the lipid in meat) contributes chemically to the development of meaty flavor aromatics, and the lipid stored as triglycerides are not essential to the development of meat flavor, but contribute to cooked fat-specific flavors. It is thought that heating of lipids results in volitilization of compounds contained in adipose cells that are species-specific. Therefore, the flavors that are specific to beef, lamb, pork, poultry, or fish are derived from adipose tissue. Additionally, adipose cells serve as reservoirs for storage of aroma compounds derived from external sources, such as the environment, feedstuffs, or for compounds that contribute toward flavor-forming reactions. The seasonal development of off-flavor in catfish is associated with the concentration of aromatic compounds in plants found at the bottom of lakes where catfish reside. For example, flavor aromatics of 2-methylisoborneol/lagoon/bluegreen, geosmin/musty/woody, rotten plants, and grainy/corn/green vegetable can be found in catfish. These aromatic flavors are associated with blue-green algae growth in pond water, decaying wet wood, decaying vegetation and feed grains, and corn husks, respectively. As catfish consume what is in their environment, off-flavors associated with increasing concentrations of these materials will be reflected in the harvested catfish meat. Similarly, cattle grazing on plants high in selenium will have off-flavors associated with the flavor of selenium and its by-products.

Lipids and Off-Flavor Development

Lipids also contribute to meat flavor through their contribution to the development of off-flavors, mainly through autooxidation or oxidation of lipids. Autooxidation is a three-step chemical process. The first step, or the initiation step, involves the attack of oxygen on unsaturated fatty acid double bonds to form highly reactive free-radical compounds and hydroperoxide. Phospholipds in muscle fibers have been shown to be the lipids most susceptible to lipid oxidation due to their high concentration of polyunsaturated fatty acids. Initiation reactions can be catalyzed by heme compounds but the predominant thought is that nonheme compounds most likely act as catalysts in meat systems. However, heme-containing compounds found in meat, such as hemoglobin, myoglobin, and cytochromes, also have been shown to contribute toward catalyzing the initiation of lipid oxidation. These highly reactive free-radicals then react in multiple potential reactions during the second step, or propagation. In propagation, multiple new peroxy radicals, hydroperoxides, and new hydrocarbon radicals are formed. Products of propagation interreact to form stable end-products in the third step, or termination. Some products from propagation and termination are volatile and contribute to off-flavor. These flavors have been described as rancid or the flavor in general has been called warmed-over flavor in cooked meats. More

specifically these off-flavor aromatics have been described as cardboardy, painty, fishy, and livery.

Other off-flavors in meat are also associated with species-specific issues such as mutton flavor in lamb that is a result of concentration of 4-methyloctanoic and 4-methylnonanoic acids or boar-odor in intact swine that is due to the presence of 5α androst-16ene-3one, a metabolite of testoterone.

Measuring Meat Flavor

Meat flavor can be detected either chemically or by using humans, defined as 'sensory evaluation.' Sensory evaluation may include either trained or consumer methods. Chemical detection of meat flavor identifies the volatile compounds from meat using gas chromatography. The compounds are separated, usually by molecular weight, and can be identified using mass spectroscopy. Ideally, as compounds come from the gas chromatography to the mass spectrometer, a portion of the compounds are directed to a sniff port. A trained evaluator can smell the compounds as they are eluded and identify the odor characteristics and intensity of volatile compounds. Because all volatile compounds not are detectable by humans, this information can be used to identify compounds that contribute to the flavor of meat. Multivariate techniques, mainly principal component analyses, can be used to identify compounds that contribute toward flavor attributes in the meat.

Flavor attributes in meat are commonly defined by trained sensory panels. Trained sensory panels can use flavor attributes defined in a flavor lexicon. The beef lexicon was developed after an expert panel evaluated beef under a multitude of conditions. The panelist determined descriptors of beef flavor and developed standardized references that could be used to uniformly and consistently identify and quantitate beef flavor attributes. The beef lexicon provides a list of attributes, definition of the attributes, and references to maintain a standard of identify across sensory panelists. Flavor attributes defined by trained, descriptive sensory panelists can then be used in principle component analyses to understand the relationship between chemical volatile compounds and human perception of flavor.

See also: Boar Taint: Biological Causes and Practical Means to Alleviate It. Carcass Chilling and Boning. Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Adipose Tissue; Chemical Composition; Color and Pigment; pH Measurement; Protein Functionality; Water-Holding Capacity. Classification of Carcasses: Beef Carcass Classification and Grading; Pig Carcass Classification. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Rigor Mortis, Cold, and Rigor Shortening; Slaughter-Line Operation and Pig Meat Quality. Cooking of Meat: Flavor Development; Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor. Electrical Stimulation. Growth of Meat Animals: Adipose Tissue Development; Muscle. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Modeling in Meat Science: Meat Quality. Muscle Fiber Types and Meat Quality. On-Line Measurement of Meat Quality. Prediction of Meat Attributes From Intact Muscle Using Near-Infrared Spectroscopy. Sensory and Meat Quality, Optimization of. Sensory Assessment of Meat. Spoilage, Factors Affecting: Microbiological. Tenderness Measurement

Further Reading

Aberle, E.D., Forrest, J.C., Gerrard, D.E., Mills, E.W., 2001. Principles of Meat Science, fourth ed. Debuque, IA: Kendall/Hunt Publishing Company.

Aberle, E.D., Reeves, E.S., Judge, M.D., Hunsley, R.E., Perry, T.W., 1981. Palatability and muscle characteristics of cattle with controlled weight gain. Time on a high energy diet. Journal of Animal Science 52, 757–763.

Adhikari, K., Chanbers IV, E., Miller, R., *et al.*, 2011. Development of a lexicon for beef flavor in intact muscle. Journal of Sensory Studies 26, 413–420.

AMSA, 1995. Research Guidelines for Cookery, Sensory Evaluation and Instrumental Measurements of Fresh Meat. Chicago, IL: American Meat Science Association and National Livestock and Meat Board.

Bailey, A.J., Light, N.D., 1989. Connective Tissue in Meat and Meat Products. London: Elsevier Applied Science.

Goll, D.E., Geesink, G.H., Taylor, R.G., Thompson, V.F., 1995. Does proteolysis cause all postmortem tenderization, or are changes in the actin/myosin interaction involved? Proceedings International Congress of Meat Science and Technology 41, 537–544.

Hafs, H.D., Zimbelman, R.G., 1994. Low-Fat Meats: Design Strategies and Human Implications. New York, NY: Academic Press.

Johnsen, P.B., Civille, G.V., 1986. A standardized lexicon of meat WOF descriptors. Journal of Sensory Studies 1, 99–104.

Koohmaraie, M., 1988. The role of endogenous proteases in meat tenderness. Proceedings Reciprocal Meat Conference, pp. 89–100. Champaign, IL: American Meat Science Association.

Koohmaraie, M., 1992. The role of Ca^{2+}-dependent proteases (calpains) in post mortem proteolysis and meat tendernss. Biochimie 74, 239–245.

Koohmaraie, M., Babiker, A.S., Merkel, R.A., Dutson, T.R., 1988. Role of Ca-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. Journal of Food Science 53, 1253–1257.

Locker, R.H., 1960. Degree of muscular contraction as a factor in tenderness of beef. Journal of Food Science 25, 304–307.

Lorenzen, C.L., Neely, T.R., Miller, R.K., *et al.*, 1999. Beef customer satisfaction: Cooking method and degree of doneness effects on the top loin steak. Journal of Animal Science 77, 637–644.

Lyon, B.G., 1987. Development of chicken flavor descriptive attribute terms aided by multivariate statistical procedures. Journal of Sensory Studies 2, 55–67.

Marsh, B.B., Leet, N.G., 1966. Studies in Meat Tenderness: 3. The effect of cold shortening on tenderness. Journal of Food Science 31, 450.

Marsh, B.B., Woodhams, P.R., Leet, N.G., 1968. Studies in meat tenderness. 5. The effect on tenderness of carcass cooling and freezing before the completion of rigor mortis. Journal of Food Science 33, 12–18.

Miller, R.K., Moeller, S.J., Goodwin, R.N., Lorenzen, C.L., Savell, J.W., 2000. Consistency in meat quality. International Congress of Meat Science and Technology 46, 566–580.

Neely, T.R., Lorenzen, C.L., Miller, R.K., *et al.*, 1998. Beef customer satisfaction: Role of cut, USDA quality grade, and city on in-home consumer ratings. Journal of Animal Science 76, 1027–1032.

Pearson, A.M., Dutson, T.R., Bailey, A.J., 1985. Advances in Meat Research: Vol. 4. Collagen as a Food. New York, NY: Van Nostrand Reinhold Company.

Savell, J.W., Cross, H.R., 1988. The role of fat in the palatability of beef, pork, and lamb. Designing Foods: Animal Product Options in the Marketplace. Washington, DC: National Academy Press.

Savell, J.W., Lorenzen, C.L., Neely, T.R., *et al.*, 1999. Beef customer satisfaction: Cooking method and degree of doneness effects on the top sirloin steak. Journal of Animal Science 77, 645–652.

Shahidi, F., 1994. Flavor of Meat and Meat Products. New York, NY: Blackie Academic and Professional.

Smith, G.C., Carpenter, Z.L., 1974. Eating quality of animal products and their fat content. In: Proceeding of the Symposium on Changing the Fat Content and Composition of Animal Products. Washington, DC: National Academy of Science.

Smith, G.C., Savell, J.W., Cross, H.R., *et al*., 1987. Relationship of USDA quality grades to palatability of cooked beef. Journal of Food Quality 10, 269–286.

Relevant Websites

http://www.extension.umn.edu/distribution/nutrition/DJ0856.html
University of Minnesota Extenstion.

http://bovine.unl.edu/eng/credits.html
University of Nebraska at Lincoln Muscle Myology website.

pH Measurement

KO Honikel[†]

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Glossary

pH buffer There exist strong, medium, and weak acids, and their counterparts strong, medium, and weak bases. The acids dissociate in aqueous solutions into H^+ (proton) and A^- (anion). Strong acids such as HCl dissociate to more than 99%, medium acids such as lactic acid to 1–5%, and weak acids to lesser than 0.1%. In water, the undissociated molecules of medium and weak acids will further dissociate with increasing pH. With declining pH, more undissociated molecules will be formed. In this way they provide more or take up protons and the increase or decrease of pH will slow down. These compounds buffer the pH change. The counteractive bases will perform in a similar way but the other way around.

Introduction

The pH of meat influences its color, water-holding capacity, flavor, tenderness, and shelf life. Its measurement at different times postmortem provides information about the forthcoming quality characteristics. The pH value at the full development of rigor mortis is termed as the ultimate pH. To monitor ultimate pH in a meaningful way, a proper measurement is necessary. However, because of the inherent characteristics of such measurements, it is not always easy to achieve accuracy and reliability.

What is pH?

The pH of a solution is defined as the negative base 10 logarithm ($\log_{10}$) of the concentration of hydrogen (H^+) ions or, to be more precise, the concentration of the reaction of H^+ with a water molecule to produce a hydroxonium ion (H_3O^+). For example, a concentration of 10^{-5} mol l^{-1} H_3O^+ in an aqueous solution has a pH of 5. The pH scale in aqueous solutions ranges between 0 and 14. Pure water has a pH of 7.0. In animal bodies most of the organs have similar neutral pH values, for example, blood has a pH value of approximately 7.3–7.4, muscles are in the pH range of 6.9–7.0.

Hydrogen ions are formed when acids such as lactic acid (CH_3–CHOH–COOH) dissociate in water according to eqn [1].

$$CH_3\text{–}CHOH\text{–}COOH + H_2O \leftrightarrows CH_3\text{–}CHOH\text{–}COO^- + H_3O^+ \quad [1]$$

The dissociation of acids can be more or less complete in strong acids such as hydrogen chloride, or it can be in equilibrium in medium strong acids such as lactic acid. Similar to pH, the degree of dissociation is defined as the pK of an acid (HA), where K is the dissociation constant of the reaction. The relationship between pK, concentration of hydrogen ions (C_{H+}), concentration of anion (C_{A-}), and concentration of acid (C_{HA}) is shown in eqn [2].

$$pK = -\log\left(\frac{C_{H+} \times C_{A-}}{C_{HA}}\right) \quad [2]$$

pK values are found in published tables. For example, for a 0.1 mol l^{-1} solution at 20–25 °C, the given pK value for lactic acid is 3.1. Using the pK value, the pH value of a solution of a weak or medium strong acid can be easily calculated. Using lactic acid as an example, the pH of a pure 0.1 mol l^{-1} lactic acid solution in water can be calculated using eqn [2], where it can be assumed that the concentration of H^+ is equal to the concentration of A^- (anion CH_3–CHOH-COO^-). Thus $C_{H+} = C_{A-}$, substituting the published pK value into eqn [2], results in eqn [3], which can be solved to give a pH value of 2.04.

$$\begin{aligned} 3.1 &= -\log\left(C_{H+} \times C_{H+}/10^{-1}\right) \\ 8.4 \times 10^{-4} &= (C_{H+})^2/10^{-1} \\ 8.4 \times 10^{-5} &= (C_{H+})^2 \\ C_{H+} &= \left(8.4 \times 10^{-5}\right)^{1/2} \\ C_{H+} &= 9.16 \times 10^{-3} \\ \text{pH} &= 2.04 \end{aligned} \quad [3]$$

The pH of an aqueous solution of 0.1 mol l^{-1} lactic acid is therefore approximately 2.0.

pH Changes from Muscle to Meat

During the early postmortem changes in muscles of slaughtered animals, the pH declines from approximately 7.0 in the muscle of a live animal to 5.3–5.8 as its final value, termed as ultimate pH (pH_u). The reason for the pH decline is the formation of approximately 0.1 mol l^{-1} lactic acid from glycogen in the anaerobic glycogenolytic pathway.

Buffering Capacity

The discrepancy between the pH of an aqueous 0.1 mol l^{-1} lactic acid solution of approximately pH 2 (see above) and the pH of approximately 0.1 mol l^{-1} lactic acid in meat (5.3–5.8) is due to the buffering capacity of other constituents in meat

[†]Deceased.

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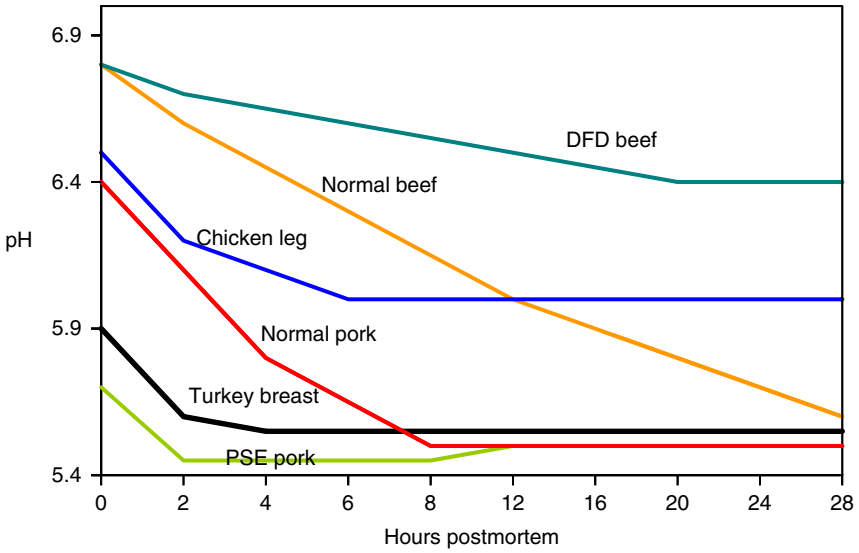

Figure 1 pH changes of individual muscles of different species. Muscles of different animals and species can differ from those shown here.

that modify the ultimate pH. Buffers are composed of either moderately strong or weak acids or bases (p*K* values between 3 and 9) and their soluble salts. These buffering substances in meat bind H^+ ions from the lactic acid formed and lactate anions prevail. It has been calculated, expressed in formed lactate units, by the breakdown of glycogen, that the buffering capacity in meat is between 35 and 50 mmol lactate/pH unit × kg of meat in the range of pH 7–5.

In a simple buffer of an acid and its corresponding salt, eqn [2] also applies. Owing to the addition of the soluble salt to the acid solution, the concentration of A^- is increased. This means that for the equilibrium to be maintained, C_{H+} must become smaller. This means in practice that both lactic acid and lactate mixtures have a greater pH than lactic acid solution alone. This happens with meat in a similar way, as expressed above in 'lactate terms' but it is much more complicated because there are many other constituents involved. Side chains of amino acids, peptides such as carnosine and anserine, phosphate ions, etc. serve as buffering substances and lead to ultimate pH values of 5.3–5.8 – more than 3 pH units greater than that of a pure lactic acid solution. In other words, less than 0.1% of the lactic acid formed is present in its dissociated form.

Importance of pH Changes and Ultimate pH in Meat

The ultimate pH of meat is reached at different times postmortem depending on species, muscle type, and stress over the preslaughter period and immediately preslaughter and can range from 5.3–7.0. The elevation of ultimate pH through stress affects tenderness and keeping quality. In pork muscles, a condition arises where the ultimate pH (5.3–5.5) is reached within 1–2 h postmortem termed pale, soft, exudative (PSE).

In normal pork muscles, pH values of 5.5–5.8 are reached approximately 6–8 h postmortem. In beef muscles, the ultimate pH of 5.5–5.6 occurs at 18–36 h postmortem. In chicken, ultimate pH values are usually greater, ending at pH 6.0 or more at 2–4 h postmortem under commercial chilling conditions (Figure 1). The rate of pH decline can be reduced by increasing the rate of carcass chilling; however, the extent of pH decline is dictated specifically by the glycogen content in the muscle at the time of exsanguination.

Quite often in bovine muscles, as a result of preslaughter stress, the pH decline stops at pH values above 6.0 owing to the lack of glycogen. The reduced glycogen content will result in less lactic acid. The so-called dark, firm, dry (DFD) beef or dark-cutting beef is the result (Figure 1). In such cases the pH values affect many meat properties including color and drip loss.

A low ultimate pH of approximately 5.5 has two effects on meat. The low pH prevents or retards microbial growth and the lactic acid/lactate gives the meat a positive flavor component. Also, at this pH the aging process results in tender meat. The rate of pH change postmortem also influences meat quality. Proteins in muscles before death are 'native' due to the prevailing salt concentration, pH value, and temperature. Falling pH values at prevailing high temperatures like those in PSE-prone muscles lead to the denaturation of proteins. These changes are counterbalanced by the rate of chilling of meat. If chilling and normal pH decline take place in a controlled way, as is achieved in many slaughterhouses, then the denaturation of proteins is limited and there is limited inactivation or activation of enzymes (e.g., calpains).

In PSE muscles of pork, the chilling of a carcass cannot keep pace with the fast pH decline. The final pH value can be reached at temperatures in the muscle well above 30 °C. Consequently many proteins denature – the meat becomes pale due to the denaturation of dissolved sarcoplasmic proteins including the meat-color-producing myoglobin. Also, proteins in membranes denature and intramuscular fluid can leak out into the extracellular space. The exudate (also termed drip loss) increases rapidly and the muscles become soft.

Time of pH Measurement

pH measurements early postmortem, at the end of the slaughterline at 30–45 min, are very helpful. A pH value of 5.8

and lower at 45 min postmortem indicates that pork muscles will very likely become PSE meat. An ultimate pH value above 6.0, as in DFD meat, indicates that an early microbial spoilage of the meat can be anticipated. Thus pH measurements are important for detecting meat quality characteristics.

Measurement of pH in Meat

Principal Methods

Until the end of the 1950s, the measurement of pH was carried out using a wide range of coloring substances (pH indicators) in a solution or bound to paper strips that changed color at various pH values. Although this was very helpful in an otherwise colorless solution, in meat or meat extracts, with their red color, colored solutions could not be used. Paper strips with pH indicators bound to the paper could be used, but they were still rather inaccurate with an error of approximately ±0.2 pH units. Indicator strips with a range of different indicator substances later enhanced the accuracy to ±0.05 pH units.

Glass electrodes were developed in parallel to metal electrodes in the 1950s. Glass electrodes are based on the principle that if a glass surface is immersed in a solution, the electrical potential developed depends on the hydrogen ion concentration. This potential can then be compared with that of another (a reference e.g., a metal) electrode so that, for example, the pH of a meat slurry can be measured with a good reproducibility. A glass electrode is a proton (H^+)-sensitive electrode. The proton sensor is a glass surface (membrane) that consists of a swollen surface layer (depth 5–500 nm) on both sides of the glass body of the membrane (Figure 2). On the outside surface of the membrane, hydrogen ions are replaced with sodium ions (Na^+). On the inside, a buffer solution with a constant pH is used. An electrical potential is built up, which is compared with a pH-insensitive reference electrode, usually a silver–silver chloride or calomel electrode, which is connected with a wick or diaphragm to the solution to be measured. This means that two electrodes need to be inserted into the same solution.

As this design was rather complicated, the so-called combination electrode was designed with glass and reference electrode combined in one shaft. The connection between the outside (the solution where the pH is measured) and the reference electrode inside is achieved via a porous ceramic membrane (Figure 3).

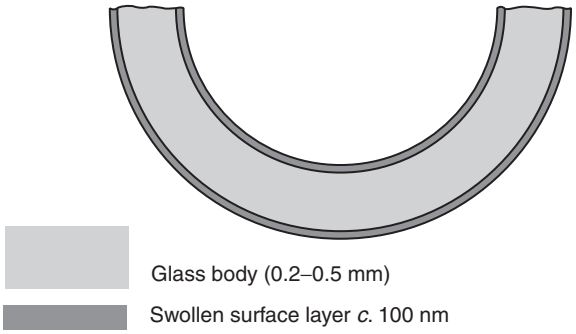

Figure 2 Structure of the glass membrane of a pH electrode.

The early glass electrodes were very fragile and could not be inserted into a piece of meat. The membrane and the diaphragm became rapidly contaminated with fat and protein particles and had to be cleaned rather frequently. Later a noble metal wire like platinum was fused in the glass shaft instead of a porous diaphragm to provide more reproducible results.

Between the outside and inside of the glass membrane, there is a high resistance of approximately $10^{10}\,\Omega$. The electrical potential between the inside and outside, measured through the diaphragm or another reference junction, must remain undisturbed. Fat or protein particles must not plug the junction. Furthermore, all parts (measuring solution or meat (carcass)) must be earthed to avoid false readings. As the glass membrane exchanges H^+ against Na^+ ions, pH measurements are influenced by a number of ions. Measuring in pure (distilled) water or in a high-salt solution (e.g., in brine with approximately 5% salt or more or in a salted batter) gives misreadings. Likewise concentrated protein solutions have a similar effect. In meat, the misreadings are not significant but an accuracy below 0.05 pH units is not possible. Temperature also has a greater influence on readings for the solution or meat.

In the 1990s, the so-called ion-sensitive field effect transistor electrodes appeared in the market. With these electrodes, the surface of a solid-state silicon chip binds H^+ ions and changes its conductivity. However, these surfaces also bind other charged particles such as proteins and have to be cleaned very frequently.

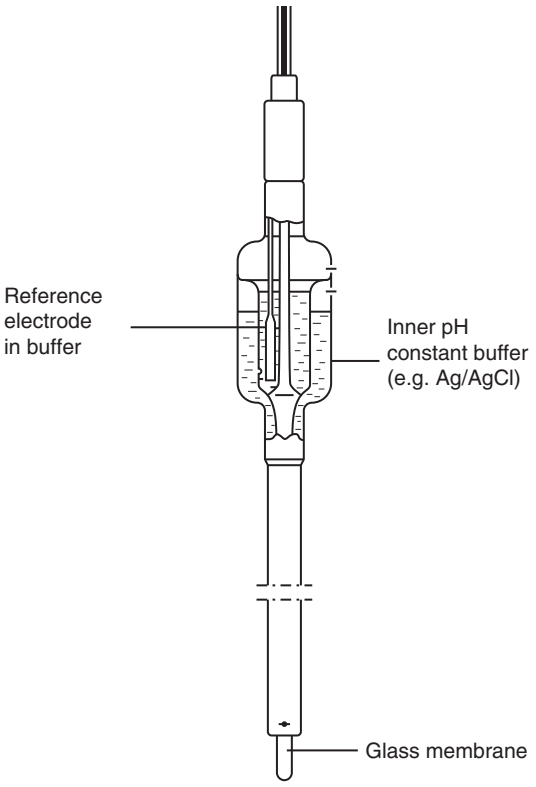

Figure 3 A modern combination of glass electrode. The connecting porous membrane or the noble metal wire is indicated by the black spot just above the glass membrane.

Today, glass electrodes are mechanically robust and stable enough to be inserted into meat. They measure pH in meat in a reproducible manner, if calibration and cleaning is done properly.

Conditions for Measurement

Most chemical reactions are temperature dependent, including the dissociation of acids and bases. Internationally, pH is recommended to be measured at ambient temperature of 20–25 °C. However, in meat, temperatures between 0 and 43 °C are common, so this must be kept in mind in interpreting pH measurements in meat, as in most cases calibration is done at approximately 20 °C, yet the meat may be at a considerably different temperature. Even a temperature adjustment at the glass electrode equipment itself corrects only the temperature dependence of the glass electrode and not the changes within the meat matrix or meat product, which may follow a different temperature dependence. Therefore, it is recommended to accept pH readings of meat to no more than one decimal place, if the calibration temperature and the temperature of the meat differ by more than 10 °C. Greater accuracy demands a calibration at the temperature of the meat. In these cases the temperature drift of calibration buffers must also be taken into account (Figure 4).

Calibration

Calibration and associated maintenance are the keys to meaningful pH measurements. A glass electrode has to be kept wet during storage. A dry glass electrode has to be conditioned (hydrated) for approximately 24 h. Electrodes should be kept in neutral buffer solution or in water. After 30–50 measurements a calibration must be carried out. At least once a day a calibration must take place.

For calibration, two buffer solutions, usually pH 7.0 and 4.0 or near these values, are taken from the refrigerator (where they should be stored to prevent microbial growth that affects the pH value). The solution has to be warmed up to the ambient temperature of the calibration pH solution. After initial cleaning of the combined glass electrode with distilled water, the electrode is inserted into the standard pH 7 buffer solution with agitation and the instrument is adjusted, taking approximately 20 s to reach the pH of the calibration buffer (e.g., 6.98).

The glass electrode is removed from the first buffer and cleaned thoroughly with distilled water, then it is inserted into the second standard pH solution, the pH 4 buffer. After 20 s of agitation and a stable reading, the pH meter is then set to this second standard buffer value.

The glass electrode is taken out, cleaned again with water, and the procedure starting with the pH 7 buffer is repeated until the values of both calibrating buffers read stable. In many modern instruments, clear stepwise instructions are displayed to facilitate calibration.

In most cases two buffers are absolutely necessary. A linear calibration curve – the linearity between pH 4 and 7 can be assumed – is defined either by two points (two calibration buffers) or one point and a constant slope. However, a constant slope recommended sometimes by the manufacturers cannot be guaranteed with an electrode in regular use as an electrode ages. This is why calibration with two buffers is strongly recommended.

Electrode deterioration is noted when the electrode cannot be calibrated by the process described above. Then it is either too old or it should be reactivated. To do this, the electrode surface is first cleaned with warm water (<45 °C) to dissolve fat films, then the electrode is inserted into a protease solution (pepsin, chymotrypsin, papain) and left there at room temperature for 1 h. After cleaning with water, the calibration procedure should be repeated. If the result is not satisfactory, repeat the protease cleaning step. If successful calibration is not achieved after carrying out the cleaning process three times, the electrode should be changed.

Although modern solid-state chip electrodes are robust, they still need cleaning and the recommendations of the manufacturer must be followed. Calibration must also be carried out with these electrodes.

It should be kept in mind that a pH electrode does not have the same reliability and stability as a calibrated thermometer, which merely has to be checked once in a while. pH electrodes

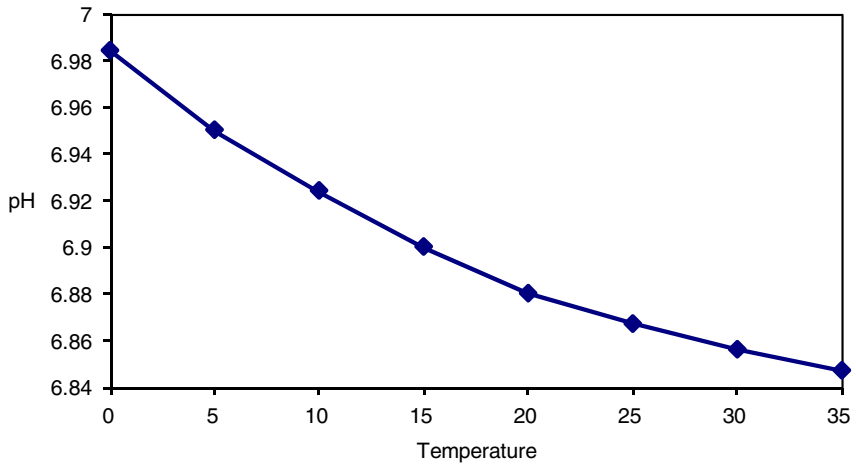

Figure 4 The effect of temperature on a phosphate buffer.

must be calibrated regularly as many factors may influence the ability of the measuring glass surface to exchange H^+ against other cations.

Conclusion

The pH of meat is an important indicator of meat quality characteristics. It can be used to detect PSE and DFD meat and it can monitor manufacturing processes. Nowadays, pH measurements are done with either glass electrodes or solid-state chip electrodes, but they need frequent calibration. Owing to temperature differences during measurement, the accuracy of a measurement is no more than to one decimal place.

See also: Conversion of Muscle to Meat: Color and Texture Deviations; Glycolysis. Electrical Stimulation. Preslaughter Handling: Preslaughter Handling

Further Reading

Bendall, J.R., 1979. Relationship between muscle pH and important biochemical parameters during the post-mortem changes in mammalian. Meat Science 3, 143–157.

Decker, E.A., 2001. The role of histidine-containing compounds on the buffering capacity of muscle. Proceedings of the 54th Reciprocal Meat Conference, pp. 161–164. Indianapolis, IN: American Meat Science Association.

Honikel, K.O., Fischer, C., 1977. Vergleichende Messung des pH-Wertes mit Glaselektrode und Spezialindikatorstäbchen bei Rindfleisch. Die Fleischwirtschaft 57 (12), 2175–2177.

Kylä-Puhju, M., Ruusunen, M., Kivikari, R., Puolanne, E., 2004. The buffering capacity of porcine muscles. Meat Science 67, 587–593.

Maribo, H., Støier, S., Jörgensen, P.F., 1999. Procedure for determination of glycolytic potential in porcine M. longissimus dorsi. Meat Science 51, 191–193.

Puolanne, E., Kivikari, R., 2000. Determination of the buffering capacity of postrigor meat. Meat Science 56 (1), 7–13.

Puolanne, E., Reeta Pösö, A., Ruusunen, M.H., *et al.*, 2002. Lactic acid in muscle and its effects on meat quality. Proceedings of the 55th Annual Reciprocal Meat Conference, pp. 57–62. Savoy, IL: American Meat Science Association.

Van Laack, R.L.J.M., 2001. Metabolic factors influencing ultimate pH. Proceedings of the 54th Reciprocal Meat Conference, pp. 158–160. Indianapolis, IN: American Meat Science Association.

Van Laack, R.L.J.M., Yang, J., Spencer, E., 2001. Determinants of ultimate pH of pork. Proceedings of the Annual IFT Meeting, New Orleans, LA (Abstract).

Relevant Websites

http://en.wikipedia.org/wiki/PH
http://en.wikipedia.org/wiki/Glass_electrode

Protein Functionality

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Glossary

Adhesion/binding A process that binds meat particles together as in restructured products.
Emulsification A process that disrupts fat into small particles, which are stabilized by a protein coating and dispersed in a heterogeneous aqueous matrix consisting of salt-soluble myofibrillar proteins, segments of muscle fibers and myofibrils, connective tissue fibers, collagen fragments, and various ingredients.
Functionality Physicochemical performance of muscle proteins in meat processing.
Gelation A process by which proteins interact to form a three-dimensional aggregated network that entraps a large amount of water.
Meat batter A meat emulsion that is multiphasic and heterogeneous, which differs from the simple oil-in-water or water-in-oil emulsion.
Solubility The percentage (w/w) of the original protein dissolved in an aqueous solution under specific extraction conditions.
Surimi A washed muscle mince or alkali/acid extract that is concentrated with myofibrillar protein.
Water binding The immobilization of water within muscle fiber or meta-homogenate through interaction of proteins with water or physical entrapment in protein matrixes.

Introduction

Definition of Functionality

The term 'functionality,' as used in meat science and discussed in this article, generally refers to the physicochemical performance of muscle proteins in meat processing. It should be distinguished from nutritional functionality, which describes the health-promoting effect of certain proteins and various other components indigenous to food materials. Thus, functional properties of muscle proteins include texture-forming and related properties, namely, water binding, fiber swelling, solubility/extractability, gelation, emulsification, and meat particle adhesion/binding. Although muscle proteins, particularly those soluble in the cytosol, can be excellent surfactants and, hence, are capable of stabilizing foams, their foaming behavior is generally considered a nuisance because, except in some ethnic foods (e.g., mousses), the foams produced hinder the operation of processing equipment. Therefore, foaming properties will be excluded from this article.

Types and Roles of Muscle Proteins

The three groups of muscle proteins – sarcoplasmic proteins (water soluble), myofibrillar proteins (soluble in solutions of >0.5 ionic strength), and stromal proteins (soluble in alkaline or acid solution) – play different roles in meat processing. Generally, sarcoplasmic proteins are relatively small in size. They are of high surface activity and, therefore, can act as emulsifiers and have a strong tendency to generate foams. Stromal proteins, which consist mainly of collagen molecules found in the connective tissues epimysium, perimysium, and endomysium, have a limited role in the structure and texture formation in comminuted meats. However, when sufficiently hydrated through slow, moist cooking, collagen is converted into gelatin, which exhibits remarkable water-binding capacity and readily forms a cold-set gel. Myofibrillar proteins, however, have the ability to impart multiple functionalities, including gelation, emulsification, water binding, and adhesion. The versatility of this protein group lends itself to the development of a variety of texturized, restructured meat products, such as luncheon meats, boneless turkey hams, and various frankfurter-type meat items.

Factors Affecting Protein Functionality

Large variations in quality attributes exist between muscle foods produced by different processors, or even within the same type of products (e.g., frankfurters). Much of the variation is caused not only by the use of different nonmeat ingredients but also by the specific functionality that muscle proteins impart under given processing conditions. The amino acid composition and peptide sequence, the spatial arrangement of the protein structure, the size (mass) and shape of the protein molecules, the solubility characteristics, the concentration of extracted proteins in the aqueous phase, surface hydrophobicity, and muscle fiber types are some of the most important intrinsic factors that can influence protein functionality. However, pH, temperature, cooking rate, degree of comminution, oxidation, and the presence of various nonprotein components such as lipids, carbohydrates, minerals, acids, as well as many other processing factors that influence protein structures and interactions can all profoundly affect protein functionality. Hence, to obtain a desirable product quality, it is imperative to maintain a delicate balance between different intrinsic and extrinsic factors.

Water Binding

Water Compartments in Muscle Foods

Proteins play a critical role in immobilizing water in meat and meat products and, hence, contribute to overall juiciness and tenderness of cooked meat. Water binding, as one of the important functional properties of muscle proteins, will be described briefly. The water present in meat or meat products can be both indigenous and extraneous (pumped, injected, etc.). Physicochemically, the water in meat exists either in a bound form or in a free state. Bound water is tightly associated with proteins through hydrogen bonds and ion–dipole interactions, which are influenced by the surface charge and polarity of the proteins. In intact muscle, free water is held via capillarity in different compartments, namely, in the spaces between myofilaments, between myofibrils, and outside the fibers. During postmortem aging, the proteolytic degradation of cytoskeleton (titin), while improving meat tenderness, can weaken water binding in muscle and increase drip loss. In comminuted products, a large portion of water is also retained via entrapment in the matrix of myofibrillar protein gels and, in some cases, gelatin gels. The interfilamental spacing, measuring approximately 320 Å between thick (myosin) filaments, is not constant. It varies with fiber type, sarcomere length, the contractile state, pH, ionic strength, the presence of certain divalent cations and phosphates, the redox potential, and many other factors. Denaturing conditions, such as rapid acid accumulation (postmortem glycolysis) while the muscle temperature remains high, lead to reduced water binding in meat; an example of meat having a poor water-binding capacity is the so-called 'pale, soft, exudative' pork, turkey, and chicken.

Effect of Salts on Water Binding

Because the bulk of water in muscle is confined within the myofibrils in the spaces between the thick (myosin) and thin (actin) filaments, any chemical, physical, or enzymatic means that increase the interfilamental spaces, i.e., expand the myofibril lattices, can bring about enhanced water binding in meat. The use of salt (NaCl or KCl) and phosphates to improve water retention in cooked meats is based on this principle. As a conventional practice, extraneous water, together with salt and phosphates, is incorporated into fresh meat through injection, marination, or tumbling to improve the product cooking yield and juiciness. Hydration and retention of added water are made possible through the NaCl- and phosphate-induced myofibril expansion and, hence, transverse swelling.

Effect of Phosphates on Water Binding

High concentrations of NaCl (usually greater than 0.6 mol l^{-1}), impart high ionic strengths (Γ), depolarize myosin filaments, whereas low concentrations of phosphates (5–15 mmol l^{-1}), notably sodium pyrophosphate and tripolyphosphate, are capable of dissociating actomyosin. Different types of phosphates, used singly or in combinations at a total level not exceeding 0.5% of the final product weight, are commonly incorporated into processed meats. Depending on the country, the use of specific phosphates is subjected to different regulations. In the presence of magnesium, the dissociation effect of pyrophosphate is very similar to that exerted by adenosine tripolyphosphate (ATP). These biochemical processes result in the weakening of the myofibril structure, thereby allowing water molecules to more readily diffuse into the interfilamental spaces to increase the extent of fiber hydration. Substantial muscle fiber swelling occurs as the NaCl concentration is raised to approximately 0.6 mol l^{-1} in the absence of phosphate, or 0.5 mol l^{-1} in the presence of as little as 10 mmol l^{-1} pyrophosphate (Figure 1). In many cases, myofibrils start to swell even at 0.4 mol l^{-1} NaCl when pyrophosphate or tripolyphosphate is also present, and this will depend on the degree of muscle fiber contraction (actomyosin cross-linking) and freshness of meat, and can vary between muscle types. In general, the extent of swelling and hydration continues until 1.0 mol l^{-1} NaCl (approximately 4.0% of muscle weight) is incorporated where the swollen fiber will start to shrink due to a salting-out effect.

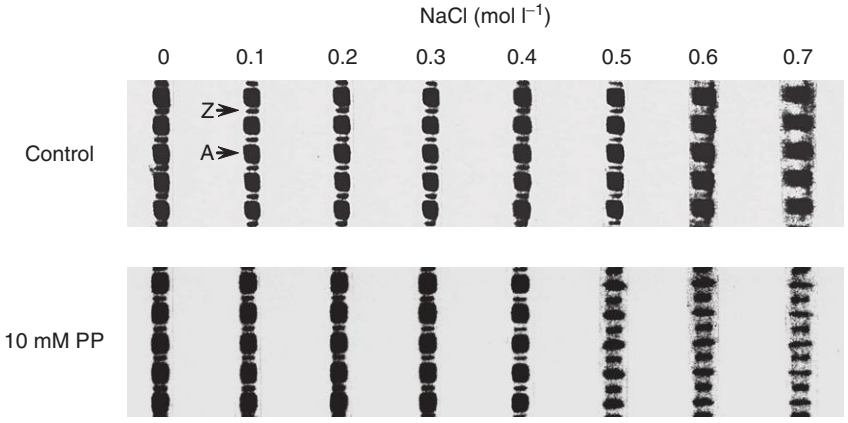

Figure 1 Ultrastructural changes in myofibrils treated by irrigation with NaCl solutions in the presence or absence of sodium pyrophosphate (PP) at pH 6.0. The A-band (A) and Z-line (Z) are indicated.

Solubility

Definition

Solubility of muscle proteins refers to the percentage (w/w) of the original protein that dissolves into the aqueous solution under specific extraction conditions. It is a manifestation of the equilibrium between the solute (protein) and the solvent (water). Solubility of proteins is of primary importance in meat processing as it is closely related to many other functional processes of proteins. For example, gelation, emulsification, adhesion, and water immobilization in comminuted and restructured muscle foods are the results of interactions of soluble myofibrillar proteins with various meat components. In the meat science literature, the term 'protein solubility' is often used interchangeably with 'protein extractability', assuming that proteins, once solubilized in a certain buffer, can be readily extracted.

Protein Structure and Solubility

Protein structure is one of the important determinants for its solubility characteristics. Most proteins belonging to the sarcoplasmic protein family are of a globular structure and are relatively small in size (most between 30 and 65 kDa) with a single cooperative domain. The surface of these protein molecules is made up of charged and noncharged polar amino acid side-chain groups, giving an isoelectric point (pI, where protein solubility is minimal) close to neutrality, far from the typical pH of meat (5.5–5.8). These structural features enable sarcoplasmic proteins to be naturally soluble in water, independently of ionic strength. However, myofibrillar proteins in muscle are associated with one another into a highly organized structural unit – the myofibril. Their isoelectric points are relatively low (pH 5.0–6.0) to allow for the unique structural arrangement and interaction between different segments of the polypeptides. Thus, myofibrillar proteins are insoluble at the physiological ionic strength. Collagen, which is insoluble in salt solution under normal meat-processing conditions, can be rendered soluble either through long-time moist heating or through limited acid/alkaline hydrolysis. These treatment conditions permit the disruption of collagen fibrils and dissociation of tropocollagen complexes, thereby facilitating the interaction of the individual collagen peptides with water.

Effect of Ionic Strength on Protein Solubility

The relationship between ionic strength (Γ, approximately equivalent to the molar concentration of NaCl) and solubility of myofibrillar protein is illustrated in Figure 2. When minced muscle is washed extensively to remove most ions through the dilution effect, a great net surface charge is established, attributed mainly to the ionizable side-chain groups and the minute quantity of small ions present. A further dilution in excessive amounts of deionized water to a Γ of 0.0003–0.001 will disintegrate the myofibrils. Although this will give myofibrillar proteins a high extractability and solubility, an ionic strength this low is rarely encountered in meat processing and is therefore of little practical significance. The solubility reaches

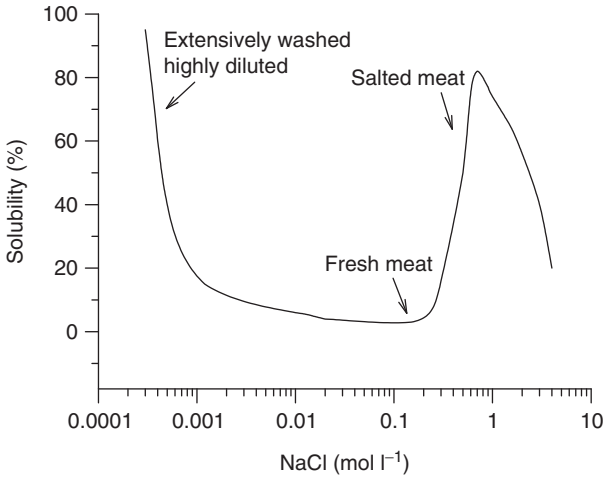

Figure 2 Schematic representation of the ionic strength–solubility relationship of muscle myofibrillar protein. The ionic strength is expressed as the molar concentration of NaCl.

a minimum within the 0.03–0.2 ionic strength range where protein surface charges are shielded by the surrounding small ionic elements (e.g., Na^+ and Cl^-). As the ionic strength is increased further, myofibrillar proteins will again reach a soluble state owing to weakening of protein–protein interactions and dissociation of myofilaments. In muscle food processing, extraction of myofibrillar proteins into the aqueous solution is achieved through the addition of salt and polyphosphates to the meat formulation, raising the pH of the meat ingredient solution, and prolonging the mixing time (massaging, tumbling, or chopping). Aside from its effect to depolymerize myosin filaments, NaCl at elevated concentrations lowers the isoelectric point of myosin so that it carries a greater net charge within the normal range of meat pH. An increased electric double layer surrounding protein molecules allows for a better hydration and solubility.

Effect of Phosphates on Protein Solubility

The effect of pyrophosphate and tripolyphosphate on myofobrillar protein solubility is remarkable. In the absence of phosphate, myosin extraction begins at the center of the A-bands (the dark regions or blocks in the myofibril). When sodium pyrophosphate is present, protein extraction begins at both ends of the A-band (Figure 1). Because the actomyosin cross-bridges are absent at the center of the A-band but are located at the ends of the A-band, it can be inferred that pyrophosphate functions as a lubrication agent, that is, it dissociates the actomyosin complex in a way similar to ATP, leading to a separation of thick and thin myofilaments. Sodium tripolyphoshate has an effect very similar to that of sodium pyrophosphate, and it is believed that tripolyphosphate is hydrolyzed to pyrophosphate by endogenous phosphatase before it becomes functionally active. There is evidence that myosin also has tripolyphosphatase activity. Other phosphate compounds, especially those with a cyclic or ring structure, are generally less efficient than the shorter-chain phosphates for protein extraction.

Effect of Muscle Fiber Type on Protein Solubility

The design of meat-processing protocols should be done in the context of muscle or fiber types. In general, myofibrillar proteins from fast-twitch glycolytic (white) fibers are more readily extracted than those from slow-twitch oxidative (red) fibers, and the former also respond more effectively to phosphate treatment. The disparity is attributed to several factors. First, proteins located in the myofibrils must overcome structural barriers and hindrances. Histologically, white myofibrils contain narrower Z-disks than red myofibrils, and the major structural protein in the Z-disk, α-actinin, has different morphology (isoforms) for red and white myofibrils. Several other minor structural proteins, for example, M-protein, C-protein, H-protein, and X-protein, also differ morphologically between fiber types. Furthermore, some proteins that make up the Z-disks in white muscle are more susceptible to early postmortem proteolytic degradation, especially in the presence of salt. The structural changes are accompanied by increased extraction of both thick and thin filaments. Experimental evidence suggests that the M-protein may serve as a transverse structural constraint to myosin extraction and is crucial for the extraction of total myofibrillar protein. Second, myosin, the most prevalent protein component inside the muscle, exists in various isoforms that are fiber-type-specific. Different myosin isoforms are known to differ in physicochemical characteristics, morphology, and solubility. Hence, even if the thick filaments are depolymerized, the different solubilities between white and red myosin constitute another conspicuous factor that influences the total amount of extractable protein from white and red muscles.

Gelation

Definition

The ability to form a gel (gelation) is one of the most important physicochemical features of muscle proteins occurring in meat processing. Gelation in muscle foods is a result of unfolding and subsequent association of extracted (soluble) protein molecules to form aggregates and strands. When aggregation reaches a certain critical level, a continuous, three-dimensional gel network structure is created, which consists of cross-linked peptides or aggregates with a large amount of entrapped water:

$$\chi P_N \overset{\text{Heating}}{\Longrightarrow} \underset{\text{(Denaturation)}}{\chi P_D} \overset{\text{Heating}}{\Longrightarrow} \underset{\text{(Aggregation)}}{(P_D)_\phi,\ (P_D)_P} \overset{\text{Heating/cooling}}{\Longrightarrow} \underset{\text{(Cross-linking)}}{(P_D)_\phi -\ (P_D)_P -}$$

where χ is the total number of native (P_N) or denatured (P_D) protein molecules, and ϕ and P (ϕ+P+$\cdots$=χ) are the number of molecules that are aggregated at a certain point of the gelation process.

Adhesive Role of Gels

The gels formed act as adhesive to bind comminuted meat particles and offer a three-dimensional network to stabilize emulsified fat globules and entrap flavor compounds and other meat ingredients, as well as a complex capillary system to immobilize moisture. Hence, protein gelation is responsible for an array of texture-related quality traits in products such as sausage, frankfurters, boneless hams, meat rolls, and various luncheon meats. For processed muscle foods with a large amount of added water, protein gelation is obviously of particular importance.

Role of Different Muscle Proteins

As is true of water binding and hydration, myofibrillar proteins are largely responsible for gelation that takes place in processed muscle foods. In comparison, sarcoplasmic proteins exhibit a poor gelling ability, although in some cases they may reinforce the myofibrillar protein gels. Collagen and elastin, which comprise the connective tissue in meat, have a minimal role in gelation because normal meat-processing conditions rarely allow them to be sufficiently hydrated and extracted. The right-handed triple α-helix of collagen molecules does not unfold into single strands until approximately 65 °C (approaching the end temperature of cooking). However, once extracted, collagen can form a rubbery cold-set gel. Therefore, pregelatinized collagen or collagen extracts from pork and chicken skins, as well as finely minced connective tissue, are sometimes utilized in the manufacture of cold delicatessen meats.

Myosin and Mixed Myofibrillar Protein Gels

Myofibrillar protein gels formed in muscle foods are generally heat induced. Two types of gels can be produced from myofibrillar proteins – the myosin gel and the mixed myofibrillar protein gel. Myosin forms filaments at ionic strengths close to the physiological condition. Hence, it can form a somewhat brittle gel at low concentrations of salt (0.15–0.20 mol l^{-1} NaCl, or ~0.6–0.8% in meat). Because this ionic condition is seldom employed in the process of producing 'texturized' meat (which requires at least 0.5 mol l^{-1} NaCl (~2% in meat) to ensure an adequate protein extraction and solubility, myosin gelation at the low salt condition is of little practical significance. However, mixed myofibrillar protein gels, also referred to as 'myofibrillar protein gels' and sometimes as 'actomyosin gels' or 'salt-soluble protein gels,' are the gels formed in most processed meats. However, even in the mixed-protein system, the myosin portion of the actomyosin remains the most important gelling protein. The contribution to the rigidity of myosin or actomyosin gels by the different segments of myosin is believed to follow the order: rod > light meromyosin > heavy meromyosin > the S-1 subunit.

Mechanism of Gel Formation

The mechanism for myofibrillar protein gelation under meat-processing conditions (0.5–0.6 mol l^{-1} NaCl, pH 6.0–6.5) has been researched extensively. Using extracted myosin as a model protein, the following sequential changes have been established for heat-induced myosin gelation (Figure 3). Unfolding of myosin head (S1 subfragment) commences at 35 °C and leads to the formation of dimers and oligomers via head–head interactions. Disulfide linkages are not required for

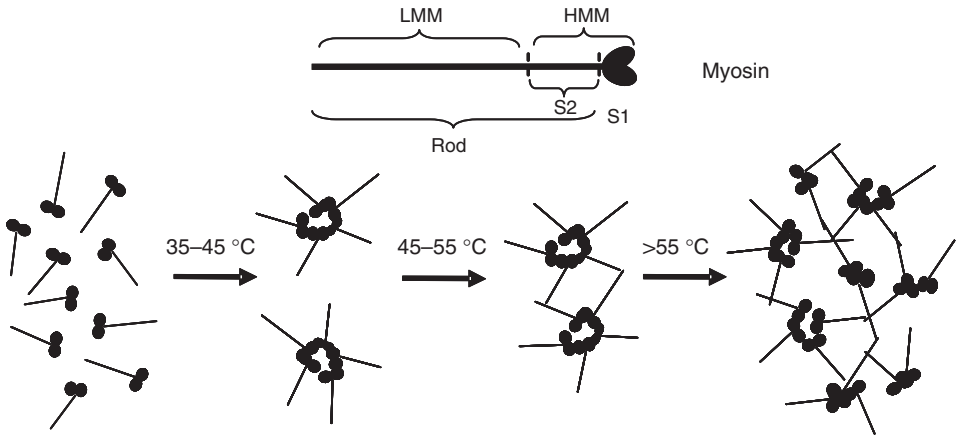

Figure 3 Schematic representation of heat-induced aggregation of myosin during gelation in the presence of 0.6 mol l^{-1} NaCl at pH 6.0–6.5. Myosin parts obtained by enzymatic hydrolysis are indicated (light meromyosin, LMM; heavy meromyosin, HMM; rod; subfragment 1, S1; and subfragment 2, S2).

the initiation of the interactions. As the temperature increases to 40–45 °C, a globular mass is formed that consists of a tightly associated head clump with tails radiating outward, resembling the shape of a Black Widow spider. At above 45 °C, oligomers coexist with the aggregates formed by the coalescence of two or more oligomers. From 55 °C, these oligomers aggregate further, apparently involving tail–tail (rod) cross-linking to form particles that make up the strands of the gel networks. The microstructure and viscoelasticity of the ultimate gels are dictated by the specific protein aggregation pattern, which is influenced by the heating rate and other processing and ingredient factors.

Effect of Processing Protocols on Gelation

Because the continuous network system in the gel is a result of ordered aggregation of soluble proteins, meat-processing protocols, including both the processing condition and the product formulation, must be carefully controlled. Myofibrillar protein gel strength increases exponentially with the protein concentration. Thus, factors that affect the extraction of myofibrillar proteins, including pH, temperature, ionic strength, muscle rigor state and postmortem aging time, polyphosphates, and time of blending of meat with salt, can all influence protein gelation. Many of the same factors, as well as others such as rate of heating, reducing or oxidizing compounds, polysaccharides, lipids, nonmeat proteins, divalent cations, the myosin:actin ratio in the actomyosin complex, endogenous proteases, and muscle fiber types, also affect protein–protein interaction and, hence, play an active role in protein gelation. In general, mixed myofibrillar proteins have an optimum gelling capacity at pH approximately 6.0, a Γ of 0.6–0.8, and a temperature approximately 65 °C. A slow-heating process is desirable because it allows for progressive protein denaturation, which favors ordered protein–protein association. Cross-bridging agents or factors, such as minute quantities of Ca^{2+} (<5 mmol l^{-1}), dehydroascorbate, and hydrogen peroxide (which induce limited protein oxidation to promote disulfide and carbonyl-amine bond formation), and the enzyme transglutaminase (E.C. 2.3.2.13, which catalyzes lysine–glutamine cross-linking through an acyl transfer reaction), have been widely observed to enhance the gelation of myofibrillar proteins.

Gelling Properties from Different Muscles and Fiber Types

The gelling properties of myofibrillar proteins are fiber-type-dependent. Proteins from white fibers (e.g., pectoralis major from chicken and cutaneous trunci from beef) have a greater tendency to gel on heating, and the gel storage modulus (G′, the elastic component of gel) is generally higher when compared with proteins from red fibers (e.g., gastrocnemius from chicken; masseter from beef) under similar processing conditions (Figure 4). The discrepancy is attributed to structural and solubility differences that are exhibited by different protein isomers, particularly myosin, and can be explained by the fact that white fiber myosin usually forms elongated filaments rather than short aggregates seen in red fiber myosin in 0.1–0.6 mol l^{-1} NaCl solutions. For some animal species and muscle types (e.g., the fish Pacific Whiting and Alaska Pollock, and beef cardiac muscle), the presence of endogenous catheptic enzymes (H, B, L, etc.) can cause considerable gel weakening when the product is cooked through the 45–60 °C temperature range. This gel-weakening phenomenon is a particular nuisance in some surimi (washed muscle mince, a crude myofibrillar protein concentrate) or surimi-based foods. Protease inhibitors such as egg white and potato extract are therefore desirable and can be used to inhibit the deleterious effect. Although beef plasma powder provides strong protease inhibition, it is no longer used in the surmi industry due to the concern with bovine spongiform encephalopathy (or commonly referred to as the 'mad cow disease'). Furthermore, sodium pyrophosphate and tripolyphosphate, though facilitating protein extraction, tend to interfere with protein gel matrix formation for both white and red muscle fiber types. Hence, the use of these phosphates should be minimized in products that already contain high concentrations of NaCl where protein extraction is not a limiting factor to gel formation.

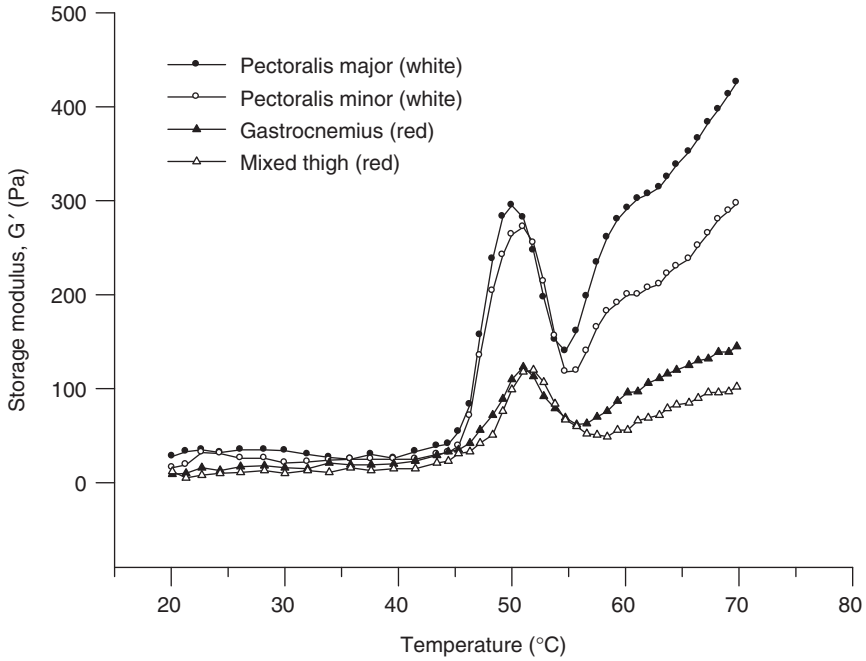

Figure 4 Typical rheology curves of chicken white (fast-twitch) and red (slow-twitch) muscle myofibrillar proteins in 0.6 mol l^{-1} at pH 6.0. The storage modulus, *G′*, represents elasticity of the gels.

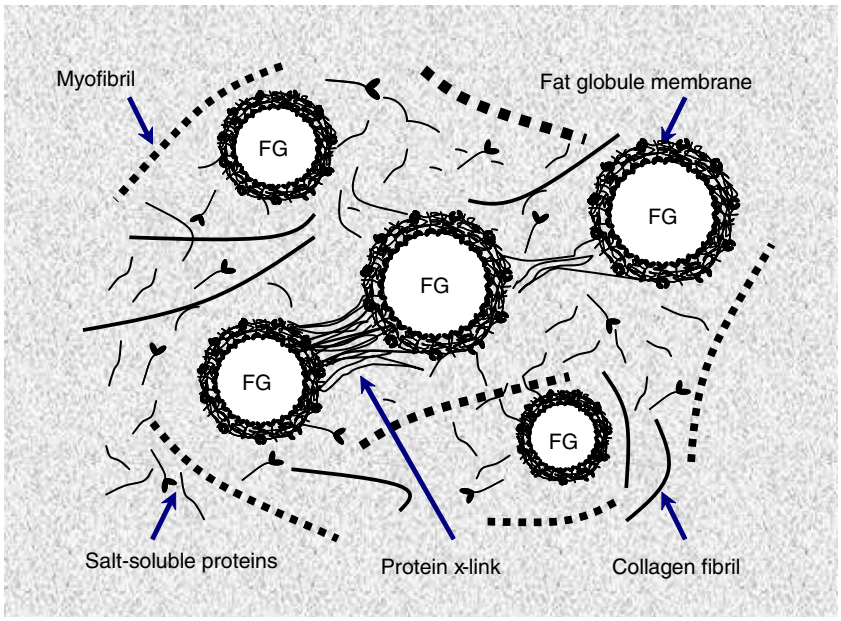

Figure 5 Schematic representation of a typical meat emulsion (batter). FG, fat globule.

Emulsification

Characteristics of Meat Emulsion

Muscle foods made from finely chopped or comminuted meat in the presence of fat are regarded as emulsion-type products. A meat emulsion differs from the classical emulsion in that the fat globules are dispersed and stabilized in an aqueous matrix system consisting of salt-soluble myofibrillar proteins, segments of muscle fibers and myofibrils, connective tissue fibers, collagen fragments, and various ingredients (Figure 5). Thus, a meat emulsion is commonly referred to as a 'meat batter' to reflect its multiphasic, multicomponent nature.

How an Emulsion is Formed

As with most other proteins, muscle proteins are amphoteric molecules possessing both polar and nonpolar groups or

structural segments. Hence, on the input of mechanical energy through the shearing process known as 'emulsification', proteins can adsorb at the fat–water interface where the hydrophobic groups will anchor into the fat and the hydrophilic groups will extend into the aqueous phase. Such structural orientation at the fat–water interface is thermodynamically favored because it leads to a reduction of total free energy of the meat batter. A slow, continuous comminution (chopping) process is usually adequate to reduce the fat particle size to the micrometer range while extracting myosin or actomyosin to form a coating surrounding the fat particles. To aid in protein adsorption, the meat batter during chopping should reach a certain elevated temperature (generally 15–20 °C, depending on animal species or degree of saturation of lipids) to soften fat, which allows efficient fat particle size reductions.

Proteins Involved in Emulsion Formation and Stabilization

The ability of muscle proteins to form a viscoelastic and flexible membrane (i.e., a coating) around the fat globule is a critical factor for emulsion formation and stabilization. The contribution of individual muscle proteins to meat emulsion stability follows the order: myosin > actomyosin > sarcoplasmic protein > actin > collagen. During emulsification, myosin (prerigor) or actomyosin (postrigor) is rapidly and preferentially adsorbed at the fat–water interface. The superior emulsifying ability of myosin to any other proteins, especially at low protein concentrations, is attributed to myosin's unique structural characteristics. First, the unequal distribution of polar versus nonpolar amino acids in different segments of myosin, i.e., a prevalence of hydrophobic residues in the globular head region or the S1 subfragment versus a preponderance of hydrophilic groups in the rod-shaped tail portion, makes myosin an ideal emulsifier. Moreover, myosin has a high length-to-diameter ratio (roughly 40:1), a structure that is conducive to protein–protein interaction and molecular flexibility at the interface. Because of its insolubility, collagen does not directly participate in the meat emulsification process. The presence of collagen fibrils may help strengthen the protein encapsulation; however, on cooking to above 60 °C, collagen fibrils start to shrink, resulting in a disruption of the emulsion matrix and rupture of the fat droplets. Hence, an excess amount of connective tissue should be avoided in the manufacture of emulsified muscle foods.

Another mechanism for meat batter stabilization is physical entrapment of fat particles in the protein matrix formed largely through protein–protein interactions. Although fat particles without an integral protein coating can be immobilized in protein gels, those with a thick and flexible membrane can interact, through the adsorbed proteins at the membrane, with proteins in the continuous phase, thereby further enhancing the stability of the meat batter. Thus, physicochemical and rheological properties of the fat globule membrane and the viscoelastic characteristics of the continuous protein matrices both contribute to emulsion stability in comminuted muscle foods.

See also: Chemical and Physical Characteristics of Meat: Water-Holding Capacity. Chemistry and Physics of Comminuted Products: Emulsions and Batters

Further Reading

Gordon, A., Barbut, S., 1994. Mechanisms of meat batter stabilization. Critical Review in Food Science and Nutrition 32, 299–332.

Hamm, R., 1960. Biochemistry of meat hydration. Advanced Food Research 10, 355–463.

Lefevre, F., Culioli, J., Joandel-Monier, S., Quali, A., 1999. Muscle polymorphism and gelling properties of myofibrillar proteins from poultry, mammals, and fish. In: Xiong, Y.L., Ho, C.-T., Shahidi, F. (Eds.), Quality Attributes of Muscle Foods. New York: Kluwer Academic/Plenum Publishers, pp. 319–334.

Offer, G., Trinick, J., 1983. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. Meat Science 8, 245–281.

Samejima, K., Kuwayama, K., Yamamoto, K., Asghar, A., Yasui, T., 1989. Influence of reconstituted dark and light chicken muscle myosin filaments on the morphology and strength of heat-induced gels. Journal of Food Science 54, 1158–1162, 1168.

Sharp, A., Offer, G., 1992. The mechanism of formation of gels from myosin molecules. Journal of the Science of Food and Agriculture 58, 63–73.

Sun, X.D., Holley, R.A., 2011. Factors influencing gel formation by myofibrillar proteins in muscle foods. Comprehensive Reviews in Food Science and Food Safety 10, 33–51.

Xiong, Y.L., 1995. Myofibrillar protein from different muscle fiber types: implication of biochemical and functional properties in meat processing. Critical Review in Food Science and Nutrition 34, 293–320.

Xiong, Y.L., 1997. Structure–functionality relationships of muscle proteins. In: Damodaran, S., Paraf, A. (Eds.), Food Proteins and Their Applications. New York: Marcel Dekker, Inc., pp. 341–392.

Xiong, Y.L., 1999. Phosphate-mediated water uptake, swelling, and functionality of the myofibril architecture. In: Xiong, Y.L., Ho, C.-T., Shahidi, F. (Eds.), Quality Attributes of Muscle Foods. New York: Kluwer Academic/Plenum Publishers, pp. 319–334.

Yamamoto, K., 1990. Electron microscopy of thermal aggregation of myosin. Journal of Biochemistry 108, 896–898.

Water-Holding Capacity

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Glossary

Calpains (*μ*-calpain, *m*-calpain, and calpain 3) Cysteine proteinases that are activated by calcium and degrade myofibrillar proteins during the postrigor phase.

Electrical stimulation (ES) Subjecting a carcass to electrical input to increase post mortem glycolysis rate and pH decline. It decreases WHC.

Enhancement Direct injection of brine containing salt, phosphates, ammonium hydroxide, and/or other ingredients for the purpose of improving flavor, tenderness, and juiciness.

Magnesium adenosine tripolyphosphate (Mg-ATP) The presence or absence of Mg-ATP controls whether or not there is energy for contraction (myosin-actin interaction).

Water-holding capacity (WHC) The ability of meat to hold onto its own or added water.

Introduction

Water is the major component of muscle tissue – striated muscle is approximately 75% water. This water is structurally arranged in layers around polar molecules and between layers of cellular materials. It has restricted movement due to a variety of forces. Approximately 5% of the water contained in muscle tissue exists as the 'true hydration water' bound to proteins by monomolecular or multimolecular adsorption. This water is not 'free'; it has an ice-like structure (liquid crystal), is unfreezable, is unaffected by charges on the muscle protein (pH), and unavailable to participate in reactions. The remaining water is free to move around with some restrictions. Water immobilized in one compartment can move to another compartment under various types of stress (Figure 1). Water immobilized within the network of cellular protein membranes (intracellular water) resides either between the proteins composing the contractile units or in the sarcoplasm. Water also exists in the extracellular spaces.

'Water-holding capacity (WHC)' is the ability of meat to hold onto its own or added water when force (heat, pressure) is applied. The majority of WHC resides in the water located in the intermolecular spaces between the salt-soluble proteins (actin and myosin) of muscle tissue where it is held in place by capillary force. The space between myosin and actin/tropomyosin varies between 320 and 570 Å depending on pH, ionic strength, osmotic pressure, and sarcomere length. The interfilamental space is maintained by electrostatic forces that operate over relatively long distances. Factors that alter the spatial arrangement of the myofilaments can alter the amount of water immobilized in this compartment. Factors, both intrinsic and extrinsic, that alter the spatial arrangement of the myofilaments include alterations in net charge induced by pH changes, screening of charges by anions/cations, presence of divalent cations (Mg^{2+} and Ca^{2+}), denaturing conditions that alter protein conformation (rapid pH decline while temperature is still high), and presence and condition of plasticizing agents such as adenosine tripolyphosphate (ATP) as well as enzymes (ATPase) and cofactors necessary for plasticizer function in prevention of myofibrillar protein crosslinking.

Contractile Proteins – Actin and Myosin

Divalent cations (Mg^{2+} and Ca^{2+}) alone can combine with two negatively charged reactive groups on the contractile proteins pulling them more closely together (Figure 2). The reactive groups are prevented from interacting with water because the charged groups are screened and because the reduction in interprotein space sterically precludes the presence of substantial numbers of water molecules. Replacing these

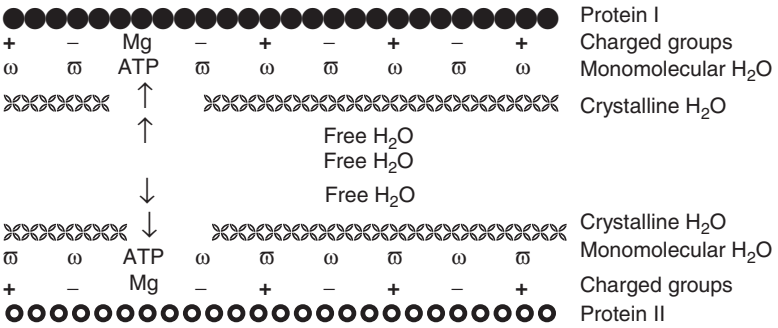

Figure 1 Arrangement of interfilamental water.

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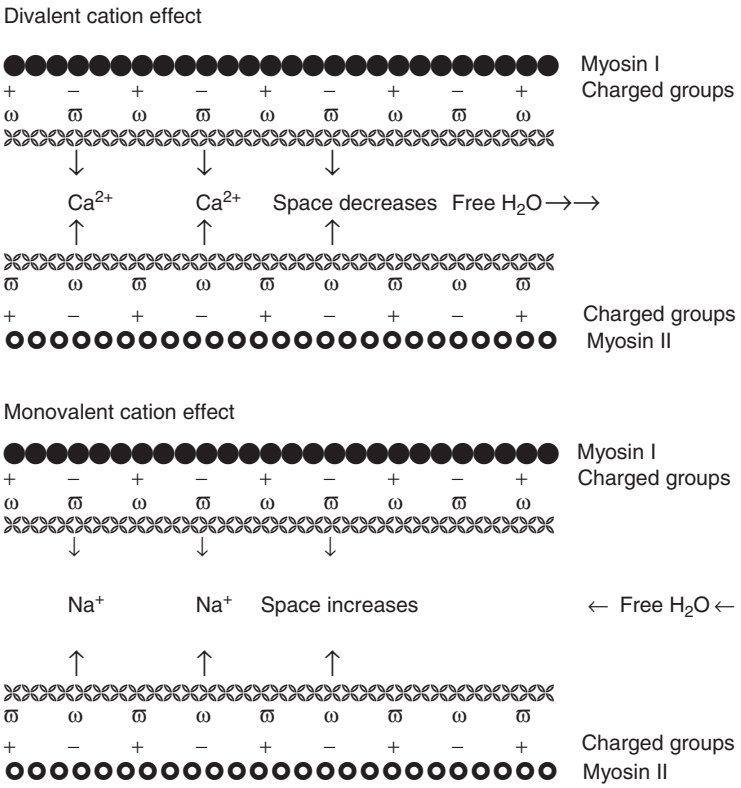


Figure 2 Divalent and monovalent cation effects on free water.

divalent cations with monovalent cations (Na^+ and K^+) substantially reduces this effect (see 'Effect of Salt').

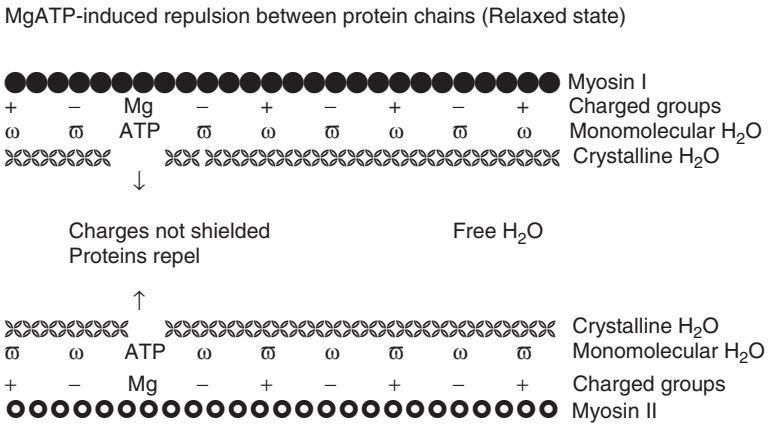
In living tissue, Mg-ATP bound to myosin allows electrostatic repulsion within the protein preventing release of ATP, and myosin binding to actin (Figure 3). Stimulation by nerve impulse causes the sarcoplasmic reticulum (SR) to release Ca^{2+}, which is bound by troponin-C. The presence of Ca^{2+} cancels or shields the negative charge on Mg-ATP, which allows water to move into the space between the protein. Ca^{2+} allows a conformational change in the protein, which activates Mg-ATP ase catalysis of Mg-ATP to Mg–ADP^- providing energy for muscle contraction. ATP is also consumed when the SR pumps Ca^{2+} back into the organelle. ATP is regenerated via aerobic glycolysis of glycogen and fatty acids. In prerigor meat, cross-linking between actin and myosin is prevented by Mg-ATP. In this situation, pH alterations can easily increase electrostatic repulsion between proteins (Figure 4), and increases in ionic strength can then attract water by osmotic force. Even so, the degree to which these proteins can move apart is constrained by actin filament attachment to the Z-line and myosin filament attachment to the M-line. During the postmortem period, when oxygen is no longer available, cells metabolize glycogen via anaerobic pathways producing lactic acid-lowering tissue pH, and ATP is regenerated by creatine kinase. Once creatine phosphate is consumed, ATP can no longer be regenerated, and actin and myosin cross-link resulting in sarcomere shortening. Contraction and rigor result.

In postrigor meat, introduction of neutral-pH solutions can introduce sufficient –OH and –H ions to shield most of the charged groups on the amino acids such that the proteins repel each other allowing free water to enter (Figure 5).

Temperature has major effects on the rate of these reactions and the degree to which they proceed because (1) many are enzymatic and (2) alterations in pH in combination with temperature can denature proteins, both structural and enzyme, resulting in loss of functionality. Protein functionality is discussed in detail in another part of this encyclopedia. Muscle does not shorten at prerigor temperatures of ≥20 °C because the activity of the Ca^{2+} pump in the SR is relatively high, maintaining sufficiently low Ca^{2+} concentrations around the myofilament, blocking the effect of the troponin-tropomyosin system on actin-myosin interaction.

Effect of pH

The pH of minimum WHC of the principle muscle proteins is 5.4–5.5, which coincides with their isoelectric points. The minimum WHC of meat occurs approximately at pH 5.0, which corresponds to the isoelectric point of actomyosin (Figure 6). Salt-soluble proteins are completely soluble above pH 5.9 but are 95% insoluble below pH 4.9 with peak solubility occurring between 5.7 and 6.0. When water is added to muscle tissue, between pH 5.1 and 4.4, swelling occurs across and along the muscle fiber axis. Increases in muscle fiber diameter were much more important to total muscle swelling than increases in sarcomere length. The pH is inversely correlated with fiber diameter ($r = -0.76$) but much less so with



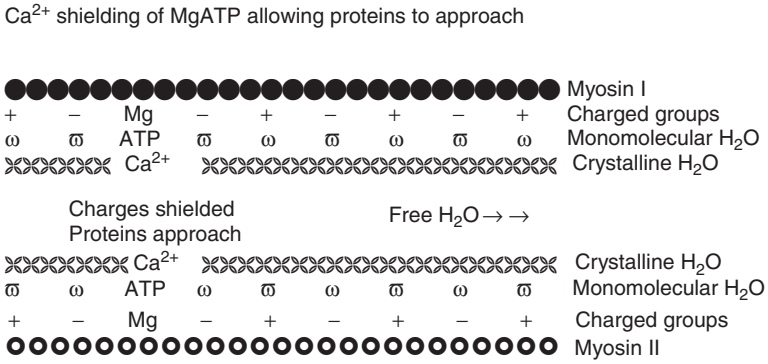


Figure 3 Cation (Ca^{2+}) shielding of mgATP and its effect on water held between proteins.

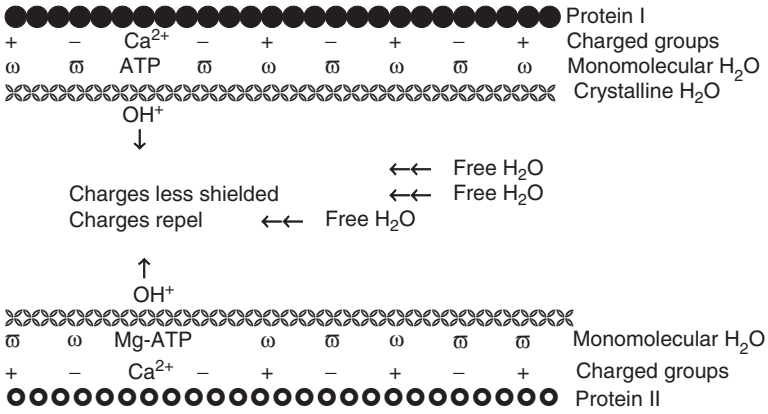


Figure 4 Effect of hydroxyl ion shielding of calcium–magnesium–ATP on water located in the myofibril.

sarcomere length (r=−0.39). As pH approaches 4.0, muscles containing predominantly white fibers continue to swell, whereas those containing predominantly red fibers shrink. This behavior has been explained by differences in buffering capacity as predominantly red fiber muscles have lower buffering capacity than white fiber muscles under acidic conditions. Maximum precipitation of sarcoplasmic proteins occurs between pH 4.8 and 5.2 regardless of temperature, however, at 37 °C and above, high pH no longer protects these proteins. The pH-induced precipitation of sarcoplasmic protein onto myofibrillar proteins decreases WHC as well as other quality characteristics of meat.

Efforts to increase WHC in meat have centered on maintaining or increasing pH during the postmortem period. Injection

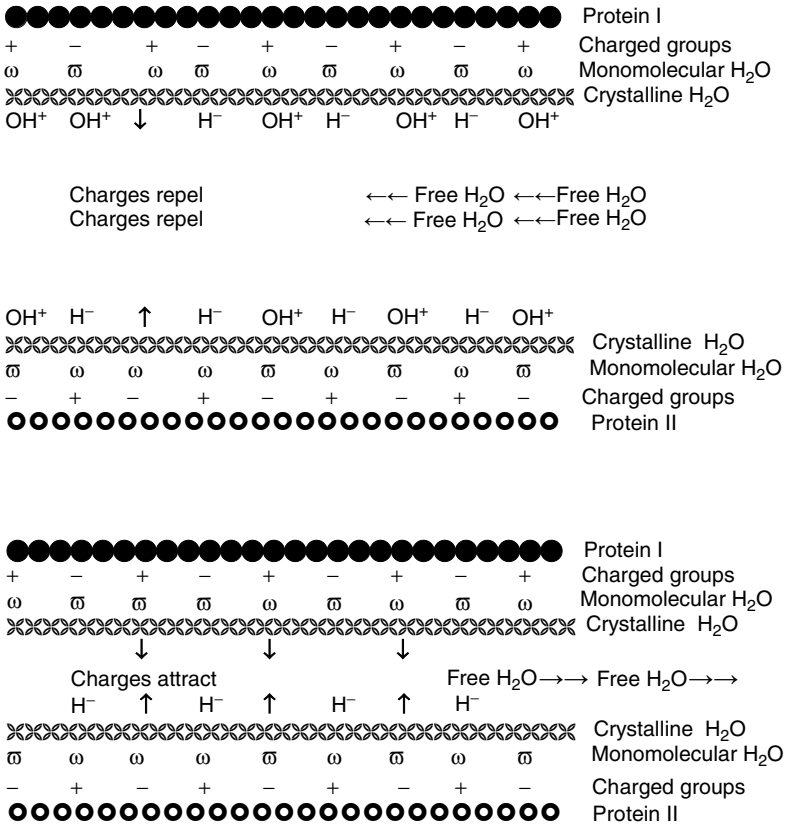


Figure 5 Effect of neutral pH (top) and low pH (bottom) on myofibrillar swelling.

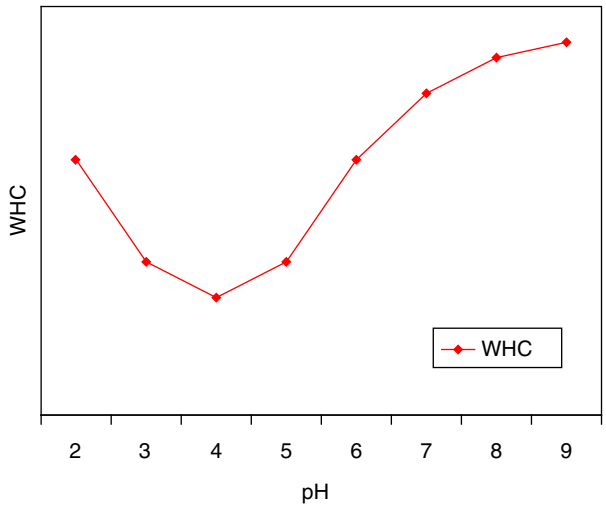


Figure 6 Effect of pH on WHC.

of adrenaline before slaughter to reduce intracellular glycogen content at slaughter such that postmortem anaerobic glycolysis to lactic acid is limited does produce meat with high postmortem pH and high WHC. Addition of pH-increasing agents such as high molecular weight phosphates and sodium bicarbonate also increase postmortem pH and WHC to some degree.

Effect of Rigor

Sarcomere shortening and pH decrease have major implications for WHC of muscle tissue. Loss of the ability to conduct aerobic metabolism results in a shift to anaerobic metabolism wherein the muscle uses glycogen to generate energy, however, it is only broken down to lactic acid and ATP is not regenerated. The significant reduction in pH is ultimately responsible for loss of protein denaturation, which makes major contributions to WHC. Immediately after slaughter, meat has excellent WHC due to the combined effects of high levels of ATP and high pH, which exist in prerigor tissue. WHC decreases during the immediate 12–24 h postmortem period during which time, ATP is enzymatically broken down and pH decreases as lactic acid forms. Rigor mortis commences in beef (bovine neck muscle) when the pH drops to 5.9 and ATP level drops to 1 μmol g^{-1}. Between pH 6.6 and 6.1 (prerigor) a small, linear decrease in myofibrillar protein solubility occurs. Protein solubility decreases dramatically as pH drops from 6.0 to 5.6. Readjusting pH to prerigor levels increases protein solubility, however, it remains much lower than that of original prerigor protein. ATP breakdown is continuous between pH 6.9 and 5.8. Taken together, this implies that the slight WHC decreases seen in the early postmortem period are a direct result of pH on muscle proteins, whereas the dramatic losses occurring during rigor are primarily due to ATP disappearance (2/3) with the remainder due to pH (1/3).

During the rigor process, muscle cells undergo both longitudinal and lateral contraction, usually within 24 h. The pressure differences between intra- and extra-cellular water compartments, which have built up during contraction are equalized very slowly.

Postmortem Glycolysis Rate

Rapid postmortem glycolysis has been associated with the high drip loss and poor WHC and color of pale, soft exudative (PSE) pork. This may be a result of myosin denaturation when a carcass experiences a low pH and a high temperature or it may be due to denaturation of sarcoplasmic proteins and subsequent precipitation onto the myofibrils. Genetic defects, such as stress susceptibility in swine, which increases postmortem glycolysis rate, produces meat with reduced WHC, which experiences high fluid losses when cooked.

Electrical stimulation (ES) of the carcass increases postmortem glycolysis rate that results in a decreased WHC as the muscle enters rigor mortis and ages rapidly. ES-induced pH decline (<6.0), while the carcass is still $>30\,^{\circ}C$, decreases Ca^{2+}-enhanced myofibrillar ATPase activity, which implies potential myosin denaturation but such denaturation stops when muscles enter rigor mortis. This can be enhanced with electrical stimulation. ATPase activity is inversely correlated with WHC.

Effect of Temperature

Immediate postmortem temperature can have significant effects on WHC. Loss of circulatory competency allows metabolic heat to accumulate allowing carcass temperatures to increase to more than 42 °C. If pH decrease is dramatic (usually during the first 45–60 min postmortem) the combined effect can produce denaturation of myofibrillar proteins such that WHC is ultimately quite low even if ultimate pH (24 h) is within normal ranges (early rigor with electrical stimulation modifies this effect in cattle). Pigs homozygotic recessive for malignant hypothermia (nn, halothane reactors) exemplify the effects of rapid postmortem temperature increase and pH decrease. Meat from these animals is commonly PSE and has poor WHC. Likewise, pigs with defects in the ryanodine receptor gene suffer from excessive pH decline, which produces abnormally acidic conditions in the meat. Ultimate pH is highly correlated with WHC and color of poultry meat as well.

At temperatures above 25 °C, sarcoplasmic proteins begin to precipitate onto myofibrillar proteins. This phenomenon appears to be related to alterations in the isoelectric point of the fibrillar proteins (from 5.0 to 7.7) allowing for intermolecular reactions between myofibrillar and sarcoplasmic proteins, which displace water and reduce solubility.

Effect of Ageing

The initial source of drip is intracellular water from myosin denaturation and loss from muscle fibers that continues through the prerigor period. Ageing commences postmortem. Water loss is driven by a pH and calcium-induced shrinkage of myofibrils during early rigor development. It is influenced primarily by the extent of sarcomere shortening (shrinkage induced by rigor). Initially, an intact cytoskeleton is necessary to translate shrinkage of myofibrils into shrinkage of the whole cell. The whole cell shrinks both lengthwise and across its diameter. Adjacent myofibrils are connected by desmin-containing filaments; peripheral myofibrils are connected to the sarcolemma by vinculin-containing structures. This highly structured, crosslinked arrangement initially leaves little room, physically, for water. During ageing, calpain proteinases degrade some of these protein interactions so that, as the cytoskeleton denatures, more water is released during tenderization. This degradation of the cytoskeleton allows the inflow of water previously expelled during rigor and released through cytoskeletal degradation. This inflow may be driven by the difference in protein concentration, which exists between intra- and extra-cellular compartments. In the prerigor intact muscle cell, all of the myofibrillar and sarcoplasmic protein exists in the intracellular compartment. During ageing, protein, primarily cytoskeletal, is lost in the drip. As ageing progresses, however, the protein concentration in the intracellular compartment appears to be the driving force attracting water back into the cell after the cytoskeleton is degraded.

Substantial shifts in electrolytes (calcium, magnesium, potassium, and phosphates) occur during ageing. Divalent ion exchange with monovalent ions frees reactive polar groups on proteins to interact with water and removes some of the steric effects that limit available space for water molecules.

Effect of Salt (NaCl)

Prerigor WHC can be retained for several days by adding NaCl to comminuted tissue in spite of the fact that NaCl and particle-size reduction increase ATP breakdown and glycolysis. Addition of up to 2% NaCl increases swelling of (beef) muscle tissue when water is added. However, addition of 3–5% NaCl causes decreased swelling followed by a rapid increase between 5% and 10% NaCl. Initial swelling has been related to replacement of calcium on meat proteins with sodium. Decreased swelling between 3% and 5% NaCl appears to be due to exchange of magnesium and potassium for sodium, whereas the rapid swelling between 5% and 10% NaCl appears to be a result of ionic effects on meat proteins. Neutral salts increase WHC of meat with high ATP levels and/or high pH to a much greater degree than after ATP depletion or after pH had declined to near the pI of myofibrillar proteins (pH 5.0). If salts are added to prerigor meat, attracting water into the interfilamental spaces, the swelling may be so great that the proximate distance between filaments is no longer conducive to actomyosin crosslinking, which normally occurs subsequent to ATP depletion. More water can be immobilized in the interfilamental spaces of higher pH tissue due to repulsion of charged proteins.

Effect of Phosphates

The sodium salts of various phosphates have long been known to increase WHC and muscle tissue swelling, and decrease both drip and cook losses. The effects of phosphates on meat

swelling appear to be related to their relative effects on pH with the exception of tetrasodium pyrophosphate (TP), which produces a swelling effect in excess of its ability to raise pH. Commercial phosphates with a pH of 9.0–10.0 can raise tissue pH above the pI by increasing the net negative charge on the myofibrillar proteins causing them to repel each other allowing water to enter. However, it must be noted that the buffering capacity of meat proteins is substantial; so much so that, in relevant quantities, phosphate with pH 10 shifts the meat pH by only 0.1–0.2 pH units, which would be expected to have negligible effects on WHC unless the tissue was at or very close to the muscle protein pI.

Low-molecular weight inorganic phosphates can react directly with actomyosin. This effect is related to breakdown of low-molecular weight phosphates to pyrophosphate (PP) and diphosphate by muscle ATPase, which has a specific swelling effect on lean meat in addition to its pH effect and its ability to split actomyosin. It has been suggested that phosphates are effective in increasing WHC to the extent that they are hydrolyzed to pyrophophate by endogenous enzymes (ATPase). PP-induced dissociation of the actomyosin complex is dependent on both pH and ionic strength. The affinity of actomyosin for tripolyphosphate (TPP) increases in the presence of high NaCl concentrations and calcium and magnesium. The mode of action is that the sodium from NaCl at high ionic strength forms an Na–myosinate complex that strengthens the affinity of actomyosin for PP and TPP. PP may then form a divalent metal-polyphosphate complex that also increases actomyosin for polyphosphates. It may be that calcium remains attached to one filament, the PP then complexes with it to prevent calcium from binding to an adjacent filament. In general, the reaction is comprised of the formation of a divalent metal-phosphate complex that acts as a normal salt with salt-free actomyosin in a way similar to organic polyphophates such as ATP.

In the presence of little or no salt, the highly polymerized inorganic phosphates, such as hexametaphosphate, bind directly to the positively charged groups on the actomyosin complex. Univalent cations compete with the phosphate for the binding site on the actomyosin molecule leaving the phosphate to behave like a normal salt increasing the ionic strength. The increase in hydration of actomyosin may occur because polyphosphates are capable of eliminating the alkaline earth metals bound to structural proteins. A significant negative correlation exists between WHC and calcium and magnesium content in beef muscle tissue. Based on enzyme studies, it appears that polyphosphate, rather than chelating calcium and magnesium, forms a divalent-metal polyphosphate complex and acts as a normal salt on actomyosin. Therefore, the presence of bound calcium facilitates the approach of polyphosphates to the protein (such that polyphosphate can be split to PP). Cleavage of the actomyosin bond allows the protein fragments to spread and water to enter.

The actions of NaCl and phosphate on actomyosin can be summarized as follows:

NaCl is a neutral salt. The first effect on muscle cells is osmotic. As membrane integrity is destroyed, semipermeability is lost and the salts migrate into the fibers. When NaCl is used in meat, which is on the basic side (pH 5.3–5.7) of the isoelectric point of actomyosin (pI = 5.0), the binding of the Cl^- anions to positively charged protein side groups screens the positive charge and breaks salt bridges allowing the protein strands to spread resulting in greater hydration. Binding of the anions shifts the pI to a lower pH. In the absence of a plasticizer such as ATP or polyphosphate, actomyosin can only be split at pH values <1.0 or >7.0. Phosphates, which are salts of weak acids, also dissociate to anions and cations. At pH values above the pI of the proteins, the anions eliminate the alkaline earth metals bound to structural proteins increasing WHC as actin and myosin are released from each other. Calcium is removed by ion exchange and the intramolecular bonds break. Salts with polyvalent anions are most effective (polyphosphates, citrate, and oxalate). Elimination of calcium inactivates ATP hydrolysis and results in hydration of actomyosin. Exchange of bound calcium is most effective at pH values above the pI because the cations crosslink carboxyl groups of the protein.

Effect of Ionic Strength

Myofibrillar proteins are more soluble at higher ionic strengths. High ionic strength extraction solutions can dissociate actomyosin complexes into higher mole ratios of myosin to actin than can lower ionic strength solutions. Hexametaphosphate, TPP, and PP all have the capacity for increasing the ionic strength. Polyphosphates provide approximately the same degree of ionic strength as NaCl on a weight percent basis; although polyphosphates have higher valence anions, they dissociate to a lesser extent. NaCl levels of 0.6 mol l^{-1} (~2.4% of muscle weight) significantly increases ionic strength and increases fiber swelling.

Phosphate solutions vary dramatically in pH. A 1% (w/v) solution of some commonly used phosphates have pH values of approximately:

Tetrasodium pyrophosphate	10.5
Sodium tripolyphosphate	9.8
Sodium hexametaphosphate	7.0
Sodium acid pyrophosphate	4.2

Addition of NaCl plus polyphosphates increases meat hydration with a maximum effect after 16 h in storage at 0 °C. Swelling is twice as great with TP at salt levels up to 10% as with NaCl alone. Tetrasodium PP is generally the most effective phosphate for increasing WHC with respect to both the presence and absence of NaCl (Table 1). Addition of NaCl slightly decreases pH and greatly increases WHC.

Effect of High-Pressure Processing

Subjecting beef to 300–650 MPa at room temperature increases expressible moisture whereas pressurizing frozen beef decreases expressible moisture. Salt addition also reduces high-pressure induced drip loss. Pressurization can improve the ability of meat samples to hold free water, however, drip losses can increase.

High pressure partially inhibits postmortem metabolism resulting in lower muscle lactate levels and higher pH values.

Table 1 Percentage of fluid retained (gram retained per 100 g absorbed) by chicken breasts marinated and tumbled for 30 min

No salt							*8% salt*						
Control	*PP*		*TP*		*HMP*		*Control*	*PP*		*TP*		*HMP*	
	Low	*High*	*Low*	*High*	*Low*	*High*		*Low*	*High*	*Low*	*High*	*Low*	*High*
Marinade retained (gram per 100 g muscle)													
4.6	29.0	35.1	29.8	25.0	16.4	15.5	26.3	37.8	41.5	34.5	29.1	28.4	27.9
Percentage retention (gram retained per 100 g absorbed)													
24.1	67.4	80.0	79.9	71.6	67.8	59.4	81.7	85.7	78.9	76.8	73.1	76.8	67.7
Cooking yield, %													
77.3	106.0	112.0	103.9	100.4	88.7	90.0	93.5	114.9	119.4	111.9	108.3	95.3	97.5

Abbreviations: PP, sodium pyrophosphate; TP, sodium tripolyphosphate; HMP, sodium hexametaphosphate.
Low = 1.6% and high = 3.2% phosphate
Source: Adapted from Xiong, Y.L., Kupski, D.R., 1999. Time dependent marinade absorption, retention, cooking yield and palatability of chicken filets marinated in various phosphate solutions. Poultry Science 78, 1053–1059.

It also reduces cook and drip loss in pork loins and hams. Subjecting enhanced pork loins to hydrodynamic pressure reduces drip losses compared to controls. Pressure-treating lamb has been shown to cause a 94% contraction in sarcomere measurements and extensive fiber disruption in addition to pH decrease. Cooking loss was also lower in pressure-treated samples.

Histological studies have shown that high pressure induces structural weakening of intramuscular connective tissue, especially the perimysium. Ageing time (to achieve beef tenderness) can be halved by subjecting the beef to pulsed electrohydraulic shock.

Heating under pressure is known to increase tenderness. It has been suggested that this improvement is a result of strengthened myfibrillar structure that, when sheared by mastication, allows the crack to pass through the meat (brittle fracture) rather than dissipating into a visco-elastic structure. Adequate cathepsin activity must have occurred to achieve this effect. However, pressure in combination with freezing induces nearly complete denaturation of actin and significant denaturation of myosin, whereas connective proteins remain practically unaltered.

Effect of Ammonium Hydroxide

Enhancement systems are generally composed of water, salt, and phosphate. Injection of these solutions has shown to increase water-binding capacity and tenderness. The chloride ions of salt are thought to bind to the myofilaments increasing the electrostatic repulsive force between them allowing additional water into the space, which is by capillary forces. Reducing NaCl concentration reduces the moisture absorptivity and increases the drip and free water loss. Phosphates increase the swelling of muscle fibers as they shield the charges allowing water to flow in and become immobilized in the myofibril lattice. Sodium polyphosphate in injection brines improves WHC, as evidenced by reduced drip loss, thaw loss, and cooking loss values. In the presence of phosphates, the concentration of chlorine needed for maximum swelling is reduced. Ammonium hydroxide has been used to replace some or all of the phosphates in injection brines in an effort to increase meat tenderness.

Adding 0.5%, 1.0%, or 2.0% ammonium hydroxide to ground beef has been shown to increase pH and WHC of ground buffalo patties. Marinating buffalo meat with 0.1–1.0% solutions of ammonium hydroxide also increased pH, WHC, collagen solubility, total and salt-soluble protein extractability, and cooking yield. Based on scanning and transmission electron microscopy, breakdown of endothelium layers surrounding muscle fibers and weakening of Z-discs was evident in samples containing 0.1% and 0.5% ammonium hydroxide. As pump level (and total ammonium hydroxide introduced) increases from 0% to 30% in beef chuck and round muscles, pH increases as does soft, mushy texture.

Using 1% ammonium hydroxide in place of 4.5% sodium-based phosphates in injection brines has been shown to have no effect on consumer evaluation of beef loin. Ammonium hydroxide produced beef loin steaks with comparable tenderness and palatability. The pH of ammonium hydroxide-containing steaks is slightly higher (pH 5.96) than that of control steaks (pH 5.86). Cook loss was slightly lower in control steaks than in ammonium hydroxide-injected steaks. Compared to alkaline-based (3.6% sodium chloride, adjusted to pH 10 with ammonium hydroxide) injection solutions, phosphate-injected steaks performed better than alkaline-injected steaks (cook yield, WHC, lipid oxidation, color stability, tenderness, juiciness, and purge loss. They also experience less purge than alkaline-injected steaks. Lamb injected with an ammonium hydroxide-containing enhancement solution exhibits higher cooked moisture retention and sensory quality characteristics. Ammonium hydroxide appears to have no effect on sarcomere length or desmin degradation.

Effect of Calpain

Much of the water in the muscle is trapped in cell structures, intra- and extra-myofibrillar spaces. Changes in the intracellular structure of the cell can influence the ability of muscle

cells to retain water. During rigor, lateral shrinkage of the myofibrils is transmitted to the entire cell if proteins linking myofibrils together and to the cell membrane (desmin) are not degraded. Degradation of cytoskeletal proteins can increase muscle cell shrinkage resulting in drip loss. In particular, desmin proteolysis contributes to greater WHC.

Calpains and cathepsins are cysteine proteinases that affect glycolysis and loss of myofilament integrity and play a key role in WHC. The calpain proteolytic system consists of at least three proteases, μ-calpain, *m*-calpain, and calpain 3, and an inhibitor of μ- and *m*-calpain, calpastatin. This system plays a key role in postmortem proteolysis. When activated by calcium, the calpains degrade substrates, but also autolyze, leading to loss of activity. Activation of μ-calpain probably occurs before meat pH levels fall to 6.1–6.2.

A sequential degradation of the structural proteins postmortem, where calpain initiates the disruption and destabilization of the myofibrillar structure, allows the enzymes that degrade myosin and actin to act. Calpains can be regulated by a nitric oxide donor S-nitrosoglutathione, which can nitrosylate calpain in both the absence and the presence of calcium especially at pH 6.5. The combination of S-nitrosoglutathione and calcium affect *m*- and μ-calpain activity and regulate μ-calpain autolysis rate. In pork, a specific CAST gene (CAST EU137105:g.76 872AA) affects both postmortem calpain activation time and drip loss.

During the postmortem period, desmin and vinculin degrade gradually whereas talin degrades rapidly, which is consistent with the hypothesis that degradation of the cytoskeleton slowly removes the linkage between lateral structures resulting in shrinkage of myofibrils and of the entire muscle fibers, which removes the force that causes flow into the extracellular space. Inflow of previously expelled water is then possible, which increases WHC.

Colocalization of active *m*-calpain with beta-1 integrin has been observed on the cytoplasmic side of the cell membrane. Calpain inhibits integrin degradation and opening of drip channels. It has been suggested that the opening of drip channels may be a consequence of calpain-mediated degradation of cell surface integrins. High levels of intact integrin at one day postmortem negatively correlates with cumulative drip loss. High levels of intact integrin at 5 days postmortem correlates negatively cumulative purge loss.

See also: Additives: Functional. Carcass Composition, Muscle Structure, and Contraction. Conversion of Muscle to Meat: Aging. Sensory and Meat Quality, Optimization of. Species of Meat Animals: Pigs

Further Reading

Baublits, R.T., Pohlman, F.W., Brown, A.H., *et al.*, 2006. Solution enhancement and post-enhancement storage effects on the quality, sensory and retail display characteristics of beef triceps brachii muscles. Journal of Food Science 71, S91–S96.

Bendall, J.R., Wismer-Pedersen, J., 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. Journal of Food Science 27, 144.

Bowker, B.C., Liu, M., Callahan, J., Solomon, M., 2010. Effect of hydrodynamic pressure processing on the processing and quality characteristics of moisture-enhanced pork loins. Journal of Food Science 75 (4), S237–S244.

Bowker, B.C., Schaefer, R.B., Grapperhaus, M.J., Solomon, M.B., 2011. Tenderization of beef loins using a high efficiency sparker. Innovative Food Science and Emerging Technologies 12 (2), 135–141.

Buczkowski, J., Ligeza, U., Mroczek, J., Pisula, A., 1990. Effect of NaCl concentration in brines and pickling media on the physico-chemical properties of the meat. Gospodarka Miesna 42 (2), 16–20.

Cerruto-Noya, C.A., VanOverbeke, D.L., DeWitt, M.C., 2009. Evaluation of 0.1% ammonium hydroxide to replace sodium tripolyphosphate in fresh meat injection brines. Journal of Food Science 74 (7), C519–C525.

Den Hertog-Meischke, M.J.A., Smulders, F.J.M., van Logtestijn, J.G., van Knaper, F., 1997. The effect of electrical stimulation on the water-holding capacity and protein denaturation of two bovine muscles. Journal of Animal Science 75, 118–124.

Everts, A.K.R., Wulf, D.M., Wheeler, T.L., *et al.*, 2010. Enhancement technology improves palatability of normal and callipyge lambs. Journal of Animal Science 88 (12), 4026–4036.

Fernandez-Martin, F., Otero, L., Solas, M.T., Sanz, P.D., 2000. Protein denaturation and structural damage during high-pressure-shift freezing of porcine and bovine muscle. Journal of Food Science 65 (6), 1002–1008.

Fernandez, P.P., Sanz, P.D., Molina-Garcia, A.D., *et al.*, 2007. Conventional freezing plus high pressure—low temperature treatment: Physical properties, microbial quality and storage stability of beef meat. Meat Science 77 (4), 616–625.

Gandolfi, G., Pomponio, L., Ertbjerg, P., *et al.*, 2011. Investigation on CAST, CAPN1 and CAPN3 porcine gene polymorphisms and expression in relation to post-mortem calpain activity in muscle and meat quality. Meat Science 88 (4), 694–700.

Hamling, A.E., Calkins, C.R., 2008. Enhancement of beef chuck and loin muscles with ammonium hydroxide and salt. Journal of Animal Science 86 (4), 967–971.

Hamm, R., 1960. Biochemistry of meat hydration. Advances in Food Research 10, 355–359.

Honikel, K.L., Fischer, C., Hamm, R., 1981. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. Postmortem storage of muscle at 20 °C. Journal of Food Science 46, 1–6.

Hopkins, D.L., Thompson, J.M., 2001a. Inhibition of protease activity. I. The effect on tenderness and indicators of proteolysis in ovine muscle. Meat Science 59 (2), 175–185.

Hopkins, D.L., Thompson, J.M., 2001b. Inhibition of protease activity. II. Degradation of myofibrillar proteins, myofibril examination and determination of free calcium levels. Meat Science 59 (2), 199–209.

Houbak, M.B., Ertbjerg, P., Therkildsen, M., 2008. *In vitro* study to evaluate the degradation of bovine muscle proteins post-mortem by proteasome and mu-calpain. Meat Science 79 (1), 77–85.

Huff-Lonergan, E., Lonergan, S., 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science 71 (1), 194–204.

Ichinoseki, S., Nishiumi, T., Suzuki, A., 2006. Tenderizing effect of high hydrostatic pressure on bovine intramuscular connective tissue. Journal of Food Science 71 (6), E276–E281.

Joo, S.T., Kauffman, R.G., Kim, B.C., Park, G.B., 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Science 52, 291–297.

Kennick W.H., Elgasim A., Holmes Z.A., Barrett E.F., 1978. The effect of ultrahydrostatic pressurization of pre-rigor muscle on tenderness and other characteristics of economic importance. Proceedings of the European Meeting of Meat Research Workers No. 24, pp. E12:1—E12:6.

Koohmaraie, M., Geesink, G.H., 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science 74 (1), 34–43.

Kristensen, L., Purslow, P.P., 2001. The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. Meat Science 58, 17–23.

Kwiatkowska, A., Jankowska, B., Cierach, M., 2002. Changes in meat hydration upon high pressure. Polish Journal of Food and Nutrition Sciences 11/52 (1), 51–54.

Lawrence, T.E., Dikeman, M.E., Hunt, M.C., Kastner, C.L., Johnson, D.E., 2004. Effects of enhancing beef longissimus with phosphate plus salt, or calcium lactate plus non-phosphate water binders plus rosemary extract. Meat Science 67, 129–137.

Lawrie, R.A. (Ed.) 1991. The eating quality of meat. In: Meat Science, fifth ed. New York: Pergamon Press pp. 190–199.
Lawson, M.A., 2004. The role of integrin degradation in post-mortem drip loss in pork. Meat Science 68 (4), 559–566.
Leach, L.M., Ellis, M., Sutton, D.S., McKeith, F.K., Wilson, E.R., 1996. Influence of slaughter weight on growth and carcass characteristics, commercial cutting and curing yields, and meat quality of barrows and gilts from two genotypes. Journal of Animal Science 75, 934–941.
Nath T.M., Hand C.D., Everts A.J., *et al*., 2006. Trained and consumer evaluations of five different beef muscles with or without pH enhancement using ammonium hydroxide. Available at: http://www.meatscience.org/Pubs/rmcarchv/2006/presentations/RMC06_Abstracts.PDF (accessed September 2011).
Naveena, B.M., Kiran, M., Reddy, K.S., *et al*., 2011. Effect of ammonium hydroxide on ultrastructure and tenderness of buffalo meat. Meat Science 88 (4), 727–732.
Naveena, B.M., Sen, A.R., Muthukumar, M., Babji, Y., Kondaiah, N., 2010. Effects of salt and ammonium hydroxide on the quality of ground buffalo meat. Meat Science 87 (4), 315–320.
Offer, G., 1991. Modelling of the formation of pale, soft, and exudative meat: Effects of chilling regime and rate and extent of glycolysis. Meat Science 30, 157–160.
Offer, G., Knight, P., 1988. The structural basis of water-holding in meat. In: Lawrie, R.A. (Ed.), Developments in Meat Science, fourth edition London: Elsevier Applied Science, pp. 63.
Parsons, A.N., VanOverbeke, D.L., Goad, C.L., Mireles DeWitt, C.A., 2011a. Retail display evaluation of steaks from select beef strip loins injected with a brine containing 1% ammonium hydroxide. Part 1: Fluid loss, oxidation, color, and microbial plate counts. Journal of Food Science 76 (1), S63–S71.
Parsons, A.N., VanOverbeke, D.L., Goad, C.L., Mireles DeWitt, C.A., 2011b. Retail display evaluation of steaks from select beef strip loins injected with a brine containing 1% ammonium hydroxide. Part 2: Cook yield, tenderness, and sensory attributes. Journal of Food Science. 76 (1), S84–S88.
Rao, M.V., Gault, N.F.S., Kennedy, S., 1989. Variations in water-holding capacity due to changes in the fibre diameter, sarcomere length and connective tissue morphology of some beef muscles under acidic conditions below the ultimate pH. Meat Science 26, 19–37.
Schultz, G.W., Russell, D.R., Wierbicki, E., 1972. Effect of condensed phosphates on pH, swelling and water-holding capacity on beef. Journal of Food Science 37, 860–866.
Scopes, R.K., 1964. The influence of post-mortem conditions on the solubilities of muscle proteins. Biochemistry Journal 91, 201–204.
Siegel, D.G., Schmidt, G.R., 1979. Ionic, pH, and temperature effects on the binding ability of myosin. Journal of Food Science 44, 1686–1691.
Sikes, A., Tornberg, E., Tume, R., 2010. A proposed mechanism of tenderising post-rigor beef using high pressure-heat treatment. Meat Science 84 (3), 390–399.
Souza, C.M., Boler, D.D., Clark, D.L., *et al*., 2011. The effects of high pressure processing on pork quality, palatability, and further processed products. Meat Science 87 (4), 419–427.
Swift, C.E., Berman, M.D., 1958. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. Food Technology 13, 365–370.
Toepfl, S., Heinz, V., 2011. Reduction of ageing time achieved. Fleischwirtschaft 91 (2), 46–48.
Vote, D.J., Platter, W.J., Tatum, J.D., *et al*., 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. Journal of Animal Science 78, 952–957.
Wynveen, E.J., Bowker, B.C., Grant, A.L., *et al*., 2001. Pork quality is affected by early postmortem phosphate and bicarbonate injection. Journal of Food Science 66 (6), 886–891.
Xiong, Y.L., Kupski, D.R., 1999. Time dependent marinade absorption, retention, cooking yield and palatability of chicken filets marinated in various phosphate solutions. Poultry Science 78, 1053–1059.
Zhang, W., 2009. Involvement of protein degradation, calpain autolysis and protein nitrosylation in fresh meat quality during early postmortem refrigerated storage. PhD Dissertation. Iowa State University, Ames, Iowa. Available at http://gradworks.umi.com/33/55/3355550.html (accessed October 2011).
Zhang, W.G., Lonergan, S.M., Gardner, M.A., Huff-Lonergan, E., 2006. Contribution of postmortem changes of integrin, desmin and μ-calpain to variation in water holding capacity of pork. Meat Science 74 (3), 578–585.

Relevant Websites

www.ncbi.nlm.nih.gov/pubmed/18274969
Critical Reviews in Food Science and Technology.
http://gradworks.umi.com/33/55/3355550.html
Iowa State University.
www.uco.es/zootecniaygestion/img/pictorex/10_10_27_ATT00013.pdf
Iowa State University.
www.extension.org/pages/.../water-holding-capacity-of-fresh-meat
Iowa State University Extension Service.
www.ncbi.nlm.nih.gov/pubmed/9413116
National Cattlemen's Beef Association.
www.ansc.purdue.edu/swine/swineday/sday02/18.pdf
Purdue University.
http://www.meatscience.org/Pubs/rmcarchv/2006/
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CHEMISTRY AND PHYSICS OF COMMINUTED PRODUCTS

Contents

Emulsions and Batters

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Glossary

Emulsifier A protein or other compound with polar and nonpolar regions able to form a stable matrix of two immiscible compounds.

Emulsion An intimate mixture (colloidal suspension) of two immiscible liquids (oil and water), one dispersed in the other to form tiny droplets with the aid of an emulsifier.

Interfacial protein film (IPF) A stabilizing protein film containing myosin, which acts as an emulsifier.

Meat emulsions or batters Systems with proteins present in three different phases: the protein matrix, the aqueous phase, and the interfacial protein film, held together by a variety of attractive forces acting between them.

Introduction

General Description

The classical definition of an emulsion is an intimate mixture (colloidal suspension) of two immiscible liquids (oil and water), one dispersed in the other to form tiny droplets with the aid of an emulsifier. The best examples of products within this definition are mayonnaise or salad dressings based on vinegar and oil where the oil and water phases do not separate on standing. A less strict description of an emulsion is a two-phase system of a dispersion of solids in a liquid in which the solids are not miscible. The second description more accurately defines what is found in a meat system. The liquid phase is water and dissolved components whereas the solid phase is nondissolved meat components and added ingredients. In a meat system, the solid phase contains the muscle, fat, connective tissue from the meat, as well as other food ingredients such as cheese, nuts, fruits, and vegetables. Although meat products do not fit the more classical definitions of emulsions, meat emulsions and meat emulsifiers are similar in many ways and the terms are often applied.

The emulsifier in a meat system is a solubilized muscle protein. The sarcoplasmic proteins have very weak binding properties, whereas the solubilized myofibrillar proteins, including actin, myosin, and actomyosin, have powerful binding properties in a meat system. Stromal proteins from connective tissue sources have some properties resembling those of the myofibrillar proteins, but they also have certain properties that are often very difficult to manage in meat systems.

Importance of Emulsified Comminuted Products

Since early in recorded history, it has been a practice to use salt to control spoilage and to extend the food supply. There is evidence of this practice worldwide. The next step beyond salt curing for preservation was probably an early understanding of what we now know as salt solubilization of myofibrillar proteins and the ability to bind small pieces of muscle to other small pieces of muscle – early sausage making. Sometimes by word of mouth and at other times by independent discovery, people in various regions of the world, of different cultures and religions, found ways to use the available meat, often combined with various available flavor modifiers including fruits, vegetables, spices, and the local salt with its inherent contaminants. Most importantly, the muscles of animals, birds, and fish respond similarly to the technology. Larger pieces of muscle became salt cured; whole-muscle products and small pieces of meat (muscle, connective tissue and fat) became sausage products. Although the names of meat products may vary widely, the common thread has been the need

for preservation, the requirement for a variety of flavors and products, and the need to ensure the availability of the meat over extended periods, with great environmental and survival challenges. Virtually all historical records make references to what we now call cured meats and sausages products.

Emulsification – the act of applying the emulsifier to the comminuted meat (of fine or minute particle size) – is what sausage and processed meat processors have done for centuries. Understanding these techniques and having a thorough understanding of protein solubilization and binding to other proteins, fat, water, and other inclusions, are essential for the production of processed meat products ranging from sausages to hams, corned beef, pastrami, and bacon. Regardless of whether tiny particles or large muscles and portions of muscles, it would not be possible to provide consumers with a wide array of processed meat products available worldwide having nearly endless variations and representing ethnic cultures and constantly changing consumer interests. This variety results from the understanding of how to produce emulsified meat products.

Meat Proteins

General Classification

Myofibrillar proteins, many of which can be solubilized with salt, are clearly the proteins of main interest in the development of emulsifiers to bind together muscle pieces in processed meats. The water-soluble sarcoplasmic proteins take part in the binding of meat but are usually considered weak in terms of binding strength. The amount of salt needed to solubilize myofibrillar proteins, such as actin, myosin, and actomyosin, is usually either that of a 7% brine solution or a dry salt addition of between 4.5% and 5% based on the weight of the meat. Solubilization of these proteins in water from the meat or added water provides an emulsifier that, through mechanical action, coats the surface of the fat particles, muscle particles, connective tissue, and any other nonmeat inclusions in the meat mix. When all particles of the meat mix are completely coated with the emulsifier, the mix is placed into restraints such as fibrous casings or metal moulds for large whole-muscle products and in natural, cellulose, collagen, or plastic casings for small-diameter sausages. During the subsequent cooking and other processing steps, the solubilized or emulsified proteins that are coating the meat pieces are heat denatured and bind the particles together to form sausages or processed meat products.

Protein Extraction

The salt-soluble proteins that are solubilized during the mincing, mixing, and mechanical processing steps become the binder to hold the meat mixture or emulsion together. A sufficient amount of emulsifier is important, because too little will result in insufficient binding, often resulting in a soft texture or meat that appears to crumble and fall apart. Excessive production of the emulsifier followed by heat processing may result in a hard texture, often characterized as tough and rubber like. Because the proteins have been made soluble and are used functionally, but not created or degraded, the amount or quality of protein in the sausage or processed meat is not altered.

Myofibrillar Protein Functionality

Under most commercial conditions, the functionality of the salt-soluble proteins may be altered by a number of processing steps. Denaturation of these proteins is similar to any form of denaturation. Heat denaturation is the most common, but the influence of dehydration, salt addition, acidity changes from pH shifts during fermentation, moisture loss, and protein denaturation during freezing may also lead to loss of functionality. These may influence the further processing of the meat during steps such as fermentation, smoking, drying, and chilling.

Meat Emulsion Matrix

Meat emulsions are made by mixing or chopping meat and water with the addition of salt until a fine, protein-rich slurry is formed. This matrix or emulsifier is then capable of binding fat, water, and other inclusions. During cooking, the salt-soluble proteins (emulsifier) cross-link and coagulate, and this results in an immobilization of the fat, water, and other constituents.

Many attempts have been made to define meat emulsions or batters, and several different viewpoints exist. It is generally accepted that finely comminuted meat products are multiphase systems containing various elements: true solutions, gels, emulsified fat, and air. These components are held together by a variety of attractive forces acting between them, i.e., the meat emulsion or meat batter. In such a system, proteins are present in three different phases: the protein matrix, the aqueous phase, and the interfacial protein film (IPF), and the types and amounts of proteins present in each phase influence the textural properties and stability of the cooked product.

Two main models have been proposed to explain the structure of these food systems: the emulsion theory and the physical entrapment theory.

Emulsion Theory

Classic emulsions consist of two immiscible liquid phases, one dispersed in the other as a colloidal suspension. Another less strict approach defines an emulsion as the system formed by two immiscible phases, one being discontinuous and the other being continuous. In meat emulsions, fat globules and other inclusions of different sizes and composition are the solid discontinuous part, which is dispersed in a continuous aqueous solution containing salt-soluble myofibrillar proteins and other particles such as insoluble proteins, muscle fibers, and connective tissue.

During meat comminution and mixing, a considerable amount of energy goes into the system. This can create a thermodynamically unstable condition. The salt-soluble proteins surround the fat globules, forming a stabilizing protein

film or IPF membrane. The main protein involved in the formation of the IPF is myosin, which acts as an emulsifier, thereby reducing the interfacial tension and stabilizing the emulsion. Myosin orients itself with the heavy meromyosin (HMM) head facing the hydrophobic phase (fat globules) and the light meromyosin tail oriented toward the aqueous phase.

The formation of the protein emulsifier film is an entropy-driven process that reduces the free energy of the system. The alignment of the HMM heads around the fat globules allows for a greater entropy of the surrounding water molecules, thus decreasing the free energy. This film formation around the fat globules has been demonstrated with microphotographs, which provide clear evidence of an emulsification process and of the importance of the IPF for meat batter stabilization.

Physical Entrapment Theory

This theory proposes that the salt-soluble myofibrillar proteins in the continuous phase exist in a sol form, which turns into a gel on cooking. Soluble myofibrillar proteins present in the continuous phase may exhibit three types of interactions or attraction forces: protein–protein, protein–water, and protein–fat. When balanced protein–protein and protein–solute interactions exist, protein molecules may create an ordered network governed by a combination of intermolecular interactions including hydrogen bonding, electrostatic attractions, van der Waals forces, and hydrophobic interactions. During heating, myofibrillar proteins start to denature, leaving several hydrophobic domains exposed. Hydrophobic interactions are then initiated and further aggregation, seeking thermodynamic stability, leads to gelation and to the formation of the characteristic network of protein fibers. This protein aggregation during cooking immobilizes the fat globules by binding them to the matrix and preventing coalescence. Physical entrapment of fat and other particles therefore occurs as a result of this phase change during thermal processing.

Comparing these two theories, it appears that formation of the meat emulsion or batter is a combination of an effective emulsifying film surrounding all particles including the fat globules, connective tissue protein, and other solid inclusions with the physical restriction and binding given by the ordered protein network. Many factors influence the extent to which either of these two mechanisms is involved in the structural properties and stability of the system.

Emulsion/Batter Stability

Meat emulsions/batters are stabilized after cooking when fat globules and other elements are immobilized by gelation of the protein matrix and the characteristic properties of the finished product are attained. The stabilization of fat, water, and other elements in the system is therefore essential for the sensory acceptability of the products.

Fat loss from a meat batter can be associated with initial moisture losses during cooking due to the formation of channels through the matrix that allow migration of water and melted fat to the surface of the product. Emulsion stability can be measured by the amount of moisture loss or fat coalescence. In successful products, these two defects are minimal when all particles, regardless of particle size, are completely covered with sufficient quantities of the myofibrillar protein emulsifier. Three major factors contribute to fat stabilization: the biophysical properties of the protein film surrounding the fat globules, the gelation properties of the protein matrix, and the physical characteristics and cell integrity of the fat. Water retention, however, is influenced by the pH and the amount of extracted salt-soluble protein in the solution. Batter stability is commonly influenced by the thermal process, the biochemical state of the muscle proteins, the ionic environment, and the pH. Variations of these factors beyond relatively restricted boundaries may cause instability.

Interfacial Protein Film Formation and Gelation

Although the emulsifying properties of the protein film play a major role in fat stabilization, the gel formed by the protein matrix has a high water-binding capacity and strong elastic properties. Protein hydrophobicity is particularly important for effective formation of the IPF, and protein–protein interactions are important during the formation of a successful soluble protein network. Myofibrillar proteins, which are interfacially absorbed, lose their ability to gel but bind to the protein matrix of the meat batter. Irreversible heat setting of this protein network ultimately stabilizes the system. The ionic environment of the meat system is important for IPF formation and heat-induced gelation. Pale, soft, and exudative (PSE) pork reduces the extractability and functionality of myofibrillar proteins. Disintegration of the muscle structure and solubilization of proteins as a result of high ionic strength are required for the creation of a three-dimensional gel structure, which then supports water binding through capillary forces and immobilizes protein emulsifier-coated fat globules and other inclusions, such as vegetables, fruits, nuts, cheese, salt, or vinegar-cured inclusions. The concentration and type of salt in the formula for the comminution process affect the amount of actin, actomyosin, myosin, and other proteins extracted from the lean meat, and this has a direct influence on batter stability. Studies have shown that reducing sodium chloride (NaCl) levels from 2.5% to 1.5% resulted in a reduced stability with an unacceptable texture due to a reduction of nearly 50% of the total proteins extracted to form the IPF. In general, NaCl and lithium chloride (LiCl) are capable of extracting myofibrillar proteins more efficiently than other chlorides such as potassium chloride (KCl), or the divalent salts magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$).

A significant factor for batter stability is the size of the relative interfacial surface, which is determined by the degree of fat disintegration and the amount of fat added. Thus both the quantity and particle size of fat are critically important, as an adequate amount of the emulsifier is needed to coat the complete surface area of the fat. Provided that enough soluble myofibrillar protein is present in the aqueous phase, the stability is greater when the size of the fat particles is smaller. Short chopping times result in interfacial surfaces with thick layers of myofibrillar segments around the fat globules and without efficient distribution of protein and fat throughout the interface. This is not favorable for stability. As the

interfacial surface increases, the protein layer and fat particles are more evenly distributed and balanced protein–water, protein–lipid, and protein–protein interactions are established. This leads to optimum stability. If the relative interfacial surface is too large (e.g., owing to high fat addition levels or too small particle sizes of the fat), the protein film around the fat globules becomes thinner and its mechanical strength decreases, which may lead to instability when the fat expands during thermal processing. Also, excessive amounts of protein could be withdrawn from the continuous phase, reducing protein–protein interactions and proper gelation during heating. Protein aggregates might be absorbed at the interface of more than one fat globule, resulting in a decrease in the system's flexibility and shear resistance. The result is poor fat stability and a loose noncohesive product after cooking.

If enough soluble myofibrillar protein is present in the aqueous phase, the presence of sufficient fat seems to play an important role. In fat-reduced batters, a relatively large proportion of the hydrophobic regions of the protein molecules are not utilized; this causes a shift in the absorption pattern, with reduced water-holding ability, and purge after cooking can be significantly increased. The higher the amount of fat – up to approximately 30% – the smaller is the degree of shrinkage of the protein structure, resulting in better processing yields. Reduced-fat products therefore require increased nonmeat ingredients for improved functionality, particularly for the binding of additional water.

The thickness of the IPF may also influence the emulsion stability. High endpoint chopping temperatures can result in an overly thick and inflexible protein envelope, which ruptures owing to thermal fat expansion. Thinner protein films have pores through which small amounts of melted fat can escape. Batter stability is therefore related to the thickness of the IPF coating, the fat content, and the integrity and density of the surrounding matrix and its response to thermal processing.

Particle size

The texture of sausage or processed meat is a function of the interaction of meat and nonmeat ingredients in addition to the particle and the amount of emulsifying capacity of the solubilized proteins. All components in the meat mixture must be completely covered with the salt-solubilized myofibrillar proteins or the emulsifier. The amount of emulsifier needed is thus determined by the amount of surface area of all particles. The amount of emulsifier increases dramatically, and the particles become smaller through the mechanical actions of the cutting of the particles and the increasing surface area that is developed.

Fat Characteristics and Processing Conditions

Several aspects of the characteristics of fat are important for emulsion stability. Reports indicate that shorter-chain saturated fatty acids and triacylglycerol are easier to emulsify than longer-chain counterparts and that the degree of saturation also plays a role. At similar chain lengths, less-saturated fatty acids are easier to emulsify than saturated fatty acids. There seems to be a relationship between the fat melting point and the degree of fat dispersion and absorption by the IPF at the fat–water interface.

Batter viscosity decreases at temperatures above the fat melting point, and as the fat particles are less dense than the aqueous phase they tend to float to the surface. This could prevent the fat globules from being coated by the IPF. The IPF-coated fat particles are less prone to coalesce during heat processing. Experiments evaluating fats of different melting characteristics at various temperatures reveal that at low-fat additions, all types of fats can produce stable emulsions, whereas at higher fat levels, high-melting fats produced more stable emulsions than that of low-melting fats, irrespective of temperature reached during emulsification. As a general rule, it is recommended to attain endpoint chopping temperatures below 18, 12, and 8 °C for beef, pork, and poultry fats, respectively.

Batter failure may occur when unfavorable conditions are present in the raw state or when changes taking place during cooking create unstable conditions. It is, for example, very important to reduce the particle size of both the myofibrillar structure, connective tissue, and the fat tissue and to achieve an even distribution of the different components of the system with the mechanical action. However, excessive chopping is detrimental to batter stability, mainly because of melting of fat, denaturation of the salt-soluble proteins, and disruption of the protein matrix. Extreme shear action may also result in a too large surface with a need for more salt-soluble myofibrillar protein. There is, therefore, a thinner and less-stable protein film around the fat globules. During thermal processing, the protein matrix undergoes several transition stages. At 40–50 °C myosin starts to denature and gelation begins; approximately 60 °C, when collagen melts, the major thermal transition takes place, and the structure is further consolidated between 72 and 83 °C when the actin is denatured. Fat within the system starts melting before any thermal protein denaturation occurs and as a consequence it is molten before the protein matrix gelation is completed. The presence of sufficient emulsifier is therefore essential to stabilize the fat in the system before protein gelation. Both the IPF and the protein matrix structure must be stable and cohesive, so that the expanding liquefied fat does not exude from the batter and coalesce in pockets.

Stabilization Mechanisms

Protein functionality characteristics such as emulsification, gelation, and water binding are determined by molecular interactions involving disulfide bonds, hydrogen bonds, electrostatic attractions, and hydrophobic interactions. Studies, utilizing chemical agents that modify the type of molecular force developed in the system, have been done to help understand their role and influence in the product attributes and acceptability. Disulfide bonds are not essential for fat and water binding in meat batters, but they are important in the raw state for development of an acceptable texture, influencing the springiness, cohesiveness, and hardness of the product. Protein hydrophobicity results in entropy-driven protein–protein interactions and aggregation required for the formation of a sufficiently cohesive and flexible gelling structure. Hydrophobic interactions are crucial in the IPF formation and

stabilization of the protein matrix, being the major force behind raw batter gelation. Hydrogen bonds and electrostatic attractions appear to have some importance and seem to participate in the binding of the IPF-coated fat globules to the protein matrix. However, if these forces are increased, the result may be excessive matrix aggregation in the raw state with well-interconnected channels throughout the batter. These can serve as routes for water losses during cooking.

Temperature of the meat mixture at stuffing or final handling before heat processing can have significant impact on the eating quality of the product, particularly the fat component in the finished sausage product. Variations in fatty composition and degree of saturation are influenced by source species. Final stuffing temperature for all beef emulsified products should be at either 4 °C or 20 °C for all beef formulations whereas the mixed meat final stuffing temperature should be between 13 and 16 °C. All pork or all poultry emulsions should remain at or below 13 °C. These temperatures reflect the heat of crystallization temperatures for all the beef fats compared to heat of crystallization temperatures for pork, poultry, and mixed species fats. Failure to follow these temperatures result in poor emulsion stability, product quality, and yields.

Stability Assessment

Emulsion breakdown can occur during the cooking process as a result of meat batter instability. Emulsion stability can be determined by the amount of fat and/or water released from the matrix after cooking. Unfortunately, there are no visible signs before cooking to indicate to the commercial processor that the problem will occur. Measuring the electrical conductivity of the raw batter can give some idea of emulsion stability, as a good continuous aqueous phase with the presence of salt will conduct electricity better than a batter in which fat separation has started to occur. Plotting electrical conductivity against time during the chopping process has been suggested as a way to visualize structural changes in the emulsion. Some authors have even found that fiber-optic probes can be used to predict the point at which an emulsion becomes unstable. However, even if such a point can be measured, it might be too late for the processor to correct the problem. A method commonly used for research requires that the raw batter is stuffed into polycarbonate syringe tubes and cooked under controlled conditions. Released fluids (fat, gelatinous liquid, and proteinaceous particles) after cooking and centrifugation are measured and the stability is related to the total loss as a percentage of the raw batter weight. This technique creates a performance track record of production and can be used to predict performance and expectations.

Emerging Trends

Low-Fat Meat Batter

Considerable work has been reported on a variety of approaches and potential nonmeat ingredients for low-fat and fat free sausage emulsions. Fat can be physically removed from the meat raw materials, although the cost and labor expenses are significant. Depending on the fat target in the formulation, there are serious limitations for cost and availability of very low-fat content raw materials. What appears to be nearly fat-free to the eye will in fact contain at least 3–5% fat. Thus, most low-fat strategies involve the binding of extra amounts of water or water-based broth solutions. This is usually accomplished with plant proteins, starches, or hydrocolloids.

Low-Salt Meat Batters

Low-salt concerns are usually described as low-sodium issues. Some success has been achieved with sodium-replaced salts including 'lite salt.' These salts are typically a 50% potassium chloride replacement. A serious concern should be focused on the impact of lowering the amount of salt in processed meat. From the basic processing standpoint, in order to extract enough protein to produce a heat-stable gel, a minimum of 1.4% or 1.75% sodium chloride (with added phosphates) is required for cooked sausages and lean meat products, respectively. Salt is one of the interventions used to control microbial growth, and nitrite salt is one of the most effective antibotulinum interventions. When salt levels decrease, the concern for safety increases and shelf life can decrease especially when product is abused with poor refrigeration temperatures. To help offset this potential problem, increased endpoint cooking temperatures for microbial control and more rapid chilling rates are being implemented. Processors must also address changes in flavor due to reducing the salt content and may be achieved through increasing spice content or through the use of other flavor enhancers.

Added Nonmeat Ingredients

Many nonmeat ingredients are being evaluated worldwide for their application to sausage emulsions and processed meats. The largest group involves products providing additional fiber, starch, protein, and hydrocolloid contents to control and maintain higher moisture levels in the finished products and to lower the amount of fat for nutritional claims. Added plant-based proteins, such as soy, have different gelation properties than meat proteins, and processes may need to be slightly modified to achieve desired product characteristics. A second group of newer ingredients involves intervention with sodium lactate, sodium diacetate, and bacteriocins as well as processing steps to improve postpackaging pasteurization. Both areas are being studied and evaluated for inclusion in processed meats in many countries.

Processing strategies to build strong emulsions with lower- or low-salt formulations

To achieve the ionic strength of the salt solution needed to solubilize sufficient myofibrillar protein to properly emulsify the meat system and all the inclusions as well as to develop the proper texture of the finished product, the sausage emulsification procedures need to be changed with lower final salt concentrations in the finished products. Salt concentrations historically were 4–5%. Today, the salt content is commonly at or near 2%, and pressure to reduce the salt content to even lower levels is desired.

Research indicates that to achieve the necessary level of emulsifier to bind the particles and create the desired eating texture, 4–5% salt is needed – clearly much higher than desired. Assuming 1.8–2.0% salt in the finished product, the strategy of using 30–40% of the leanest meat with all the available salt (formulated at 1.8–2.0%) would, for a short time during initial mixing, provide a temporary 4–5% salt strength, which would adequately create the needed emulsifier for the entire meat block. Then, the addition of the other nonmeat ingredients and the remaining 60–70% of the total meat in the formula would bring the final formulation to the desired salt content of 1.8–2.0%. Although this strategy allows good emulsifier development for current products, there is a point where other strategies will be required to develop sufficient emulsifiers for bind and eating texture.

See also: Additives: Functional. Chemistry and Physics of Comminuted Products: Nonmeat Proteins. Cooking of Meat: Heat Processing Methods. Extrusion Technology. Minced Meats. Sausage Casings

Further Reading

Barbut, S., 1995. Importance of fat emulsification and protein matrix characteristics in meat batter stability. Journal of Muscle Foods 6 (2), 161–177.

Bater, B., Maurer, A.J., 1991. Effect of fat source and final comminution temperature on fat particle dispersion emulsion stability and textural characteristics on turkey frankfurters. Poultry Science 70, 1424–1429.

Colmeneros, F.J., 2002. Muscle protein gelation by combined use of high pressure/temperature. Trends in Food Science and Technology 13 (1), 22–30.

Crehan, C.M., Troy, D.J., Buckley, D.J., 2000. Effect of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5% and 2.5% salt. Meat Science 55 (1), 123–130.

Curt, C., 1996. Characterization of stability of meat emulsions. Use of electrical conductimetry to optimize comminution. Viandes et Produits Carnes 17 (6), 347–349.

Fernandez-Martin, F., Cofrades, S., Carballo, J., *et al*., 2002. Salt and phosphate effects on the gelling process of pressure/heat treated pork batters. Meat Science 61 (1), 15–23.

Gordon, A., Barbut, S., 1992. Mechanisms of meat batter stabilization: A review. Critical Reviews in Food Science and Nutrition 32 (4), 299–332.

Gordon, A., Barbut, S., 1997. Meat batters: Effect of chemical modification on protein recovery and functionality. Food Research International 30 (1), 5–11.

Hoogenkamp, H., 2001. Soy Protein and Meat Formulations. St. Louis, MO: Protein Technologies International.

Jones, K.W., Mandigo, R.W., 1982. Effect of chopping temperature on the microstructure of meat emulsions. Journal of Food Science 47, 1930.

Ruusunen, M., Puolanne, E., 2005. Reducing sodium intake from meat products. Meat Science 70, 531–541.

Schut, J., 1976. Meat emulsions. In: Friberg, S. (Ed.), Food Emulsions. New York: Marcel Dekker, pp. 385–451.

Tornberg, E., Olsson, A., Perrson, K., 1997. The structural and interfacial properties of food proteins in relation to their function in emulsions. In: Friberg, S., Larsson, K. (Eds.), Food Emulsions. New York: Marcel Dekker, pp. 279–349.

Xiong, Y.L., 2010. Meat binding: Emulsions and batters. Meat Processing Technology Series. Champaign, IL: American Meat Science Association, 28 pp.

Nonmeat Proteins

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Glossary

Functional foods Term relating to the implications of the different 'functional ingredients or functional components' (including proteins) in human health (enhancing health or reducing risk of disease), dealt with specifically in the section on 'Composition for Nutrition and Health Purposes.' That terminology must be clearly differentiated from the terminology defined below.

Functional properties and functionality Terms widely used throughout this article referring to any physicochemical property of a protein that allows protein molecules to interact among themselves and with their environment to produce or improve the quality and stability of the final meat product. Protein functionality determines the technological suitability of the protein and has an important role in food behavior during processing and storage, affecting many of the desirable physicochemical and sensory attributes of muscle foods.

Introduction

Many nonmeat ingredients are used in the manufacture of comminuted meat products; of these, protein-based ingredients are among the most important. They are very widely used, and in many cases there are specific norms regulating the amounts that can be used and which products they can be used in. The range of applicable nonmeat proteins from animal and plant sources is very large (Table 1).

This article discusses the use of various nonmeat proteins, their functionality, and their impact on the characteristics of comminuted meat products.

Functions of Nonmeat Proteins in Comminuted Meat Products

Nonmeat proteins are used as ingredients in meat products essentially for purposes of economy, functionality, and composition (nutrition and health).

Economics

In this connection, nonmeat proteins are used essentially to reduce costs and improve the processing yields of products to which they are added – economic dictates guide the right selection.

Functional Properties

Nonmeat proteins can be added to meat products for functional purposes. The characteristic qualities of comminuted processed meats (emulsion, particulate, sectioned, shaped, and restructured products) depend on the functional properties of the protein matrix (from meat and nonmeat sources). In the comminution process, the meat is mixed with salt and reduced to varying degrees of particle size to partially extract salt-soluble components. Subsequently, depending on the type of product, any fat is dispersed or emulsified within the protein sol matrix, which is then heated to produce setting or gelling of the emulsion and the protein matrix. Water-binding, fat-binding, emulsifying, and gelling properties play an important role in such meat systems. Nonmeat proteins are used to enhance one or more of these properties, although the functional benefits they confer will never equal those of high-quality lean meat. Additionally, some proteins can also be injected into whole muscle meats to achieve textural integrity. Tenderizing enzymes (of plant, fungal, or bacterial origin) have been used for their ability to make tough meat more palatable. The choice of nonmeat proteins in meat processing, how they are used and how they affect the characteristics of comminuted meat products, depends on a number of factors (Figure 1).

Water- and fat-holding properties

The ability of proteins to hold water and fat, as well as to retain these two components when heated and stored, is

Table 1 Nonmeat proteins used in the manufacture of comminuted meat products

Protein Source	
Animal	Milk
	Fish (surimi)
	Meat coproducts: connective tissue, blood, mechanically separated meat, and surimi-like materials
	Egg
Plant	Oilseeds: soy, sunflower, peanuts, cottonseed, and rapeseed
	Cereals/grains: corn, wheat, oat, and rice
	Legumes: peas, beans
Microbial	Yeast
	Streptoverticillium mobaraense (Transglutaminase, EC 2.3.2.13)

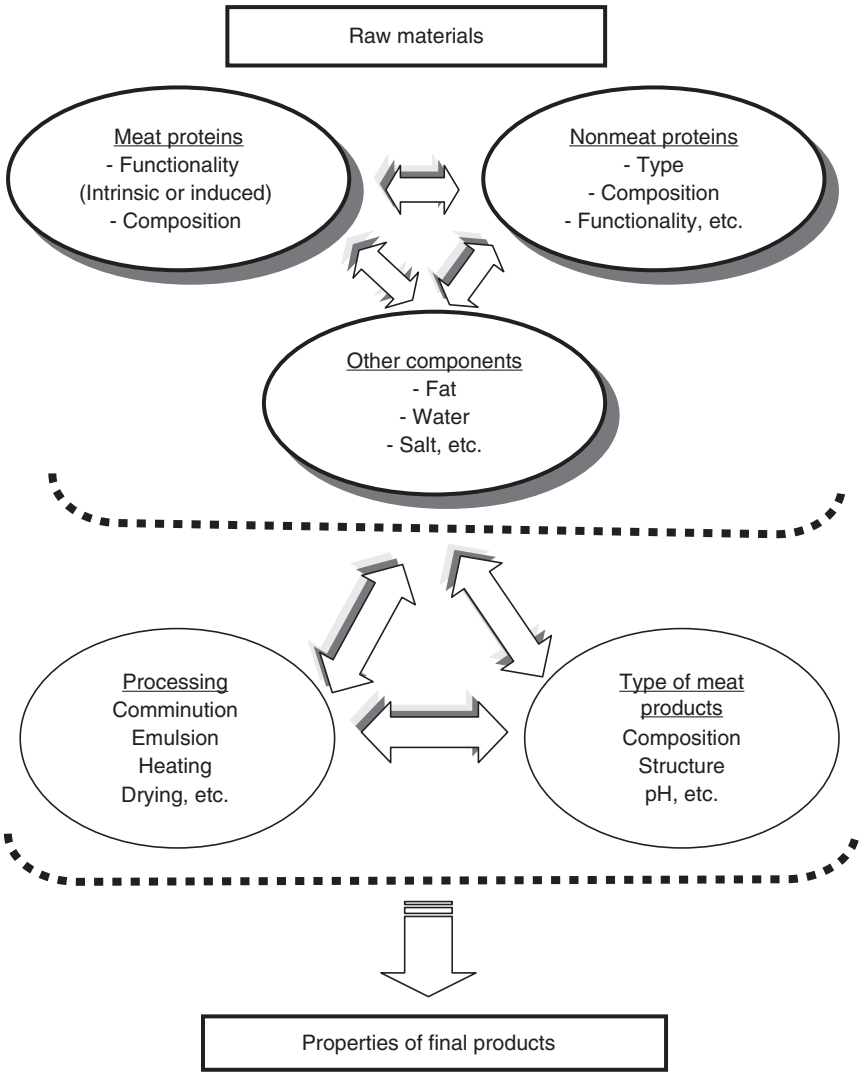

Figure 1 Relationship between the factors determining choice of nonmeat proteins and their effect on comminuted meat products.

crucial in the manufacture of processed meat products. These properties determine not only the final cook yield and the purge or drip loss on freezing and thawing, but also the final quality of the product (appearance, texture, color, juiciness, flavor, etc.). Different nonmeat proteins can be used to hold fat or water. The relative importance of holding water or fat depends on the kind of product involved. For example, in a full-fat meat emulsion, fat holding is the more important of the two, whereas in low-fat meat products, where part of the fat is replaced by water to reduce the calorific density, water-holding capacity is the key factor.

Emulsifying properties

A meat emulsion is a multiphase system consisting of a complex colloidal aqueous system (or matrix) of salts, proteins, and other soluble components in which solid components, including insoluble proteins and fat particles, are dispersed. The stability of meat emulsions, especially after heat processing, depends on the formation of a stable protein matrix gel in the continuous phase that entraps water as well as fat (gel/emulsion system). Nonmeat proteins can assist the formation and stabilization of meat gel/emulsion systems.

Gelation

The formation of a stable gel network is important for a range of functional properties of muscled-based foods, including holding of water, fat and particles, and texturization. There is a variety of ways in which two or more proteins can interact that will affect the properties of a multicomponent system. A number of possible models have been described for the spatial partitioning of a gelling protein (e.g., from meat) and a gelling or nongelling coingredient. These are: filled gels (where components are interspersed throughout the primary gel network); complex gels (where components are physically associated); and multicomponent gels (having an interpenetrating polymer network). When nonmuscle proteins (generally globular proteins, unlike the fibrillar proteins of muscle) are added, the resulting system may be considered qualitatively incompatible, semicompatible, or compatible. Depending on the degree of compatibility, the consequences may range from weakening

of the texture of the product through dilution of the meat protein (which is a highly functional component) or interference in gel formation, to strengthening of gel texture through reinforcement of the gel structure. Nonmeat proteins (e.g., transglutaminase) are also used as cold gelling agents for processed meat.

Proteins may be modified to change functionality for specific applications in meat processing. A number of nonmeat proteins (soy, wheat, and others) are texturized by various procedures to give specific textures and shapes, often so as to mimic the structure or appearance of meat. A number of nonmeat proteins (whey, milk, and egg proteins) are microparticulated to mimic certain properties of fat and are used to replace fats in low-fat processed meats. Flavor enhancers derived from nonmeat proteins (such as hydrolyzed vegetable protein or autolyzed yeast protein) are used to lend a more meat-like flavor. Animal and plant proteins (casein, whey protein, gelatin/collagen, fibrinogen, soy protein, wheat gluten, corn zein- water-insoluble prolamine from corn gluten, and egg albumen) have been used in edible films.

Therefore, the incorporation of nonmeat proteins influences the processing and physicochemical properties of comminuted meat products (Table 2).

Composition for Nutrition and Health Purposes

Nonmeat proteins can be used to provide nutritional benefits by lowering the calorie and cholesterol contents (when used as fat replacers) and by increasing the protein level and balancing the amino acid profile. Some nonmeat proteins (soy, sunflower, etc.) also contain health-enhancing components that make for reputedly healthier processed meats. For example, soy protein has been used to make a pork sausage whose consumption reportedly helps to maintain a proper blood cholesterol level (a functional meat product).

Animal Proteins

Milk Proteins

Milk yields a number of forms of milk proteins with a range of functional and nutritional attributes. These proteins are used as fillers, binders, and extenders in comminuted meat products (with normal and low fat content). The functional ability of such nonmeat protein ingredients derives from casein and whey, the two principal protein components of milk. In conjunction with various factors (composition, processing conditions in which they are derived, etc.), milk proteins can offer excellent functional properties such as solubility, water and fat binding, gelation, emulsion stability, and others, and they are widely accepted in the meat industry.

Nonfat dry milk (NFDM) is produced from pasteurized skim milk that is vacuum concentrated and spray-dried (skim milk powder). NFDM contains both casein and whey proteins along with lactose and minerals. It has been used as a functional protein ingredient in different comminuted meat products (frankfurter, bologna, roast, etc.) to improve emulsion stability, sensory characteristics (flavor, color, or juiciness) and water-binding properties (reduced cooking yields). Because calcium can influence the binding properties negatively, the low-calcium form of NFDM may improve functionality.

Caseinates are made from acid casein. Sodium caseinate is the most widely used in processed meats, although calcium and potassium caseinates are used when lower sodium formulation is required. The functional ability of caseinates lies in their molecular structure, a unique combination of electric charge and amino acid content (high-proline and low-sulphur), which prevents heat gelation and denaturation of caseinates and ensures high viscosity of caseinates in solution. They are not capable of binding meat pieces together because they do not gel during heating, but they do increase the gel strength. Caseinates, which are used usually in processed meats as emulsifiers to improve moistness and smoothness, are preferentially absorbed by the meat proteins at the fat–water interface. Because of the larger small fat globules created by the presence of milk proteins, the water loss during heating is reduced. Unlike vulnerable meat proteins, meat-based emulsions containing casein are less sensitive to temperature changes during processing (e.g., chopping). Processed meats such as hamburgers, nuggets, liver sausages, patties, frankfurters, and so forth have been formulated with caseinate, and, although its effect depends on a number of factors, it has generally been found to improve meat emulsion stability (more than many vegetable proteins), sensory attributes, and water-binding properties. It can be used in three different forms: as a prefabricated caseinate emulsion, in dry powder form at the beginning of the comminution process, and as a prefabricated gel.

Milk coprecipitates contain both casein and whey proteins and can be produced with a wide range of functional properties

Table 2 Influence of nonmeat proteins on processing and final characteristics of processed meat products

Aspect	*Area of influence*
Processing	Preparation conditions: form of addition, comminution, emulsifying, heating, etc.
	Raw meat batter properties: viscosity, chopping temperature, pH, etc.
	Thermal gelation process: gelling temperature, network and molecular interactions
	Cooking properties: yield, emulsion stability
Product characteristics	Binding of meat particles
	Slicing characteristics
	Cooking behavior: cooking loss, shrinkage, etc.
	Sensory attributes: appearance, color, flavur, palatability, texture, etc.
	Storage properties: microbial stability, vacuum purge accumulation, lipid oxidation, freeze–thaw stability, etc.

to perform a variety of functions in different comminuted meat products. Depending on the calcium content, coprecipitates can be good emulsifiers, can improve water-binding properties, and can serve as gelling agents and thickeners. Coprecipitates have been used in luncheon meats, bologna, nuggets, frankfurters, and other products.

Whey, a coproduct of cheese and casein manufacture, can be processed into a variety of forms including dried whey (2–13% protein), concentrates (35–80% protein), and isolates (>90% protein). Whey proteins present a strongly folded and organized globular structure. Under appropriate heating conditions, they unfold and build intermolecular disulfide bonds resulting in a gel matrix whose capillaries entrap water. Hydrophilic and hydrophobic regions on the protein surface provide emulsifying properties. The value of whey proteins as functional replacers or supplements of meat proteins in processed meats lies in their high functionality, and that functionality depends both on their origin and on further chemical modification. Whey proteins can bind a considerable amount of water by physical and chemical means, thus preventing moisture loss, improving yields, and reducing purge loss in vacuum-packed meats. They are very efficient emulsifiers of fat and oil, forming stable emulsions and helping improve appearance, mouthfeel, and juiciness. Whey protein products are used in a variety of processed meats (minced meats, emulsion products, coarse-ground products, whole muscle products) to improve flavor, texture, emulsification, water binding, cook yield, and product functional performance. Edible coatings based on whey proteins can be applied to frankfurters and other processed meats.

Surimi and Surimi-Like Materials

Surimi is stabilized myofibrillar protein obtained from mechanically deboned fish flesh that is washed with water, strained, and blended with cryoprotectants. The functional properties of surimi make it eminently suitable as an intermediate product for use in a variety of applications, and its very low fat content makes it ideal for low-fat meat products. It has also been proposed as an additive to processed red meat and poultry products to improve texture and water/fat holding. Surimi has been incorporated in varying proportions as a partial replacement for meat in various comminuted meat products, including frankfurters, bologna, restructured meats, and others.

In a more recent development, processes similar to those used in fish have been applied to various meat coproducts (beef heart, mechanically separated meat (MSM), etc.) to produce a surimi-like material for use as a functional ingredient in comminuted meat products such as sausages, patties, and the like.

Blood Proteins

Blood collected from a healthy animal, usually sterile, contains approximately 18% protein, whose amino acid composition is reasonably well balanced. Blood is used in food as an emulsifier, stabilizer, clarifier, color additive, etc., and it enhances the color of cured meat. In many meat products, however, it imparts undesirable characteristics (dark color and often unpalatable flavor), and it therefore offers more applications when separated into plasma protein (60–80%) and red cell fraction (20–40%).

Plasma proteins, which are mostly used in dry form, consist of various proteins, mainly albumin, globulins, and fibrinogen. They have been used to improve functional properties, to reduce formulation costs, and as a protease inhibitor. For purposes of meat processing, the outstanding functional property of plasma proteins is their exceptional gel-forming ability (primarily because of the albumin), which helps to impart good water-binding and meat particle-binding properties. Albumin gells at 85 °C and fibrinogen gells at 50 °C. Plasma protein also has excellent emulsifying and foaming properties. Plasma proteins have been used as gelling and binding agents in minced beef, frankfurters, bologna, pâtés, and other products.

The red cell fraction (34–38% protein) is dried to form a meal, or the haem group is removed to obtain globin. Globin protein has excellent foaming and emulsifying abilities and has been used in some meat derivates, although the high iron content may increase lipid oxidation. Other aspects that need to be considered are the dark color and off-flavor that haemoglobin-rich materials impart.

A blood-based system can be used to bind comminuted and large pieces of meat. The binding mechanism works through the combined blood-clotting action of fibrinogen, thrombin, and transglutaminase (factor XIIIa). Transglutaminase catalyzes protein polymerization and cross-linking through the formation of covalent bonds between protein molecules. It has been proposed as a cold-set binder of muscle protein on the ground that it would reduce the need for added sodium chloride and phosphate.

Mechanically Separated Meat

MSM makes an ingredient of excellent nutritional and functional value for comminuted meat products. MSM has good emulsifying capacity, emulsion stability, and water-holding capacity. MSM has been used in varying proportions in a large number of meat derivatives (minced meat, sausages, and other types of products). Generally speaking, as the proportion of MSM in the formula increases, flavor and overall acceptability scores decrease, the product is darker in color, and tenderness and juiciness scores rise.

There have been two European Union decisions affecting the use of MSM in processed meats. One (Decision 2000/418/EC) prohibits the use of meat separated from ruminant bones, and the other (Directives 2001/101/EC and 2002/86/EC) excludes poultry and pork MSM from the harmonized definition of 'meat' in the labeling of products containing them.

Egg Proteins

Egg proteins, derived from whole egg, yolk, or whites, are available in different forms for use in processed foods for their functional properties, such as foaming, binding and thickening ability, emulsifying ability, and moisture retention. Whole egg and egg white have been used in the formulation of

normal and low-fat meat products (meat patties, bologna, and others) and have proved very effective as binders for meat. They also have excellent nutritional properties. However, there is a drawback to their use in processed meats as they are relatively costly.

Connective Tissue Proteins

Connective tissue is composed of two proteins, collagen and elastin. Collagen, the more abundant of the two, produces gelatin by heat-denaturalization and partial hydrolysis. Connective tissue is present in comminuted meat products either as a natural component of the meat raw material or as a nonmeat ingredient. The use of collagen in processed meats is generally limited because on heating, collagen molecules shrink and gelatinize, which causes the release of gelatin. However, when properly handled, it can be a good functional ingredient for processed meats. Typically, trimmings with high connective tissue content are minced and added to comminuted meat products. As a rule, the meat in meat products should contain no more than 25% collagen to maintain their structure and acceptability. Collagen in comminuted meat products helps to stabilize the emulsion and to impart textural properties to products like burgers and sausages. The ability of gelatin to form water/fat emulsions that gel on cooling is considered important for some meat products. Thanks to its excellent water- and fat-absorption properties, collagen improves cooking yields and juiciness. It also reduces syneresis (purge) and hence shelf-life, because purge loss is a growth medium for bacteria. Collagen can potentially be used as a fat replacer in comminuted meat products.

The success of such nonmeat proteins as ingredients in the manufacture of comminuted processed meats will depend considerably on the source (basically pig skin and hide collagen, bone collagen, offal collagen, and skeletal muscle collagen), the preparation conditions, how the ingredient is used, and the nature of the product it is to be used in. A variety of procedures to improve the functionality of connective tissue proteins have been tested with a view to enhancing their potential as ingredients in comminuted meat products. These procedures include mechanical modification, enzymatic hydrolysis, acidic or alkaline phosphate treatment, and addition in a preemulsified or precooked form. Modified connective tissue has already been used in the formulation of low-fat processed meats. Collagen is also used in edible sausage casings.

Plant Protein Sources

Soy Proteins

Soy has always been one of the most widely used proteins in comminuted meat products. A variety of processes are used to produce soy flour and grits (52–56% protein), concentrates (70% protein), and isolates (≥90% protein) from soy beans and as coproducts of oil extraction. In each case, differences in composition and processing produce different characteristics, and these characteristics determine their particular application in a wide range of comminuted meat products. Soy flour and grits are chiefly used in minced meat systems as binders; however, both flour and grits give meat products a slightly bitter taste, which limits their use. Soy protein concentrates possess good water- and fat-holding abilities and emulsification properties and are used in emulsion-type sausages, luncheon meat, and meat patties. Soy isolates are excellent water and fat binders and possess both emulsifying and emulsion-stabilizing properties. Soy protein isolates aid in forming gels, which act as matrices to retain moisture, fats, and solids. Concentrates and isolates are most frequently used in finely ground sausages or emulsified meats. Textured soy products (flours, concentrates, or isolates) are extruded under high pressure and heat to give a specific texture and shape, frequently so as to mimic the structure or appearance of meat when hydrated. A major application of textured soy protein ingredients is in coarsely chopped or minced meats to be used for pizza topping, taco meats, meatballs, meat patties, and restructured steak.

Soy protein products are used primarily for their functional characteristics. The soy protein polymer chain contains both lipophilic and hydrophilic groups, so that the protein associates readily with both fat and water. This promotes the formation of stable oil and water emulsions when a protein dispersion is mixed with oil. Soy protein can associate with many different types of compounds, adhering to solid particles and acting as a binder or as a dispersing and suspending agent in solution. The functionality of soy protein in comminuted meat products is enhanced by prehydration or by the formation of a preemulsion in which fat, water, and soy protein are finely ground and then salt is added. The degree of hydration varies widely, but as a rule the end product will be firmer the lower the level of hydration. Salt assists the extraction of salt-soluble protein, but it has the opposite effect on soy protein, because it prevents hydration. For optimum functionality, therefore, soy protein must be fully hydrated before salt is added.

Soy protein has been reported to assist in the formation of a gel that acts as a matrix to retain moisture and fat and impart a desirable texture; however, under normal meat-processing conditions (temperature 65–73 °C, pH 5.5–6.0, and ionic strength 0.1–0.6), none of the major soy globulins exhibits any appreciable structural changes or hence any interaction with muscle proteins. Such lack of interaction is one of the main drawbacks to the use of soy protein as a functional ingredient in comminuted processed meats. Indeed, soy protein in high concentrations may act as a diluent, weakening the gel-forming capacity of meat proteins and so negatively affecting the texture of the finished product. Prior heating (90 °C) of soy protein improves its interaction with meat protein.

Soy protein can impart certain residual flavors or induce dilution of the natural flavor of the meat, depending on the type of derivative concerned.

Soy protein has been used as a fat replacer in the manufacture of low-fat (high-moisture) comminuted meat products.

Wheat Proteins

Wheat protein is one of the plant proteins (along with soy) most commonly utilized in meat products. The protein level of

finished wheat gluten products is typically 75–82% (dry basis). Vital wheat gluten, texturized wheat gluten, and isolated wheat gluten can be used in meat products. When hydrated, texturized wheat gluten has a fibrous structure that can be adapted to mimic the appearance and texture of beef, chicken, or pork. It has an excellent water-binding capacity.

Gluten protein functionality depends on the wheat source and the preparation process (separation, drying, extrusion, etc.). Gluten protein is eminently suitable for use as a nonmeat additive in meat products thanks to its unique functional properties. When mixed with water it can form a viscoelastic mass. Of particular importance to the meat industry are the binding and film-forming characteristics of gluten, which has the ability to interact with myosin.

Vital wheat gluten has been used in meat products as a binder, filler, or extender. When added to comminuted meat products such as frankfurters and bologna, it improves cooking yields, water-holding capacity, and batter stability. In restructured meats it imparts several benefits, such as enhanced viscoelasticity, color stability, firmness, juiciness, and moisture retention. In other meat pieces and processed meats, the binding ability of wheat gluten improves yield, cooking loss, adhesion, structural strength, rehydration properties, sliceability, and retention of sensory attributes. Also, texturized wheat gluten has been added to various meat products (burgers, nuggets, etc.) to improve physical properties and the taste perception of reformed patties or nuggets. Wheat proteins are potentially good nonmeat ingredients for use as extenders or binders in low-fat comminuted meat systems.

Corn Proteins

Corn gluten meals are coproducts of the corn wet-milling industry. Available products include corn meal (60% protein), defatted corn germ protein, and corn protein isolate (90% protein).

Corn germ protein is potentially a suitable protein additive for use as an extender in meat products such as frankfurters, bologna, and patties. When added to comminuted meat products, it improves cooking yield, water-holding capacity, and batter stability, although it can soften the texture of the product; also, the spice formulation may need to be altered. In low-fat meat products, the water-binding and texture-softening effects of corn germ protein are desirable qualities.

Other Vegetable Proteins

Nonmeat ingredients containing proteins from other plant sources have been used as binders and extenders in comminuted meat products.

Pea proteins is a coproduct of starch extraction from peas. It has good potential as an additive for emulsions and other meat products thanks to its promising functional properties (water and fat binding, emulsifying, whippability, and foam stability). Pea proteins in the form of flours, concentrates, and isolates (up to 90% protein) have been used in both normal- and low-fat sausages.

Different oilseed (other than soy) protein ingredients (sunflower, rapeseed, peanut, and cottonseed, in the form of flours, protein concentrates, protein isolates, and extrusions) have been used experimentally in minced meats, patties, and sausages. These generally improve cooking yield and emulsion stability and retard oxidative rancidity.

Rice products (flours and protein isolates), oat proteins, and bean products (flours) have been used in sausage meat.

Microbial Proteins

Several yeast-based ingredients can be used in comminuted processed meat as extenders or flavor enhancers. Dried yeasts (45–53% protein), coproducts of the brewing industry, are good protein extenders that can be used as emulsifiers in cooked and canned comminuted meat products. Yeast extracts, produced by autolysis, are widely used in processed meats because they impart a meat-like flavor.

Microbial transglutaminase (MTGase) has many potential applications in meat processing. MTGase can be used in combination with caseinate or collagen as a cold-set binder (reducing the need for added salt) for meat products that can be sold raw in the chilled state. Consumers and processors are increasingly interested in reducing the salt content of processed meats because of the potential health benefits. MTGase can also be a useful additive in cooked meat products.

See also: Additives: Extenders; Functional. Chemistry and Physics of Comminuted Products: Emulsions and Batters; Other Ingredients. Mechanically Recovered Meat. Sausages, Types of: Cooked; Emulsion

Further Reading

Asgar, M.A., Fazilah, A., Huda, N., Bhat, R., Karim, A.A., 2010. Nonmeat protein alternatives as meat extenders and meat analogs. Comprehensive Reviews in Food Science and Food Safety 9, 513–529.

Bailey, A.J., Light, N.D., 1989. Connective Tissue in Meat and Meat Products. London: Elsevier Applied Science.

Dill, C.W., Landmann, W.A., 1988. Food grade proteins from edible blood. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research, vol. 5. London: Elsevier Applied Sciences, pp. 127–145.

Egbert, W.R., Payne, C.T., 2009. Plant proteins. In: Tarté, R. (Ed.), Ingredients in Meat Products: Properties, Functionality and Applications. New York: Springer, pp. 111–129.

Elibert, S.J., Mandigo, R.W., 1997. Use of additives from plant and animal sources in production of low fat meat and poultry products. In: Pearson, A.M., Dutson, T. R. (Eds.), Advances in Meat Research, vol. 11. London: Blackie Academic & Professional, pp. 210–225.

Endres, J.G., Monagle, C.W., 1987. Nonmeat protein additives. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research, vol. 3. New York: Van Nostrand Reinhold, pp. 331–350.

Giese, J., 1994. Proteins as ingredients: Types, functions, applications. Food Technology 10, 50–60.

Liu, D.-G., Ockerman, H.W., 2001. Meat co-products. In: Hui, Y.H., Nip, W.-K., Rogers, R.W., Young, O.A. (Eds.), Meat Science and Applications. New York: Marcel Dekker, pp. 581–603.

Maningat, C.C., DeMeritt, G.K., Chinnaswamy, R., Bassi, S.D., 1999. Properties and application of texturized wheat gluten. Cereal Foods World 44, 650–655.

Mittal, G.S., Usborne, W.R., 1985. Meat emulsion extenders. Food Technology 4, 121–130.

Motoki, M., Seguro, K., 1998. Transglutaminase and its use for food processing. Trends in Food Science and Technology 9, 204–210.
Rakosky, J., 1989. Protein Additives in Foodservice Preparations. New York. AVI, Van Nostrand Reinhold.
Rao, K.H., Rao, K.V.S.S., Singh, R.R.B., Anjaneluyu, A.S.R., Yadav, P.L., 1999. Milk proteins as emulsion stabilizers in comminuted meat products. Indian Food Industry 18, 287–298.
Tarté, R., 2009. Meat-derived protein ingredients. In: Tarté, R. (Ed.), Ingredients in Meat Products: Properties, Functionality and Applications. New York: Springer, pp. 145–171.
Xiong, Y.L., 2009. Dairy products. In: Tarté, R. (Ed.), Ingredients in Meat Products: Properties, Functionality and Applications. New York: Springer, pp. 131–144.
Ziegler, G.R., Foegending, E.A., 1990. The gelation of proteins. In: Kinsella, J.E. (Ed.), Advances in Food and Nutrition Research, vol. 34. London: Academic Press, pp. 204–298.

Other Ingredients

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Introduction

Food additives play a critical role in the manufacture of comminuted and whole muscle meat products. These products are frequently cured, and salt (sodium chloride, NaCl) is almost always a critical part of the cure. Other salts approved in the United States are potassium, calcium, and magnesium chlorides, but sodium chloride is by far the most popular in meat products, although there is a push today to reduce the salt (Na) content of meat products.

Salt

The purposes of using salt for curing can be summarized as follows:

Retardation of Bacterial Growth

Retardation of bacterial growth is the result of the cured muscles having a higher concentration of salt than bacterial cells. Most bacterial cell walls are semipermeable and allow water but not salt to pass through the cell walls. As a result of osmotic pressure, water passes this barrier from lower salt concentration (in the bacterial cell) to greater salt concentration (in the muscle tissue). Thus, the bacterial cell dehydrates, shrivels, and dies due to the lack of moisture.

Other reported effects of salt on bacterial growth in muscle tissue include the following:

- Chlorine ion is toxic to bacterial cells.
- Salt reduces oxygen solubility in muscle tissue and, because some microorganisms require oxygen to survive, this creates an environment that retards microbial growth.
- Salt is also an enzyme inhibitor and reduces the effectiveness of some bacterial photolytic enzymes.

Salt is quite toxic to most microorganisms, but molds and yeasts are not affected to the same extent. Upper limits for microbial growth in salt have been reported as:

Clostridium botulinum	10% NaCl
Staphylococcus aureus	15% NaCl
Most other bacteria	8% NaCl
Fermentative yeasts	10% NaCl
Oxidative yeasts up to	25% NaCl
Molds	18% NaCl
	some up to 22% NaCl

The preservative action of salt is considered to relate to the brine concentration (not the percentage of NaCl). This is calculated from eqn [1].

$$\text{Brine concentration} = \frac{\%\text{salt}}{\%\text{moisture} + \%\text{salt}} \times 100 \qquad [1]$$

The influence of brine concentration on microorganisms in muscle tissue is often listed as:

- 4–5% – Brine concentration is sufficient to protect properly treated sausages
- 5–6% – Brine concentration is sufficient to inhibit most bacteria
- 15% – Brine concentration can be tolerated by only a few bacteria

Other conditions in meat can also affect the influence of salt on microorganisms:

- Lower water content (as a result of drying): see brine concentration formula (eqn [1])
- Increased nitrite and nitrate levels (nitrate has less influence)
- Refrigerated storage: synergistic effect
- Lower pH: synergistic effect

Salt was the original food additive and its primary purpose was to preserve meat for future use as food. With refrigeration, it has become less important, but it is still useful as an extra hurdle in refrigerated storage of meat products. It is also essential in the extraction of salt-soluble protein that is essential in emulsion products.

Role of Salt in Emulsion Formation

Salt and water form a solution for extracting myosin from muscle fibers, and this protein solubilization increases emulsion stability, processing stability, water binding, and yield. It also reduces thawing and cooking losses. This is accomplished by the salt, water, and protein solution encapsulating fat in the mixture and forming an emulsion (which appears like a sponge that has absorbed fat). The smaller the fat particles (created by more chopping), the greater the surface area they contain, and this requires more protein coating material. If the fat is well coated and combined with a protein solution of high viscosity, the emulsion will be more stable. Salt contact time and concentration are both important to obtain maximum protein extraction. For example, a dwell time of 2 h results in approximately 60% of the potential protein extraction, 8 h results in 92%, and 24 h results in 97%. This is the purpose of 'preblending' (combining salt with muscle), thus increasing the dwell time and obtaining greater percentage of protein extraction. The advantage of preblending, in addition to the increased protein extraction, is that it allows utilization of some lower binding raw materials and allows time for chemical analysis to be completed. The salt level providing the greatest protein extraction is approximately 6–7%, but this salt level would make the final product too salty. Therefore, higher salt levels are usually used for high-protein tissue and lower levels for lower protein tissue; by mixing high- and low-binding ingredients, one can obtain an

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appropriate final product salt content. Tumbling or massaging success also depends on salt-soluble extracted protein to hold these very small muscle pieces together to construct a solid appearing tissue.

Reduction of Heat Treatment for Canning

Salt and heat both reduce bacterial load, and the combination (the hurdle effect) of these two treatments allows the same bacterial reduction with reduced levels of either treatment. High canning temperatures reduce meat quality, and an additive (e.g., salt) that allows a reduction in temperature treatment is important. Therefore, most canned meat products contain salt and are also cured.

Flavor

Salt is a pleasant-tasting compound that is necessary in the diet (at $\sim$0.5 g day^{-1}) and is instinctively craved by most animals. Human consumption levels average 5 g day^{-1} with a range of 2–12 g. Perception of salt varies tremendously among individuals, and the salty flavor can be diminished by not chopping the product finely (coarse chop), by diluting the flavor as a result of consuming it with less salty foods, and by adding sugar.

Tenderization

If salt is placed on the surface of meat during aging or cooking, it dehydrates the meat and causes the tissue to toughen; if it is incorporated into the tissue, it increases tenderization dramatically.

Water Binding

Salt increases water binding, which increases yield and reduces thawing and cooking losses. This is accomplished by uncoiling the protein helix and by hydration of the protein, and salt-soluble protein solutions in an emulsion that coat finely formed globules of fat, providing binding that consist of meat, fat, and moisture.

Other Functions of Salt

- Variety: The addition of salt and most other additives offers a variety of meat products.
- Chilling and cooking: Salt is used in chilling water for chilling poultry and sausage products. When salt is mixed with water, it lowers the freezing point of the solution and allows chilling at temperatures below 0 °C. When salt is added to a cooking liquid, it raises the boiling point of the liquid and allows cooking at temperatures above 100 °C, which produces a tenderizing effect on the tissue.

Disadvantages of the Use of Salt

Salt promotes rancidity and thus shortens the shelf life of meat products (particularly frozen products). Salt also synergistically increases the catalytic effects of iron on oxidation. Aluminum clips on sausage casing oxidizes in a salt solution.

The salt level in comminuted products is not restricted by US regulations. Some dried cured pork products that are stored without refrigeration can have salt levels approximately 5.5%. Products that are stored under refrigeration will have lower salt levels. Levels of salt in brine-cured product in the United States average 2–3% (1.7–2% in Europe); bacon levels are 1.25–2%; Wiltshire sides 2.5%; hams 2.2–2.7%; fresh sausages 1.5–2% (2–2.5% when consumed after cooking); bologna or frankfurters 2.2–2.6%; restructured products 0.5–0.75%; and cured poultry products 2.8–3.3%. Brine concentration injected into a product that is going to be refrigerated is normally 5% and into a shelf-stable product is 17%. Salt used in meat products should be of high purity and should dissolve at a moderate rate.

Sweeteners

Sugars (normally sucrose, glucose, corn syrup solids, corn syrup, glucose syrup, malt syrup, and sometimes dextrose) are used in cured meats to protect color and to act as a synergist with antioxidants. Sorbitol can be used in frankfurters and knockwürst to sweeten, to facilitate removal of casings, and to reduce charring. The functions and effects of sugars include the following:

- Flavor: Sugar provides a sweetening and flavor-enhancing property. Approximately 0.5% sucrose (0.6% dextrose) is the taste threshold level. In cured meats, sucrose also reduces the harshness of salt. Sometimes special sugars (e.g., brown or maple) are added for their distinctive flavor properties. Various sugars have different sweetening levels and they are usually expressed as glucose or dextrose equivalents. Sugars with low glucose or dextrose level can be added at a higher level because the product will not taste sweet. Lactose (milk sugar) tends to reduce the harshness of liver in liver sausage.
- Color: Dextrose and corn syrup solids tend to caramelize and turn brown when heated; they char on prolonged heating. Cane and beet sugars are nonbrowning and are often used in fresh sausages and grilled products that are exposed to high heat. Sorbitol also does not brown with heat. Sweeteners are sometimes added to promote reducing conditions and improve cured color, but good cured color can be obtained in a product containing no sugar.
- Peelability: Peelability is improved by sugar because it attracts moisture.
- Retardation of bacterial growth: For sugar to retard bacterial growth, an extremely high level (e.g., 20–80%) is needed, and these levels are rarely found in western meat products. In fact, the level found in meat usually encourages bacterial growth. The level inhibiting growth for most bacteria is 50–60%, for food-poisoning bacteria 60%, for most yeasts 60%, for molds 80%, and for osmophilic yeasts 85%. Dextrose is often added to fermented products as an energy source for growth of desirable bacteria, which encourages acid production.
- Gas fermentation: In perishable canned products, sugars are often added (1% sucrose or 0.5% dextrose) to promote gas formation by anaerobic and aerobic spores, causing

cans to swell and thus giving an indication of improper processing or storage.

The level of sugar in cured products ranges from 0 to ~2% (some oriental products might have more than 20%). Hams usually contain 2% and frankfurters 0.5–1.5%. Dextrose addition ranges from 0.75% to 3.5%, and sugar should be adjusted to a lower level when milk products (lactose) are added and adjusted to a higher level when soy or other nonmeat proteins are added. Neither dextrose nor sucrose is restricted in the United States.

Nitrite or Nitrate

Nitrite is an essential ingredient in cured meat and may be supplied in the form of sodium nitrite ($NaNO_2$) or potassium nitrite (KNO_2) or may be derived by reduction of sodium or potassium nitrate compounds. Nitrate today is normally used only on long-cured products (e.g., dry-cured hams) and can be supplied in the form of sodium nitrate ($NaNO_3$; Chile saltpetre) or potassium nitrate (KNO_3; saltpetre or Bengal saltpetre). Nitrate can supply nitrite when it is reduced by bacterial action, but today it is used much less than in the past when long curing processes were normally employed. In the United States, both nitrite and nitrate must be kept under lock and key (bond room). Both nitrite and nitrate are sensitive to decomposition, and brines should be made with water below 4.5 °C. The major functions of nitrite include the following:

- Cured color development: Nitric oxide, a breakdown product of nitrite, combines with the principal meat pigment (myoglobin) to produce the typical cured meat color with the aid of heat. It is estimated that 20–30 ppm is needed to produce the commercial meat color.
- Safety and quality: Nitrite is bacteriostatic and there is a strong synergistic effect between salt and nitrite; these two preservatives provide an important aspect of protection against botulism. Nitrite also serves as a wholesomeness indicator, because a green color in cured products is almost always an indicator of spoilage.
- Flavor: Nitrite is thought to be responsible for the typical cured meat flavor. It is a powerful antioxidant and retards rancidity in meat products. This aids in the prevention of warmed-over flavor. It is postulated that nitric oxide combines with the iron of myoglobin and removes the iron catalyst from the oxidation cycle.

  If too much nitrite is added, or if it is added in a low-pH environment, a condition of nitrite burn (green discoloration) is evident. High levels of nitrite are toxic; therefore, maximum levels are established by regulations. In the United States, this is 156 ppm in most products, but only 120 ppm in bacon. Some countries impose considerably lower nitrite levels. Nitrite levels decrease drastically on processing, particularly with heat. Nitrous acid (HNO_2; a breakdown product of nitrite) can combine (particularly at high temperature and low pH) with secondary amines (RNH_2; found in muscle tissue and spices) to form nitrosamines ($R_2N–NO$), which have been shown to be carcinogenic if consumed at high levels. To date, nitrosamines have been found only at very low levels in meat products; inspection services continue to monitor this reaction, and processing techniques have been established to reduce even these low levels.

Reducing Compounds

Ascorbic acid and similar compounds (reductants) are used in cured meat and poultry products (not in fresh products) and may be supplied in the following forms.

- Ascorbic acid (vitamin C)
- Erythorbic acid (isoascorbic acid and D-araboascorbic acid)
- Sodium ascorbate
- Sodium erythorbate (sodium isoascorbate), a salt of erythorbate acid, is obtained from sugar; also a color fixative.

These compounds promote reducing conditions and encourage the following effects.

- Faster color development, important if time from chopping to cooking is less than 45 min.
- Resistance to color fading (antioxidative activity) during retail storage.
- Protection against rancidity (water-soluble antioxidant).
- Reduction of residual nitrite level.
- Conversion of nitrite to nitric oxide and of nitric dioxide (produced when nitrite reacts with oxygen) to nitric oxide.
- Reduction of metmyoglobin to myoglobin.
- Reduction of the amount of occluded oxygen.
- Possible aid in bacterial protection and retardation of nitrosamine formation.
- Reduction (ascorbic acid or its salt) of formation of green pigment, which might occur where meat touches metal smoke sticks.

In general, 470 ppm of the acid or 550 ppm of the salt can be utilized. In bacon, 550 ppm of the salt is utilized.

Cure Accelerators

Compounds that lower pH (acidulants) in cured meat alter the flavor, reduce bacterial growth, retard the action of many enzymes, increase the reaction rate of reducing compounds, aid peelability, produce a product with a better sliced shelf life, and accelerate a better meat color development. Compounds sometimes utilized in meat include:

- Citric acid, 547 ppm
- Sodium citrate, 547 ppm
- Glucono delta lactone (GDL; converts to gluconic acid), 0.5% (8 ounces per 100 pounds) in the United States; some countries use higher levels
- Fumaric acid (allows higher cooking temperature), 625 ppm
- Pyrophosphate, 5000 ppm

Sodium (or Potassium) Lactate

Lactate is reported to extend shelf life, enhance flavor, lower water activity, increase water-holding capacity, and have an

antimicrobial effect. It is sometimes used in the United States at up to12.5 g kg^{-1} of product; however, it is often used at lower levels due to its sodium content (1% sodium lactate = 0.6% chloride).

Phosphates

In many countries, phosphates are permitted and used for processed meat products to improve water-holding capacity and juiciness. A variety of sodium and potassium phosphates (tripolyphosphate, hexametaphosphate, and orthophosphate) are permitted at 0.5% in fresh beef, beef for further cooking, and cooked beef in the United States. Properties of basic phosphates include the following:

- Increasing pH of meat, which causes an increase in water-holding capacity and flavor protection. Phosphate and salt have a synergistic effect on water holding.
- Increasing juiciness, reducing refrigerator and thawing losses, decreasing cooking losses and increasing yield, making the product easier to slice, and reducing purge in canned products.
- Increasing emulsion stability by increasing extraction of salt-soluble proteins and reducing fat separation.
- Reducing rancidity and warmed-over flavor by acting as antioxidants due to metal (catalyst) chelating ability.
- Increasing water-holding capacity and reducing shrinkage during cooking by addition of sodium hydroxide (but not often used) with alkaline phosphates (four parts phosphate to one part sodium hydroxide; the combination not to exceed 0.5% of product).
- Reducing meat batter viscosity, which results in more uniform stuffing and reduced temperature rise on chopping.

Acid phosphates (e.g., sodium acid pyrophosphate) lower pH, accelerate color reaction rate, increase color and color retention, and protect from fading; however, the tissue has less water-holding capacity.

Water or Ice

Moisture is sometimes added to meat to distribute curing ingredients. This accelerates curing and helps to extract myosin, which increases binding, emulsion stability, processing stability, water-holding capacity, and yield. It can also be used to lower or to raise the temperature of the product during processing. It increases juiciness and lowers the per-unit-weight cost and is, therefore, controlled by regulation according to labeling utilized in the United States and many other countries. Added water is regulated in the United States via a calculation of the protein content multiplied by 4 – this is considered the natural water content in muscle tissue, and chemically analyzed water in excess of this quantity must be declared as added water. In Europe, the factor (nitrogen factor) is used to calculate added water. Hard water can cause discoloration. Overshowering of cooked products can remove nitrite and salt, which can encourage color fading and bacterial growth.

Binders, Emulsifiers, Extenders, or Fillers

A number of additives are permitted and used as binders, emulsifiers, extenders, or fillers in emulsion products. The labeling requirements vary and such additions are subject to different limits in various countries.

- Cereal and flour products often contain approximately 8% protein and absorb 2–3 times their weight of water. Such additives include: (1) corn and stone-ground flour hard gel; low protein (0.7%); (2) corn gluten (90% protein isolate); (3) dried whey (in some countries a dextrose minimum is required) used as a binder or extender; (4) oats, rolled or steel-cut types (the highest protein cereals); (5) oatmeal (12% protein); (6) rye; (7) rice (soft gel; 6% protein; absorbs 3–4 times its weight of water); (8) sunflower meal, used as a textured vegetable protein; (9) wheat (14% protein; rehydrates at 1.25 parts water to 1 part flour); (10) vital wheat gluten, retains viscoelastic properties; (11) potatoes, provide good binding properties; (12) soya (products with low protein (50%) that often have a bean-like flavor, protein isolate (90% protein) has less flavor problems and hydration rate is usually 2.5 to 1; (13) peanut (28% protein, which can be increased by defattening); (14) cottenseed; (15) tapioca; (16) barley (8% protein); (17) 'rusk' – stale, baked, spongy cracker that is ground (12–15% protein); (18) bread, baked and allowed to become stale; and (18) carrageen (source – seaweed).
- Lecithin and mono- and diglycerides act as emulsifiers and retard separation.
- Milk products give a smoothing effect and sheen to the surface and improve sliceability. Care is needed in their use because of lactose intolerance in some people. Milk products cannot be used in kosher meat products. Typical products used include (1) nonfat dry milk (38% protein; 8% calcium; high in reducing sugars, which may help color retention; good flavor; and light color); (2) sodium caseinate (90% protein or more; bland; light color; does not contain high levels of reducing sugars; some emulsifying capacity; and absorbs 4 times its weight of water); (3) calcium-reduced dried skim milk (calcium replaced by sodium, which increases the water-binding properties); and (4) dried whey (18–75% protein; enhances flavor and browning).
- Gelatin is a thickener obtained from collagen and is used in jelly loaves and canned hams, where it helps to contain purge and improve slicing
- Yeast protein, has a meat-like flavor
- Gums
- Blood (both whole and fractions)
- Single-cell protein (mixed protein extracted from pure or mixed cultures of algae, yeasts, fungi, or bacteria (grown on agricultural wastes))
- Hydrolyzed (source) protein (from plant or animal sources) alters flavor
- Modified (source not required) food starch thickens meat products

When incorporated into meat products, these additives (1) improve economy; (2) bind the product and absorb fat and water; (3) reduce shrink during cooking; (4) alter color

(usually lightening it unless a browning reaction occurs); (5) alter the nutritional value (amount depends on the product used and the quantity); (6) improve slicing and texture in some cases; (7) improve yield; and (8) alter flavor (depending on the additive).

Disadvantages are associated with the use of some additives: (1) soy can bind iron, making it less available; (2) vegetable proteins are often low in B vitamins, some trace minerals, calcium, and methionine; (3) trypsin inhibitors are present in uncooked soybeans; and (4) flatulence is sometimes associated with vegetable proteins.

The following additive quantity limitations are permitted in the United States (some countries permit higher amounts): (1) maximum of 3.5% in some sausages or meatloaves; (2) imitation sausage (US sausages not regulated by 'standard of identity' rules) may exceed 3.5%; (3) maximum 2% isolated soy protein in some sausages or meat loaves; (4) some cured ham items with appropriate labeling can contain 2% modified food starch, 2% sodium caseinate, 2% isolated soy protein, or 1.5% carrageenan.

Breading

Coating of meat with a batter or breading before cooking is becoming more popular.

- Polysaccharides give viscosity control: materials include (1) flours (wheat, corn, and potato) and (2) starches (wheat, corn, and pregelatinized starch to absorb water).
- Proteins used include (1) milk or whey proteins (increase adhesion, fry color, and flavor) and (2) egg white or albumin (provide body, structure, and crispness).
- Egg yolk is used for body, softness, and color.
- Fats or oils are used for tenderness and crispness.
- Leavening agents puff and increase fat absorption, which increases crispness.
- Water is used for suspension of ingredients, adjustment of viscosity, and adhesion.
- Seasonings are incorporated for flavoring.
- Sodium acid pyrophosphate can be included as a reducing compound.

In general, the US regulations allow 30% breading (some countries differ).

Flavoring Agents

Additives also influence flavor, but some are added primarily for the following purposes:

- Hydrolyzed plant protein (HPP) – produced from wheat, corn gluten, yeast, rice, soybean meal, or casein and often has a meat flavor. Usually used at the rate of 125–625 g per 100 kg.
- Monosodium glutamate (MSG) – 'flavor potentiator' or 'flavor body,' which makes taste buds more sensitive. Normal level of use is 60–180 g per 100 kg. Some people are sensitive to MSG.
- Smoke, natural or liquid, generated from wood – used for flavor, color, and increased preservation; forms a skin on the surface and acts as an antioxidant. Liquid has a less rounded flavor because some of the ingredients are not captured in the solvent utilized.

Nucleotides

Disodium guanylate and disodium inosinate are used as flavor potentiators at very low levels (0.01%; 100 ppm).

Spices

A multitude of natural, whole, ground, cracked, and rubbed spices, along with sterilized spices, oleoresins, and some dehydrated vegetables, are allowed in meat products. Spices are added for flavor, color, and texture, and because some of them have antioxidant and antibacterial properties. There is no US limit on quantities except for paprika, turmeric and saffron (coloring materials), and mustard and hydrolyzed protein (protein fillers). Spices are usually added at the rate of 250–500 g per 100 kg of meat.

Problems with spices can include discoloration of fresh products, short shelf life of spices (6–8 months with proper storage), high bacterial loads (mainly spores), and nonstandardization of flavoring potential.

Coloring

Surface coloring might be added to some products by using agents such as alkanet (brownish red), annatto (bixon-yellow to orange-red), carotene (yellow), saffron (orange-brown), chlorophyll (green), turmeric (yellow), coal tar dyes (a variety of colors; in the United States they must be approved by the FDA and are called certified food colors), titanium dioxide (white), and caramelized or extra dark brown sugar or caramel color (brown).

Starter Cultures (Lactic Acid Bacteria) or Added Acids

Low pH is sometimes used to give meat a distinctive 'tangy' flavor and to aid preservation. Lower pH can be obtained by adding live lactic acid bacteria with high salt and nitrite tolerance, which ferment sugars to acids (the preferred method), or by directly adding acids. Starter cultures are often stored in the freeze-dried or frozen state before use. These starter cultures are usually added at approximately 0.5%, and different microorganisms or mixtures of microorganisms have different optimum temperatures for growth. Dextrose is usually also added as a source of sugar. The final pH of fermented dried or semidried sausages is usually 4.6–5.4.

Antioxidants

Tocopherol (vitamin E) is a natural antioxidant. Butylated hydroxyanisole, butylated hydroxytoluene, glycine, nordihydroguaiaretic acid, propyl gallate, and resin guaiac are useful food antioxidants that in small concentrations help to retard

oxidation and rancidity and sometimes help to protect natural ingredients (e.g., vitamin A). Acids are sometimes combined with antioxidants to act as synergists by chelating metals that are catalysts for oxidation.

Enzymes

Plant enzymes, such as pepsin (from the papaya tree; most popular, degrades collagen and elastin), ficin (from figs; helps to degrade collagen and elastin), and bromelain (from pineapples), are sometimes used to tenderize meat tissue. Transglutaminase is an enzyme that is often described as 'meat glue' which is useful in connecting small meat pieces together to make a larger one very similar to the nonenzymatic effect of tumbling. Each of the enzymes has an optimum time/temperature and active concentration and each enzyme affects different structural components of the muscle fibers. Distribution of the enzyme in tissue is sometimes difficult, and a variety of techniques are used to assist in distribution. Overtenderization and mushiness may be encountered.

Preservatives

Salt and nitrite are often used in cured products. Much less used, and only in specific products, are ascorbate, benzoic acid, potassium sorbate, borates, and propylparaben. Additional preservatives are permitted in other countries.

Miscellaneous

Treatment with gases, irradiation, and application of packaging materials and casings are not strictly additives, but they do affect muscle tissue. Some examples are given below:

- Glycerin (humectants) helps to retain water.
- Carbon dioxide (CO_2, dry ice) is used primarily to chill meat ($-78.5\ ^{\circ}C$), but it has also been used to inhibit growth of microorganisms and to reduce fat oxidation. Ozone (O_3), produced by ultraviolet irradiation of oxygen, is an oxidizing agent and is bactericidal to airborne organisms. Unfortunately, it has little effect on organisms on the surface of meat products and accelerates rancidity in fat and formation of brown metmyoglobin on the muscle. It can be dangerous to personnel at high levels.
- Liquid nitrogen (N_2) is used to lower the temperature of, or to freeze, meat ($-195.8\ ^{\circ}C$).
- Vacuum and nitrogen are used in packaging systems to exclude oxygen, to reduce rancidity, and to retard growth of oxygen-requiring organisms.
- Microwave or radiofrequency heating, using waves at a frequency of 200 MHz or greater, used in cooking meat.
- Ionizing radiation from machines or radioactive compounds might be used for both pasteurization and sterilization.
- High hydrostatic pressure (high-pressure pasteurization) is a nonthermal process using water under very high hydrostatic pressure to produce foods that are safer, longer lasting, and have a more original flavor.
- Packaging materials contribute various properties: (1) Oxygen permeability allows oxygen to oxygenate the myoglobin pigment and produce a bright red color in fresh meat; polyvinyl chloride (PVC) is often used. (2) Low oxygen permeability in films used with cured meat excludes oxygen, so nitrosomyoglobin will not be oxidized to metmyoglobin, and it aids in controlling bacterial growth and oxidation. Polyvinylidene chloride (PVdC) is often used. (3) Heat shrinkability is exploited to exclude oxygen from irregular-shaped products, such as poultry.

Comminuted meat products start with the natural intact meat tissue and subdivide into smaller units and combine it with a large number of additives to change its physical and flavor profile, extending its shelf life and restructuring it into a semisolid structure. This changes consumer meat choices from approximately hundreds of choices to many thousands of meat items that have a variety of flavor combinations.

See also: Chemical Analysis: Analysis of Final Product Composition for Labeling. Chemical Analysis for Specific Components: Major Meat Components. Chemical and Physical Characteristics of Meat: Chemical Composition; Water-Holding Capacity. Chemistry and Physics of Comminuted Products: Emulsions and Batters; Nonmeat Proteins; Spices and Flavorings. Sausage Casings

Further Reading

Additives in Meat and Poultry, 2011. USDA Food Safety and Inspection Service. Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/additives-in-meat-and-poultry-products/additives-in-meat-and-poultry-products (accessed 22.05.14).

American Meat Institute Foundation, 1960. Chapters 8–11. San Francisco and London: WH Freeman.

Lawrie, R.A., 1979. Meat Science. New York, NY: Pergamon Press.

Ockerman, H.W., 1996. Chemistry of Meat Tissue. Columbus, OH: Department of Animal Sciences, The Ohio State University.

Relevant Websites

http://www.globalmeatnews.com/Product-Categories/Ingredients-additives
Global Meat News.

http://www.malabarsuperspice.com/ref_meatproadd.htm
Malabar, Canada.

http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/additives-in-meat-and-poultry-products/additives-in-meat-and-poultry-products
USDA.

Spices and Flavorings

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Introduction

Psychological and physiological responses when meat is eaten are from the flavor and aroma that stimulate saliva and gastric juices, which aid in digestion. The total sensation of taste is a combination of gustatory (taste) and olfactory (aroma) stimuli. Animal muscles that have a more intense flavor or game animals that have a more intense flavor are used more than muscles or species that have less intense flavor. An example is the comparison between white meat and dark meat in poultry. Carcass aging not only increases tenderness but also alters (usually improves) flavor. Species differences in flavor are usually attributed to the fat portion of the muscle. Flavor intensity increases with animal age and amount of marbling in the muscle. Cooking methods also alter flavor (e.g., moist vs. dry). Fat loss during cooking reduces not only juiciness and tenderness but also meat flavor. Flavor is also influenced by the animal's diets, with high concentrate diets being preferred (at least by Americans) to grass-fed diets. Some plants consumed by animals can also affect tissue flavor. Mature male (sometimes female) animals, primarily in swine, have an off-flavor referred to as 'boar odor.' Storage of precooked meat causes development of an undesirable flavor, referred to as 'warmed-over flavor.'

In addition to animal muscles' contribution to flavor, spices can drastically alter this flavor and are used in most sausage products. Culture and country of consumers frequently determine the spice quantity and combination preferred, and spices themselves are intertwined with world history. India, the major producer of spices, has a spice history that dates back 7000 years. As far back as 2600 BC, Egyptians added spices obtained from Asia to food for laborers building the great pyramids. Syria used cloves, which came from Indonesia, not long after that. Europe imported spices before Rome was founded, and in AD 40 Rome was obtaining spices from India. In AD 408, the Visigoths demanded a ransom in gold, silver, and pepper to call off the siege of Rome. Before the sixth century, Confucius in China was advocating the use of ginger, which came from the tropics. In the fifteenth century, Venice held a monopoly on the spice trade between the Orient and Europe. To counteract this, Spain and Portugal each financed many exploration parties to find a sea route to the spice world. Vasco da Gama rounded Africa's Cape of Good Hope and in 1489 arrived in Kozhikode, India, the world's greatest pepper-growing region. Spain financed Columbus to sail west, but instead of finding India, he discovered America and also found chilies that he called 'red peppers.' The Spanish also financed Magellan and a crew to circumnavigate the earth, and his crew found the Spice Islands and returned to Europe with a load of spices on board. In 1511, the Portuguese found the Banda Islands (Indonesia), the source of nutmeg and mace and, later, cloves. The Dutch East India Company formed in 1602 dealt primarily in spices and by 1670 was the richest corporation in the world.

In early history, pepper was often used as a portable method of transporting wealth. Currently, it is estimated that 500 000 tons of spices and herbs valued at US$1.5 billion (2008) are imported globally every year, and 46% of this supply comes from India. The leading producers of some of the major spices can be found in Table 1.

Today, spices are used primarily for food, but historical demand suggests that they often had other uses. In Egypt, for example, cassia and cinnamon were used in the embalming process, and anis, marjoram, and cumin were used to clean the innards of the dead. In Rome, many combinations of spices were used in food and beverages and also in medicine. In Asia, spices were used as an antiseptic and for cosmetic purposes. Like jewels they often were presented as gifts of state, collected like precious objects, used in some religious rituals, and used as a means of payment. Incense also became popular and spices were the main ingredients. In Europe, the early demand for spices was primarily for food, particularly when poor harvests and cold winters restricted the food supply. The only way to prevent starvation was to consume poorly preserved food in which spices, particularly pepper, masked the decay, especially in meat.

Characteristics and Components of Spices and Flavoring Agents

Specialized flavor taste buds are located in different regions of the tongue, and the four basic flavors can be sensed in the following areas:

- Sweetness: tip of the tongue
- Saltiness: front side of the tongue
- Sourness: rear side of tongue
- Bitterness: across the rear of the tongue

In addition to the flavor of spices, much of the total spice sensation comes from the aromatic component of the spice, which is detected by smell through the nose and throat. This flavor of spice can be altered by the amount of grinding (which

Table 1 Leading producers of some of the major spices

Spice; part of plant used	*Leading producers*
Allspice; ripe fruit	Jamaica
Bay Leaves; leaves	Turkey
Cardamom; ripe seed	India and Guatemala
Mustard; ripe seed	Northern United States and Canada
Nutmeg; ripe seed	Indonesia and Grenada
Paprika; ripe fruit	Hungary and Spain
Pepper; unripe fruit	India, Indonesia, and Brazil
Turmeric; rhizome	Southeast Asia, India, Peru, Australia, and West Indies
Vanilla; fruit	Madagascar and Uganda

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Table 2 Spices and flavorings often used with animal products

Spice or flavoring (might be used in several forms)	*Properties when used with meat items or food items, cuisines in which they are incorporated, or origin of spices*	*Meats and products in which spice is sometimes used (others are possible) or purpose of the spice*[a]
Alcohol, cider, rum, and wine	Sharp; acidic; antimicrobial; and flavor	Most products
Allspice	Barbecue sauce; small quantities or extract might be used in ham, bacon, corned beef, and Canadian bacon	Beef, pork, and veal
Anis	Aromatic herb of carrot family	Beef, pork, poultry, and veal
Smoked bacon	Braunschweiger	Pork and pork liver
Basil	Popular cooking herb	Beef and poultry
Bay leaves	Laurel shrub with an aromatic oil	Beef, fish, pork, and veal
Cascarilla, cassia, gentian, orange peel, and quinine	Bitter	Beef, pork, and veal
Capsicum	Herb or shrub of nightshade family; peppers	Beef, pork, poultry, and veal
Caraway	Aniseed; fennel flavor	Beef, pork, poultry, and veal
Cardamom	Curries	Beef, pork, and veal
Cassia	Sweet, acidic, and prune flavor	Beef and pork
Cayenne	A pepper or pimento	Beef, pork, poultry, and veal
Celery seed	Roast, meatloaf, and stew	Beef, fish, and pork
Chilli pepper	Sweet, pungent, and burnt; Tex-Mex, chilli, and curry powder	Beef, cheese, fish, and poultry
Chives	Garden freshness	Beef, cheese, eggs, fish, pork, and veal
Cinnamon	Ham, bacon, corned beef, and Canadian bacon; small quantities or extract may be used	Beef, lamb, pork, poultry, and veal
Cloves	Sweet and aromatic; small quantities or extract may be used in ham, bacon, pork, beef, corned beef, and Canadian bacon	Pork and beef
Coriander	Sweet, aromatic, rose like; used in some frankfurters, smoked sausage, Polish cooking, sausage, dry sausage, and Wisconsin smoked sausage	Beef, pork, and veal
Corn syrup	Browning of bratwurst	Pork, beef, and veal
Cumin	Strong and musty; Tex-Mex and chilli powder	Beef, lamb, and veal
Curacao	Liqueur made from orange; brandy and gin	Beef and pork
Dextrose	Used in smoked sausage for browning	All species
Dill	Northern and Eastern Europe cooking	Beef, fish, pork, and veal
Fennel	Sweet and liquorice like; pepperoni, Italian sausage, and flavored meat items	Beef, pork, and poultry
Garlic	Strong, hearty, odored, and pungent; Polish sausage, summer sausage, beef summer sausage, mild Italian sausage, dry sausage, Italian and Chinese cooking, mild Italian sausage, beef frankfurters, bologna, salami, pepperoni, Tex-Mex, corned beef, small quantities or extract in ham, bacon, corned beef, and Canadian bacon; used to reduce tallow flavor in beef and used in sausage served cold	Pork and pork lever
Ginger	Strong and warm; root used as a condiment, often used with sage to prevent burning and burping	Beef, pork, and veal
Honey	Sweet nectar; flavoring, breakfast sausage, and ham	Pork
Horseradish	Tangy; condiment	Beef
Lactic acid	Produced by starter or natural fermentation; dry sausage, pepperoni, and summer sausage	Beef and pork
Lemon grass or fruit	Southeast Asian cooking	Beef, pork, poultry, and veal
Juniper	Used with venison, pheasant, and rabbit	Beef, game, and pork
Mace	Sweeter than nutmeg, stronger flavor, lighter color, and pungent; frankfurter and bologna	Beef and pork
Maple	Sweet; ham and bacon	Cured product
Marjoram	Polish, Italian, French, and Mexican cooking	Beef, lamb, pork, poultry, and veal
Mint	Many varieties of fragrant plant	Fish and lamb
Monosodium glutamate	Flavor enhancer; Chinese cooking; some are allergic to it	Chicken and pork
Mustard, permitted limit of 1.5%	Bitter, pungent (allyl isothiocyanate), and acrid. If heat treated (150° F) has no flavor but adds protein (28%) when incorporated into meat means that extra water can be added (each 1% added will give 0.75–1 cents saving/pound of product; Practical limit 1.5%). Frankfurter, bologna, salami, summer sausage, and rubs	
Nitrate and nitrite	Curing ingredient, inhibits bacterial growth, and also alters flavor	Nitrite is used in almost all cured products

(*Continued*)

Table 2 Continued

Spice or flavoring (might be used in several forms)	*Properties when used with meat items or food items, cuisines in which they are incorporated, or origin of spices*	*Meats and products in which spice is sometimes used (others are possible) or purpose of the spice*[a]
Nonfat dried milk	Sweet flavor; liverwurst	Liver products
Nutmeg	Sweet and pungent; used in frankfurters, dry sausage, bologna, and other sausages (cotto, cooked salami liverwurst, salami, bratwurst, and dry sausage); lighter flavor and darker color than that of mace	Beef, cheese, pork, and veal
Onion	Used in poultry and frankfurters	Beef, pork, veal, and poultry
Oregano	European and Mexican cooking	Beef, cheese, pork, poultry, and veal
Paprika	Sweet but in meat contributes little flavor; adds color; pepperoni, mild Italian	Beef, fish, lamb, pork, poultry, and veal
Parsley	Popular American herb	Beef, eggs, fish, pork, and veal
Pepper, black	Hot (mouth), spicy, and pungent; used in most sausage (2–8 oz; average 4/100 lbs. meat), smoked sausage, Polish and Italian cooking, fresh pork sausage, salami, cotto, pepperoni, bratwurst, summer sausage, dry sausage, Tex-Mex, and cotto (cooked salami, whole or cracked in salami)	Beef, fish, pork, poultry, and veal
Peppercorns	Salami	Beef
Pepper, red	Hot (throat), biting, and pungent; used at low levels in smoked sausage and Polish cooking, hot link fresh pork sausage, mild link, mild and hot Italian sausage, and pepperoni; often used in crushed form	Beef, pork, and veal
Pepper, white	Less pungent than black pepper and no black specks in light-colored emulsions; musty flavor; liverwurst	Beef, pork, poultry, and veal
Pimiento	A variety of red pepper	Beef, pork, and veal
Rosemary	Minty and sweet	Beef, pork, poultry, and lamb
Saffron	Very expensive	Fish
Sage	Bitter and aromatic; fresh pork sausage; hot, linked, and smoked sausage	Beef, cheese, fish, pork, and veal
Salt	NaCl: flavor and preservative	All cured products
Savory	Combination of mint, thyme, and pepper flavor	Beef and pork
Shallots	Garlic and onion; sweet flavor	Beef, eggs, fish, pork, poultry, and veal
Smoke, liquid, or natural	Used in frankfurters, smoked sausage, Wisconsin hot links, Polish products, and smoked bacon in liverwurst	Beef, pork, poultry, and veal
Soy sauce	Savory and heightens flavor; Eastern cooking	Beef, pork, poultry, and veal
Star anise	Strong liquorice flavor	Poultry
Sugar, corn syrup, dextrose, fructose, glucose, nonfat dry milk	Sweet and burnt; used in smoked sausage and Polish cooking; dextrose used for surface browning; nonfat dry milk used in liverwurst	Any product needing sweetening or a fermented or canned product needing a substrate for microorganisms
Tarragon	Rich robust flavor	Cheese, eggs, fish, and poultry
Thyme	Popular cooking herb	Beef, cheese, fish, lamb, pork, poultry, and veal
Turmeric	Southern Indian curry powders	Beef, fish, pork, lamb, and veal
Vegetables, many used		Used with many products
Vinegar, citric acid, and lactic acid		Beef, pork, poultry, and veal
Wine	Unique flavor of dry sausage	

[a]The same spices are used for wild or game animals as those appropriate for beef, pork, poultry, or veal (Lamb is not mentioned at all!) ('Properties' are not mentioned for all spices. For example, no property is mentioned for celery).

Source: Reproduced from Maine Made Sausage, 2002. Down east spice blends. Available at: http://waldo.villagesoup.com/p/rfd-maine-the-wonderful-world-of-sausage/916691 (accessed 15.07.12) and Ockerman, H.W., 1989. Sausages and Processed Meat Formulations. New York, NY: Van Nostrand Reinhold. Available at: http://kb.osu.edu/dspace/handle/1811/25224?show=full (accessed 15.07.13).

also influences the appearance of a light-colored product) and storage (time, temperature, sealed environment, relative humidity, and light).

By definition, spices (tropical aromatics) and herbs (temperate aromatics) are derived from plants and are used for preservation and during cooking for seasoning of food. They can be made from bark, blossoms, bulbs or tubers, fruits (berries), leaves, roots, seeds, and stems.

Natural spices or herbs are obtained from fruits and seeds and are sometimes processed only by drying and cleaning. They can be left whole or further processed by grinding, chopping, or dicing, in which case they contain a full complement of the spice flavor, color, and appearance. Whole or ground spices are usually added to the product when visual appearance is important. The processed particle size of spices determines the release and distribution of flavor. Whole spices,

however, are not soluble in brine solutions. A spice's flavor, flavor strength, and quality are subject to variation, depending on season, where and how it is grown, handling, transportation, and processing. Spices can also be a source of microorganisms, which can be incorporated into the food along with the spices. Methods of decontamination include radiation techniques, such as gamma, electronic, or UV; heating, microwave processing, or exposure to steam; treatment with ethylene oxide gas; or high-pressure processing. Some spices also have antimicrobial and antioxidant properties and can be incorporated into food to exploit these properties. However, spices alone cannot preserve meat products.

'Essential oils' are volatile, odoriferous components that are present in many plants and are normally obtained by steam distillation. Most components of essential oils are hydrocarbons (terpenes and sequiterpenes), oxygenated compounds (alcohol, esters, aldehydes, and ketones), and nonvolatile residues (waxes and paraffins). They contain only part of the spice profile and are often deficient in bitterness, spice hotness, sweetness, and other flavor components.

'Oleoresins' are prepared from spices or herbs by extraction with organic solvents (that are removed later in processing) and contain both the volatile portion (essential oils) and the nonvolatile extract that includes resins. For this reason, they are considered to contain a more complete flavor profile than essential oils. Both oleoresins and essential oils are microbiologically sterile and can be standardized for strength and flavor profile. Oleoresins can be incorporated with emulsifiers to make them water soluble or can be placed on soluble carriers, such as dextrose, flour, salt, or yeast to produce a dry product and to obtain a better distribution during mixing, thus avoiding 'hot spots.'

'Compounded flavors' are produced when ingredients are combined with amino acids to achieve a desired flavor result. 'Processed flavors' (e.g., Maillard reaction or nonenzymatic browning) are manufactured when meat, vegetable proteins, and reducing sugars (fructose, glucose, maltose, sucrose, or xylose) are combined in specific ratios and then reacted under controlled conditions, such as incorporating a catalyst and controlled pH, temperature, time, and water activity, to achieve the desired flavor product.

Applications of Spices

'Topical rubs' may be used alone or in conjunction with a brine and are employed to add color (paprika, turmeric, and annatto), visual appearance (which can also be developed by browning agents, such as caramel coloring, dextrose, or Maillose®), and flavor to the surface of meat products. They are usually used with other bulking ingredients, including corn syrup and dairy solids. Rubs are usually used at a rate of 2–5% of the meat product weight. Coextrusion has also been used to coat spices onto the surface of shaped meat products. Spices are sometimes adhered to paper, which is then wrapped around the meat tissue; on cooking, the spice is released to coat the external surface of the meat, and the paper is removed before consumption.

A 'brine' or 'marinade' system distributes flavors and functional ingredients into products. Flavoring ingredients must be water soluble (or emulsifiers must be used), must have fine particle size, and must be easily diffusible in the product for proper distribution of flavor and functional properties.

Other products can be added to meat as flavoring and these can be used for characterization purposes (lemon–pepper chicken), for process enhancement or replacement of a processing step (smoking and roasting), or for general flavor enhancement (hydrolyzed vegetable protein, yeast, and meaty flavors). Fermentation (natural or starter culture) drastically alters flavor due to the production of lactic acid and other metabolites of the microorganisms, because different bacteria produce different flavors. Lactic acid is the major chemical that alters flavor, but addition of it alone does not produce the same desirable flavor. A mixture of several microorganisms seems to produce a more desirable flavor than a single-strain starter culture.

Spices that are often added to animal products are identified in Table 2. Because the use of spices with meat is almost an art rather than a science; many thousands of combinations have been used by various processors and chefs to convert meat tissue into a tremendous variety of 'value-added' products. The levels often used for meat products are indicated in Table 3.

Table 3 Spice limits for meats

Spice	*Usage per 45.4 kg (100 lb) of meat product*	
	Minimum	*Maximum*
Salt (% of meat block)	0.45 kg (1%)	1.1 kg (2.5%) In cooked products, for example, bacon and dried products, this level can increase up to ~7%
Chili pepper	226 g (8 oz)	453 g (16 oz)
Coriander	28 g (1 oz)	170 g (6 oz)
Cumin	113 g (4 oz)	226 g (8 oz)
Fennel	84 g (3 oz)	340 g (12 oz)
Garlic	21 g (3/4 oz)	57 g (2 oz)
Ginger	1.8 g (1/16 oz)	14 g (1/2 oz)
Mustard	14 g (1/2 oz)	453 g (1%) heat treated
Nutmeg	7 g (1/4 oz)	57 g (2 oz)
Paprika	226 g (8 oz)	340 g (12 oz)
Pepper, black	57 g (2 oz)	226–453 g (8–16 oz)
Pepper, red	14 g (1/2 oz)	113–170 g (4–6 oz)
Sage	14 g (1/2 oz)	57 g (2 oz)
Sugar	None	0.5 kg (1%) (with 1.1 kg (2.5%) salt), often higher in dried Oriental products
Secondary spices	1.8 g (1/16 oz)	14 g (1/2 oz)

Source: Adapted from Knipe, 2002. Spices for meat products. Available at: http://www.ag.ohio-state.edu/meatsci/as550/Spices.htm (accessed 15.07.13) and Ockerman, H.W., 1989. Sausages and Processed Meat Formulations. New York, NY: Van Nostrand Reinhold. Available at: http://kb.osu.edu/dspace/handle/1811/25224?show=full (accessed 15.07.13).

Usage

For spice combinations or recipes used with meat items, a complete list would run into the thousands, but some of the

Table 4 Spices used in some of the most popular items (per 45 kg meat/liver/bread)

Meat and spices used	*Fresh pork sausage*	*Frankfurters*	*Bologna*	*Liver sausage*
Beef		31 kg (70 lb)	36 kg (80 lb)	9 kg (20 lb)
Pork	45 kg (100 lb)	14 kg (30 lb)	9 kg (20 lb)	22 kg (50 lb)
Pork liver				9 kg (20 lb)
Bread or wheat flour				5 kg (10 lb)
Water		7–9 kg (15–20 lb)	9–13 kg (20–30 lb)	
Salt (% of meat block)	1.1 kg (2.5%)	1.1 kg (2.5%)	1.1 kg (2.5%)	1.1 kg (2.5%)
Sugar		226 g (8 oz)		
Nonfat dry milk				1 kg (2 lb)
Sodium nitrite		7 g (0.25 oz)	7 g (0.25 oz)	
Cardamom				28 g (1 oz)
Celery seed				28 g (1 oz)
Coriander		57 g (2 oz)	57 g (2 oz)	14 g (0.5 oz)
Mace		57 g (2 oz)		28 g (1 oz)
Onions				1–2 kg (2–4 lb)
Pepper, black	170 g (6 oz)	170 g (6 oz)	170 g (6 oz)	40 g (1.4 oz)
Sage	57 g (2 oz)			

Source: Adapted from Ockerman, H.W., 1989. Sausages and Processed Meat Formulations. New York, NY: Van Nostrand Reinhold. Available at: http://kb.osu.edu/dspace/handle/1811/25224?show=full (accessed 15.07.13).

basic combinations can be found on the American Meat Institute web site.

Printed sources are listed under Further Reading.

Some of the combinations of spices used (thousands of variations are used) and a few of the most popular sausage types are listed in **Table 4**.

Labeling (United States)

The ingredient list shall include all meat items and the true names of spices and flavorings as well as other additives, such as monosodium glutamate, hydrolyzed vegetable, salt, sugar, flour, vegetable starch, etc. The ingredients are arranged in order of predominance except that spices might be designated either as spices or flavorings, and flavoring (including essential oils, oleoresins, and other spice extractives) might be designated as flavorings without naming each. Spices can be grouped as flavorings, but flavorings cannot be grouped as spices. Because labeling rules change periodically, a more detailed list can be found at the USDA, FSIS.

See also: Chemistry and Physics of Comminuted Products: Emulsions and Batters; Nonmeat Proteins; Other Ingredients

Further Reading

American Meat Institute, 2002. How hot dogs are made: The real story. Available at: http://www.hotdog.org./hd_made.htm (accessed 15.07.12).

Ames Company, 2002. Sausage formulations. Available at: http://www.amescompany.com/sausage_formulas.htm (accessed 15.07.10).

Bailey, J.W., 1970. Encyclopedia of Labeling Meat and Poultry Products. St. Louis, MO: Freezer Provisioning.

Frey, W., 1998. Sterilization and degermination of spices and herbs – What is possible, necessary and desirable? Food Marketing and Technology 11 (3), 10–12.

Grohs, B.M., Kunz, B., 2000. Use of spice mixtures for the stabilization of fresh portioned pork. Food Control 11 (6), 433–436.

Hadsen, H.L., 1997. Spices as antioxidants (Thesis). Dissertation Abstracts International C58 (1), 76.

Jayathilakan, K., Vasundhara, T.S., Kumudavally, K.V., 1997. Effect of spices and Maillard reaction products on rancidity development in precooked refrigerated meat. Journal of Food Science and Technology, India 34 (2), 128–131.

Knipe, L., 2002. Spices for meat products. Available at: http://www.ag.ohio-state.edu/meatsci/as550/Spices.htm (accessed 15.07.13).

Maine Made Sausage, 2002. Down east spice blends. Available at: http://www.downeastspiceblends.com/meat.htm (accessed 15.07.12).

Matulis, R., 2002. Spices and Flavors for Meat Products. Cincinnati, OH: Givaudan Flavors Corporation Mimeograph.

Molins, R., 2001. Food Irradiation: Principles and Applications. New York, NY: Wiley.

Morin, P.G., Gale-Barrow, III, W.N., Gust, J.I., Vandyke, W.G., 1999. Method of Application of a Coating of an Improving Agent to a Shaped Meat Product. Spanish Patent Application. Application Number: ES19990000265 19990209. Kraft Foods Inc. (USA).

Ockerman, H.W., 1989. Sausage and Processed Meat Formulations. New York, NY: Van Nostrand Reinhold. Available at: http://kb.osu.edu/dspace/handle/1811/25224?show=full (accessed 15.07.13).

Spice Board India, 2002. Indian spices – All the purity of mother nature. Available at: http://www.indianspices.com/ (accessed 15.07.13).

The Epicentre, 2002a. The spice trade, a taste of adventure. Available at: http://www.theepicentre.com/Spices/spicetrd.html (accessed 15.07.12).

The Epicentre, 2002b. Spices, or the dawn of the modern age. Available at: http://www.theepicentre.com/Spices/excerpt2.html (accessed 15.07.12).

Relevant Websites

http://www.fao.org/docrep/010/ai407e/AI407E07.htm
FAO.

https://www.d.umn.edu/~alphanu/cookery/herbs.html
University of Minnesota.

http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/natural-flavorings-on-meat-and-poultry-labels
USDA.

CLASSIFICATION OF CARCASSES

Contents

Beef Carcass Classification and Grading

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Glossary

Carcass classification The sorting of carcasses into classes according to descriptive criteria.
Carcass grading Assigning values to different classes.
Conformation The shape of a carcass taking account of the contours and the amount of soft tissue development.
Intramuscular fat (IMF) Fat deposits within a muscle.
Rib eye Common term for the longissimus muscle group.
Video image analysis (VIA) The process of taking images and deriving numerical and color data from them to predict features of interest.
Warner Bratzler A device fitted to a material's testing machine to determine the maximum resistance of a meat sample to shearing (hence Warner Bratzler Shear Force (WBSF)).

Purpose of Beef Carcass Classification

The main purposes of beef classification and grading schemes are to reward producers according to the quality of the carcasses they produce and to facilitate trade in carcasses by providing a common and unambiguous language for describing the commercially important attributes of carcasses and meat. Another purpose arises from the need for regulatory authorities to collect price information to enable market transparency and this requires a definition of a standard carcass on which comparisons may be based. Provided the attributes have a real value in trade and the classification is operated with sufficient accuracy for all sectors of the industry to have confidence in it, quality-based payments to producers are then linked to the classes or grades. This then becomes an effective means of giving signals about the type of carcasses desired by the market, and this should really mean eventual consumers. The extra returns that are available to producers who make adjustments to their production systems, for instance by using different breeds or improved lines or by making changes to the feeding system, should have the effect over time of changing the population of slaughter cattle in the direction of improved quality as defined by the market. This is most clearly demonstrated by the continuous reduction in fatness of pork carcasses over several decades in those countries that introduced effective grading schemes. Due to the longer generation time and the less clear signals being sent to producers, the effectiveness of beef carcass classification and grading schemes is less easily demonstrated, but the principle that effective schemes allied to meaningful price differentials will bring about change in the desired direction is nonetheless true.

Beef Carcass Classification in Europe

In 1980, a common system of classifying beef carcasses was agreed upon in the European Union (EU), the so-called EUROP system (Figure 1). The regulations state that beef

	Conformation				
Fat class	E	U	R	O	P
1					
2					
3					
4					
5					

Figure 1 The EU classification grid.

carcasses must be classified according to their conformation and fat cover by trained classifiers. For conformation classes, the letters E U R O P are used with E denoting carcasses with excellent conformation and P denoting poor conformation. There is an option to use an extra S (superior) class for carcasses with extremely good muscle development, such as double-muscled individuals. Fat cover is assessed on a five-point scale using the numbers 1–5, with 1 denoting low fat covering and 5 denoting high. Some countries subdivide each of the categories for conformation and fat into 3 subclasses to give 15 classes for conformation and fat. In other countries, the most common fat class or classes are subdivided into 2 or 3 subclasses. The category of carcass (sex/age) must also be specified in the classification and all carcasses must be dressed according to a detailed specification. Carcasses must be marked with the classification results. The classification results must be made available to producers and there are provisions for appeals by producers. Although the EUROP system does not attempt to estimate saleable yield, there is an underlying relationship between the descriptive scales for conformation and fat cover such that better conformed and leaner carcasses are likely to have a higher yield. Unlike many other classification or grading schemes, the EUROP system does not relate to meat quality.

National legislation governs the operation of the scheme in each member country. These include descriptions for conformation, fat cover and category classes (**Tables 1–3** for examples), and photographic standards. In most member countries classifiers are employees of the abattoir but in others they are employed either by a public authority (e.g., Greece, Ireland, and Portugal) or by a private company (e.g., Austria, Germany, The Netherlands, Great Britain, and Northern Ireland). In all cases the classifiers are highly trained and must be regularly monitored and retrained if necessary. Standards throughout the EU are maintained by an expert panel who visit each country on a regular basis to check that the classification results are in line with the EU standards. The classification scheme is used by the EU for price reporting and market intervention purposes, and by the industry for quality-based payments to producers and for carcass trading.

A significant development in the operation of the EUROP scheme was the decision in principle in May 2000 to allow mechanical grading using Video Image Analysis (VIA). This was followed in June 2003 by agreement on the rules for conducting calibration trials for mechanical systems and criteria for determining their accuracy. A scoring system was devised whereby scores are given for each carcass according to the size of the deviation of the score by the mechanical system from the median score of a panel of at least 5 classifiers, with no more than 2 classifiers coming from the country where the trial is conducted. Carcasses with zero deviation score 10. As the deviations increase, the scores awarded decrease and become negative. The scales are different for conformation and fat, recognizing the greater difficulty of assessing fat cover compared to conformation. The mechanical systems must score more than 600 points for both conformation and fat cover on a representative sample of 1000 carcasses. They also have to pass targets for bias and the slope of the regression line. Ireland was the first country to conduct a validation trial, which resulted in 25 VIA systems (VBS2000, E+V gmbh; Germany) being installed in their main export factories in 2004. Calibration trials followed in some countries and VIA systems have been installed in several countries. Classification information is returned to the supplier by the factory. In Ireland, over 90% of carcasses are classified by machine classification using VIA to carry out various measurements of the carcasses. As the determination of classification in this case is objective, no appeal is possible. In smaller plants,

Table 1 Conformation – Description of carcass profiles, in particular, the essential parts (round, back, and shoulder) in the Irish Statutory Instrument

Class	*Description*	
E – Excellent	All profiles convex to superconvex and exceptional muscle development	Round: very rounded and topside spreads markedly over the symphysis (symphysis pelvis) Back: wide and very thick, up to the shoulder; and rump very rounded
U – Very good	Profiles on the whole straight and very good muscle development	Round: rounded and topside spreads over the symphysis Back: wide and thick up to the shoulder and rump rounded Shoulder: rounded
R – Good	Profiles on the whole straight and good muscle development	Round: well developed and topside and rump are slightly rounded Back: still thick but less wide at the shoulder Shoulder: fairly well developed
O – Fair	Profiles straight to concave and average muscle development	Round: average development to lacking development Back: average thickness to lacking thickness, rump, and straight profile Shoulder: average development to almost flat
P^+ – Poor	Profiles straight to concave and poor muscle development	Round: elongated and poorly developed Back: narrow and thin Shoulder: flat and poorly fleshed spina of the scapula evident
P – Very poor	All profiles very concave and very poor muscle development	The round, back, and shoulder are very poorly developed with the outlines
P^- – Extremely poor	All profiles extremely concave and extremely poor muscle development	Little or no flesh covering on the round, back, and shoulder. All the bones of the skeleton are very apparent

Table 2 Degree of fat cover – Description of fat classes in the Irish Statutory Instrument

Class	*Description of amount of fat on the outside of carcass*	*Description of amount of fat in the thoracic cavity of carcass*
1 – Low	None to low fat cover	No fat
2 – Slight	Slight fat cover and flesh visible almost everywhere	Intercostal muscle clearly visible
3 – Average	Flesh, with the exception of the round and shoulder and almost everywhere covered with fat	Slight deposits of fat but intercostal muscles still visible
4L – Above average	Flesh covered with fat, but still partly visible on the round and shoulder and seams of fat prominent on the round	Distinctive fat deposits and intercostal muscles may be infiltrated with fat
4H – High	Flesh covered with fat, but small areas partly visible on the round, back, and shoulder and seams of fat very prominent on the round	Prominent fat deposits and intercostal muscles may be infiltrated with fat
5 – Very high	Entire carcass covered with fat and the round is almost completely covered with fat, so that the seams of fat are no longer clearly visible	Heavy fat deposits and intercostal muscles infiltrated with fat

Table 3 Categories of carcass in the Irish Statutory Instrument

Category	*Code*	*Description*
Young bull	A	Carcasses of uncastrated young male bovine animals of less than two years of age in which the cartilaginous extremities of the spinous processes of the first nine dorsal vertebrae do not show signs of ossification
Bull	B	Carcasses of other uncastrated male bovine animals
Steer	C	Carcasses of castrated male bovine animals
Cow	D	Carcasses of female bovine animals that have calved
Heifer	E	Carcasses of other female bovine animals

classification is carried out by factory employees who have been licensed by the Department of Agriculture, Food and the Marine. In these cases, the supplier can appeal the decision of the classifier to the slaughter plant.

Grading in the United States

A federal system of beef carcass grading was first introduced in 1927. The industry has changed considerably since then and the scheme has evolved to reflect these changes. The purposes of the scheme are to aid quality-based marketing of cattle and to provide a common language for carcass trading. Grading is voluntary and the service is provided by the US Department of Agriculture (USDA) on a cost-recovery basis.

Beef carcasses are graded according to quality and yield grades. The yield grades estimate the percentage of boneless, closely trimmed retail cuts from the high-value parts of the carcass – the round, loin, rib, and chuck. The grades are numbered 1–5, YG1 having the highest expected yield and YG5 the lowest (Table 4). The grades are calculated from a formula that includes the fat depth over the rib eye; the percentage kidney, pelvic, and heart fat (KPH); carcass weight; and rib eye area. The rib eye fat depth is measured at the 12th rib, three-quarters of the length of the rib eye from the chine bone, but skilled graders make an adjustment of this measurement to reflect unusual amounts of fat in other parts of the carcass. In other words, they assess how representative this fat depth is of total carcass fat. The amount of KPH fat is evaluated subjectively and expressed as a percentage of carcass weight, although one large processing company weighs carcasses before and after KPH fat removal so that actual percentage of KPH is determined. The area of the rib eye muscle is measured using a dot-grid. The US industry developed and the US Department of Agriculture's Agricultural Marketing Service approved the use of VIA for both yield grading and marbling assessment. Most beef processors have implemented this technology because of greater accuracy and uniformity in application of the grading standards. Figure 2 shows that fat thickness is measured at three locations rather than a single measurement.

The yield grades have descriptions in terms of the external and internal fat deposits and a stepwise procedure is used to determine the yield grade. First, the preliminary yield grade is determined from the fat measurement over the rib eye. This

Table 4 USDA yield grades

Yield grade	*% BCTRC*[a]
1	$\geq$52.3
2	52.3–50.0
3	50.0–47.7
4	47.7–45.4
5	$<$45.5

[a]BCTRC = 51.34–5.78 (fat opposite rib eye, in.) – 0.46 (percentage KPH fat) – 0.0093 (carcass weight, pounds) + 0.74 (rib eye area, in.^{2}).
Abbreviation: BCTRC, Boneless, closely trimmed retail cuts.

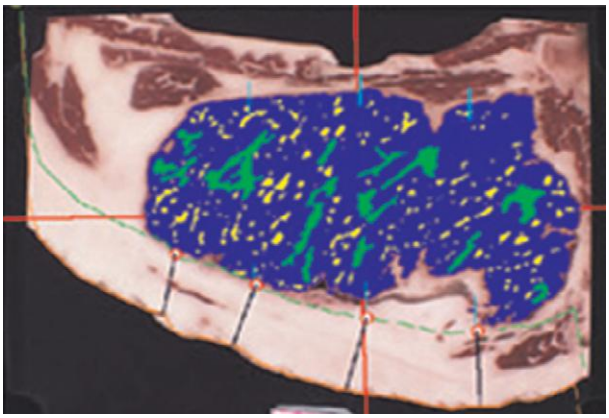

Figure 2 Example of an image captured by a video image analysis instrument used in instrument grading in the US Four fat thickness measurements, longissimus muscle area, and marbling are measured and interpreted by computer software.

Table 5 USDA marbling grades

Quality grade	*Marbling score*
Prime^{+}	Abundant$^{00–100}$
Prime^{0}	Moderately Abundant$^{00–100}$
Prime^{-}	Slightly Abundant$^{00–100}$
Choice^{+}	Moderate$^{00–100}$
Choice^{0}	Modest$^{00–100}$
Choice^{-}	Small$^{00–100}$
Select^{+}	Slight$^{50–100}$
Select^{-}	Slight$^{00–49}$
Standard^{+}	Traces$^{34–100}$
Standard^{0}	Practically Devoid$^{67–100}$ to Traces$^{00–33}$
Standard^{-}	Practically Devoid$^{00–66}$

Table 6 USDA maturity grades

Carcass maturity	*Approximate live age*
A	9–30 months
B	30–42 months
C	42–72 months
D	72–96 months
E	>96 months

is then adjusted for rib eye area relative to the carcass weight, then for the percentage KPH fat. The VIA system also predicts the percentage meat yield in addition to the yield grade, but price adjustments mostly are based on yield grades.

Beef quality grades are designed to sort carcasses according to their expected meat color and palatability, which is a combination of tenderness, juiciness, and flavor. Quality grading is based primarily not only on visual marbling, reflecting the amount of intramuscular fat, but also on maturity. Graders evaluate the amount of marbling fat in the rib eye muscle after carcasses have been ribbed between the 12th and 13th ribs. Quality grades are called Prime (most marbling), Choice, Select, and Standard (least marbling) (Table 5). Each quality grade is divided into one to three marbling score subclasses, for example, Prime is divided into Abundant, Moderately Abundant, and Slightly Abundant. Each degree of marbling is divided into 100 subunits, but in practice marbling scores are generally referred to in tenths within each marbling grade, for example, Slightly Abundant^{90}, Moderately Abundant^{50}, etc. Most large beef processing plants utilize VIA instrument assessment of marbling using hand-held rib eye cameras. Extensive study and testing by the USDA has shown greater precision in VIA assessment of marbling than when graded by trained graders.

Maturity is the second criterion of beef quality grading. Maturity refers to the physiological age of animals rather than the chronological age and is used mainly because the latter is generally not available but also because physiological age can be affected by factors such as nutrition, gender, and biological type of cattle. The indicators of maturity are the bone characteristics (the degree of ossification of the cartilage of the sacral and lumbar vertebrae) and the spinous processes of the thoracic vertebrae (greater ossification relates to older physiological age). Maturity is also based on the color of the rib eye muscle (darkens with age) and texture (becomes more coarse with age). Carcass maturity grades are labeled A–E, A being 9–30 months and E being over 96 months (Table 6). Carcasses are separately assessed for maturity based on ossification (Table 7) and on lean color and texture (Table 8) and, when the two do not agree, a balancing is carried out with more weighting on the bone score.

The final quality grade is determined by combining the marbling and maturity scores according to a detailed plan (Table 9). A stepwise procedure is used to determine the final quality grades of Prime, Choice, Select, Standard, Commercial,

Table 7 USDA skeletal ossification grades

Maturity group					
Vertebrae	*A*	*B*	*C*	*D*	*E*
Sacral	Distinct separation	Completely fused	Completely fused	Completely fused	Completely fused
Lumbar	No ossification	Nearly completely ossified	Completely ossified	Completely ossified	Completely ossified
Thoracic	No ossification	Some ossification	Partly ossified	Considerable ossification (outlines of buttons are still visible)	Extensive ossification (outlines of buttons are barely visible)
Thoracic buttons	0–10%	10–35%	35–70%	70–90%	>90%

Utility, and Cutter. Grades of Commercial, Utility, and Cutter typically are from cows that are more than 42 months of age (C, D, or E maturity).

The USDA recently approved (2013) a system by which processors, retailers, or purveyors can label beef cuts as 'guaranteed tender' or 'guaranteed very tender' based on either Warner–Bratzler or slice shear force. It is anticipated that this system will be utilized in conjunction with grades to assure acceptable eating experiences for consumers a high percentage of the time, or it could diminish the importance of grades.

Grading in Australia

Meat and Livestock Australia (MLA) has conducted extensive literature research and testing in development of the VIAscan objective grading instruments and more recently the Meat Standards Australia (MSA) grading program. The former involves the use of VIA technology to predict the saleable yield of carcasses from measurements taken from a video image captured as carcasses pass the system. The MSA program is a totally different approach and merits further attention here.

The MSA grading aims to ensure the quality of beef rather than the quantity. This is driven by a desire to meet the requirements of consumers for a guaranteed positive eating quality experience every time they buy a certain cut of beef. The approach taken was to analyze the factors along the meat

Figure 3 Stamp that will be placed on meat cuts/packages that meet specifications of MSA 'Optimization' and MSA 'Index' to enhance the value of MSA to the supply chain and to support continual growth and development of the MSA program.

Table 8 USDA lean maturity descriptions

Maturity	*Lean color*	*Lean texture*
A	Light cherry red	Very fine
B	Light cherry red to slightly dark red	Fine
C	Moderately light red to moderately dark red	Moderately fine
D	Moderately dark red to dark red	Slightly coarse
E	Dark red to very dark red	Coarse

Table 10 Classification of yield score

Grade	*Yield estimated percentage*	*Specification*
A	72% and above	Yield of total cuts is above average range
B	69% and above and under 72%	Average
C	Under 69%	Below average range

Table 9 Relationship between marbling, maturity, and carcass quality grade[a]

Degree of marbling	*Maturity*[b] *A*[c]	*B*	*C*	*D*	*E*	*Degree of marbling*
Abundant						Abundant
Moderately Abundant	Prime					Moderately Abundant
Slightly Abundant						Slightly Abundant
Moderate			Commercial			Moderate
Modest	Choice					Modest
Small						Small
Slight	Select			Utility		Slight
Traces					Cutter	Traces
Practically Devoid	Standard					Practically Devoid

[a]Assumes that firmness of lean is comparably developed with the degree of marbling and that the carcass is not a 'dark cutter.'
[b]Maturity increases from left to right (A through E).
[c]The A maturity portion of the figure is the only portion applicable to bullock carcasses.
Source: Reproduced from USDA, 1996. Standards for Grades of Slaughter Cattle and Standards for Grades of Carcass Beef. Washington, DC: Government Printing Office.

supply chain that affect eating quality, from conception right through to consumption. MSA call this a PACCP (Palatability Assessed Critical Control Points) approach, adapting the terminology from food safety (HACCP). Considerable research has been carried out to determine the individual and combined effects of these factors on the four main eating quality traits of tenderness, juiciness, flavor, and overall liking as assessed by consumers. Consumers were asked to rate cooked samples for these four attributes on a scale of 0–100. A combined meat quality score (MQS) is then determined using appropriate weightings for each attribute. These scores are then converted to quality ratings using thresholds. The ratings are 2-star (ungraded), 3-star (good everyday quality), 4-star (better than everyday quality), and 5-star (premium quality). Using a large database of samples of known background (production, carcass characteristics, and processing factors) cooked by different methods to strict protocols and tasted by a large number of consumers, a mathematical model was developed to predict the quality grade for each cut, cooked by a number of methods, from the recorded factors. The database is continually added to and revised versions of the model are derived at intervals. Both producers and processors must sign up to the scheme in order for the meat to be MSA graded. Trained graders are based in the factory to record pH, hump height (to estimate percentage of *Bos indicus*), ossification, marbling, fat depth over rib eye, rib eye area, and color of the lean and fat.

The MSA scheme is voluntary and the number of MSA graded carcasses has grown steadily each year since its introduction. It is more than a carcass grading scheme; rather it is more of a quality assurance program. Because it is consumer driven, it indicates the direction that grading schemes must follow in the future if beef is to compete successfully with other meats. MSA is designed to take the guesswork out of buying and cooking Australian red meat. MSA involves all sectors of the supply chain from paddock to plate. A wide range of cattle management practices, processing systems, cuts, aging periods, and cooking methods have been researched to

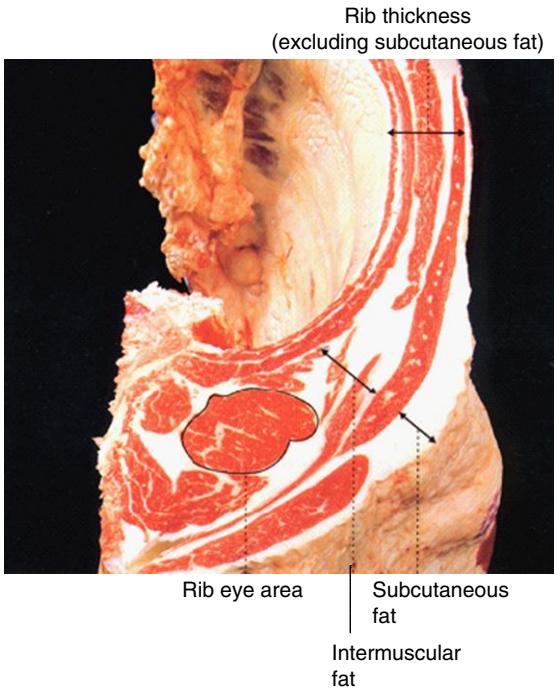

Figure 4 Carcass measurements on the 6th to 7th rib.

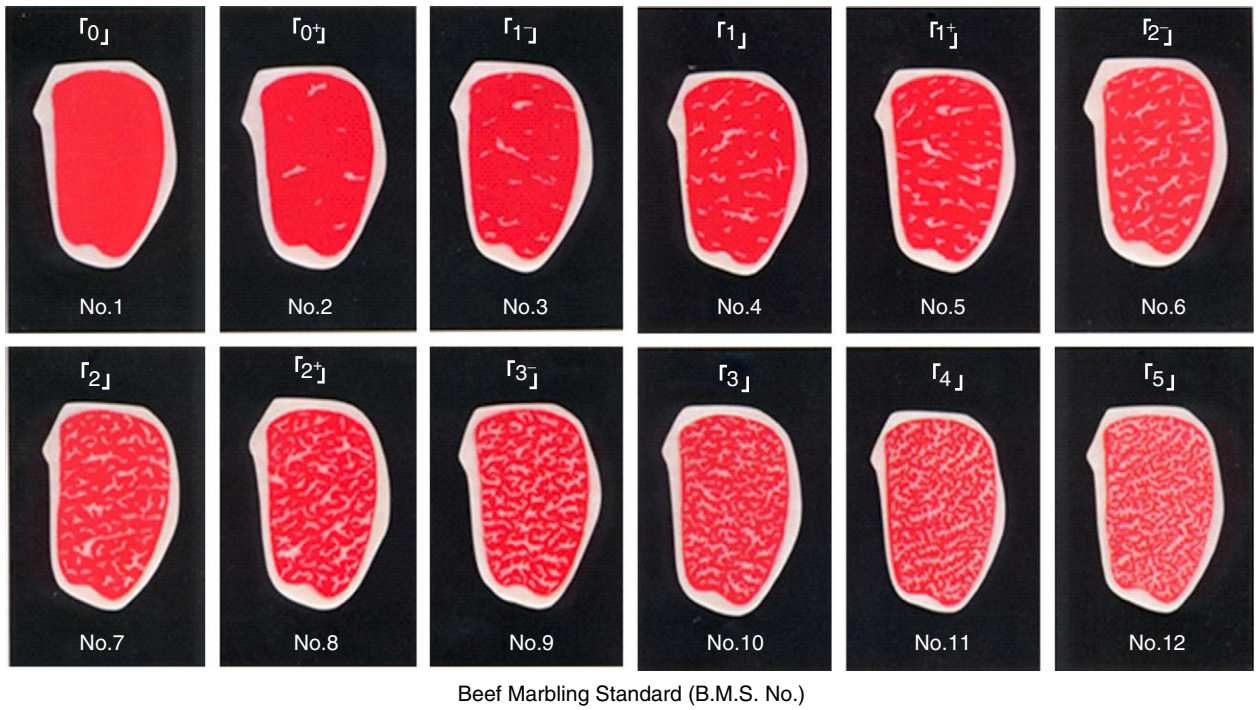

Figure 5 Beef Marbling Standard (B.M.S. No.).

determine the impact each has on eating quality. There were two significant updates occurring to the MSA beef program in 2013 to be rolled out over the next 18 months. These are called (1) MSA optimization and (2) MSA index. Both of these initiatives are aimed at enhancing the value of MSA to the supply chain and support the continual growth and development of the MSA program (Figure 3).

Indeed, other countries have appreciated this and have tested the MSA model. Even though the model is based on Australian beef and Australian consumers, it has proved to be quite robust with trials in several countries (e.g., Ireland, France, and Korea) being favorable.

Grading in Japan

The beef carcass grading system in Japan is implemented according to the guidelines of the 'Beef Carcass Grading Standard' established by the Japan Meat Grading Association. This standard was established in 1988 and consists of the evaluation of 'yield' and 'meat quality.'

Table 11 Classification of beef marbling grade

Grade		*Beef Marbling Standard (B.M.S.) No.*
5	Excellent	No. 8–12
4	Good	No. 5–7
3	Average	No. 3–4
2	Below average	No. 2
1	Poor	No. 1

Table 12 Fat area ratio and crude fat content of beef marbling standard model

Beef Marbling Standard (B.M.S.) No.	*Fat area ratio (X%)*	*Crude fat (Y%)*[a]
12	45.4	33.0
11	41.4	29.8
10	35.1	24.8
9	31.8	22.2
8	25.2	16.9
7	22.5	14.8
6	19.1	12.1
5	14.9	8.8
4	9.6	4.6
3	6.2	1.9
2	2.4	–
1	0.0	–

[a]Crude fat content was calculated by the regression equation ($Y = 0.793X - 3.04$, Kuchida, K., Kono, S., Konishi, K., *et al.*, 2000. Prediction of crude fat content of longissimus muscle of beef using the ratio of fat area calculated from computer image analysis: Comparison of regression equations for prediction using different input devices at different stations. Journal of Animal Science 78 (4), 799–803).

Grading Procedure

Yield score

The yield score is classified into three grades – A, B, and C, determined by the estimated percentage yield (Table 10). This percentage is calculated by a multiple regression equation as follows:

$$\text{Yield estimated percentage } (\%) = 67.37 + (0.130 \times \text{rib eye area } (\text{cm}^2)) + (0.667 \times \text{rib thickness } (\text{cm})) - (0.025 \times \text{cold left side weight } (\text{kg})) - (0.896 \times \text{subcutaneous fat thickness } (\text{cm}))$$

where rib eye area, rib thickness, and subcutaneous fat thickness must be measured on the 6th to 7th rib section of the left side (Figure 4). Note: Add 2.049% for Wagyu (Japanese Black breed) carcasses.

The yield score can be reduced by one rank if the intermuscular fat thickness is rather thick compared to the left side weight and rib eye area, or if the round is too thin and the proportions of the fore and hind quarters appears to be undesirable.

Meat quality score

The MQS is classified into five grades, No. 1–5. The grades are determined in terms of (1) beef marbling, (2) meat color and brightness, (3) firmness and texture of the meat, and (4) color, luster, and quality of the fat. Each item is classified into five grades as follows, and the MQS is graded down to the lowest grade among these items.

(1) Beef marbling
Marbling is evaluated by the Beef Marbling Standard (B.M.S.) prepared as twelve continuous models (No. 1: poor, No. 12: very abundant, Figure 5), and graded according to Table 11. The evaluation sites are the longissimus thoracis, dorsal semispinalis dorsi, and semispinalis capitis muscles on the 6th to 7th rib cross section.

The relationship between B.M.S. number and fat concentration is shown in Table 12.

(2) Color and brightness of meat
The meat color is evaluated by the Beef Color Standard (B.C.S.), represented by seven standards (No. 1: light, No. 7: dark, Figure 6). The brightness of meat is evaluated by visual appraisal. Both factors are considered in the final decision of this item (Table 13). The evaluation sites are the same as for the determination of marbling.

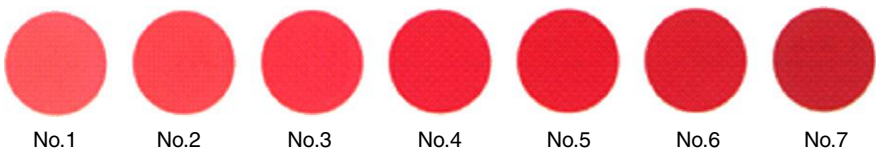

Figure 6 Beef color standard.

(3) Firmness and texture of the meat
The firmness and texture of the meat are evaluated by visual appraisal and both factors are considered in the final decision of this item (Table 14). Evaluation sites are the same as for marbling.

(4) Color, luster, and quality of the fat
The fat color is evaluated by the Beef Fat Standard (B.F.S.), represented by seven standards (No. 1: white, No. 7: yellow, Figure 7). The luster and quality of the fat are evaluated simultaneously by visual appraisal. These three factors are considered in the final decision of this item (Table 15). The evaluation sites are the subcutaneous fat and intermuscular fat on the 6th to 7th rib cross section and the external and internal fat of the carcass.

Stamping of the yield and meat quality score on the carcass

Three grades (A, B, and C) for yield and five grades (No. 1–5) for meat quality make a total of 15 grades, hence the mark of one class from A5 to C1 is stamped on the carcasses.

Damage indication by superscript stamp

A carcass recognized as having any damage is stamped with a superscript mark classified according to the type of damage (Table 16).

Table 13 Classification of color and brightness grade

Grade		Beef Color Standard (B.C.S.) No.	Brightness
5	Very good	No. 3–5	Very good
4	Good	No. 2–6	Good
3	Average	No. 1–6	Average
2	Below average	No. 1–7	Below average
1	Inferior	A grade except grade 2–5	

Table 14 Classification of firmness and texture grade

Grade	Firmness	Texture
5	Very good	Very fine
4	Good	Fine
3	Average	Average
2	Below average	Below average
1	Inferior	Coarse

Cumulated records on graded carcasses

Cumulated records on graded carcasses of 2002 are indicated in Table 17. Grade B was the most common yield grade, while Grade 2 was the most common meat quality grade.

Relationship between carcass price and meat quality grade

Fatty beef, such as 'Kobe beef,' is highly valued in Japan. Thus beef carcass prices are mainly determined by the degree of marbling, although breed also has an effect (Figure 8).

Feeding system for the production of highly marbled beef

Beef marbling is an important factor in determining the palatability of beef. It is affected by several factors, including breed, feed, and finishing period. Vitamin A is also known to

Table 15 Classification of fat color, luster, and quality grade

Grade		Beef Fat Standard (B.F.S.) No.	Luster and quality
5	Excellent	No. 1–4	Excellent
4	Good	No. 1–5	Good
3	Average	No. 1–6	Average
2	Below average	No. 1–7	Below average
1	Inferior	A grade except grade 2–5	

Table 16 Classification of the type of damage

Type of damage	Mark
Muscle bleeding (stain)	ア
Muscle edema	イ
Inflammation of muscle	ウ
External wound	エ
Part missing	オ
Other	カ

Table 17 Cumulated records on graded carcasses of 2002 (%)

Yield grade	Meat quality grade					
	5	4	3	2	1	Total
A	4.9	12.0	12.7	6.1	0	35.8
B	0.4	3.2	15.5	30.5	0.8	50.3
C	0.0	0.1	1.7	8.1	4.0	13.9
Total	5.3	15.2	29.9	44.7	4.8	100.0[a]

[a]Total number of graded carcasses is 1 029 593.

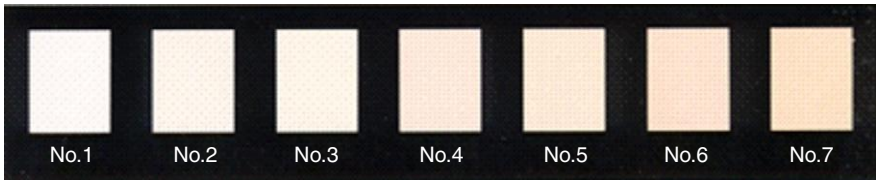

Figure 7 Beef Fat Standard (B.F.S.).

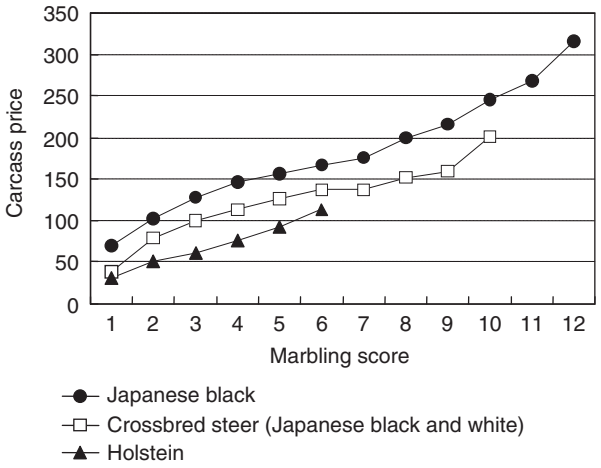

Figure 8 Relationship between marbling score and carcass price in a major meat wholesale market at.

affect marbling as evidenced by reports of retinal suppression of adipogenesis and by a report that a low level of serum Vitamin A results in a high degree of marbling. Vitamin A intake is, therefore, restricted during the late-early to middle-fattening stages, which is about 15–23 months of age in the case of Japanese Black steers.

Further Reading

Japan Meat Grading Association, 1988. New Beef Carcass Grading Standards. Tokyo, Japan: Japan Meat Grading Association.

Jones, S.D.M. (Ed.), 1995. Quality and Grading of Carcasses of Meat Animals. New York, NY: CRC Press, Inc.

Kempster, A.J., Cuthbertson, A., Harrington, G., 1982. Carcase Evaluation in Livestock Breeding, Production and Marketing. London: Granada.

Lazzaroni, C., Gigli, S., Gabiana, D., 2007. Evaluation of Carcass and Meat Quality in Cattle and Sheep. Netherlands: Wageningen.

Mermelstein, N.H., 2013. Instrumental grading of meat. Food Technology 67 (3), 67–69.

Polkinghorne, R., Thompson, J.M., 2010. Meat standards and grading – A world view. Meat Science 86, 227–235.

Polkinghorne, R., Thompson, J.M., Watson, R., Gee, A., Porter, M., 2008. Evolution of the Meat Standards Australia (MSA) beef grading system. Journal of Animal Science 80, 3211–3221.

Smith, G.C., Tatum, J.D., Belk, K.E., 2009. International perspective: Characterisation of United States Department of Agriculture and Meat Standards Australia systems for assessing beef quality. Australian Journal of Experimental Agriculture 48 (11), 1465–1480.

Strong, J., 2004. Differences in carcass grading schemes used in the USA, Japan and Australia. Australian Journal of Experimental Agriculture 44 (7), 675–680.

Relevant Website

http://www.teagasc.ie/
Agriculture and Food Development Authority.

Pig Carcass Classification

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This article is a revision of the previous edition article by EV Olsen, volume 1, pp 301–306, © 2004, Elsevier Ltd.

Glossary

Accuracy Common term for both repeatability and reproducibility.
Calibration A method to transform online measurements to an estimate value of lean meat content.
Computed tomography Computed tomography (CT) is a method that involves digital image processing. In this context the different signals related to fat, meat, and bone are utilized.
Lean meat content The amount of muscle of a carcass or cut that can be estimated by dissection carried out manually or by CT.
Online instrumentation Instrumentation to measure meat content attributes in real time.
Reference method Method that is used by agreement as defining the attribute such as lean meat.

Introduction

When Britain became an industrialized society and thus obtained a higher standard of living, high-quality bacon and similar products were required. This is indirectly the main reason for the classification system that is prevalent today. The complete system includes several interacting elements. Although the agricultural structure varies from one country to another, the principles are the same and can be described very briefly. Basically, pig producers deliver their pigs to a slaughterhouse producing primary products. The products are sold to customers, including a mixture of processing companies, retail shops, and ultimately to the consumers. Higher standards of living have resulted in a demand for high-quality products, and in this connection quality is identified as leanness. To meet the demand, farmers will require a breeding system, and a payment system reflecting the quality demand will motivate farmers to produce the lean pigs desired by the market. Consequently, the quality – the leanness – needs to be evaluated. Initially, classifiers at slaughterhouses in Europe performed the evaluation visually, but later online instruments were implemented to ensure objective evaluation. The instruments were used to measure the visible backfat thickness, which was initially the primary measure together with a subjective evaluation of the proportion between lean meat and fat in the carcass. However, it was observed that the thicknesses of the loin muscle and the backfat above the muscle were correlated with the lean meat content. The precision was acceptable, and the aim of the measurements was to express the percentage of lean meat rather than a subjective grading result. Consequently, the measurements became indirect measurements, and a calibration of the instruments was needed, together with a reference method.

There was a considerable trade in pigmeat between the European countries. A quotation for pig carcasses on a common basis was therefore established within the European Community (EC) so as to have a comparable basis for the price of a standard quality. The European Commission has specified a common scale, which expresses the leanness by intervals of percentages for the lean meat content, the so-called EUROP Community scale. To ensure that the scale is estimated correctly, common rules are established for calibrating the online measuring equipment. It is also specified that a monitoring system must be in place to ensure that the methods for assessing the lean meat content are applied correctly.

In what is now the European Union (EU), the reference for leanness is expressed by the amount of lean meat in a carcass as estimated by dissection. In other pig-producing countries around the world, the amount of saleable meat is often used as the reference. However, the concept is the same. This article deals only with the EU classification methods.

Reference

Subjective Assessments

The first EU classification scheme was based on the assessments carried out visually by trained staff inspecting the midline of the half-carcass and the complete carcass. The scheme reflected the proportion of lean meat by a subjective assessment of the meat-bearing parts of the carcass and the thickness of the backfat. No information exists about the precision of the method. Although an effective monitoring system might have existed, only the repeatability would have been acceptable. The reproducibility of subjective systems will always cause problems, and this was also the case in the EU system. Consequently, the Commission accepted classification based on measurements with online instruments in 1984 and defined the EUROP Community scale. Instrumental classification became obligatory in 1989.

Reference for Instrument Calibration

A precondition for instrumental classification is a reference, and the first problem is to define the carcass presentation. In the EU, the standard presentation of a carcass is defined as 'the

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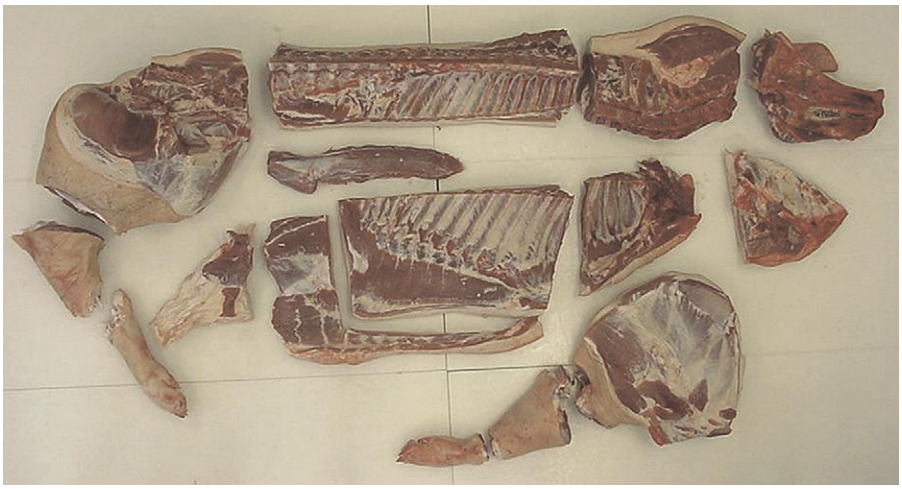

Figure 1 Cutting of the carcass before dissection according to the EU reference method. EC project G6RD-CT-1999-00127 EUPIGCLASS. Photo: Reinhard Höreth, Max Rubner-Institut.

body of a slaughtered pig, bled and eviscerated, whole or divided down the midline, without tongue, bristles, hooves, genital organs, flare fat, kidneys, and diaphragm'. The next step is to estimate the content of lean meat. The first reference method was based on dissection into lean meat, fat, bones, sinews, and hide, and the lean meat was defined as 'all red transverse-striped muscle meat from all parts of the carcass, which can be dissected with a knife'. The method is probably relatively precise, although the reproducibility has never been estimated, but it is also very time-consuming and costly. This was the reason for considering a new reference. In 1996, a new reference method called partial dissection was established based on dissection of four main cuts (shoulder, loin, belly, and hind leg), and the reference lean meat content was defined as the weight of lean meat in the four cuts plus the weight of the tenderloin in relation to the total weight of the half-carcass. In addition, a scale factor of 1.3 was used to obtain approximately the same level as before.

In 2008, the definition was changed again because of the results from a joint European experiment. By changing the divisor in the defining equation to be the weight of only the four cuts and the tenderloin instead of the total carcass weight, the accuracy is improved considerably. It was estimated that a new scale factor of 0.89 would yield the same level as a total dissection. At the same time, both total dissection and partial dissection (new definition) became legal reference methods.

Accuracy of Reference

The accuracy of the EU reference method has been estimated by a European research team. The term 'accuracy' is the common term for both repeatability and reproducibility of the dissection method and refers to the ISO standard. The estimation is complicated because only two replicates are possible – the left and right sides of the carcass. Natural variation and inaccurate carcass splitting result in the two sides not being completely identical, and identical replicates are needed to estimate the accuracy. However, no alternatives exist and the problem has to be dealt with carefully. The experiment consisted of a sample selected in each of four countries. In each country, two slaughterhouses were involved to ensure a representative sample for the country. At each slaughterhouse 16 carcasses were selected in three fat groups and balanced with respect to gender. A team of eight butchers, representing eight EU countries, dissected the carcasses. The butchers worked in pairs (all combinations of pairs) and they dissected the two sides independently of each other, that is, they divided the half carcass into 12 pieces (Figure 1) and dissected the four main cuts only.

The repeatability standard deviation of the 1996 definition was estimated at 0.9 lean% and the standard deviation related to the butchers was estimated at 0.7 lean%, giving an estimate of the reproducibility standard deviation of 1.1 lean%. Although the reproducibility is relatively good compared with the population, which typically varies approximately 3 lean% (standard deviation), the variation between butchers can cause a systematic difference between different calibrations of online instruments of up to 2 or 3 units of lean%. The procedure for dividing the half carcass into 12 pieces seems to be the most sensitive part of the process, whereas the dissection itself was rather uniform. The 2008 definition reduced the reproducibility standard deviation to 0.8 lean% (repeatability standard deviation to 0.5 lean%).

Alternative Reference Method

In 2008, it became possible to replace the assessment of the lean meat percentage by dissection using a method based on computer tomography (CT) (Figure 2). This was possible on the condition that satisfactory comparative dissection results are provided. Two approved methods have been developed in Denmark and Germany, respectively, based on complete CT scanning of carcasses. The two methods are basically different regarding the representation of data from CT scanning. Data representation is by either images (contextual segmentation) or spectra (multivariate methods). Both methods are comparable to manual dissection either because of high correlation except from a scaling factor or because the method

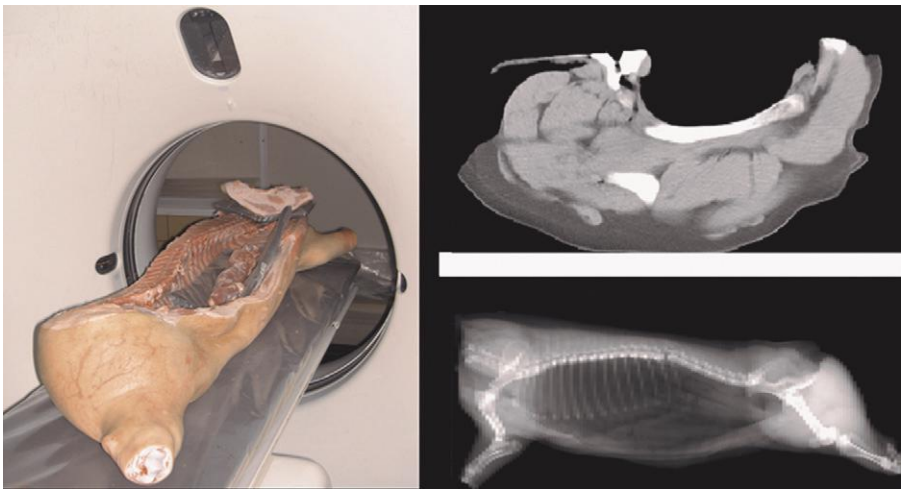

Figure 2 Computed Tomography as reference method. To the right an example of a tomogram and a topogram.

includes multivariate regression using the results from manual dissection as depended variable. Both the methods are valid as references for the lean meat percentage in pig populations similar to the population used to estimate the methods.

Intermediate Methods

Although the partial dissection method is less costly than the total dissection, some users have been looking for cheaper alternative calibration methods. These methods are characterized as cost-effective methods or intermediate methods, and special statistical methods (double regression and similar methods) have been developed to utilize the cheaper methods in an appropriate way.

Online Instruments

These methods can be characterized in various ways: invasive/noninvasive, split-line/back measurement, and manual/automatic measurement. The best method for a specific situation depends on the number of pigs slaughtered per day, the slaughter rate of the killing line, the desired accuracy and possibly the technological strategy at the slaughterhouse.

Noninvasive/Split-Line Methods

The simplest instrument is a measuring ruler, which some slaughterhouses made use of in the 1930s. The method is in use even today, and is generally known as the 'Zwei-Punkt' (ZP) procedure. The main principle of the method is to measure the thickness of fat and muscle, but various procedures exist together with different types of instruments such as callipers both for reading and for electronic data capture (Figure 3). Methods based on vision technology are also available.

Invasive/Back Measurement Methods (Probe Instruments)

The first probe instrument was developed in the 1950s (in Denmark). It was an optical probe, a metal tube mounted in a pistol grip and with a knife at the end. Inside the tube was a system of mirrors and, after inserting the probe into the carcass, it was possible visually to identify the line between fat and muscle and thus the thickness of subcutaneous fat at a specific location. In the next generation of probe instruments, the mirrors were replaced with a measuring technique based on electrical conductivity, utilizing the fact that fat and meat conduct electricity differently. Not only the fat thickness, but also the thickness of the loin muscle, was measured automatically, and it was soon also possible to capture the data electronically. In the third generation (Figure 4), the measuring technique was light reflection utilizing the fact that fat and muscle reflect light, especially light in the near infrared area, differently; this technology is used in all types of probe instruments today.

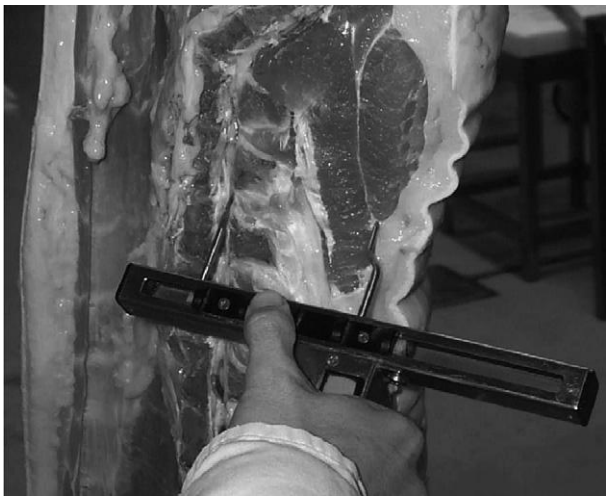

Figure 3 Example of Zwei-Punkt (ZP) instrument.

Noninvasive/Back Measurement Methods

The latest generation of instruments is based on ultrasound technology. The probe is replaced by a number of transducers and the technique exploits that an ultrasound reflection is

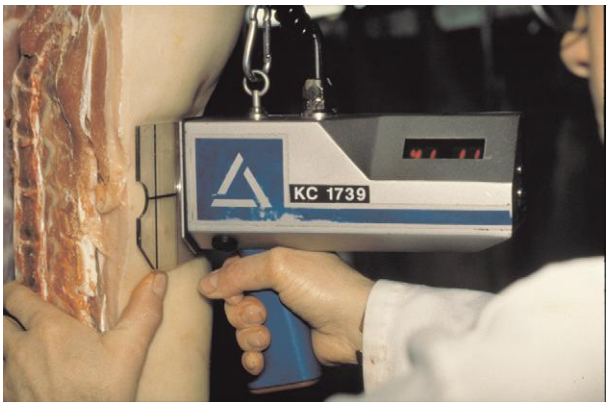

Figure 4 Example of a probe instrument. Photo: Danish Meat Research Institute.

produced each time a transition between two adjacent tissue areas occurs. Unfortunately, this will also happen from two adjacent areas of the same type of tissue, such as the layers of subcutaneous backfat on pigs. Consequently, the signal analysis must include a priori knowledge about the probable fat and meat proportions. The application is limited to slaughterhouses using a weak singeing technique, because strong singeing leaves air bubbles in the skin. The bubbles reflect strongly the ultrasound waves, and consequently no echoes can be detected from fat and muscle.

Manual/Automatic

There exist fully automatic systems based on probes, ultrasound, and vision. Prerequisites for these instruments are fast data processing together with advanced signal interpretation, such as artificial neural networks. The challenge is to establish a system, where data are handled automatically resulting in prediction of the lean meat content with high reliability. Often, a huge amount of data is obtained and only a small part of the data contains the information in question. The rest of the data is used to identify the position and validate the result.

Accuracy

The accuracy of online instruments depends on the prediction ability of the instrument, which is evaluated in the calibration procedure (see next section, Calibration). However, it also depends on the maintenance, the manual handling, and the measuring conditions. The European research team has estimated the accuracy of a variety of online methods. Investigations have been carried out in 12 countries, resulting in estimates of repeatability and reproducibility of ZP, ultrasound, and probe instruments. The reproducibility includes possible variation between different copies of the same type of instruments and differences between operators. It has been concluded that the variation between operators is generally more important than the variation between instruments. However, it is advisable to establish a monitoring system to ensure proper maintenance of the instruments and support and training of the staff.

Calibration

Online measuring methods only create indirect information about the content of lean meat, and it is therefore necessary to calibrate the instruments, i.e., to transform the information from the online measurements to an estimated value of the lean meat content. In the 1970s, research teams in Europe observed that a weighted average of the fat and muscle thicknesses at the middle of the back combined with one or two extra measurements estimated the lean meat content relatively precisely. In principle, the same method is used today, although it has been adapted to the new technologies.

To ensure that the calibration of the various instruments is carried out properly in the EU, the Commission has established certain requirements, and before a new type of instrument can be used in a member state or region, the Commission must approve the calibration. The procedure includes a number of steps:

- preparation of an experimental plan to be presented to the Commission;
- selection of sample of connected online and reference data;
- choosing an appropriate statistical method;
- establishment and assessment of a prediction formula, which must be reported to the Commission.

A team of European statisticians has prepared a handbook as a support for the preparation of new proposals.

Sampling

In general, calibration is based on a sample, but it is not obvious how the sampling should be done. It could be a representative and random sample or a stratified sample, and the reason for choosing a specific method would be to obtain a more robust prediction formula. The handbook mentioned in the Calibration section includes best practice and a number of examples.

Authorization of Prediction Formula

In 2008, the whole set of EU regulations were revised, including the requirements for the prediction ability of online methods. Classification (grading) methods shall be authorized only if the root mean squared error of prediction (RMSEP), computed by a full cross-validation technique or by a test set validation on a representative sample of at least 60 carcasses, is less than 2.5 lean% units. In addition, any outliers shall be included in the calculation of RMSEP.

Summary

In the beginning of the 1980s, the European Commission decided to harmonize the classification (grading) methods in the member states. The aim was to ensure uniform grading of pig carcasses to guarantee producers a fair payment based

on the weight and composition of their pigs and to make the market for the trade in pig carcasses more transparent. The Commission equated lean meat content with value and defined a Community scale, which is used to establish quotations for pig carcasses on a common basis and to make them comparable to the basic price valid for the standard quality.

Researchers in Europe are working with appropriate reference methods with respect to cost and accuracy. New technologies for both online methods and reference methods imply advanced data handling. The range of methods vary from very simple to highly advanced methods and it is a challenge to establish principles for authorization ensuring comparable results all over the EU.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Chemical and Physical Characteristics of Meat: Adipose Tissue; Chemical Composition; Color and Pigment; pH Measurement; Protein Functionality; Water-Holding Capacity. Classification of Carcasses: Beef Carcass Classification and Grading. Conversion of Muscle to Meat: Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Equipment Cleaning. Growth of Meat Animals: Growth Patterns. Meat Pricing Systems. Nutrition of Meat Animals: Pigs. On-Line Measurement of Meat Quality. Preslaughter Handling: Preslaughter Handling. Quality Management: Abattoirs and Processing Plants; Farm Level: Pork Quality. Slaughter-Line Operation: Pigs

Further Reading

Branscheid, W., Judas, M., Hoereth, R., 2011a. Zur Klassifizierung von Schweinehälften: Neue Schätzformeln und neue Geräte. Teil 1. Versuchsdurchführung und Schätzformeln für die Einstufung in die EUROP-Handelsklassen. Fleischwirtschaft 91 (4), 106–111.

Branscheid, W., Judas, M., Hoereth, R., 2011b. Zur Klassifizierung von Schweinehälften: Neue Schätzformeln und neue Geräte. Teil 2: Schätzformeln für die Einstufung nach Handelswert und Diskussion. Fleischwirtschaft 91 (5), 104–108.

Causeur, D., Dhorne, T., 2003. Linear regression models under conditional independence restrictions. Scandinavian Journal of Statistics 30, 637–650.

Dobrowolski, A., Romvari, R., Allen, P., Branscheid, W., Horn, P., 2003. X-ray computed tomography as an objective method of measuring the lean content of a pig carcass: A study in the framework of the European EUPIGCLASS project. Congress Proceedings, 49th International Congress of Meat Science and Technology, Brazil, pp. 371–372.

Engel, B., 1987. Increasing precision or reducing expense in regression experiments by using information from a concomitant variable. Biometrics 47, 1011–1023.

Engel, B., Buist, W.G., Font i. Furnols, M., Lambooij, E., 2004. Sub-populations and accuracy of prediction in pig carcass classification. Animal Science 78, 37–52.

Engel, B., Buist, W.G., Walstra, P., Olsen, E., Daumas, G., 2003. Accuracy of prediction of percentage lean meat and authorization of carcass measurement instruments: Adverse effects of incorrect sampling of carcasses in pig classification. Animal Science 76, 199–209.

Font i. Furnols, M., Gispert, M., 2009. Estimation of lean meat content in pig carcasses using X-ray computed tomography and PLS regression. Chemometrics and Intelligent Laboratory Systems 98, 31–37.

ISO 5725 (1994/Correction and amendment: 1998). Accuracy (trueness and precision) of measurement methods and results.

Marty-Mahé, P., Loisel, P., Brossard, D., Allen, P., Daumas, G., 2003. Vision method tested to predict the lean meat percentage of a pig carcass in relation to the reference dissection method. Congress Proceedings, 49th International Congress of Meat Science and Technology, Brazil, pp. 369–370.

Nissen, P.M., Busk, H., Oksama, M., *et al*., 2006. The estimated accuracy of the EU reference dissection method for pig carcass classification. Meat Science 73, 22–28.

Olsen, E.V., Candek-Potokar, M., Oksama, M., *et al*., 2007. On-line measurements in pig carcass classification: Repeatability and variation caused by the operator and the copy of instrument. Meat Science 75, 29–38.

Vester-Christensen, M., Erbou, S., Hansen, M.F., *et al*., 2009. Virtual dissection of pig carcasses. Meat Science 81, 699–704.

Walstra, P., Merkus, G.S.M., 1996. Procedure for assessment of the lean meat percentage as a consequence of the new EU reference dissection method in pig carcass classification. Report ID-DLO 96.014, March, 22p.

Relevant Websites

http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2008:337:SOM:EN:HTML
Commission Regulation (EC) No 1249/2008.

http://w3.cost.eu/index.php?id=181&action_number=FA1102
Cost Action: Optimising and Standardising Non-Destructive Imaging and Spectroscopic Methods to Improve the Determination of Body Composition and Meat Quality in Farm Animals Acronym: FAIM.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32007R1234:en:NOT
Council Regulation (EC) No 1234/2007.

CONNECTIVE TISSUE: STRUCTURE, FUNCTION, AND INFLUENCE ON MEAT QUALITY

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Glossary

Collagen It is the main component of connective tissue. There are many types of collagen, of which the main ones in muscle are type I and type III, which are the focus of this article. In muscle, collagen forms the endomysium, perimysium, and the epimysium.

Collagen content With approximately 25–35% of the whole body protein content, collagen is the most abundant protein in mammals. It generally constitutes 1–2% of muscle tissue but may represent up to 6% of the weight in high tendinous muscles.

Collagen cross-linking The individual protein subunits of collagen are assembled together by covalent intermolecular cross-links, which increase the tensile strength of collagen. The number of cross-links generally increases with the age of the animals.

Collagen solubility While heating, molecular chains of collagen separate and solubilizes to gelatin. The solubilization of collagen decrease when the number of cross-links increase.

Proteoglycans These are heavily glycosolated proteins that are a filler substance between cells that regulate movement of molecules through the matrix and affect activity and stability of proteins and signaling molecules.

Introduction

Connective tissue is produced by fibroblasts and is the most abundant protein in mammals, making up approximately 30% of the total protein content. When mineralized, it is found in skeletal tissues including bone, teeth, and cartilage. The non-mineralized soft connective tissue is found in skin, tendon, ligament, adipose tissue, blood and lymphatic tissue, and the connective fiber framework in muscles. It contains a mixture of polysaccharides and fibrous proteins. The most abundant protein in connective tissue is collagen, but it also contains various other proteins (such as elastin, fibronectin, and laminin) and proteoglycans in varying proportions, depending on the organ. Connective tissue represents a small proportion of skeletal muscle and is mainly found in extracellular matrix of muscle fibers. However, the meat is the result of transformation of the muscle after slaughter of the livestock animal. Connective tissue content and composition significantly affects the quality of meat and meat products, particularly texture and water-holding capacity. For this reason, numerous scientific studies have been led to characterize its structure, organization, and composition in order to understand its role in determining meat quality.

Distribution, Composition, and Structure of Muscle Connective Tissue

Muscle connective tissue content varies from 1.5 to 15% of dry matter depending on muscle and muscle function. Intramuscular connective tissue is principally comprised of collagen and elastin proteins within a proteoglycan matrix. Muscle elastin content is approximately 0.1–0.2% of dry matter, except in semitendinosus and latissimus dorsi muscles, where it reaches approximately 2%. The vast majority of this connective tissue is composed of collagen in amounts ranging from 1.5% to approximately 10% of dry weight. Collagen I and III are the predominant types in muscle, but collagens IV, V, VI, XII, XIV, XV, and XIX are also present in minor quantities (Figure 1).

Collagen is composed of three polypeptide alpha chains with a simple repeating primary structure Gly–X–Y, where X is often proline and Y is often hydroxyproline. The three chains are assembled together to form a triple helix of tropocollagen. The tropocollagen molecules are assembled together to form fibrils that are then assembled to form collagen fibers (Figure 2).

The fibrous collagen is cross-linked by a mechanism based on aldehyde formation from lysine or hydroxylysine side chains. Fibrils and fibers are stabilized by intermolecular lysine-derived cross-links formed between lysine aldehyde or hydroxylysine aldehyde and hydroxylysine to form an aldimine or oxo-imine bond. These bonds are replaced during aging by stable multivalent mature cross-links, which are thought to link microfibrils and increase matrix stability. Thus, higher collagen cross-linking translates into higher mechanical strength.

From a structural standpoint, intramuscular connective tissue is divided into three main structures (see Figure 3).

Epimysium is a layer of dense irregular connective tissue that surrounds whole skeletal muscle. It protects muscles from

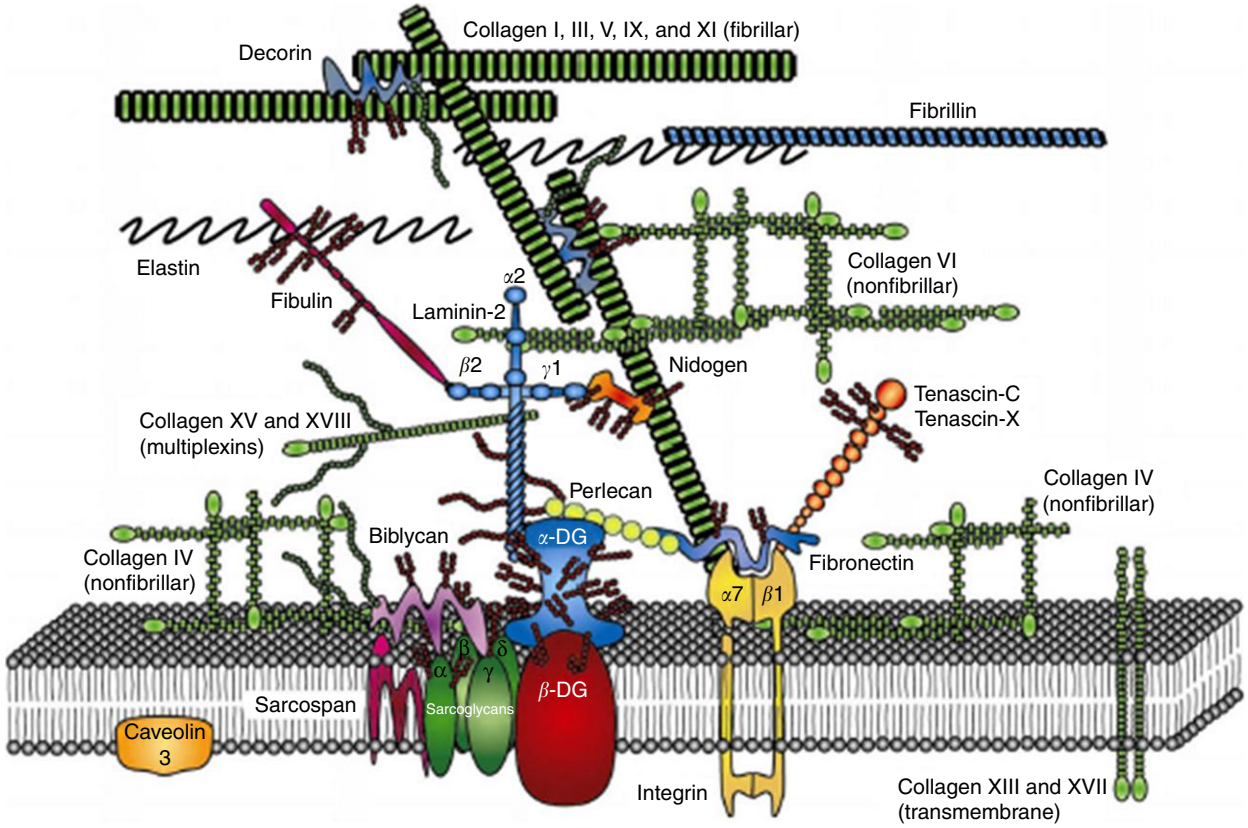

Figure 1 Representation of the muscle fiber extracellular matrix. Reproduced from Voermans, N.C., Bönnemann, C.G., Huijing, P.A., *et al.*, 2010. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscular Disorder 18, 843–856.

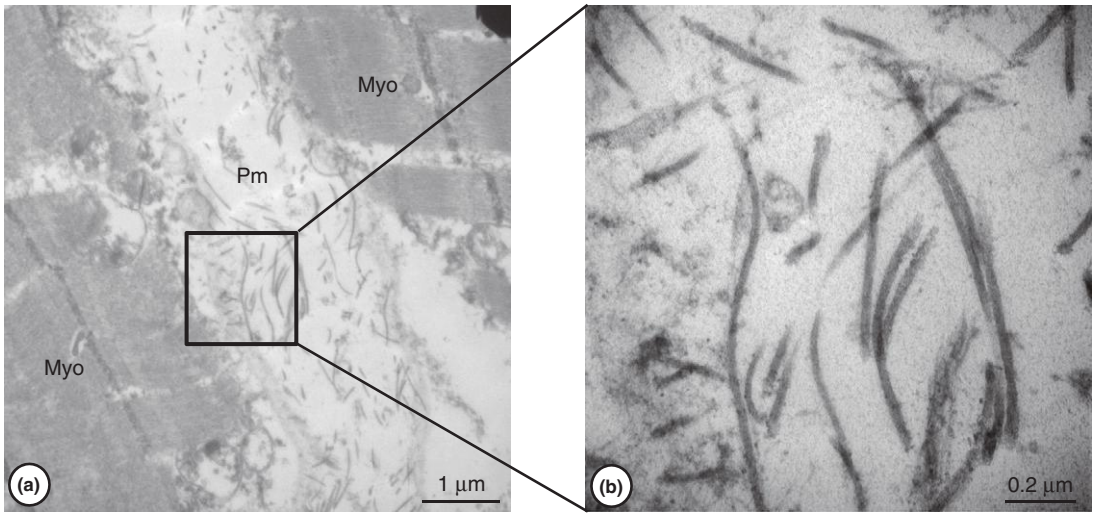

Figure 2 Collagen fibers (bovine semitendinosus muscle). (a) Collagen fibers seen in perimysium. (b) Longitudinal section of collagen fibers. Myo, myofibrils; Pm, perimysium.

friction against other muscles and bones. The epimysium is generally separated from the body of the muscle and need not be considered as a factor in meat texture, except in some meat products such as dry and cooked ham where muscles are intact.

Perimysium is a layer that separates bundles of muscle fibers within the muscle. It represents approximately 90% of total muscle connective tissue, where it is organized as a network of interconnected segments that vary extensively according to muscle type, species, age, and region in the

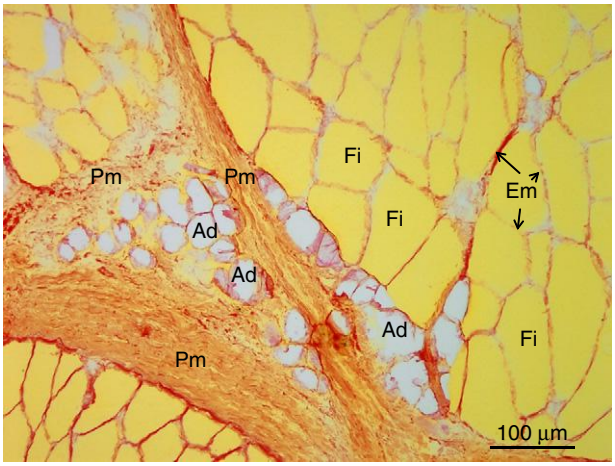

Figure 3 Transversal section of porcine longissimus thoracis muscle. Ad, Adipocyte; Em, endomysium; Fi, muscle fiber; Pm, perimysium.

muscle. The perimysium is usually arranged in large circular or pentagon-shaped fascicles several millimeters in diameter, with the smallest bundles, or primary perimysium, grouped within larger bundles of thicker perimysium, the secondary fascicles, which may be further organized by tertiary fascicles. Perimysium composition is highly variable and can change with nutrition and exercise. Strength muscles tend to have more collagen than postural muscles have, and collagen from old animals has significantly more cross-links than collagen from young ones.

Endomysium surrounds each muscle fiber. It is composed of basal lamina proteins, proteoglycans, and laminin, plus collagens I, III, and IV, and is very similar in all muscle types and even across species.

'*In vivo*' Function of Connective Tissue

Skeletal muscle connective tissue forms a network that plays a dynamic role in muscle differentiation and development. It serves as a supportive structure in skeletal muscle.

Connective tissue adheres to myofibers and fiber bundles, providing a scaffold that maintains muscle structure during contraction, and acts as an interface allowing fiber bundle sliding. Connective tissue provides the cell-to-cell connections both between individual muscle cells and between muscle cells and neighboring small blood vessels and nerves. It gives coherence and mechanical strength, functions as an elastic, stress-tolerant system, and distributes the forces of muscular contractions in both muscle and tendon. The muscle connective tissue participates in cell growth and tissue regeneration after damage.

The organization of muscle into fascicles separated by the perimysium reflects the need to accommodate shape changes as the muscle contracts, which is achieved by allowing fascicles to slide past each other. Functionally, different muscles have very different requirements in terms of accommodating the shear strains that necessarily occur as the muscle contracts and changes shape, which explains why the amounts and distribution of perimysial connective tissue varies so widely between functionally different muscles.

Connective Tissue Properties Related to Meat Texture

The content, nature, and heat solubility of collagen are key factor of meat tenderness.

Technological processes help to reduce the impact of connective tissue on the texture of meat products. Mincing, for example, can decrease the sensation of toughness in meat, whereas cooking, by solubilizing collagen, significantly reduces the mechanical strength of the connective tissue. Acidic marinating can also partially degrade the connective tissue, which may tenderize meat. However, some meats, like roasts and grilled meat, are consumed after maturation and short-term cooking, in which case cooking temperature at core of the meat piece remains too low to solubilize the collagen. As collagen content is relatively unaffected by the action of proteases during maturation, it ultimately defines the basal hardness of raw or undercooked meat. Thus, the less tender muscles, like pectoralis profundus, generally have high collagen concentrations and a high number of cross-links, whereas a tender muscle like Longissimus thoracis has a lower collagen concentration (1.86%) and fewer cross-links. It is assumed that collagen concentration and number of cross-links have a cumulative effect, leading to a negative impact on meat tenderness. Collagen type also plays a role in meat tenderness. Particularly, type-III collagen, which is more susceptible to proteases, is associated with tenderness, whereas type-XII and type-XIV collagen are thought to decrease total collagen solubility and therefore overall meat tenderness.

Method of Measuring Meat Texture

An important consideration when determining the connective tissue properties tied to texture is the texture measurement method used. In evaluating overall texture quality, and especially the contribution made by connective tissue, the best tests include adhesion force between fibers, peak shear force minus initial yield, and compression, which correlates well to sensory chewiness and texture. For example, old animals show increased adhesion force, decreased collagen solubility, and tougher meat. Understanding the mechanical properties of meat requires an assessment of large amounts of information about sample muscle fiber orientation, cooking temperature, fiber size, and the amount of connective tissue. Compression tests at 20–80% of the initial height of the sample with fibers perpendicular and parallel to the test tool can be used to estimate the relative contribution of each myofiber and connective tissue to the texture. These types of tests have shown that amount of connective tissue goes a long way toward explaining the between-breed and between-muscle differences in texture.

Sensory evaluation of the contribution of connective tissue to texture is not only the most sensitive and accurate but also the most laborious method. Results using this method follow the same trend as mechanical measures, indicating that collagen quantity is not a major factor in animals of the same age when comparing the same muscle type. Collagen content does explain some of the differences in sensory texture between different types of beef muscles, with the psoas and longissimus having low collagen content and tender texture, whereas biceps and pectoralis having high collagen content and tough texture. In

general, pork has similar connective tissue properties to beef, with a weak but significant relationship between hydroxyproline content and sensory tenderness, connective tissue, and flavor.

Biological Factors, Connective Tissue, and Meat Tenderness

In general, the content or quality of the connective tissue varies with biological factors.

Species

Structural characteristics are similar from one species to another. Longissimus muscle from young cattle, sheep, and pigs do not differ in collagen content and solubility, indicating that longissimus has similar collagen properties across species.

Breed

Textural differences can be related to connective tissue properties as well as aging potential. For example, *Bos indicus* breeds have a high collagen content, low collagen solubility, and a tough texture. However, a comparison between *B. indicus* and *Bos taurus* crosses did not find significant differences in collagen content or solubility, which may suggest that connective tissue only makes a significant contribution to texture in purebred *B. indicus* lines. Similarly, a comparison of six European and African breeds found little difference in collagen content and solubility. Compared with normal animals, double-muscled cattle have less collagen, higher collagen solubility, and low raw meat texture scores but similar cooked texture scores. *Bos taurus* breeds that mature late have high collagen solubility, and the rheology of the connective tissue is variable in raw samples but not in cooked ones. In conclusion, it seems that no consistent differences amongst breeds for texture-related connective tissue properties are evidenced.

Sex

The sex of animal could have an effect on the connective tissue content and quality. Comparisons on animals at the same age beyond puberty show that meat from uncastrated males is less tender than meat from castrated males, which, in turn, is less tender than meat from females. These variations can be partly explained by differences in collagen solubility. Thus, the lower collagen content in muscle from heifers at 13 and 24 months compared with young bulls is a key determinant of differences in tenderness between the two types of animals. Moreover, the meat of females is softer than that of bulls, as it has a lower collagen content and higher collagen solubility.

Age

Tenderness is highest in meat from very young animals but subsequently declines with age and physical activity, along with an increase in the amount of collagen and its degree of complexity. The increase in toughness is more linked to a reduction in collagen solubility than an increase in collagen content. The water solubility of collagen under the action of heat decreases gradually with increasing age, with the result that the meat progressively becomes less tender. These changes are usually attributed to a gradual increase in the number of stable intermolecular cross-links.

Many studies have shown a progressive age-related decrease in muscle collagen solubility and tenderness, with little or no change in total collagen content. Although most of these studies were done on ruminants, the pattern is a general feature of connective tissue. The change in solubility results in increased meat toughness in old animals and is the major factor shaping the contribution of connective tissue to meat quality. Very young animals have more collagen and elastin than mature animals have, but veal and lamb have a very soluble collagen and consequently a very tender meat. There is no evidence that elastin per se is associated with texture, despite the fact that its content varies.

Several studies on the effect of animal age on connective tissue-related meat texture showed that from 1 to 60 months, the textural properties of meat were significantly related to collagen content and its solubility. In the same studies, comparisons of muscle types showed that all 12 types were acceptably tender in animals of approximately 24 months of age or less. Myofiber toughness did not change markedly after 24 months of age, but the connective tissue toughness increased markedly in 10 of the 12 meat types in animals aged 3 years upward. In addition, an increased cross-sectional area of perimysium is observed with age. Perimysium becomes either thicker or has more branched bundles, provided no change in content occurs. Increasing age also results in firmer fiber adhesion by connective tissue. Furthermore, fiber adhesiveness is lost at 60 °C in meat from young animals but not old animals. This may be the property linking collagen solubility to cooked toughness, as it is also related to sensory chewiness.

Muscle Type and Connective Tissue Organization

Collagen and elastin contents differ severalfold between different muscle types. In general, the force muscles such as the biceps have more collagen than postural muscles such as the psoas have. In addition, perimysium volume and organization vary greatly between muscle types (see Figure 4). In the 1930s and 1940s, structural classification of muscle suggested that muscle type differences in texture were correlated to connective tissue content and organization. Muscle grain and muscle fascicles are known to vary significantly between muscle types. Fascicles vary in size, from approximately 1–10 mm, and shape and are hard to visualize and measure. A larger bundle size of fibers ensheathed in primary perimysium would presumably translate into a firmer meat texture, especially when the perimysium is thick and has extensive collagen cross-links that will not melt at cooking temperatures. Fascicle size is correlated to sensory tenderness and shear force texture. Thickness of the perimysium has been shown to be correlated to shear force in chicken and pork. Thicker perimysium melts more slowly and thicker regions have a different composition such as more type-I collagen, more heat-stable elastin, or more cross-links. However, veal also has thicker perimysium than that from mature animals, but the meat stays tender due to its high collagen solubility and small fiber size, which rules out a

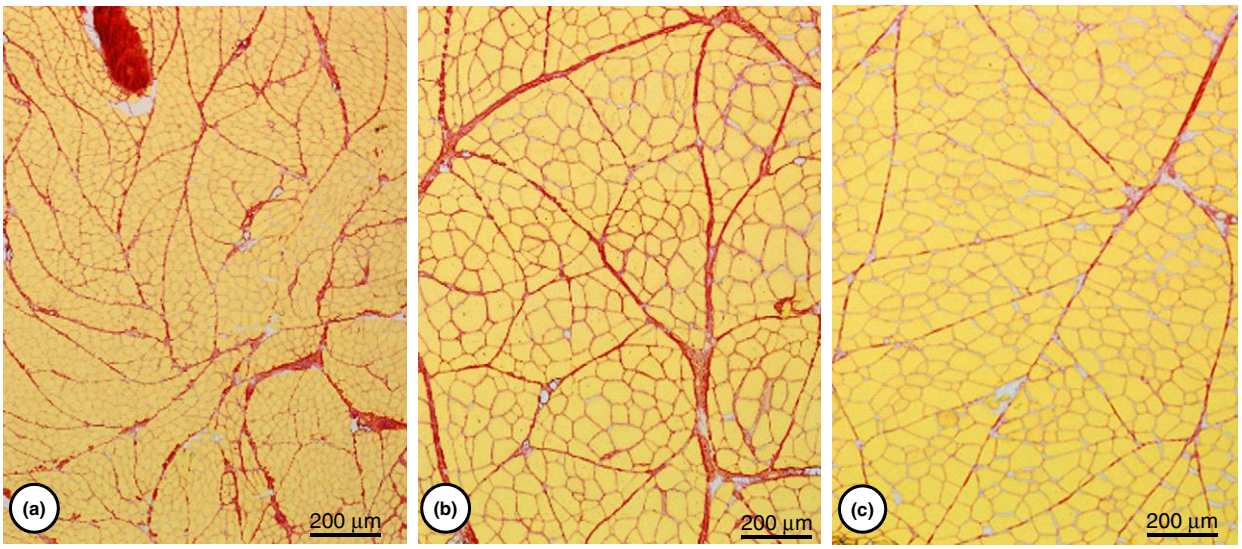

Figure 4 Differences in structural organization of pig muscles. (a) Masseter, (b) semitendinosus, and (c) longissimus thoracis. Connective tissue is stained in red and muscle fibers in yellow. The content and distribution of connective tissue depends on the muscle. Longissimus thoracis, which have less thicker perimysium than semitendinosus and masseter muscles, is more tender. Fiber size is smaller in masseter than in semitendinosus and longissimus thoracis.

systematic direct relationship between perimysium thickness and texture. As stated above, collagen can only influence shear force if it has more cross-links and is in greater quantity.

There have been attempts to link perimysium organization to meat quality, and a relationship has been found. Several research teams have used a qualitative grading system to show that perimysium organization varies with muscle type, species, and age. Semiquantification by image analysis on digital images coming from magnetic resonance imaging and histology techniques show that tough muscles have smaller fiber bundles.

The last property to discuss in this section is myofiber adhesion to perimysium and fiber–fiber adhesions. These are the most fragile structures in cooked meat. The initial fracture plane is usually at the endomysium–perimysium junction. When stress is applied parallel to the fiber plane, the fracture occurs in the endomysium, whereas when stress is applied perpendicular to the fiber plane, the fracture occurs at the perimysium–endomysium junction.

Postmortem Changes in Connective Tissue

There are changes in connective tissue that could play a role in tenderness and thus require further study.

Endomysium

It has been shown that the endomysium starts to detach from fibers as early as 6 h postmortem, and at least half of the endomysium is detached within 24 h.

The detachment of the endomysium may be related to the very rapid drop in shear force observed soon after rigor and may be a general aspect shaping the development of texture. The detachment of endomysium in callipyge sheep, even though the meat is very tough, demonstrates that it is marginally involved in meat texture properties. Nevertheless, endomysium detachment from sarcomeres remains a necessary first step for texture development. However, mechanical measures show that lateral adhesion of fibers by endomysium is stable postmortem and accounts for approximately a tenth of the longitudinal force of fibers. The contribution of endomysium to meat texture is, therefore, stable postmortem and very minor compared with the contribution of myofibers.

Perimysium

Perimysial collagen is degraded during storage. Transmission and scanning electron microscopy studies show that the structural integrity of the intramuscular connective tissue decreases during postmortem aging. The major postmortem change in perimysium is that myofibers separate from perimysium within 6 h. Perimysium seems to be the structure most vulnerable to meat shearing action. It has also been shown that the isometric tension of intramuscular collagen decreases at 21 days postmortem in beef. Furthermore, the breaking strength of the perimysial connective tissue in raw beef decreases during postmortem aging. The thermal shrinkage temperature of bovine intramuscular collagen decreases by 7–8 °C within 7 days postmortem. These structural changes are strongly related to the mechanical strength of meat, as demonstrated by shear measurements on raw muscle or uncooked intramuscular connective tissue structures. Proteoglycan degradation occurs at the same time frame as structural changes in connective tissue (Figure 5), but further studies are required to better understand the role of proteoglycans in texture.

Changes in Connective Tissue with Cooking

Elastin is very heat stable, and its properties are generally unaffected by cooking. However, elastin content in the

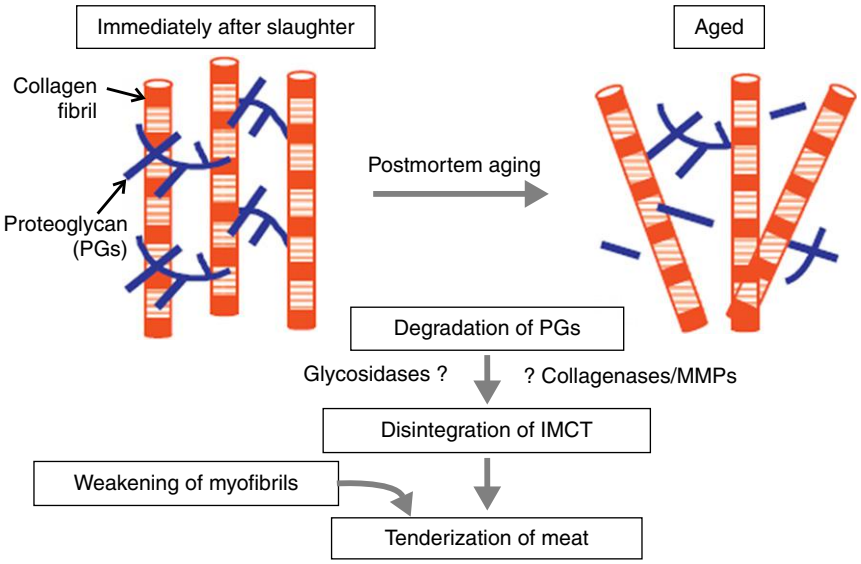

Figure 5 Proteoglycan involvment on intramuscular connective tissue changes during postmortem ageing. IMCT, Intramuscular connective tissue; PGs, Proteoglycans. Reproduced from Nishimura, T., 2010. The role of intramuscular connective tissue in meat texture. Animal Science Journal 81, 21–27, with permission from Japanese Society of Animal Science, John Wiley & Sons Ltd.

perimysium is generally low (0.1–0.2% of the total connective tissue) and its impact on meat texture is, therefore, considered as not significant. However, elastin may contribute to resistance to shear in the semitendinosus muscle, where elastin accounts for up to 40% of total connective tissue.

In contrast, collagens and proteoglycans are unstable to heat, and their properties are affected by cooking. The thermal denaturation of proteoglycans does not play a direct structural role in mechanical terms, but it can play a role in the thermal stability of collagen due to molecular interactions with collagen fibers.

Although, connective tissue makes the largest contribution to texture in raw or undercooked meat, myofibers make the largest contribution to texture in cooked meat. Cooking at temperatures of 60–70 °C causes gelation of most of the perimysium. The temperature for structural changes tends to vary between studies: Some authors report beef perimysium gelation at 70 °C and above, whereas others cite 60–63 °C. Using isolated fibers and small muscle samples it was demonstrated that perimysium changes its thermal mechanical property at 50–60 °C, before the myofibers toughen. These changes are mostly due to collagen melting. The contribution of connective tissue to meat texture declines as temperature increases.

Although thermal stability is well established as the major property determining the role of connective tissue in cooked meat texture, it is less clear how structure and composition variability affect the structural changes involved. Perimysium attributes like thickness are highly variable in cooked meat. Furthermore, elastin distribution as well as collagen type varies greatly within small regions of perimysium. The relationship of connective tissue structure and composition to thermal and mechanical properties of meat requires further study in order to better evaluate the specific influence of collagen type, elastin, or perimysium thickness.

When heated at more than 60–65 °C, the collagen molecule changes from its native helical ordered state to a randomly coiled structure, and fibrous collagen is converted from a fibrillar to a rubber-like amorphous structure. The destruction of hydrogen bonds in the molecule is accompanied by shrinkage of the collagen fibers. If collagen is free to shorten, it shrinks to one-quarter of its original length. The contraction of collagenous networks during cooking is a major mechanism by which collagen influences meat texture. The thermal contraction of collagen fibers and fibrils starts with a free contraction, after which there is a forced contraction where collagen fibers and fibrils apply pressure on muscles fibers and muscle fiber bundles. The more the collagen fiber contracts during heating, the lower its mechanical strength. As the thermal contraction of meat collagen fibers is limited by muscle fibers, the resistance of muscle fibers or muscle fiber bundles influences the ultimate elastic modulus of the collagen fibers. Moreover, the nature of the cross-links determines contraction strength, contraction amplitude, collagen solubilization, and the final mechanical properties of the connective tissue after heating. The thermal stability of collagen is related to the presence of heat-stable mature cross-links, which increase with age and contribute to toughness. At 70 °C, 42% of veal collagen is soluble compared with 2% solubility in beef from 10-year-old animals, and the thermal shrinkage temperature is 55 °C for veal and 70 °C for beef from 10-year-old animals. Collagenase digests 21% of the collagen in veal, whereas the percentage is approximately 10% in 10-year-old animals. These results indicate that the age-related toughness of cooked meat is directly associated with the change in collagen thermal stability.

The role of cross-links in meat quality is less clear in same-age animals. Stable cross-links do vary significantly by muscle type, with sheep longissimus having few and biceps many. But the tenderest muscle, the psoas, has a high number of

cross-links, yet it remains tender even at normal sarcomere lengths. These results suggest that both high collagen content and cross-link formation are needed for connective tissue in order to be able to influence toughness, as is the case in biceps muscle. Between-muscle differences in cross-link quantity have also been reported for beef, sheep, and goat. Several studies have failed to demonstrate a relationship between number of cross-links and collagen solubility. In addition, numerous studies have failed to find a relationship between cross-links and shear force in same-age animals, including two studies that measured five different types of cross-links. Consequently, a relationship can be ruled out between these cross-link types and shear force, especially as meat from callipyge sheep has relatively few cross-links but is still extremely tough. Therefore, it is not yet clear which cross-links are responsible for the age-related change in meat texture.

One hypothesis is that heat stability is the result of collagen interaction with decorin, which is found at every D-line (every 64 nm) along the collagen fibril, is heat stable, and cross-links collagen fibers. There is evidence that decorin is stable post-mortem, and there is little evidence that it has mechanical properties. However, decorin has not been yet sufficiently investigated to determine its importance in connective tissue stability at cooking temperatures, despite the fact that this may prove an important factor.

Conclusion

Skeletal muscle connective tissue is a supportive matrix composed of various fibrous proteins, primarily collagen. Many molecular interactions converge to stiffen the matrix, whose properties vary with the physiological and biological characteristics of source animals. The quantity, quality, and organization of this connective tissue depend on the muscle and its function in the body. Connective tissue changes as the animal matures, and the quantity and degree of cross-linking in muscle connective tissue are generally related to the basic toughness of animal meat. As it is impossible to fully control the characteristics of the muscle tissue, several strategies are employed in order to meet consumer expectations. The first is to sort the muscles according to their position on the carcass and their function, both of which are typically associated with connective tissue characteristics. Strength muscles are generally richer in cross-linked collagen and are oriented toward the production of minced meat or for long-term moist cooking processes to dissolve the collagen and tenderize the meats. Postural muscles are generally less collagen rich, and some are used as meat to grill. Therefore, it is possible to optimize meat tenderness by selecting breeds known for their low connective tissue content or by using young animals with lightly cross-linked collagen. Research to date has provided some insight to help elucidate the role and function of connective tissue in the muscle, to determine its composition and organization from the tissue level down to molecular level, and to assess its contribution to the organoleptic properties of the meat end product. Although the role of connective tissue in the mechanical properties of meat is generally understood, the properties of meat textures are complex and multifactorial, resulting from the behavior of connective tissue, muscle fibers, and interactions between these two muscle components. The future development of innovative technological processes that can degrade or even partially dissolve the connective tissue could improve the price and market value of those meat cuts known for their high mechanical strength.

Finally, food science research to date has mainly focused on connective tissue to understand its role in shaping meat texture, and it is only recently that the focus has shifted to also look at the nutritional qualities of meat. Although connective tissue by itself is not known for its nutritional value, its structural organization at different levels in whole tissue muscles may play a role in the distribution of small molecules of nutritional interest during various processes such as cooking, mincing, and cutting, thus resulting in a loss of micronutrients into exudates and/or cooking juices. The endomysium and perimysium envelopes are also more likely to limit the accessibility of digestive enzymes (pepsin, trypsin, and chymotrypsin) to the high nutritional value myofibrillar proteins. Such transformation processes, especially cooking, dissolve all or part of collagen depending on its degree of cross-linking and on the heating conditions. However, there is still a lack of data on the role played by the overall whole muscle tissue in the transfer of micronutrients and small molecules, and on the bioaccessibility of digestive enzymes in myofibrillar proteins.

See also: Carcass Composition, Muscle Structure, and Contraction. Classification of Carcasses: Beef Carcass Classification and Grading. Cooking of Meat: Physics and Chemistry. On-Line Measurement of Meat Quality

Further Reading

Bailey, A.J., Light, N.D., 1989. Connective Tissue in Meat and Meat Products. London: Elsevier Applied Science.
Bendall, J.R., 1967. The elastin content of various muscles of beef animals. Journal of Agricultural and Food Science 18, 553–558.
Bloom, W., Fawcett, D.W., 1975. A Textbook of Histology. London: WB Saunders.
Gillies, A.R., Lieber, R.L., 2011. Structure and function of the skeletal muscle extracellular matrix. Muscle & Nerve 44, 318–331.
Lepetit, J., 2008. Collagen contribution to meat toughness: Theoretical aspect. Meat Science 80, 960–967.
Light, N.D., 1987. The role of collagen in determining the texture of meat. In: Pearson, A.M., Dutson, T.R., Bailey, A.L. (Eds.), Advances in Meat Research, Collagen as Food, vol. 4. New York: Van Nostrand Reinhold, pp. 87–107.
McCormick, R.J., 1994. The flexibility of the collagen compartment of muscle. Meat Science 36, 79–91.
McCormick, R.J., Phillips, A.L., 1999. Muscle extracellular matrix. Role in growth, development, and meat tenderness. In: Xiong, Y.L., Ho, C.T., Shahidi, F. (Eds.), Quality Attributes of Muscle Foods. New York: Kluwer Academic/Plenum Publishers, pp. 219–227.
Nishimura, T., 2010. The role of intramuscular connective tissue in meat texture. Animal Science Journal 81, 21–27.
Purslow, P.P., 1987. The fracture properties and thermal analysis of collagenous tissues. In: Pearson, A.M., Dutson, T.R., Bailey, A.L. (Eds.), Advances in Meat Research, Collagen as Food, vol. 4. New York: Van Nostrand Reinhold, pp. 187–209.
Purslow, P.P., 2004. Physiology and biochemistry of collagen Proceedings of the 57th American Meat Science Association Reciprocal Meat Conference. Kentucky: Lexington, pp. 81–85.
Purslow, P.P., 2005. Intramuscular connective tissue and its role in meat quality. Meat Science 70, 435–447.

Sifre, L., Berge, P., Engel, E., *et al*., 2005. Influence of the spatial organization of the perimysium on beef tenderness. Journal of Agricultural and Food Chemistry 53, 8390–8399.
Takala, T.E., Virtanen, P., 2000. Biochemical composition of muscle extracellular matrix: The effect of loading. Scandinavian Journal of Medicine & Science in Sports 10, 321–325.
Voermans, N.C., Bönnemann, C.G., Huijing, P.A., *et al*., 2010. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscular Disorder 18, 843–856.

Relevant Websites

http://www.ivy-rose.co.uk/HumanBody/Muscles/Muscle_Structure.php
Ivy-Rose.
http://en.wikipedia.org/wiki/Collagen
Wikipedia.
http://en.wikipedia.org/wiki/Connective_tissue_in_skeletal_muscle
Wikipedia.
http://en.wikipedia.org/wiki/Elastin
Wikipedia.
http://en.wikipedia.org/wiki/Proteoglycan
Wikipedia.
http://www.wisc-online.com/Objects/ViewObject.aspx?ID=AP14504
Wisc-online.

CONVERSION OF MUSCLE TO MEAT

Contents

Aging

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Glossary

Calpains Components of the enzyme system that act on cytoskeletal proteins during meat tenderization.
Conditioning One of the terms used as meat enters rigor mortis.
Connective tissue/collagen Noncontractile fibrous proteins (epimysium, endomysium, and perimysium) that surround each muscle fiber – the amount and extent of cross-linking dictate the basal tenderness. Shrinkage occurs as a result of heating (e.g., during cooking) above the collagen shrink temperature.
Cooking Application of heat to meat that denatures the various proteins at different rates depending on the temperature that results in various textures of cooked meat.
Cytoskeletal proteins A set of structural proteins in muscle (includes titin, nebulin, desmin) that are denatured by calpains.
Drip or purge Water that increases over time arising from the cytoskeletal protein denaturation as meat tenderizes – it is different to that from myosin denaturation, which may also occur.
Electrical stimulation Application of an electric current through a carcass or primal cut post mortem that accelerates the rigor process.
Hot boning A process when the meat is removed from the carcass before rigor mortis in contrast to cold boning when meat is removed after rigor mortis.
Myofibrillar proteins The muscle contractile proteins actin and myosin.
Post mortem The period after slaughter when the pH falls until rigor mortis and subsequent aging.
Preslaughter The period before slaughter when factors such as stress can affect meat quality.
Protein denaturation A process whereby proteins lose their tertiary and secondary structure such as by application of acids or heat – water that is part of the tertiary structure may be released.
Rigor Occurs when individual muscle fibres are depleted of ATP; rigor mortis applies when all muscles that enter rigor become stiff.
Shear force The force (N) applied to a standardized piece of meat to shear it.
Shortening A process that occurs when prerigor muscle is cooled below 10 °C – additionally it also occurs as muscles enter rigor at high temperatures (rigor shortening).
Tenderization The enzymatic process that takes place after rigor mortis, which makes meat tender.
Ultimate pH The pH that is reached when muscles reach rigor mortis.

Introduction

Aging is the name given to the process of meat tenderization. The tenderization occurs through the action of endogenous muscle enzymes present in living muscles that are still available in postmortem meat. The extent and rate of aging can be influenced by virtually all aspects of production and processing, thus the term aging by itself needs to be qualified in some way. Aging is affected by the live animal's history before slaughter, including preslaughter stress effects; and it can be influenced to an even greater extent by the muscle's prerigor temperature, muscle shortening, electrical stimulation, and the

temperature and duration of aging ('rigor' is a term applied to the process of individual muscle fibres becoming depleted of adenosine triphosphate (ATP), whereas 'rigor mortis' is a term that refers to the muscle stiffness that occurs after all muscle fibres enter rigor). The rate of aging is different for different muscles of the same animal and for different animal species. The temperature at which meat ages and the packaging required are determined in a practical way by the need to ensure low levels of pathogenic and spoilage bacteria.

Meat, apart from fermented and specialty raw or dried products, is usually cooked before it is eaten with cooking temperatures also having a bearing on the final tenderness but the tenderness changes due to aging are independent of this. This article uses two modes of presenting data from meat cooking. Water bath cooking is an experimental procedure involving cooking meat in a bag in a water bath to a defined temperature, whereas dry heating has a heat source applied to steaks from above or below by a hot plate or hot air – this procedure is closer to what a consumer is used to. Raw meat is relatively tender but toughens slightly on initial cooking. Cooking at temperatures from 55 to 67 °C (various grades of rareness) reduces the red color and the meat then becomes tender (see Figure 1). At higher temperatures, the myofibrillar and connective-tissue proteins denature at different rates, and it is the properties of these denatured proteins that determine the texture of the different cuts of meat as well as changes related to cooking temperature. For example, at temperatures between 40 and 65 °C (various degrees of 'rareness') there is first a decrease in toughness then an increase in toughness caused by increasing aggregation of the denatured myofibrillar proteins, which is accompanied by a loss of fluid and shrinkage of the muscle fibres within the endomysial sheath. Connective-tissue shrinkage occurs at approximately 65–67 °C and is one of the causes of increasing toughness as the collagen in the endomysium and perimysium denatures further and more water is squeezed out. There is a steady rise in toughness up to 80 °C and with further increases in temperature above 80 °C, prolonged heating, as in stewing or casseroling, solubilizes collagen and there is a reduction in shear values. Collagen can be solubilized at lower temperatures, but this requires considerably longer cooking times. At temperatures from 70 °C upwards, the meat shifts from 'medium to well done.' The method of cooking chosen, from grilling to stewing, therefore depends on the type of cut and the connective-tissue content.

The process whereby muscle goes into rigor mortis is termed 'conditioning' by some workers and subsequent holding periods are termed 'aging.' Other workers use the term 'conditioning,' or alternatively 'aging,' for the whole process of going into rigor mortis together with further postmortem holding. Aging is the term used for the postrigor process throughout this article.

It has probably been known for millennia that, following the death of an animal, the muscle enters rigor mortis and becomes firm. Over time, rigor mortis 'resolves' somewhat and the meat becomes less firm and increasingly tender when

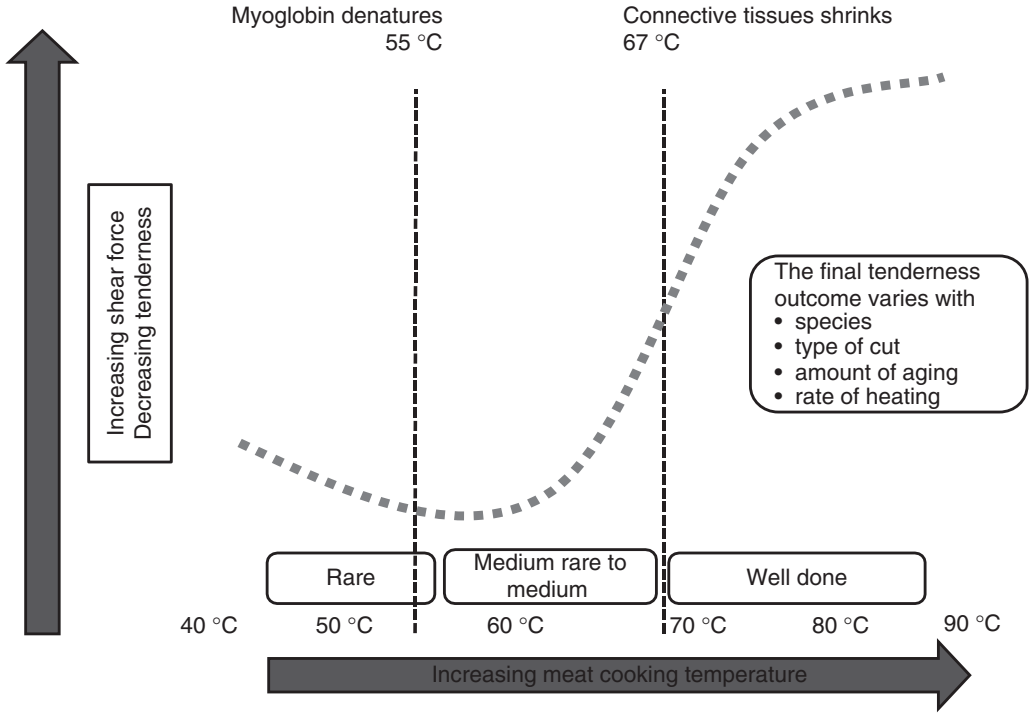

Figure 1 Aging tenderizes meat, but this tenderization is superimposed on other factors that also affect tenderness. This diagram considers the effects of cooking and broadly shows how the changes in shear value (an objective measure) or sensory panel score (a subjective measure) occur with increased heating temperatures (cooking). As the temperature rises, there is a slight increase in tenderness before toughness increases (dashed line). The final outcomes depend on species, type of cut, amount of aging, and rate of heating. Modified from Obuz, E., Dikeman, M.E., Grobbel, J.P., Stephens, J.W., Loughin, T.M., 2004. Beef Longissimus lumborum, and deep pectoralis Warner–Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. Meat Science 68, 243–248.

cooked. It is perhaps because this takes time that the term aging arose. However, the time required depends on the relatively low ambient temperature conditions that are required to ensure that spoilage is not an issue.

Mechanism of Aging

Aging involves the breakdown of the muscle structural proteins (e.g., C-protein, M-protein, and the cytoskeletal proteins titin, nebulin, desmin, dystrophin, and vinculin) by endogenous enzymes termed 'calpains.' Structural proteins are those holding the contractile proteins together. The major contractile proteins, actin and myosin, however, are minimally involved in aging.

Among the potential enzymes involved, the calpains seem to be the best candidates for tenderizing meat (although other enzymes may also be involved). However, although the optimum pH is around neutral, they retain sufficient activity at the low pH of rigor mortis to be effective. Another group of compounds termed 'cathepsins,' which are contained in lysosomes that eventually rupture following rigor mortis, might also be involved. Once released from the lysosomes, cathepsins are optimally active at more acidic pH levels (pH 5.4–5.6) and may be involved in long-term aging.

Calpains, by acting on the structural proteins, can be regarded as the initiators of muscle degradation and remodelling in living animals, which is a normal process. The calpains first act on structural proteins, followed by the ATP-dependent ubiquitin/ proteasome pathway in muscle. The bulk of extracellular and intracellular protein is then degraded through other pathways such as the lysosomal pathway.

Calpains exist in two forms, μ- and m-calpain, which differ in their calcium requirements. μ-Calpain requires 1–30 μmol of calcium and m-calpain requires 100–750 μmol of calcium for half-maximal activity (this latter level of calcium is hardly ever reached throughout the whole cell and suggests that local high concentrations achieved in some way might be just as important). These levels of calcium also do not exist in the sarcoplasm of living cells except during a contraction, and the calpain is presumably not activated in this situation (see the discussion of calpastatin below).

Calpains are also autolyzed (broken down by their own activity), reducing their activity at high prerigor temperatures, especially above 30 °C; this activity is also initiated by calcium, the agent that initiates calpain activity. The activity of calpain in the living muscle is inhibited by 'calpastatin.' It has been suggested that, in meat, high levels of calpastatin inhibit aging, but this appears to be simplistic, especially as calpastatin is always present in excess and is clearly compartmentalized away from calpains in some way.

There is some evidence that the amount of calpastatin determines the speed of tenderization through regulation of calpain activity, and the ratio of calpain to calpastatin could be considered as an indicator of the activity of calpain and possibly of tenderization. However, the relationships may be merely associative, especially when pH effects are taken into account. The inhibition of calpain by calpastatin is pH-dependent, so that the calpastatin activity, measured under optimal conditions (pH 7.5), always exceeds the activity of μ-calpain. Under pH conditions prevailing in postmortem muscle (pH < 5.8), the calpain activity is reduced, but the effective activity of calpastatin is probably reduced much more, which may explain why μ-calpain can be active in postmortem muscle with an ultimate pH (pH_u) of 5.5–5.8. Even so, this does not explain the rapid tenderization of meat of a high pH_u. There is also a time-scale difference between the decline of calpain activity and tenderization. For example, at storage temperatures of 0–2 °C, calpain levels are low by 2 days postmortem and a significant amount of tenderization still occurs after that point.

Although enzyme action is considered to be the main mechanism in meat aging, some factors, such as the increase in ionic strength to twice the postmortem values, might also contribute to aging or at least influence it. Calcium weakening of the myofibrillar structure has been reported to do the same thing, but it has not yet been established as a significant mechanism; however, it could influence destabilization of myofibrillar structure, thus facilitating aging. There does not seem to be any large effect on the connective-tissue proteins over time, so aging is predominantly a myofibrillar activity.

When Does Aging Start?

After rigor mortis, aging mechanisms become fully activated across all fibres, but some aging will have taken place earlier in those fibres that have already reached rigor (i.e., ATP production ceases). Normal calpain-inhibiting mechanisms are in place before rigor in the remaining muscle fibres. Depending on initial glycogen concentrations, individual muscle fibres enter rigor sequentially, so aging commences gradually and some muscle fibres are aging while their neighbors have not yet entered rigor. The interplay of factors such as the rapid pH fall following electrical stimulation and the high temperatures maintained after rigor can dramatically change the commencement, extent, and subsequent speed of aging (aging enzymes appear to be protected from degradation at rigor mortis, so the earlier it occurs the better).

Effects of Temperature on Aging

The major influence on the rate of aging is the temperature of the meat. Whatever is the temperature of the meat, it ages rapidly at the start and then more slowly over time at an exponentially falling rate. This is shown in the exponential decay equation (eqn [1]).

$$F_t = F_\infty + (F_0 - F_\infty)e^{-kt} \quad [1]$$

In eqn [1], F_∞ = final aging shear force, F_0 = initial shear force, F_t = shear force at a given time, and k is the temperature-dependent aging rate constant, which is different for each muscle type. This implies that there is an initial shear value F_0, from which meat starts to tenderize (Figure 2). It is even different from one animal to another and, in the case of cattle and sheep, for a constant temperature, the value of the aging constant k can have a fourfold range. For many reasons, F_0 varies widely, depending on muscle shortening, the amount of connective tissue, the species involved, and procedures

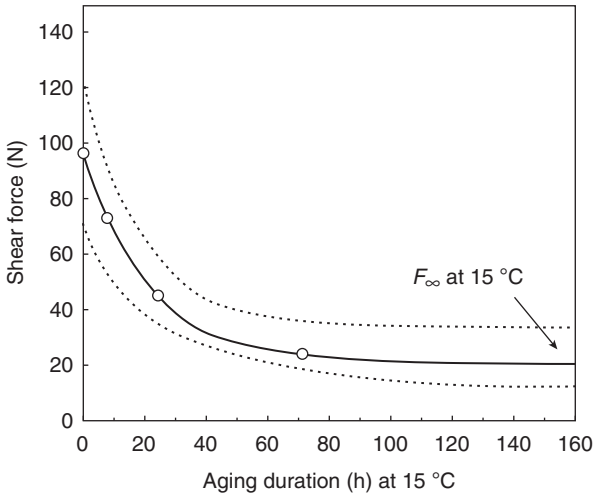

Figure 2 Curves for 64 electrically stimulated lamb m. longissimus thoracis et lumborum that entered full rigor mortis at 18 °C and aged at 15 °C (0 = mean values at each sampling time). The meat was cooked to 75 °C and sheared in a MIRINZ tenderometer. The mean aging curve (solid line) fits an exponential equation. The mean initial shear force value was 97 N, $F_\infty = 22$ N, aging constant k was 0.048. There is still a range of aging rates (also exponential) as shown by the two extremes (- - -) and as seen in **Figure 6** for stimulated and nonstimulated muscles. After 24 h, there is still a 25 N range in shear force. The aging is rapid in this figure because of the high temperature used; and in practice with temperatures of 0–4 °C the rate would be significantly slower (see **Figure 3**).

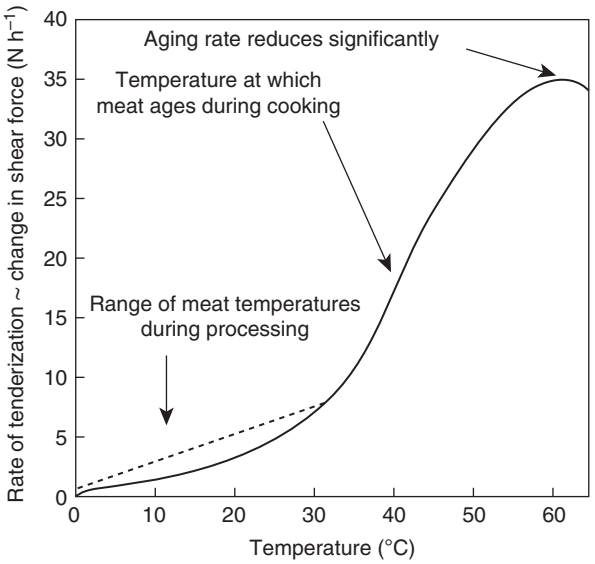

Figure 3 This diagram illustrates how the rate of tenderization of nonstimulated, unaged beef m. sternomandibularis proceeds with aging temperature (it will be different for other muscles, but the trends will be similar). The muscle went into rigor mortis at 15 °C, so that inhibition of aging due to high rigor mortis temperatures did not occur and then the meat was held at the required temperature. Aging rate increases with holding temperature. At 0 °C, the rate of tenderization is relatively slow and long holding periods are required. During carcass cooling, when temperatures initially higher than those finally attained during storage are encountered, or at even higher temperatures during cooking, the degree of aging has an important part to play in the final tenderness. However, at temperatures above approximately 65 °C, aging rate reduces significantly. Modified with permission from Davey, C.L., Gilbert, K.V., 1976. The temperature coefficient of beef aging. Journal of the Sciences of Food and Agriculture 27, 244–250, © John Wiley and Sons Ltd.

utilized, such as electrical stimulation. The final shear force F_∞ is influenced by, among other things, the rigor mortis temperature and pH_u, which are not addressed by the parameters of the equation.

In a typical meat-processing plant, the muscle temperature falls, depending on the chilling regimen, and the rate of aging is gradually reducing throughout processing until a stable storage temperature is reached. Aging continues and, given enough time, finally reaches the value for F_∞. The actual expected tenderness achieved can be modelled using eqn [1] in conjunction with the fall in temperature and taking into account the other prerigor conditions that modify glycolytic rate, including electrical stimulation.

As aging is temperature-dependent and carcasses are in a cooling environment, processes that produce an early rigor mortis while the carcasses are still relatively warm, such as electrical stimulation, ensure early rapid aging and also avoid the effects of cold shortening. However, there are other temperature effects that modify the rate and extent of aging.

At high constant prerigor mortis temperatures of approximately 35 °C, aging is inhibited in nonstimulated meat compared with rigor mortis at 15 °C. This situation does not apply following sufficient electrical stimulation to achieve a significant pH fall (e.g., to pH 6.3 approximately). The inhibiting mechanisms appear to involve inactivation of calpain known to be caused by holding meat at prolonged low pH and high temperatures preceding rigor mortis (see Biochemical factors affecting aging below): however, following rigor mortis they are unaffected.

The aging rate of meat (assuming aging potential has not been reduced by elevated prerigor temperature conditions) increases dramatically as the temperature rises and, for a 10 °C temperature rise, the rate increases 2.4 times. As expected, additional aging takes place during cooking (if F_∞ has not been reached), but ceases dramatically at a temperature of just above 65 °C (**Figure 3**).

In general, meat ages as it moves along the distribution system from harvest to consumer, but the optimal tenderness is not achieved unless aging is long enough in duration or takes place at elevated temperatures. High temperatures facilitate increased bacterial growth as well as resulting in faster meat aging: however, the deleterious effects of bacteria dominate, so low aging temperatures from −1.5 to 5 °C are used together with packaging that reduces the growth rate of pathogenic and spoilage bacteria. In practical terms, surface drying prevents microbial growth on the meat surfaces of whole carcasses, but it is not always practical. Vacuum packaging or modified-atmosphere packaging of cuts can ensure that bacterial growth is low under these circumstances. In certain species, such as deer and other game animals, extreme aging is often regarded as desirable because of the development of flavors that are not related to spoilage.

Variability in Tenderness and Aging

The initial tenderness of meat before significant aging that has taken place varies from one muscle to another, among individuals of the same species, and for different species. The tenderness of unaged meat and the extent of aging that take place on completion also depend on factors such as muscle shortening, which is usually hard to determine routinely. Highly shortened meat does not age and tenderize effectively, so that, in the worst case scenario of severe cold shortening, the meat is tough and appears not to be aged at all. Although tenderization has apparently been reduced depending on degree of shortening, many of the chemical changes associated with aging via the calpain system have still taken place (i.e., proteolysis) but, when cooked, the shortened muscle is tough. Even in cold-shortened meat, some muscle fibres will have entered rigor early and will be protected from cold shortening, so there will still be some small changes in tenderness over time. There are other factors adding to variability such as temperature variation across muscles during processing. As meat ages, tenderness variability across a single muscle reduces.

The sarcomere length of some commercially important muscles increases significantly when the carcass is hung from the pelvis rather than from the Achilles tendon. With pelvic suspension, there are dramatic early changes in tenderness with the increased sarcomere lengths, but the differences often even out over long aging durations, possibly due to connective tissue limitations. Such meat is consumer-ready much earlier than that from normally hung carcasses.

The extreme situation in the other direction is that of hot or warm boning, where prerigor meat is removed from the carcass skeletal attachments, so rigor shortening can occur if the temperature falls below 10 °C prerigor. Rigor shortening is least in the range 10–20 °C; therefore, if temperatures in this range are achieved prerigor mortis, then hot boned meat ages in a similar way to meat left on the carcass under skeletal restraint. Electrical stimulation lowers pH rapidly and significantly changes rigor rate with early commencement of aging, so any negative effects are minimized.

Physical Factors Influencing Aging

Tenderness depends not only on the extent of aging, but also on other compositional (connective tissue) and physical (shortening) factors such as pelvic suspension mentioned above and when muscles are restrained by wrapping. Thus, it is evident that the tenderness of meat or the extent of aging cannot be assessed visually. However, as meat ages, microscopic differences can be observed, such as the disintegration of Z-lines (normally in register in unaged meat) and the appearance of gaps at the A–I junction and between fibres; these changes are consistent with degradation of the structural or cytoskeletal proteins, such as titin, nebulin, desmin, dystrophin, and vinculin (Figure 4).

As mentioned above, proteolysis clearly occurs in both cold-shortened and unshortened meat, but interactions of the muscle proteins of shortened sarcomeres during meat cooking ensures the meat is tough. Whereas pelvic suspension ensures that tenderness commences early in the stretched muscles, the opposing muscles are slightly tougher, but the differences tend to even out over long aging durations.

The connective-tissue content of muscle also affects its tenderness. Connective tissue varies across muscles in a carcass and is hydrolyzed in certain cooking procedures, such as roasting and stewing. Thus, the connective tissue of various muscles of a carcass (type of cut) affects the end use of the various muscles or muscle groups. It appears that the changes in connective tissue during aging are small, and certainly not large enough to shift a given cut from a low-value to a high-value cut. However, connective-tissue changes may be significant for long aging durations.

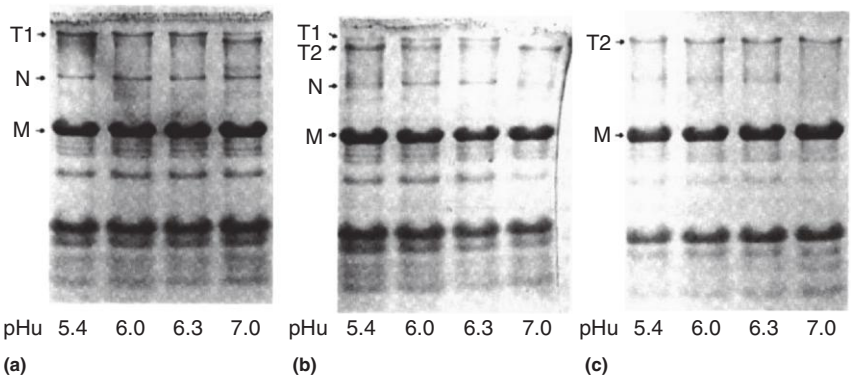

Figure 4 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) of muscle sample of sheep m. longissimus thoracis et lumborum at different ultimate pH (pH_u), obtained after storage at 10 °C for 12 h (a), 24 h (b), and 48 h (c) after slaughter, shows the disappearance of the structural or cytoskeletal proteins titin (T) and nebulin (N) rather than the contractile proteins (Actin not indicated). Myosin (M) is unchanged. The titin band splits at high pH_u values, indicating more rapid aging. At 24 h, titin 2 has disappeared at pH 7 and the remaining titin bands are split. At 48 h, no titin 1 remains. The nebulin band takes longer to disappear at pH_u values of 6.0 and 6.3. The nebulin band disappears completely in (c). Reproduced from Watanabe, A., Devine, C.E., 1996. The effect of meat ultimate pH on rate of titin and nebulin degradation. Meat Science 43, 407–413.

Biochemical Factors Influencing Aging

There is some inhibition of aging following high prerigor mortis temperatures, and this appears to be greatest at 35 °C and least at 15 °C; it appears to be inactivation of the aging enzymes. This is shown in Figure 5, where it is also suggested that calpain levels change little in the early stages of pH fall during progression into rigor mortis and that it is only in the later stages of rigor mortis that a combination of elevated temperatures and low pH, acting in concert, reduce calpain activity. With electrical stimulation, these effects appear to be negligible commercially, although experimentally the greatest tenderness of meat is still achieved with careful temperature control when rigor mortis occurs close to 15 °C.

The degradation of the cytoskeletal proteins by calpain has consequences other than tenderization. As the meat ages, free water (e.g., released during centrifugation in experimental situations) increases exponentially from 6% to 16% throughout the aging period, whereas bound water, dry matter, and shear force decrease exponentially. Such observations were shown to be independent of prerigor holding temperatures from 4 to 35 °C. The water released over time by centrifugation studies can be correlated with studies where normal drip loss or purge increases over time as beef ages in a vacuum package and where drip loss increases exponentially in case-ready pork over a 7-day storage period at 6 °C. There are various water compartments in meat and such water loss from cytoskeletal proteins during aging should not be confused with the water produced from myosin denaturation in PSE pork that is related to prerigor temperatures and can mask that arising from aging. The degradation of the cytoskeletal proteins (and indeed denatured myosin) is also associated with a decrease in water binding. It has been suggested that the changes in water binding to the cytoskeletal proteins are responsible for the change in near-infrared (NIR) spectra related to tenderness. NIR is being developed as a nondestructive method to measure meat tenderness and other meat quality attributes online. A corollary is that as meat ages (tenderizes) there is an increase in drip. When aging is rapid, such as after electrical stimulation and the higher temperatures, drip increases more rapidly but there is no more drip for the equivalent tenderness achieved.

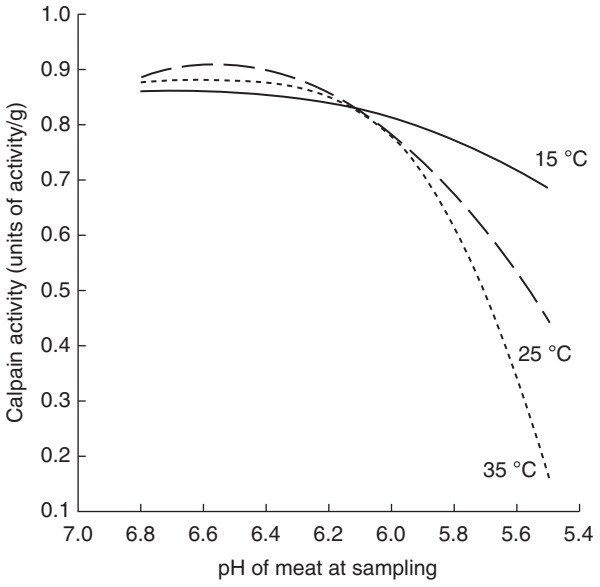

Figure 5 The changes in activity of calpain (the enzyme that appears to be responsible for meat aging) are related to the temperature of the meat while it is entering rigor mortis. In meat from unstimulated cattle held in different temperature conditions postslaughter, the μ-calpain postmortem activity begins to fall with the high-temperature and low-pH conditions of the meat before rigor mortis, the optimum temperature being 15 °C. This figure shows that, below pH 6.2 prerigor, high processing temperature conditions can reduce the enzymes available for aging, presumably owing to increased autolysis of the calpains, and hence will limit the rate of aging and final tenderness achieved. Modified from Simmons, N.J., Singh, K., Dobbie, P., Devine, C.E., 1996. The effect of prerigor holding temperature on calpain and calpastatin activity and meat tenderness. Proceedings of the 42nd International Congress of Meat Science and Technology, pp. 414–415. Lillehammer: International Congress of Meat Science and Technology (ICoMST).

Other Factors Influencing Aging

The rate of aging depends on the temperature postrigor; the extent of aging depends on preslaughter conditions (pH_u) and the immediate post rigor conditions. These all interact to affect meat tenderness at any given sampling time. It is clear that meat from different species have different degrees of tenderness and age in different ways. There are also various perceptions that stress, breed, sex, growth paths, different types of feeding, and the amount of marbling also affect tenderness and aging. There is no doubt that, with increasing amounts of fat (higher marbling score), there is less meat per bite, the meat has a lower shear force, and the consumer appreciation is greater because the meat slides down the throat more easily, but in the normal range of intramuscular fat there is likely to be little difference. Aging rate does not change merely because of fat.

When animals have been subjected to the same treatment, the measured initial tenderness, rate of aging, and final tenderness are hardly ever the same (see range in Figures 6 and 7). Although the sex of the animal does not directly affect aging rate, other characteristics, such as elevated pH_u (Figure 6), which might occur in bulls more often than in steers, will affect aging rate.

The aging constant is not the same for all animals in any experiment, suggesting that there are still other factors influencing aging rate. It has also been suggested that animals with higher protein turnover, i.e., faster-growing animals, are more tender and age to a higher degree of tenderness. It has been difficult to establish the veracity of these suggestions because many other factors such as animals being of different weights at the same age, may contribute by modifying chilling rates. Other factors could relate to preharvest effects, including level of nutrition and low levels of stress that are not enough to measurably influence pH_u.

Inclusion of β-agonists in a diet increases shear force, and certain genetic muscle developments, such as the callipyge condition in sheep, also distinctly reduce the rate and

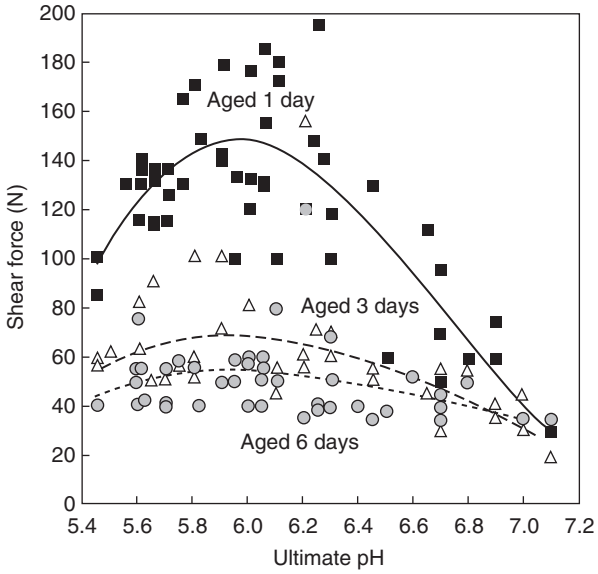

Figure 6 There are changes in rate of tenderization of meat resulting from preslaughter stress and this figure shows how wide the range of shear force values can be. Preslaughter stress results in glycogen depletion that raises ultimate pH (pH_u). The shear force values (from nonstimulated sheep m. longissimus thoracis et lumborum) are related to the pH_u values. After 1 day of aging, both high and low pH_u values result in tender meat, but meat with a moderate pH_u is tougher. However, after 6 days of aging at 10 °C, all meat eventually becomes tender. The higher shear force of the intermediate pH_u meat is a result of slower aging rather than other factors. In the pH range 5.5–5.9, a small increase in pH results in large increases in toughness. Modified from Watanabe, A., Daly, C.C., Devine, C.E., 1995. The effects of ultimate pH of meat on the tenderness changes during aging. Meat Science 42, 67–78.

extent of aging. However, a situation with excessive muscle development, termed 'double muscling' in cattle, seems to have the opposite effect in the Piedmontese breed and the meat is tender. *Bos indicus* cattle have higher levels of calpastatin than most *Bos Taurus* breeds, which results in less tender meat (but this effect is reduced with electricalstimulation). Addition of vitamin D to the diet, which raises the calcium levels that modify calpain activity, is also reported to accelerate aging, but there is no difference in tenderness at 14–21 days.

Hot Boning

Rapid chilling during prerigor-boning processing exacerbates shortening, and cold shortening in particular needs to be prevented to avoid toughness. This is not an issue if the meat is to be used for manufacturing. Without electrical stimulation, chilling cannot be too rapid and prerigor temperatures need to be close to 15 °C to minimize rigor shortening and maximize tenderness. Wrapping the meat tightly, or surrounding the meat in an elastic packaging material to limit expansion during contraction, also prevents shortening. Electrical stimulation ensures rapid rigor entry and the toughness due to enzyme inhibition at elevated temperatures is minimal.

Electrical Stimulation

If electrically stimulated and nonstimulated muscles enter rigor mortis at the same temperature and are aged at the same temperature, taking into account the fact that aging commences at rigor mortis (predictably reached at different times), the stimulated meat ages at a higher rate and is more tender (Figure 7). In commercial procedures, where stimulated muscles enter rigor mortis rapidly and immediately commence to age, the increases in tenderness are even more dramatic. It had been hypothesized that following electrical stimulation, when rigor mortis takes place at relatively high temperatures, there would be increased drip and a reduction of tenderization. Such high temperatures where they occur appear to be so transitory that they are of little concern and, in addition, once the meat is in rigor mortis, enzymes responsible for aging appear to be protected. Not only is stimulated meat more tender than nonstimulated meat, but several studies have shown that tropical breeds that are normally less tender than British and continental breeds also respond favorably to stimulation.

Following electrical stimulation, changes in the ultrastructure of the muscle shown in electron micrographs suggest that contracture nodes are formed, accompanied by fibre disruption that may facilitate the degradation of the myofibrillar structure during aging. This may be more important in red muscle fibres, where the disruption is greatest.

Different Species and Breeds

The mechanism of aging is likely to be the same for all species, but there are differences because of different glycolytic rates preceding aging and the differences in the rate of aging itself, even at the same temperature. The rate of aging in various meats decreases in the order

poultry→pork→lamb→beef

In the case of pork, there are possible differences in aging as a consequence of prerigor myosin denaturation.

In the case of poultry, the aging-rate constant (k) is approximately 0.2 at 4 °C and such a value is not reached even at 35 °C in the case of sheep and cattle. In poultry, cold shortening is not an issue, occurring around 2 °C, so poultry can be cooled in iced-water slushes but are not exposed long enough to fall below 2 °C. Even at these temperatures, optimum tenderness through aging occurs in 6–8 h (for chickens) and 12–24 h for turkeys and they can be frozen at this time. Rigor mortis at temperatures above 20 °C is even more detrimental to tenderness in poultry than in the case of other meats.

Factors That Reduce Aging

The aging of meat can be prevented by injection of zinc salts and certain enzymes that inhibit calpains. The final shear-force values obtained in such cases represent those of unaged meat.

Meat of an intermediate pH_u (pH 5.8–6.1) has been shown to be tougher than meat with a higher or lower pH_u (Preslaughter handling) so the relationship between shear force

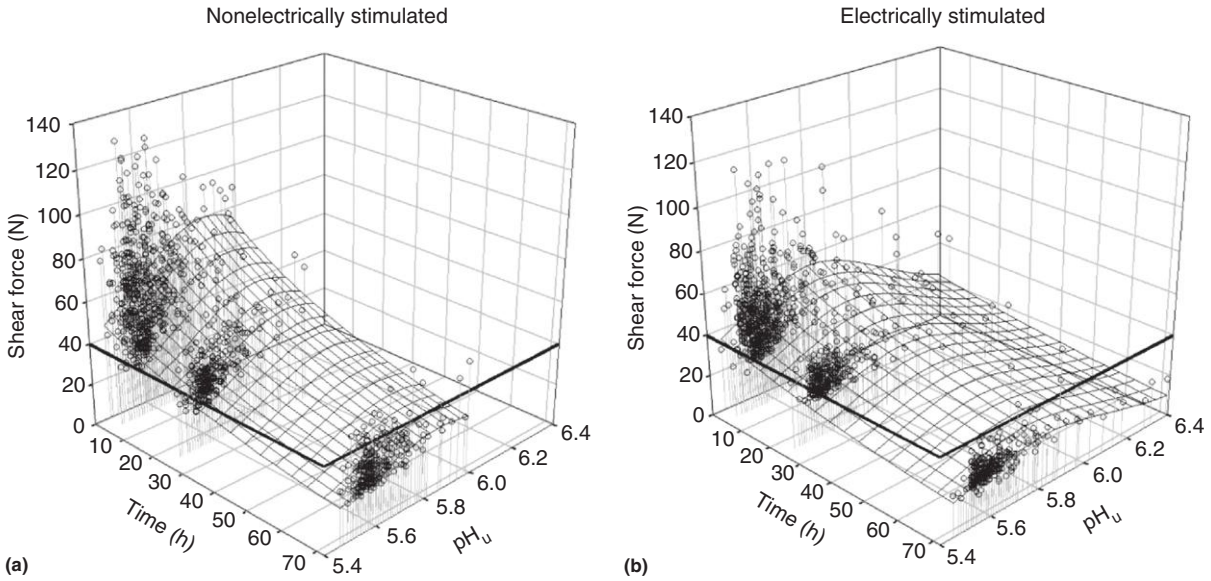

Figure 7 Three-dimensional plots showing the interrelationship between hours of aging (0, 4, 24, and 72) versus shear force and pH_u, for the longissimus et thoracis muscle of nonelectrically stimulated lambs (a) and electrically stimulated lambs (b) held at 15 °C for 72 h duration post rigor (at lower temperatures aging takes longer and equivalent values are reached at 4 °C for 8 days or up to 6 weeks at −1 °C). The individual shear values and drop lines onto ultimate pH are superimposed on the 3D mesh plot mean values. The mean shear force in the electrically stimulated group (b) is significantly lower and is less variable than the nonstimulated lambs (a) ($P<0.05$). A line is drawn at shear values of 40 N (moderate acceptability); superimposed and acceptable values are reached in 25–30 h for nonstimulated low pH_u muscles and within 10 h for stimulated muscles. High pH_u muscles tenderized very rapidly. At 72 h, all muscles became highly tender with the exception of those with intermediate pH_u values. The tenderness measurements (obtained with an Australian Warner–Bratzler device) commenced at rigor mortis – this was reached in 12 h for nonstimulated muscle and in 5 h for stimulated muscles. Modified from Devine, C.E., Lowe, T.E., Wells, R.W., *et al.*, 2006. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. Meat Science 73, 304–312.

and pH_u is best described by an inverted U-shaped curve (Figure 6). The rate of aging of intermediate-pH_u meat is slower than that of meat of a high or low pH_u (approximately 75% slower) and is a major contributor to toughness so that most meat never tenderizes significantly with prolonged aging. Although high-pH_u meat is more tender than low-pH_u meat, other properties of high-pH_u meat, such as a dark color and off-flavor, are not desirable. The relationship between pH_u and shear force appears to be similar for all species.

Injection of Calcium and Other Agents

Calcium-activated tenderization appears to be a useful method to accelerate aging. Calpains require calcium for activity, but optimum calcium levels may not be available in postrigor muscle to activate them fully. Calcium (5% w/v) can be injected into meat postrigor to activate calpains and thus to induce a more rapid and extensive tenderization. Following injection, cuts are vacuum-packaged and stored before consumption. The process is not so effective in prerigor meat, but can be used up to 14 days postmortem; however, if injected too early prerigor, calcium causes shortening that toughens meat. Calcium injection does not affect meat that is already tender and so does not make tender meat 'mushy.' It is reported to have little effect on other meat-quality traits and is effective on all breeds, cuts, or classes.

Postrigor injection of other salts, such as phosphate, increases aging rate and can overcome some effects of shortening. Marinating meat with acids does not dramatically improve tenderness unless the pH is below 4.5, although the flavors added can enhance consumer appreciation. There is also difficulty of penetration and the marinade may have to be injected.

Making the meat alkaline (e.g., by sprinkling or soaking with small amounts of sodium bicarbonate) does genuinely increase tenderness, possibly by a similar mechanism to that occurring in high-pH meat, but there have been reported minor texture changes and small flavor changes, although these can be masked with spices.

Subjection of meat to a steady, high-hydrostatic pressure tenderizes it and a recently developed procedure uses an explosive charge to generate a hydrodynamic shockwave, which is transmitted through water to the packaged meat so that it tenderizes rapidly.

Freezing and Thawing

When meat is frozen it does not age, although it is possible for some aging to occur in the latent heat phase during freezing. When meat thaws, it again begins to age, at a much faster rate, but there have been limited studies to quantify this effect. The process of freezing and thawing of meat also results in drip, much of which is eventually taken up again by the meat (if it remains in contact with the drip).

Measurement of Aging

Meat tenderness is the way consumers see one aspect of the property of meat: cooked meat becoming more tender and acceptable (higher scores) as the meat ages. To replicate consumers, a common procedure is to train panellists to evaluate meat attributes. Meat is cooked to a standard end-point temperature and maintained at this temperature when served to the panellists under standard lighting conditions. Measuring appreciation of tenderness through the use of a consumer panel provides more information than just tenderness and is influenced by the type of cut, amount of fat, and connective tissue, juiciness, cooking procedure, and cooking temperature effects, such as the well-known differences that exist between a well-cooked and a rare steak. Some evaluations use untrained people but, in the absence of training, there is a very wide range of scores for the various attributes. A more objective measure is obtained by shearing meat following standardized cooking procedures, for example, 70 (American Meat Science Association) to 75 °C (Meat Industry Research Institute of New Zealand (MIRINZ)) in a water bath or an electric belt grill system – the lower the shear value, the more tender the meat. There are several types of 'tenderometers' in use, for example, the Warner–Bratzler tenderometer and MIRINZ tenderometer. The absolute shear values depend on the type of tenderness-measuring device used (e.g., Warner–Bratzler values are 0.6 times MIRINZ values), but the rate of change over time, i.e., the aging rate, will be similar for all devices.

Ideally, one would like to measure the tenderness of raw meat. Cytoskeletal proteins degrade over time and alter a meat's integrity, so the consequences of protein degradation in raw meat can be determined by using the myofibrillar fragmentation index. This involves viewing myofibrillar length changes under a microscope or determining turbidity changes. The degradation of the cytoskeletal proteins is also associated with a decrease in their water binding and the increased water release changes the NIR spectra, and this can be correlated with tenderness. NIR has been developed as a nondestructive method to measure meat tenderness and can also measure other meat quality attributes on line. A corollary is that as meat ages (tenderizes) there is an increase in drip. When aging is rapid, such as after electrical stimulation and high temperatures that occur, drip increases and meat tenderizes more rapidly as expected but there is no more drip for the equivalent tenderness achieved.

Pulling all of the discussion points above, the shear force of meat decreases exponentially over time and is significantly modified by pH_u and electrical stimulation as shown in Figure 7. In this figure, lamb meat was held at a constant 15 °C (this ensures more rapid aging than at lower temperatures). The mean shear force in the electrically stimulated group is significantly lower at all aging points than the nonstimulated lambs ($P < 0.05$). The line drawn corresponding to shear values of 40 N (moderate acceptability) shows that acceptable values are reached in 25–30 h for nonstimulated, low pH_u muscles and within 10 h for stimulated muscles. Because of the considerable variability, long aging is needed for all meat to become acceptable – consumers' appreciation of tenderness is remembered by the tough outliers.

Packaging

The type of packaging does not affect the rate of aging. However, the extended time for which meat can be held at low temperatures in specialized packaging systems, such as vacuum packaging and controlled-atmosphere packaging, allows the meat to age for a long time and become tender, and at the same time reduces the development of spoilage and pathogenic bacteria. Dry aging is that traditionally applied to whole carcasses in a chiller where the dry surfaces prevent bacterial growth and the sterile interior of the muscles is maintained and the adjacent bone may have an effect on flavor – the carcass is later broken down for sale. Wet aging occurs in meat cuts placed in a vacuum package and the bone is usually not present – the meat can then tenderize en route to distribution and sale. There are reported to be differences in flavor between dry and wet aging.

Although freshness is the aim for most food products, it is clear that 'fresh' meat may not be optimal in tenderness. To some extent, meat can be customer-ready earlier by using electrical stimulation or a process such as tenderstretch (in which the carcass is suspended by the pelvis, thus stretching some muscle groups) where the initial tenderness, before aging, is high. In many distribution systems, especially the extreme of shipping vacuum-packaged or controlled-atmosphere packaged meat chilled to −1. 5 °C to distant markets, the meat ages in transit. The absence of a reliable way of determining meat tenderness in a nondestructive way means one must adhere to a belief in the controlled operation of meat distribution to obtain tender meat.

See also: Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Palatability; Water-Holding Capacity. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening; Slaughter-Line Operation and Pig Meat Quality. Cooking of Meat: Cooking of Meat. Cutting and Boning: Hot Boning of Meat. Electrical Stimulation. Muscle Fiber Types and Meat Quality. Packaging: Modified and Controlled Atmosphere; Vacuum. Prediction of Meat Attributes From Intact Muscle Using Near-Infrared Spectroscopy. Sensory and Meat Quality, Optimization of. Species of Meat Animals: Pigs. Tenderizing Mechanisms: Chemical. Enzymatic; Mechanical. Tenderness Measurement

Further Reading

Bailey, A., 1988 Connective tissue and meat quality. Proceedings of the 34th International Congress of Meat Science and Technology, pp. 152–160. Brisbane: International Congress of Meat Science and Technology (ICoMST).

Bendall, J.R., 1973. Post mortem changes in muscle. In: Bourne, G.H. (Ed.) The Structure and Function of Muscle, vol. 2. New York: Academic Press, pp. 243–309.

Davey, C.L., Gilbert, K.V., 1974. Temperature-dependent cooking toughness in beef. Journal of the Science of Food and Agriculture 25, 931–938.

Davey, C.L., Gilbert, K.V., 1976. The temperature coefficient of beef aging. Journal of the Sciences of Food and Agriculture 27, 244–250.

Devine, C.E., Lowe, T.E., Wells, R.W., *et al.*, 2006. Pre-slaughter stress arising from on-farm handling and its interactions with electrical stimulation on tenderness of lambs. Meat Science 73, 304–312.

Devine, C., Wells, R., Lowe, T., Waller, J., 2014. Pre-rigor temperature and the relationship between lamb tenderisation, free water production, bound water and dry matter. Meat Science 96, 321–326.
Dransfield, E., Wakefield, D.K., Parkman, I.D., 1992. Modelling post-mortem meat tenderisation – I. Texture of electrically stimulated and non stimulated beef. Meat Science 31, 57–73.
Gursansky, B., O'Halloran, J.M., Egan, A., Devine, C.E., 2010. Tenderness enhancement of *Bos indicus* and *Bos taurus* cattle through electrical stimulation. Meat Science 86, 635–641.
Judge, M.D., Aberle, E.D., Forrest, J.C., Hedrick, H.B., Merkel, R.A., 1989 1991. Principles of Meat Science. Dubuque: Kendal/Hunt Lawrie, RA, 1989 1991. Meat Science. Oxford: Pergamon Press.
Obuz, E., Dikeman, M.E., Grobbel, J.P., Stephens, J.W., Loughin, T.M., 2004. Beef Longissimus lumborum, and deep pectoralis Warner-Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. Meat Science 68, 243–248.
Ouali, A., 1990. Meat tenderization: Possible causes and mechanisms. A review. Journal of Muscle Foods 1, 129–165.
Rosenvold, K., Micklander, E., Hansen, P.W., *et al*., 2009. Temporal, biochemical and structural factors that influence meat quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.
Rosenvold, K., North, M., Devine, C.E., *et al*., 2007. The protective effect of electrical stimulation and wrapping on beef tenderness at high pre-rigor temperatures. Meat Science 79, 299–306.
Simmons, N.J., Singh, K., Dobbie, P., Devine, C.E., 1996. The effect of pre-rigor holding temperature on calpain and calpastatin activity and meat tenderness. Proceedings of the 42nd International Congress of Meat Science and Technology, pp. 414–415. Lillehammer: International Congress of Meat Science and Technology (ICoMST).
Watanabe, A., Daly, C.C., Devine, C.E., 1995. The effects of ultimate pH of meat on the tenderness changes during ageing. Meat Science 42, 67–78.
Watanabe, A., Devine, C.E., 1996. The effect of meat ultimate pH on rate of titin and nebulin degradation. Meat Science 42, 407–413.

Relevant Websites

http://www.themainmeal.com.au/index.htm
Cooking (including mobile phone application).
http://en.wikipedia.org/wiki/Temperature_(meat)
Cooking Temperature.

Color and Texture Deviations

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Glossary

Color deviation Any change of color, such as browning or lightening.

Dark firm and dry Refers to the color and the texture of the meat.

HAL Halothane gene, the halothane gene increases the amount of lean in a carcass but equally increases the risk of development of PSE meat.

Pale, soft, and exudative (PSE) Refers to the color and the texture of the meat.

***PRKAG3* gene** Encodes a muscle-specific isoform of the regulatory γ subunit of adenosine monophosphate-activated protein kinase.

Introduction

Deviations from the 'normal' status resulting in an abnormal course of the conversion of muscle to meat are frequently observed, but discussion tends to focus solely on extremes. Deviation cannot be addressed without first defining normality, yet strikingly, there are no universally accepted criteria for defining 'normal' meat. Indeed, the expectations of meat consumers are roughly the same in most regions of the world – beef and mutton are expected to be red, pork more or less pink, veal very pale, and so on – and excessive drip is universally considered as undesirable. However, the thresholds for acceptability vary greatly from one region to another. Thus, it must be kept in mind that the concept of normality in meat quality is entirely relative and wholly dependent on the user's needs and habits.

Color and texture are influenced by numerous factors, both intrinsic and extrinsic to animals. Intrinsic factors include muscle features and stress reactivity; extrinsic factors include rearing conditions, preslaughter treatment, slaughter techniques, and carcass processing conditions. This article sets out to bring together the factors that produce variability.

Effects of Rigor Mortis and Aging Conditions on Texture and Color

During rigor mortis set-in, the main changes in muscle tissue influencing subsequent meat quality are stiffening and acidification due to glycogenolysis. In brief, the kinetics of acidification strongly affects water-holding capacity and color. This is well exemplified in pig muscle, which exhibits tremendous variability in both rate and extent of acidification (Figure 1).

In pigs harvested in good conditions, the white muscles reach a stable pH value (referred to as ultimate pH or pH_u) in the range 5.5–5.7 after a few hours. This leads to a decrease in water-holding capacity and color intensity, as the myofibrils shrink when pH descends to values close to the isoelectric point of most myofibrillar proteins, i.e., approximately 5. Myofibril shrinkage squeezes fluid out from the muscle cells, causing drip loss, and enhances the light-scattering power of the meat, contributing to lighter color intensity. If pH_u is increased because of a lack of muscle glycogen at slaughter, meat appears darker and drier. At normal pH_u, any increase in acidification rate is detrimental to both water-holding capacity and color intensity, as it promotes the denaturation of muscle proteins, especially myosin. Protein denaturation occurs when pH reaches low values while muscle temperature is still high. Myosin denaturation considerably increases myofibril shrinkage and also causes light scattering, thereby increasing drip loss

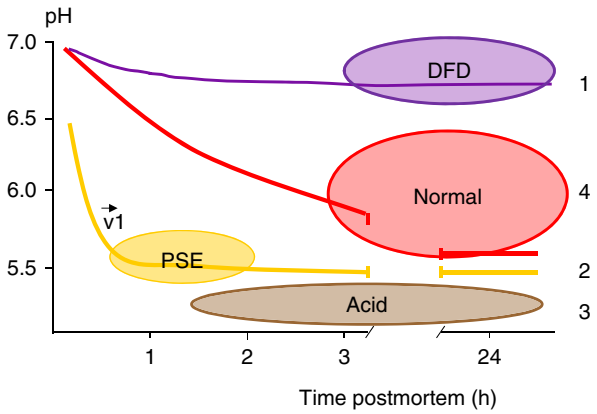

Figure 1 A schematic representation of the relationship between postmortem change of muscle pH and meat quality in pigs. When pH stays at a high value (1), irrespective of the rate of pH drop, meat is dark colored, dry, and firm to the touch, as the pH is far from the isoelectric point of myofibrillar proteins (pH = 5) and is termed dark, firm, and dry (DFD) meat. This condition is also found for bovine muscle. When the rate of pH drop is excessively fast (2) with a normal (i.e., in the range 5.5–5.7) or low ultimate pH (pH_u), extensive denaturation occurs, leading to what is termed pale, soft, and exudative (PSE) meat. When the rate of pH drop is normal but pH_u is lower than normal (3), the meat is called 'acid' or 'Hampshire-type' meat. Rate of pH drop has been considered according to French criteria as excessively fast when pH_{60} is <6, pH_u too high when >6.3, and pH too low when <5.5. Other countries may use different thresholds depending on local meat consumption patterns. Meat that is not outside of the thresholds (4) is considered 'normal.' Clearly, variation in the quality of 'normal' meat is still very high. Modified from Monin, G., 1988. Evolution post mortem du tissu musculaire et conséquences sur les qualités de la viande de porc. Journées de la recherche porcine 20, 201–214.

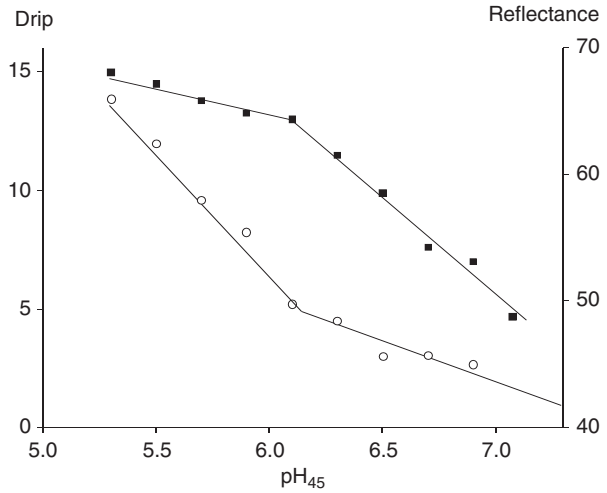

Figure 2 Relationships of drip loss (■) and reflectance (○) to pH_{45} in pig longissimus muscle. Points are the means of grouped data; the regression lines were drawn from calculations from the ungrouped data. Water-holding capacity decreases strongly at pH_{45} values above 6.1, with little effect on reflectance; the contrary is observed at pH_{45} values below 6.1. Thus, in meat with low or normal pH_u, high acidification rates give rise to PSE meat, whereas intermediate rates result in wetness but not paleness. Modified from Warriss, P.D., Brown, S.N., 1987. The relationship between initial pH, reflectance and exudation in pig muscle. Meat Science 20, 65–74.

and reducing color intensity. Moreover, myoglobin denaturation reduces the coloring strength of this pigment, which often comes with myoglobin oxidation. In extreme cases, the meat can look greyish or yellowish. The acidification rate is assessed via pH value at a given postmortem time, generally 45 or 60 min (pH values at these times are referred to as pH_{45} or pH_{60}, respectively). Water-holding capacity decreases strongly with pH_{45} values above 6.1, with little effect on reflectance; the contrary is observed at pH_{45} values below 6.1 (Figure 2). Thus, in meat with low or normal pH_u, high acidification rates give rise to paleness, softness, and exudation (PSE), whereas intermediate rates result in wetness but not paleness. Such defects are not found in meat with higher than normal pH_u, irrespective of the acidification rate, as pH does not reach the low values needed for significant protein denaturation.

The main pork quality deviations related to abnormal pH drop kinetics are schematized in Figure 1. Here, rate of acidification is considered excessive when pH_{60} is lower than 6. This high rate gives 'PSE' meat but only if pH_u is lower than 5.6. If pH_u is higher than a conventional set limit (which varies according to country between pH 5.8 and 6.3), meat is qualified as abnormal 'dark, firm, and dry (DFD)' meat. If pH_u is lower than 5.5, the meat is referred to as acid meat or 'Hampshire-type meat,' as this defect is frequent in Hampshire breed pigs.

The normal pattern of acidification varies considerably among species. The rate of postmortem pH drop in the main meat species follows the order poultry > pork > lamb > beef. Pigs show the strongest variation in acidification rates (0.2–0.5 pH units per hour). Extent of acidification is lower in poultry than in the other species, because poultry meat's pH_u is normally $\geq$5.7 against 5.4–5.7 in most muscles of the other species.

The main change occurring in meat after rigor mortis set-in is proteolysis, which results in tenderization. The among-species variation in rate of aging follows the same order as for acidification. In regard to color, discoloration occurs with time, as the red myoglobin is progressively oxidized to brown metmyoglobin. This discoloration is associated with a decline in quality.

Pale, Soft, and Exudative Meat

PSE pig carcasses have been described in Europe since as early as the nineteenth century. PSE meat is also frequently found in poultry and sometimes in beef, particularly young bulls. PSE is actually a somewhat confusing term, as it covers different kinds of defects whose only common trait is that they all result in paleness and drip. It is very important to clearly distinguish between the various kinds of PSE, because they differ on important traits, such as tenderness or flavor on top of the most evident deficiencies common to all of them. Moreover, the different PSEs result from different physiological or biochemical mechanisms and thus require different remedies.

Pork

Halothane Sensitivity

Bromochlorotrifluoroethane, or halothane, is a gaseous anesthetic widely used for human and animal surgery. Some pigs develop a malignant hyperthermia syndrome (MHS) when forced to breathe halothane or other halogenated anesthetics. The most evident symptoms of MHS are muscle rigidity, hyperthermia, metabolic acidosis, and arrhythmia. These are the expressions of an exacerbation of ATPase activity in the whole skeletal musculature. Generally, the animal dies after a few minutes. The MHS crisis can also occur during exposure to any of the numerous stresses occurring during an animal's life, such as mixing strange animals, aggressive interactions for establishment of social hierarchy, sustained muscle exercise, transportation, and slaughter.

Muscle ATPase activation is due to an explosive increase in sarcoplasmic free calcium. When occurring at slaughter, it induces a very fast postmortem drop in pH (Figure 3), leading to drip formation and discoloration (i.e., paleness) in most muscles. The loss of control of free Ca^{2+} level results from a mutation in the *HAL* gene encoding the ryanodine receptor in the calcium channel of the sarcoplasmic reticulum. The inheritance of halothane sensitivity is recessive. The *HAL* gene has two alleles: *N* (normal, dominant) and *n* (halothane sensitivity, recessive). *nn* pigs show high mortality during rearing (approximately 10-fold higher) and a high frequency of PSE meat at slaughter. The *n* allele appears to be incompletely recessive in terms of meat quality criteria, which implies that heterozygous pigs are more or less intermediate between the two homozygotes. This allele has marked positive effects on muscle development and carcass lean content (approximately +3–5% in *nn* compared with *NN* pigs), which explains its high frequency in some pig breeds. However, the halothane test does not distinguish Nn from nn. More

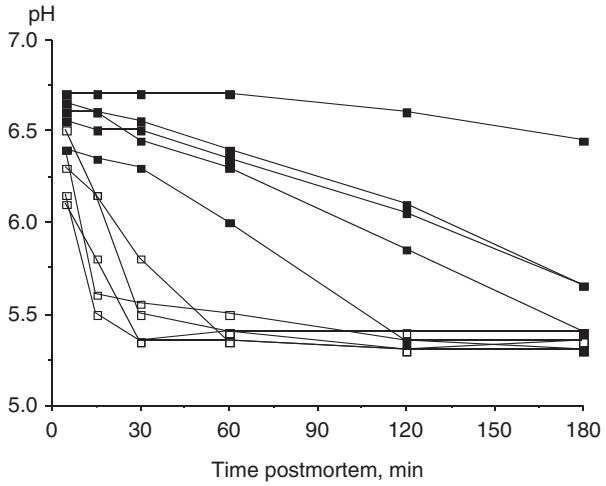

Figure 3 Postmortem pH changes in the longissimus muscle of halothane-negative (■) and halothane-positive (□) Piétrain pigs. Each curve represents one animal. All pigs came from the same herd and were killed on the same day in an experimental abattoir. Note the clear split between halothane-sensitive groups, despite the strong variation among halothane-negative pigs.

recently, a molecular genotyping method test by using a real-time PCR hybridization probe and applying it to the analysis of C1843T mutations of the Sus scrofa RYR1 gene has been developed.

In modern production systems, pigs fed for market are frequently bred by crossing specialized reproducer lines, as the sire line is especially meaty. Intense breed line selection for meatiness favors the *n* allele if no care is taken to control its frequency, which is close to 1 in some Piétrain and Landrace strains. The *HAL* genotype of the reproducers is routinely assessed using a DNA test. Mating an *nn* or *Nn* male with an *NN* female is a common practice, resulting in either 100% or 50% *Nn* market pigs, respectively, which presents some advantage in terms of carcass composition but at a cost of meat quality. Other breeders prefer to eliminate the *n* allele to produce only *NN* pigs. Choice of breeding strategy depends on the relative weights of meat quantity and quality in determining carcass value in a given marketing situation.

Fresh PSE meat from halothane-sensitive animals is less attractive than normal meat, mainly due to excessive drip and its very pale color. After cooking it is tougher than meat from *NN* pigs, primarily due to less advanced proteolysis during aging but also to higher cooking losses. Cooking loss is a matter of concern for the cooked ham industry, as processing yield is dramatically reduced, as well as for consumer appeal and eating quality.

PRKAG3 Gene

Another gene that has major effects on pork quality is the *PRKAG3* gene, also known as RN gene, which encodes a muscle-specific isoform of the regulatory γ subunit of the adenosine monophosphate-activated protein kinase, an enzyme regulating glycogen metabolism. A mutation in this gene (R200Q substitution, denoted RN^-) is responsible for a type of meat that is characterized by a normal rate of postmortem pH drop and that reaches a low ultimate pH, often called 'acid meat.' As it was first described in the Hampshire breed, where it has a very high frequency, it is sometimes known as 'Hampshire-type meat.' Acid meat is definitely pale, and somewhat soft and exudative, but less so than the PSE meat resulting from halothane sensitivity. In acid meat, paleness and exudation are related primarily to the meat pH being close to the isoelectric point of myofibrillar proteins, whereas in PSE meat they result mainly from extensive protein denaturation.

The normal allele (200R) is called rn^+. The mutated allele RN^- is dominant. It induces an accumulation of glycogen in the sarcoplasm of white muscle fibers (see muscle fiber types), with the result that muscle glycogen concentration increases with increased proportion of white fibers. Glycogen is increased by as much as 70% in the white muscles, allowing the production of more lactate during postmortem glycolysis and thus resulting in a lower pH_u. Protein content is lower, whereas lipid content is unchanged and water content is slightly higher (Table 1). Acid meat has been reported to be less tender than normal meat in France but tenderer in Sweden. In Sweden, acid meat is considered to have a higher sensory quality, as it is tastier. The residual glycogen could react with protein by cooking and thus enhance the development of Maillard reaction-like aroma. Moreover, this disagreement between these two countries might result from differences in cooking methods, such as typical cooking times and temperatures.

The main defect arising from acid meat is the weight loss occurring during meat processing by curing and cooking, particularly when no water-binding additive, such as polyphosphate, or extra protein is added. The increased weight loss results from both the lower ultimate pH and the higher water-to-protein ratio of the meat. Indeed, 1 g of glycogen binds 2–4 g of water, which is of the same order as the binding of water by proteins (approximately 3.3 g water per gram protein). Thus, in white muscle of RN^--carrier pigs, the water bound by glycogen is noticeably higher than in muscle from normal pigs. During postmortem glycolysis and further processing, glycogen is degraded and the corresponding bound water released. This results in an excess of water relative to the protein components available to hold it and in extra release of water during cooking.

Except for fresh meat marketed in a handful of countries, the RN^- gene is considered undesirable elsewhere. A DNA test is available for *RN* gene management in pig populations.

Another mutation in the *PRKAG3* gene (V199I substitution, denoted rn^*) has been shown to be associated with lower glycogen levels and higher ultimate pH, but its effects are low compared with those of the RN^- mutation. The frequency of this mutation depends on the pig lines considered but is not restricted to Hampshire pigs.

Stress-Induced Pale, Soft, and Exudative Meat

It has long been known that intense stress immediately before harvest can induce PSE meat, even in stress-resistant pigs. Stress-induced PSE lesions are generally less extensive than in the case of halothane sensitivity. In most cases, they remain limited to the deep parts of the ham, mainly the 'adductor' and the inner 'semimembranosus' muscles. Sometimes the lesions extend to other ham muscles and the 'longissimus' muscle.

Table 1 Effect of *RN* genotype on composition of *longissimus thoracis* muscle and meat quality. The acid meat resulting from the RN^- mutation (R200Q substitution in the *PRKAG3* gene) contains more glycogen and glycogen-bound water and less protein than normal meat (rn^+/rn^+). Higher glycogen levels result in lower pH_u in the most important loin and ham muscles. It is clear from the similarities between RN^-/RN^- and RN^-/rn^+ genotypes that the RN^- mutation is completely dominant

Trait	rn^+/rn^+	RN^-/RN^-	RN^-/rn^+	P^a
Glycolytic potential at rest (μmol g^{-1})[b,c]	167	304	277	<0.001
Glycolytic potential at slaughter (μmol g^{-1})[c]	110	224	196	<0.001
Moisture (% of wet tissue)	75.4	76.4	76.3	<0.001
Lipids (% of wet tissue)	1.42	1.31	1.34	0.63
Proteins (% of wet tissue)	22.2	20.5	20.6	<0.001
pH_u longissimus (loin)	5.74	5.54	5.53	<0.001
pH_u semimembranosus (ham)	5.75	5.52	5.54	<0.001
pH_u adductor femoris (ham)	5.92	5.56	5.54	<0.001

[a]Level of significance of the *RN* genotype effect.

[b]Glycolytic potential is an estimator of the content of glycolytic compounds liable to transform into lactic acid after death, expressed in %mol lactate per g; its value is close to double the actual glycogen level expressed in %mol glycosyl per gram wet tissue.

[c]At rest, determined from a biopsy on resting pigs during rearing; at slaughter, determined from a sample taken shortly after slaughter.

Source: Adapted with permission from Le Roy, P., Elsen, J.-M., Caritez, J.-C., *et al*., 2000. Comparison between the 3 porcine RN genotypes for growth, carcass composition and meat quality traits. Génétique Sélection Evolution 32, 165–186 and Lebret, B., Le Roy, P., Monin, G., *et al*., 1999. Influence of the 3 RN genotypes on chemical composition, enzyme activities, and myofiber characteristics of porcine skeletal muscle. Journal of Animal Science 77, 1482–1489.

The mechanisms underlying the occurrence of this kind of PSE meat are not totally understood. Preslaughter stress seems not always to be the cause of the lesions, as limited PSE zones are frequently observed even in pigs harvested under minimal-stress conditions. However, stress increases the extent and seriousness of the lesions. It probably acts by increasing muscle temperature and metabolic acidosis, particularly in cases involving aggressive interactions and struggling, which induce intense muscle work. In white muscle subjected to intense muscular work, intracellular pH can be reduced to values below 6.7 (Figure 4), whereas temperature is increased above the normal resting value (approximately 38 °C in the pig 'longissimus'). At harvest, muscle temperature is further increased by electrical stunning (sometimes by more than 1.5 °C in the longissimus), particularly when stunning is defective and prolonged as it prompts strong and prolonged muscle contractions. It is common to observe muscles with a temperature higher than 41 °C and a pH lower than 6.5 just after stunning, as these muscles experience extended protein denaturation after death. A deficiency in the control of free sarcoplasmic calcium is suspected, as the presence of the HAL^n allele increases the frequency and extent of lesions.

This defect is of particular concern in the cooked ham industry, as most cases are detected only at deboning, making it impossible to sort defective hams at entry into the processing lines. Ham muscles with PSE zones crumble easily during industrial slicing of cooked hams. This produces holes or splits in the slices, which make it nearly impossible to sell the meat prepacked. It has been reported that, in extreme cases, as much as half of the sliced material might be lost this way.

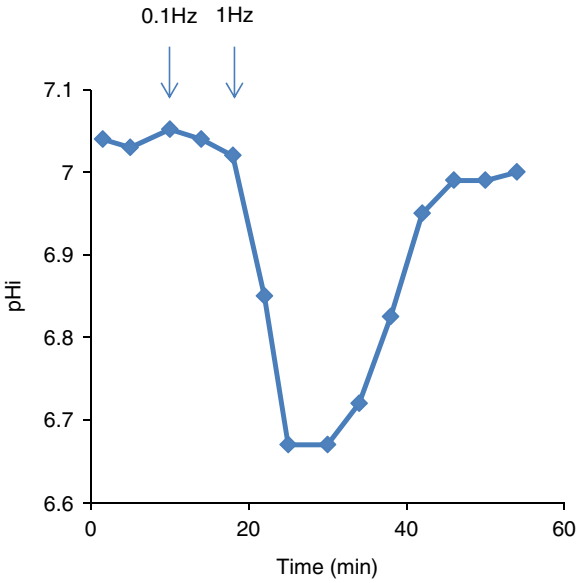

Figure 4 Change in intracellular pH (pH_i) of pig semimembranosus muscle during work: electrical stimulation. Piglets of 30 kg liveweight were anesthetized using pentobarbital; then, two platinum electrodes and an NMR (nuclear magnetic resonance) coil were fixed on the semimembranosus muscle and the animals were introduced into a NMR spectrometer. The muscle was electrically stimulated (0.1 Hz, 10 min; 1 Hz 10 min) via the electrodes, then allowed to rest for 20 min. pH was measured throughout the experiment using ^{31}P NMR spectrometry. Each point represents the average of 5 animals. Adapted from Miri, A., 1991. Application of Phosphorus-31 NMR to study of ante and post mortem metabolism in rabbit and pig muscles; relation with meat quality. PhD Thesis. University of Clermont-Ferrand.

Poor Chilling

As muscle temperature is critical for protein denaturation, slow chilling favors the occurrence of PSE meat. Modern slaughter plants are equipped with powerful chilling facilities that help to reduce the problem. However, in extreme cases it is impossible to chill the deep musculature rapidly enough to prevent protein denaturation and subsequently PSE meat and, at the same time, avoid cold shortening conditions occurring in slow-glycolysing muscle. (In the United States, most processing plants use blast chilling at -10 or -20 °F for 15–30 min followed by normal cooler chilling.)

Other Species

PSE beef has been reported to occur in carcasses of young bulls, but this defect is not frequent. The chilling of deep hind leg muscles is slow in cattle as the muscle mass is very thick. Thus, protein denaturation can occur if the rate of glycolysis is increased even slightly. However, the quality deviation of PSE beef is generally much less pronounced than that in PSE pork. For this reason, and due to its low frequency, there has been little research into PSE beef, and the physiological mechanisms underlying this defect remain unknown.

PSE meat is frequent in poultry, particularly in turkey breast muscles. As in pigs, it is associated with accelerated postmortem acidification (approximately 0.03 pH units per minute vs 0.06 in normal meat). Again, the physiological mechanisms responsible for this defect are unknown. There are some indications that preslaughter stress could play a prominent role. Some turkeys respond to halothane administration by leg stiffness, but this halothane sensitivity is not related to meat quality.

Rapid chilling after slaughter significantly reduces PSE in poultry. Chilling in ice water is particularly efficient but is banned in some countries. Acid meat is sometimes found in chicken but has not yet been tied to any gene mutation.

Dark, Firm, and Dry Meat

Carcasses with dark, purplish-red colored-meat are found in all meat species but are mainly a concern in cattle. The cuts of such meat are firm to the touch and dry, so the condition is called DFD or, specifically in cattle, dark-cutting (DC) beef. The darkness and lack of drip are related to the high pH values.

Lack of muscle glycogen at slaughter results from preslaughter stress, particularly during loading and transportation operations. Psychological stress, related to changes in physical and social environments, induces release of catecholamines, which increase glycogenolysis by activating muscle phosphorylase. Sustained muscle exercise requires mobilization of readily available muscle energy stores composed mainly of glycogen. Male animals are prone to DFD because of their excitable temperament and aggressive behavior, particularly in cattle. The in-carcass distribution of DFD meat depends on the species. In pigs, DFD is encountered mainly in the forequarters and deep ham muscles, as these body parts contain more slow red muscles, which, owing to their lower glycogen content, are more prone to DFD than white muscles. Conversely, in cattle, DFD affects mainly the hindquarters and the 'longissimus' muscle, although body distribution of muscles of different metabolic types is similar to pigs. This is probably due to the mounting behavior in this species, particularly in young bulls, which induces intense work in back muscles and hind legs. In sheep, the 'longissimus' muscle rarely shows DFD traits due to its high content of very glycogen-rich fast red fibers. In the United States, the incidence of dark-cutting beef is generally the highest in the fall when temperatures can drop rapidly from rather warm to cold within 12–24 h and cattle are then transported to harvest.

The discoloration of DFD meat is due to several factors. In high-pH meat, light scattering is reduced and muscle is more translucent at the cut surface, with the result that the color appears darker. Moreover, high pH allows mitochondria to continue respiration and thereby to consume oxygen, which enables the myoglobin to stay in its reduced form. The oxymyoglobin layer is very thin, and the purplish-red color of myoglobin predominates. Growth is optimal around neutral pH for most bacteria and so is favored in the higher range of meat pH values. This is particularly true for certain bacteria that are able to degrade sulfur amino acids, giving rise to hydrogen sulfite (H_2S), which has a very unpleasant odor. Moreover, high-pH meat has little carbohydrate, and consequently the bacteria present at its surface immediately degrade amino acids and cause early spoilage.

The main disadvantages of high-pH meats are discoloration and early spoilage when sold fresh. In beef, particularly from old animals, discoloration is accelerated by the high myoglobin content, prompting consumers to reject the product. In pigs, discoloration is only a concern in extreme cases, as most pig muscles have relatively low myoglobin content. Spoilage is particularly detrimental in beef, because aging is generally longer than in pork or lamb (weeks instead of days). A pH higher than 5.8 is generally considered unacceptable in beef, whereas pork is acceptable at up to pH 6.2, depending on local habits and ways of utilizing the meat.

Processing of meat joints by dry curing (dry hams, viande des Grisons, cecina) is successful if salt penetration is faster than microbial development in the depth of the joint. As high pH slows down salt penetration and accelerates bacterial growth, it favors putrefaction and is the main source of losses in the dry ham industry.

Cold Shortening

When carcasses are chilled too early after slaughter, some muscles contract. This effect, known as cold shortening, corresponds to a slow contracture and can exceed 50% of the muscle length when muscles are not restrained by skeletal attachments. Three conditions are needed for cold shortening to develop: temperature below 10 °C, pH above 6, and the presence of adenosine 5′ triphosphate (ATP). At lower pH and without ATP, contraction cannot occur. Cold shortening readily occurs in red muscles, mainly in beef and especially in lamb in some commercial conditions. In lamb, the acceleration of glycolysis by electrical stimulation reduces the possibility of cold-shortening temperatures occurring before rigor onset. In most pig and poultry muscles, acidification occurs too fast, and a pH <6 is generally reached before the temperature of 10 °C can be obtained. Cold-shortened meat remains tough, mainly due to its inability to tenderize even with prolonged aging, despite the fact that calpain enzyme activity remains. When beef and lamb muscle contraction is not noticeable, 'cold-induced toughening' can still occur. Thinly finished or very rapidly chilled carcasses are more susceptible to cold shortening or cold-induced toughening.

Double Muscling

Double muscling is the term used to designate a muscle hypertrophy characteristic of cattle and Texel sheep. It is due to

a mutation in the myostatin (MSTN) gene, which encodes the growth-regulating factor myostatin. Muscle hypertrophy arises from an increased total number of fibers. It is not uniform throughout the body, as some regions are isotrophic or even hypotrophic. It essentially affects the outer muscles, and it is more prominent in the hindquarters than in the forequarters.

Double-muscled animals are particularly appreciated in some European countries because of their substantial advantages in body composition/meat yield. Dressing yield is approximately 5% higher than in normal animals of the same sex, age, and breed. Carcass leanness and muscularity are superior, with percentage muscle in the carcass being approximately 10 percentage points higher (e.g., 80% vs. 70%) than in normal animals. However, double-muscled animals present some deficiencies. They are more susceptible to stress and are reputed to be more prone to dark-cutting conditions. Stress susceptibility is related to a lower overall respiratory capacity, as double-muscled animals have smaller heart and lungs and a lower blood oxygen capacity. Consequently, metabolic acidosis caused by exercise is more pronounced and more slowly compensated in double-muscled cattle than in normal cattle. This higher stress susceptibility could explain the more rapid postmortem glycolysis observed in double-muscled cattle. Proneness to DFD could be related to the higher proportion of glycolytic myofibers (particularly fast, white fibers), which are more susceptible to stress in terms of glycogen depletion.

Meat from nonstressed double-muscled animals is paler than normal due to its lower pigment content in relation to the higher proportion of fast, white fibers. It yields more drip and cooking losses, probably due to the faster postmortem pH drop. It is generally accepted that it is tenderer in some breeds mainly due to its lower connective tissue content, which might partly explain its appreciation in some European markets. However, higher shear force has been observed when cooking meat from double-muscled young bulls compared with normal individuals of the Belgian Blue breed. The cause of this difference is not known, although higher shear forces have been associated with lower calpain activity, differences in collagen solubility, and faster glycolysis.

Callipyge Sheep

The *callipyge* gene (CLPG) sheep phenotype has recently emerged in the US commercial sheep population. Callipyge lambs show extreme muscling with hypertrophy of the main hindquarter and loin muscles (in Greek, *kalli* means beautiful and *pyge* means buttock). The condition is due to the mutant allele (C) of the *callipyge* gene (normal allele *N*). The mode of inheritance of this type of trait, referred to as polar overdominance, forms a quite particular case, as only heterozygous animals (*CN*) that received the *C* allele from their sire express the callipyge phenotype, whereas the three other genotypes (*NN*, *CC*, and *NC*) are normal. Muscle hypertrophy is related to higher proportion of fast-twitch glycolytic fibers and muscle cell enlargement. Callipyge lambs have superior dressing percentages and carcass compositions. These advantages are counterbalanced by the fact that the hindquarter meat turns out much tougher. Muscles from Callipyge lambs have a higher calpastatin content, which inhibits both the rate and extent of postmortem proteolysis. Thus, tenderizing by aging is limited, and shear force values of 'longissimus' muscle from Callipyge lambs at 21 days 'postmortem' are comparable to those from normal lambs at 1 day postmortem. Moreover, callipyge animals have less marbling.

Muscle Degeneration

Muscle degeneration is sometimes observed in meat carcasses. It generally results from nutritional deficiencies, for example, in vitamin E or selenium, which give rise to serious disturbances in muscle cell metabolism and ultimately result in necrosis. A localized nonnutritional muscle degeneration known as focal myopathy occurs in turkey breast muscle, particularly the Pectoralis minor. Degenerative changes consist in necrosis, hypercontracted muscle fibers, proliferation of connective and fat tissue, and mononuclear cell infiltration into the necrotic areas. This condition has been related to local microischemia due to a low capillary-to-fiber ratio, and it is suspected to be a causative factor of PSE turkey breast muscle. In this respect, the meat shows some similarity with the PSE zones encountered in deep ham muscles of pigs, as described above.

Stress-induced muscle degeneration is sometimes found in beef skeletal muscles, particularly 'longissimus' and 'pectoralis' muscles. It affects mainly young cattle from muscular breeds. Lesions appear as zones with a pinkish to yellowish color. Texture is soft and even jelly-like in serious cases. Despite this PSE-like appearance, degenerated meat differs from PSE meat by its very high pH (>6.5). It results from long and stressful transport before harvest, as evidenced in France, where the condition proved fairly frequent in young bulls harvested after being transported several hundred kilometers by truck or train while it was lacking in similar animals killed close to the 'home' farm. It has been hypothesized that degenerative meat might be related to the action of catecholamines.

See also: Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Water-Holding Capacity; Color and Pigment. Conversion of Muscle to Meat: Aging; Glycolysis. Growth of Meat Animals: Muscle. Ham Production: Cooked Ham. Sensory and Meat Quality, Optimization of

Further Reading

Bendall, J.R., 1973. Post mortem changes in muscle. In: Bourne, G.H. (Ed.), The Structure and Function of Muscle, vol. 2. New York, NY: Academic Press, pp. 243–309.

Boccard, R., 1981. Facts and reflections on muscular hypertrophy in cattle: Double muscling or culard. Developments in Meat Science 2, 1–28.

Gregory, N.G., 1998. Animal Welfare and Meat Science. Wallingford, Oxon: CABI publishing.

Lawrie, R.A., 1991. Meat Science. Oxford: Pergamon Press.

Milan, D., Jeon, J.-T., Looft, C., *et al.*, 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science 288, 1248–1251.

Monin, G., 1988. Evolution post mortem du tissu musculaire et conséquences sur les qualités de la viande de porc. Journées de la recherche porcine 20, 201–214.

Monin, G., 1995. Effects of fresh meat quality: The effect of the RN^{-} gene. In: Lenges, J., Casteels, M., Deweghe, L., Nicolaï, T. (Eds.), Curing Technology for Cooked Pig Meat Products; An Update. Ghent Belgian: Association for Meat Science and Technology, pp. 91–103.

Offer, G., 1991. Modelling of the formation of pale, soft and exudative meat — effects of chilling regime and rate and extent of glycolysis. Meat Science 30, 157–184.

Offer, G., Knignt, P., 1988. The structural basis of water-holding in meat. Developments in Meat Science 4, 63–244.

Pearson, A.M., 1987. Muscle function and post-mortem changes. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products. Westport, CT: Food and Nutrition Press, pp. 155–192.

Sellier, P., 1998. Genetics of meat and carcass traits. In: Rothschild, M.F., Ruvinsky, A. (Eds.), The Genetics of the Pig. Wallingford, Oxon: CAB International, pp. 436–510.

Warriss, P.D., Browm, S.N., 1987. The relationship between initial pH, reflectance and exudation in pig muscle. Meat Science 20, 65–74.

Relevant Websites

http://www.fao.org/docrep/
Food and Agriculture Organization of the United Nations.

http://www.genomenewsnetwork.org
Genome News Network.

Glycogen

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Glossary

Glycogenolysis The break-down of glycogen to glucose-1-phosphate and glucose.

Glycogen debranching enzyme A double-function enzyme that moves three glucose units from the 1-6 branching point (transferase) and then 1,6-glucosidase removes the last glucose unit.

Glycogen phosphorylase A very fast enzyme that is capable to remove glucose-1-phosphate from nonbranched chains of glycogen, down to the fourth glucosyl unit from the 1-6-branching point.

Glycolysis (from *glycose*, an older term for glucose+*-lysis* degradation) The metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, $CH_3COCOO^-+H^+$.

Introduction

Most of the time, blood will provide enough nutrients for the metabolism, and especially, for the energy production of the muscles. The principal portion of the energy produced in a muscle cell originates from the main nutrients; carbohydrates, volatile and nonvolatile fatty acids plus glycerol, and proteins. Fat, as free fatty acids, is an important source of energy at rest and during light exercise. In light exercise, the plasma content of free fatty acids increases within a few minutes, whereas during heavy exercise it decreases. In ruminants, short-chain free fatty acids (mainly acetate, propionate and butyrate) form the major proportion of the energy substrates with carbohydrates having a minor role (5–15%). Oxygen is required, in long terms for all kinds of energy production.

Muscles are able to produce/use 5 μmol of adenosine triphosphate (ATP) s^{-1} g^{-1} at maximum rate, and that appears to be universal across muscle tissues. The resting level of consumption is approximately 0.02–0.05 μmol ATP s^{-1} g^{-1}, and is used for maintaining membrane potentials, and fuelling the calcium pump that returns Ca^{++} back to the sarcoplasmic reticulum, the occasional contractile reactions between actin and myosin, as well as the anabolic reactions of molecule syntheses. ATP consumption also generates body heat.

The maximal rate of ATP consumption is at least 100 times greater than that of a resting muscle. Since the production and consumption of ATP must be equal at any given moment, the portion of ATP that cannot be provided by the oxidative metabolism must be produced anaerobically. This explains the existence of two mechanisms (aerobic and anaerobic) for ATP production. If indeed the aerobic production was to be able to cover the maximal level of energy consumption, the animal would, depending on species, need to have much larger organs (lungs, heart, circulatory system, organelles and enzymes for aerobic metabolism in fibres) than it currently has for obtaining and using oxygen. Since animals seldom need maximal energetic capacity over a sustained period of time in all of their muscles, a capacity for a full aerobic coverage would be an inefficient physiological 'investment.' Another mechanism has, therefore, developed for short bursts of energy.

Depending on the oxidative capacity of a muscle, the oxidative production/usage of energy can increase due to increased activity. In pigs, for example, the increase is less than 10 times the resting level, whereas in cattle, it is 20-fold, and in horses, almost 50-fold. The above mentioned figures are of course approximates, because the oxygen consumption within species depends on the fibre type composition and the basic tone of each muscle. The oxygen consumption of various muscles depends on their activity at a given moment, as does the blood flow through the muscles, as well as the relative amount of oxygen left in the muscles. At rest, for example, the blood flow through muscles may be 20% of the total flow. At maximal activity, however, the cardiac output increases tenfold, and 80% of it is circulated through muscles, which then utilize up to 90% of the passing oxygen. Together, these values translate to a 25-fold consumption of oxygen in comparison to a resting muscle.

The oxidative capacity of a body's total muscle mass seems to be in balance with the capacity of blood circulation. Animals with a high oxidative capacity (e.g., horse, reindeer) have relatively big lungs and hearts, and muscles that have dense capillary networks, compared to animals with less oxidative capacity (e.g., pig, chicken). Furthermore, breeding has had a significant effect on the oxidative capacity of domestic animals. The proportion of oxidative fibres is higher in the muscles of the wild pig than in the domesticated pigs, as is the capillary density. In wild pigs, 1 g of heart tissue serves 85 g of muscular tissue, whereas in domestic pigs it has to serve 140 g. In poultry, severe oxygen deficiency within the poorly capillarized Pectoralis muscle results in deep pectoral myopathy. The crucial importance of sufficient oxygen supply for muscle tissues cannot, therefore, be overly emphasized.

In a muscle fibre, glycogen is the energy reserve used in case the circulatory system cannot provide enough oxygen and nutrients. Glycogen breaks down to glucose phosphates that are utilised anaerobically or aerobically, depending on animal species and their level of physical and/or psychological stress. There is indirect evidence that in stress, triggered by adrenaline, glycogen is preferred even if there were other energy substrates available, i.e., even if the stress did not involve high physical activity. The lactate formed in the glycolysing fibres will be transported into the blood via monocarboxylate transporters (MCT) that move lactate through the cell membranes from the higher concentration toward the lower. Lactate is destined to be exported to the liver to be used as a substrate for the synthesis of glycogen or glucose-6-phosphate. While on its way, some of it will probably also be aerobically

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utilized either by the oxidative muscle fibres or by the heart, if the lactate concentration in them is lower than that in blood.

Structure of Glycogen

Theoretical studies have revealed how the glycogen molecule has taken its form in the biological evolution. The glycogen molecule is optimized for storing a maximum amount of glucose in the smallest possible volume, and at the same time, for allowing a rapid release of glucose from its structure by glycogen phosphorylase (GP) (Figure 1). A 100 mM solution of glucose would create a high osmotic pressure, whereas the equivalent 0.002 mM of glycogen does not, the glycogen molecule being more a particle (granule) than an element in a solution. The maximum sized glycogen molecule/granule of a diameter of 40 nm contains approximately 55 000 glucose units, and 2100 nonreducing chain ends. The total molecular weight is approximately 10^7 Da. Each glycogen molecule has 40–50 GP dimers (or 20–25 tetramers) bound to it, facilitating the immediate and fast cleavage of the glucosyl units. Glycogen synthase (GS), glycogen branching enzyme (GBE), AMP-activated protein kinase (AMPK), as well as glycogen debranching enzyme (GDE) are also bound to the glycogen complex, and thus, readily available when needed. There are also several other proteins with regulatory roles bound to the complex (Figure 2).

In the core of the glycogen molecule is glycogenin (molecular weight (MW) 37 000), an enzyme-acting protein (glucosyltransferase) that serves as a primer for the molecule. In glycogen formation, uridine diphosphate moves the first eight glucosyl units to glycogenin after which GS takes over and extends the chain of glucosyl units via $\alpha(1\rightarrow4)$ linkages. Glycogen branching enzyme then transfers an A-chain of 6–7 glucosyl units forming an $\alpha(1\rightarrow6)$ linkage between the original chain and the transferred chain. A new branch point must be at least 4 glucosyl units apart from the previous branch point.

Theoretical calculations have shown that the optimal chain length is 13 (12–14) glucose units, and that it is optimal to have two branching points in each B-chain (Figure 1). Each level of chains forms a concentric tier, and all new tiers double the number of chains of the previous tier. After 12 tiers, the structure will turn self-limiting, eventually due to the spatial relations between the glycogen molecule and GS. The distance of four glucose units between the branching points thus leaves tails of 4 or 5 glucosyl units.

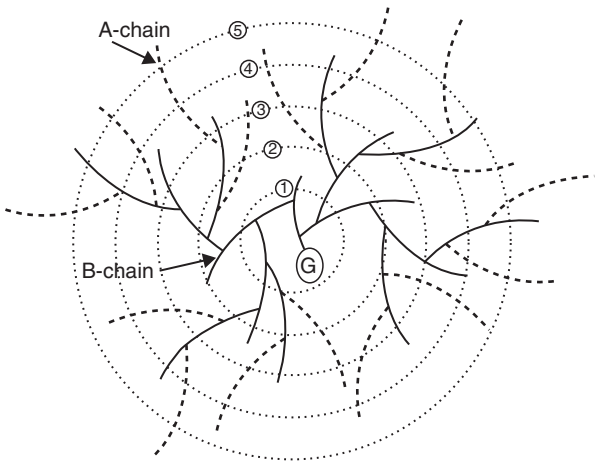


Figure 1 Glycogen molecule (5 tiers) according to Meléndez-Hevia and others. Each unbranched A (dotted line) and branched B (solid line) chain has 12–14 glucosyl residues. G = glycogenin. Adapted from Immonen, K., 2000. Bovine muscle glycogen concentration in relation to diet, slaughter and ultimate beef quality. Doctoral Thesis, University of Helsinki, EKT Series no 1203, 90 pp. Available at: https://helda.helsinki.fi/bitstream/handle/10138/20879/bovinemu.pdf?sequence=2 (accessed 15.08.13).

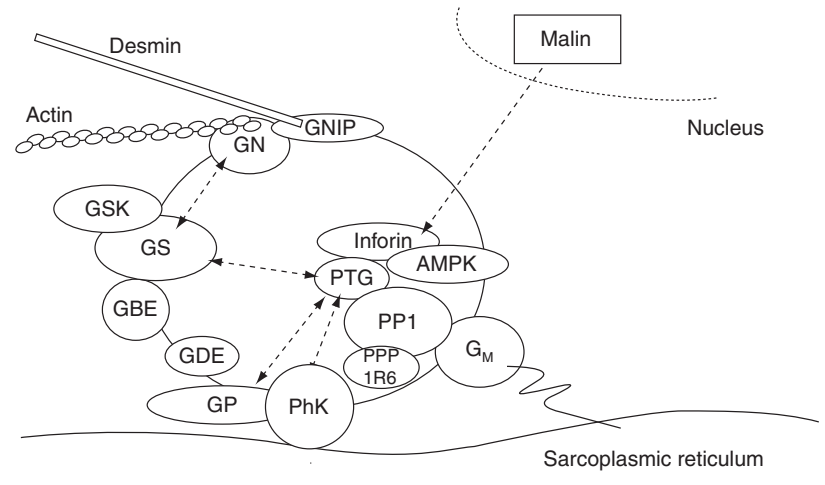


Figure 2 A schematic summary of proteins known to interact with glycogen. GDE, glycogen debranching enzyme; AMPK, AMP-activated protein kinase; GBE, glycogen branching enzyme; G_M and PP1R6, regulatory subunits of protein phosphate 1 PP1; GN, glycogenin; GNIP, glycogenin-interacting protein; GS, glycogen synthase; GSK, glycogen synthase kinase; GP, glycogen phosphatase; PhK, phosphorylase kinase; PTG, protein targeting to glycogen. Reproduced from Graham, T.E., Yuan, Z., Hill, A.K., Wilson, R.J., 2010. The regulation of muscle glycogen: The granule and its proteins. Review. Acta Physiologica 199, 489–498.

Until recently, glycogen was thought to exist as two separate forms: proglycogen and macroglycogen. Particles with molecular weight of 400 kDa are called proglycogen, which due to its protein content of approximately 10%, is insoluble in acidic solutions. Glycogen molecules of a much higher molecular weight are called macroglycogen, which in turn maintain its water-solubility even in acidic solutions due to the dominant abundance of carbohydrate. It has been postulated that proglycogen is utilised in severe stress as well as postmortem, whereas macroglycogen is used during endurance exercise, and less so postmortem. The existence of different glycogen pools has, however, been challenged. It has been concluded that instead of existing as two separate forms, there is a continuum of glycogen molecules of different size. It has also been claimed that muscle fibres accommodate glycogen in multiple sites; some free in cytosol, and some bound to the sarcoplasmic reticulum. There are, therefore, differences in both solubility and availability for use. Microscopically, the granules are most abundant between the myofibrils, but also inside the fibrils. In humans, the average diameter of a glycogen granule is 25 nm, i.e., tier 8 is mainly in use, and less than 20% of the granules have built all 12 tiers. In conclusion, glycogen particles are not of fixed molecular weight, but instead, are subject to on-going resynthesis or degradation depending on the level of physical activity and/or stress, as well as the nutritional status of the animal.

Metabolism of Glycogen

The main function of glycogen is to serve as a source of glucose for anaerobic glycolysis (includes glucose-1-P to pyruvate and, with the help of muscle-type lactate dehydrogenase, from pyruvate to lactate). Anaerobic glycolysis is a very rapid metabolic pathway producing 3 ATP of energy per glucosyl unit, the yield being much lower, however, than if pyruvate was utilised aerobically. The biochemical role of lactate formation is to regenerate NAD^+ for glycolysis, and one proton will be simultaneously bound to pyruvate when lactate is formed. There has been an intensive discussion over the source of such protons, and it seems that physiologists, dealing with the pH range of 7.2–6.4 of a dynamic living muscle, and meat and animal scientists, working with a broader range of pH, i.e., from 7.2 all the way down to the pH 5.0 of a more stable postmortem muscle, see the matter quite differently. The protons derive from earlier stages of glycolysis, so that different pK_a-values of glycolytic metabolites and phosphates at different postmortem pH-values relate to free proton formation. Irrespective of the sources of protons, and/or the buffering capacity of meat, there is an abundance of data confirming the linear negative correlation between lactate content and pH-value.

In the breakdown of glycogen, there are two enzymes directly involved: GP that releases glucose is strongly controlled by various factors, including hormonal, and GDE that disassembles the branch points. The rate of the process is limited by the availability of free HPO_4^{2-}. The pK_a value of H_2PO_4 is 7.2, and at pH 5, the pool of HPO_4^{2-} is virtually nonexistent.

GP (842 amino acids and a MW of 97.4 kDa) amounts to approximately 2% of the soluble proteins of a muscle, and has an immense acting capacity. GP is activated both by allosteric interactions as well as reversible phosphorylation. The inactive form *b*, of GP can be found in resting muscles, and is activated by a cascade involving epinephrine, calcium, cyclic AMP, AMP-activated protein kinase (which activates phosphorylase kinase), and inhibited, however, by glucose-6-P and ATP. GP is biologically active as a dimer of two identical subunits, but can also be found as a tetramer. There are dimeric GP molecules attached to each glycogen molecule/particle. Each GP monomer consists of two domains: the *C*-terminal domain (the one responsible for catalysing the reaction), and the *N*-terminal domain (responsible for regulation of the enzyme). The binding of phosphate or AMP at the effector sites leads to changes in the conformation of the protein, and increases the acceptance of glycogen at the active site. One subunit is being active whereas the other, as attached to the molecule, is regulating the activity.

When the outermost tier is full of glycosyl units, 50% of all glucose within a glycogen particle lies in the unbranched A-chains, whereas the remaining 50% constructs the B-chains, irrespective of the total number of tiers. GP can only cut 9 out of the 13 consecutive 1–4-linked glucosyl units of a linear A-chain. Thus, according to theoretical calculations, GP can initially cleave 34.6% of the glucosyl units of any glycogen particle. At the fourth glucosyl unit from the 1–6-linked branch, the activity of GP ceases.

The cascade for the activation of glycogenolysis is very effective. One epinephrine molecule bound to a receptor on a muscle fibre results in 400 000 glucose-1-P units cleaved by GP per second. This means that one molecule of GP can disassemble glycogen at a speed of 30 000 degradations per particle per second. Blood epinephrine concentration of 10^{-10} M generates an intercellular cyclic adenosine monophosphate (cAMP) concentration of 10^{-6} M. The relative concentrations of the three successive enzymes of the cascade; cAMP-dependent protein kinase, phosphorylase kinase and GP, occur as molar ratios of 1:10:240 including adrenalin at a ratio of 0.0001, indicating an effective amplification of the signal.

At the onset of muscle contraction, GP is activated from form *b* to form *a* by the increasing content of free cytosolic Ca^{++}, as well as by norepinephrine secreted from the motor-end-plates at the sarcolemma. GP then remains active in high concentrations of AMP and phosphate, and will be inhibited again when ATP and glucose-6-P levels have increased. Decreasing levels of ATP and glucose-6-P will start glycogenolysis. In case of nonintensive exercise, the increased blood supply of nutrients and oxygen quickly take over, and GP *a* is phosphorylated back to *b*. In a postmortem muscle of the pig, for example, GP stays in the form *b* for the first 10 min. It should be noted, however, that in a stressed animal, when creatine phosphate has been utilised, glycolysis starts earlier, even before slaughter. In this case, the reaction $CP^{2-}+ADP^{3-}+H^+ \rightarrow C+ATP^{4-}$ will not generate the initial buffering effect.

For the continuation of glycogenolysis, GDE must remove the 1–6 linkages. The rate of GDE is not more than 10% of that of GP. The biological significance for this may be that a controlling system is needed to stop excessive breakdown which would cause damages in the fibre. Also GDE is bound to the glycogen particle, and thus, always available. It has a

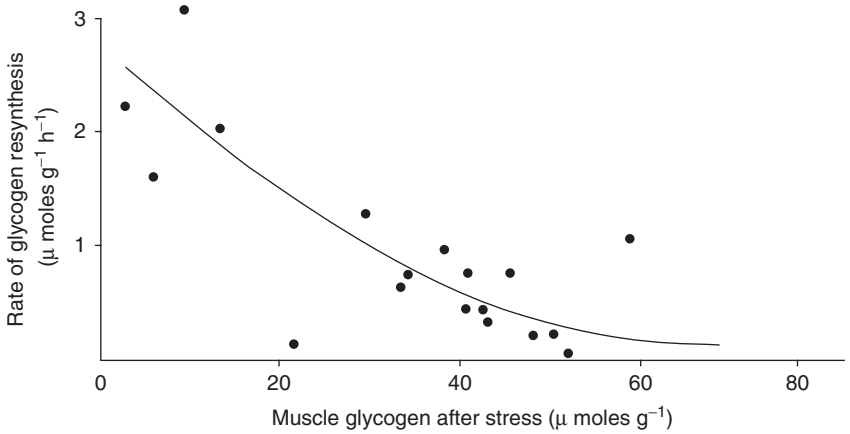


Figure 3 The relationship between muscle glycogen concentration and the rate of resynthesis in young bulls. Reproduced with permission from Tarrant, P.V., 1988. Animal behaviour and environment in the dark-cutting condition. In: Fabiansson, S.U., Shorthose, W.H., Warner, R.D. (Eds.), Dark-Cutting in Cattle and Sheep, pp. 8–18. Australian Meat and Live-stock Research & Development Corporation.

double function. In one end of the molecule, there is a transferase removing three glucosyl units and placing them at the end of another chain, most probably B, whereas in the other end of the enzyme complex there is 1,6-glucosidase activity cleaving the last remaining glucosyl unit as free glucose, which is then immediately phosphorylated. Finally, GDE will move further away from the A-chain thus allowing GP to function again. Very little is known about the control of GDE, except that it can only process A-chains of a length of four glucosyl units. It seems that glycogenolysis is independent on GDE at high glycogen concentrations, but at lower glycogen contents, GDE is of more relevance. GDE works optimally at a temperature range of 39–42 °C, and is not particularly sensitive to a lowering of pH. GDE is, however, very sensitive to low temperatures, and has hardly any activity at below 10 °C. It is, therefore, possible that postmortem, at low levels of glycogen, and in cold meat, GDE is rate-limiting.

GS is allosterically activated by glucose-6-phosphate. Contrary to GP, GS is thus active when high blood glucose concentrations lead to elevated intracellular glucose-6-phosphate. GS is inactivated by an on-going glycogenolysis, as the cAMP cascade ceases glycogen synthesis. GS is phosphorylated by protein kinase A as well as by phosphorylase kinase. Phosphorylation of GS promotes the '*b*' (less active) conformation. The cAMP cascade with AMPK having a central role thus controls the levels of glycogen. Also the intake of glucose into a fibre is controlled by AMPK. In the liver, glucose-1-phosphate may also, instead of being converted to glycogen, be converted into glucose-6-phosphate, which is then dephosphorylated for the release into the blood stream (from muscle fibres the charged glucose phosphates do not escape).

Once degraded, it takes a relatively long time to replenish the glycogen stores. There are, however, marked differences between species. In beef animals, the resynthesis is particularly slow, 1–2 mmol kg^{-1} h^{-1}, increasing only at very low glycogen stores (Figure 3). In race horses, during three consecutive bouts within 2 h, the glycogen content first decreases approximately 30%, and interestingly, an additional 20% during 4 h at rest. The recovery takes 3 days indicating that the consumption of carbohydrates is high after repetitive intensive exercises.

Effect of Glycogen Content on Meat Quality

The glycogen contents of animals at rest or just after slaughter have mostly been estimated with the glycolytic potential, i.e., the sum of glycogen, glucose, glucose-6-P and lactate, expressed as lactate equivalents. This is, in principle, a good indicator for antemortem glycogen levels. However, as one connects the relative contents of the above variables with the time postmortem, and especially, with the pH and buffering capacity, it soon becomes evident that in many cases it has not been all that significant of an indicator for meat quality due to complexity of the events. An alternative simple procedure has not, however, at least to date, been introduced for the retrospective determination of preslaughter glycogen contents. The analyses are indeed challenging, especially because glycogenolysis and glycolysis can proceed very rapidly during sample preparation and analysis. It is, therefore, very important to immediately freeze the fresh muscle samples in liquid nitrogen. A resting muscle biopsy taken before any measures of transportation, or just before slaughter, would make the most representative sample, provided that sampling and freezing are, again, carried out promptly.

The glycogen contents of resting muscles are usually at the levels of 80–100 mmol kg^{-1}, given as glucose, yet lower levels are frequently found at slaughter due to ante-mortem stress. In pigs, higher levels (of 120 or even 150 mmol kg^{-1}) are found, especially in the Hampshire, and Hampshire crossbreeds. Also in cattle, high resting levels (110–120 mmol kg^{-1}) can be found in animals that have first been depleted of glycogen, and then allowed to recover on a high energy diet. Pre-slaughter stress, such as transportation, invariably reduces glycogen contents. Although the glycogen levels of mammals are controlled by AMPK through PRKAG3 gene, the mechanism in poultry may be different. In chicken, the glycogen contents of the light Pectoralis major muscles are lower (below 100 mmol kg^{-1}) than in the light

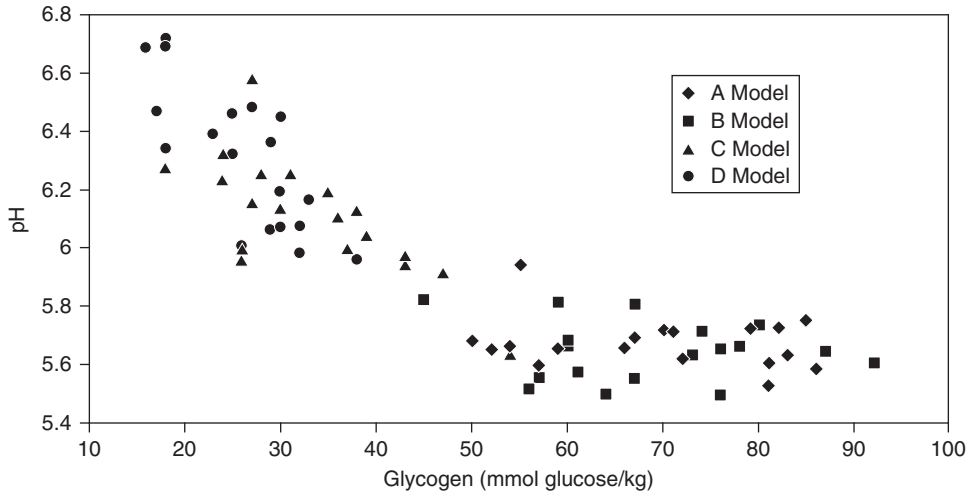


Figure 4 Ultimate pH and glycogen content immediately before slaughter in pig *M. longissimus dorsi*. A–D, various exercise intensities before slaughter. Reproduced from Henckel, P., Karlsson, A., Jensen, M.T., Oksbjerg, N., Søholm Petersen, J., 2002. Metabolic conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri- and post mortem energy metabolism. Meat Science 62 (2), 145–155.

mammalian muscles, the buffering capacity, however, being higher. This may be because chicken use the Pectoralis muscles mainly for single intensive bursts for flight, followed then by an aerobic recovery.

The threshold glycogen content resulting in ultimate pH-values above the normal of 5.5 is 53 mmol kg^{-1} in pigs (Figure 4) and 57 in cattle. This means that any induced stress can last for quite some time without affecting the ultimate pH value, even with significant glycogen depletion. Especially in beef, the pyruvate resulting from glycolysis may also be immediately used aerobically by the fibre in question, or by another fibre of better aerobic status. In a young bull, the average rate of glycogen breakdown is 0.18 mmol kg^{-1} min^{-1}, the maximum being 0.39. The corresponding hourly rates would therefore be 11 and 23 mmol kg^{-1}, should the exercise truly last for hours.

There is an abundance of literature on the effects of animal stress on meat quality, and especially on the incidence of the pale, soft and exudative (PSE) meat, as well as the dark, firm and dry (DFD) meat. These aspects will be dealt elsewhere in this book (preslaughter treatment), and are therefore not discussed here in detail. However, the quality effects of preslaughter stress manifest themselves through glycogenolysis, and consequently, through the rate of pH fall, as well as the ultimate pH. When the muscles of pigs and poultry, and more seldom cattle, coming to slaughter have a high content of glycogen accompanied with low levels of creatine phosphate, low oxygen saturation, as well as high body temperature, glycogenolysis and glycolysis will start premortally, and remain rapid for the first few hours postmortem. This will result in a low pH at temperature still being high, and usually in a very low ultimate pH, causing PSE meat. On the contrary, when glycogen content is low already at rest, or has been consumed in a long-term (hours) stress, the consequent low level at slaughter results in a lower rate of pH fall and an elevated ultimate pH. High pH lowers the keeping quality of meat, as well as causes a nontypical taste to it, while increasing the water-holding capacity and tenderness. Glycogen reserves are hardly ever totally exhausted; approximately 10–20 mmol kg^{-1} always being left over, even in severely stressed cattle. The glycogenolysis may also cease at a point where, at least in theory, a sufficient amount is left yet to accommodate an additional pH fall of substantial size (Figure 5). It is not known whether the lower keeping quality is related to pH exclusively, or if also the low content of carbohydrate contributes to the alteration of the bacterial flora and/or its metabolism.

The liver can store 10% or more of its weight as glycogen, and release it into the blood as glucose. In a slaughter-weight pig, this equates to 150 g of glucose, or 2.4 MJ of energy, and corresponds to approximately 20% of the needs of basic daily metabolism.

Concluding Remarks

Livestock live normally a peaceful life without having to deal with frequent, long-lasting physical stress. Stress, namely, the secretion of epinephrine, increases glycogenolysis, and if psychological stress/excitement is accompanied with physical strain, it results in a very rapid use of glycogen. Various preslaughter treatments (collecting, regrouping with fighting and withdrawal of feed, marking, transport, slaughterhouse lairage, and finally, driving to stunning) involve potential physical and psychological stress factors that an animal may not have ever previously experienced. Furthermore, exposure to these treatments may continue from hours to several days, and thus, depending on species as well as the length and type of the logistic channel of each individual case, seriously jeopardize both animal welfare as well as meat quality. The quantity of research in carbohydrate metabolism is overwhelming, but the majority concerns humans and/or laboratory animals, and has been focused on living organisms, i.e., at pH 7.0 and temperature 37 °C. The decreasing pH and

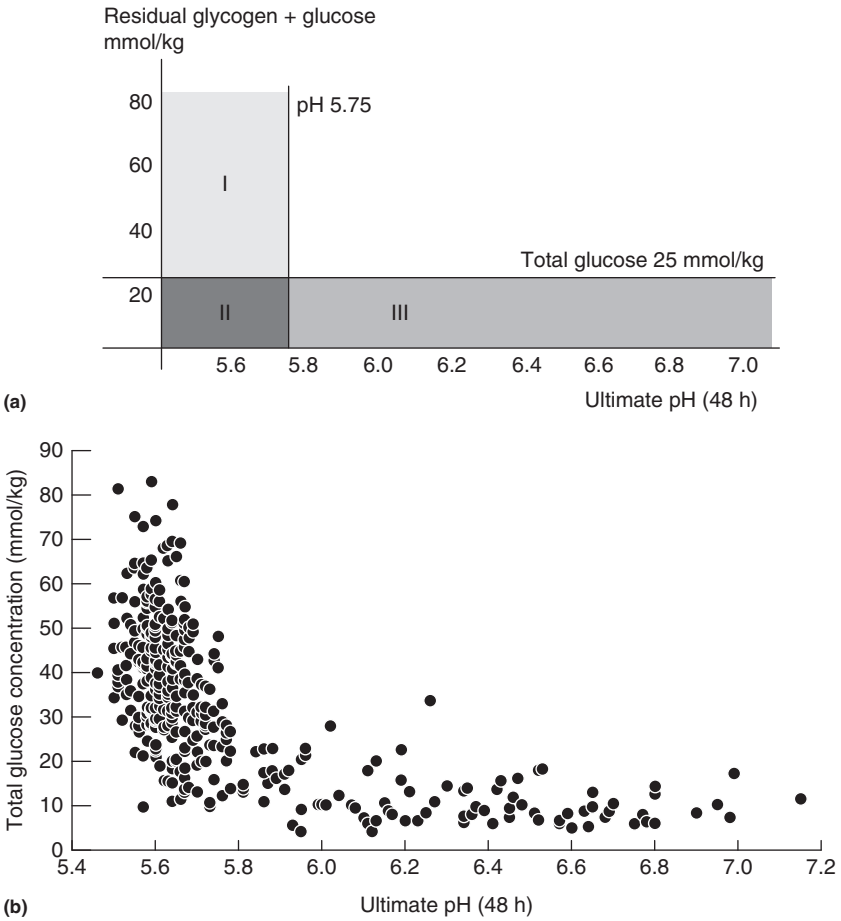


Figure 5 A suggestion (A) for beef quality categories based on the residual (48 h postmortem) glycogen+glucose content and ultimate pH (B). A I: low-stress, unproblematic; A II: medium-stress, potentially problematic; A III: high-stress, problematic. B shows that substantial amounts of glycogen may be found in chilled beef. Reproduced from Immonen, K., Puolanne, E., 2000. Variation of residual glycogen-glucose concentration at ultimate pH values below 5.75. Meat Science 55, 279–283.

temperature, however, induce changes in enzyme activities, and in the net charges of, for example, phosphates and glycolytic intermediates, thus greatly influencing the regulation of glycogenolysis and glycolysis. As examples, the scientific community does not fully agree from where protons are derived from postmortem, and what it is that stops glycolysis with more or less glycogen still left in the muscle. More research is needed to focus on postmortem biochemistry where pH and temperature are 'one-way downwards' variables, and the metabolic profile (glycogen, lipid and protein metabolisms in relation to energy supply and recovery) resulting from the various forms of animal stress needs to be studied in more detail.

See also: Conversion of Muscle to Meat: Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Electrical Stimulation. Growth of Meat Animals: Physiology. Modeling in Meat Science: Meat Quality. Muscle Fiber Types and Meat Quality. Preslaughter Handling: Preslaughter Handling; Welfare including Housing Conditions; Welfare of Animals

Further Reading

Fabiansson, S.U., Shorthose, W.R., Warner, R.D. (Eds.), 1989. Dark-cutting in cattle and sheep. Sydney: Australian Meat & Live-stock Research & Development Corporation.

Fernandez, X., 1991. A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. Journal of Muscle Foods 2, 209–235.

Graham, T.E., Yuan, Z., Hill, A.K., Wilson, R.J., 2010. The regulation of muscle glycogen: the granule and its proteins. Review. Acta Physiologica 199, 489–498.

Hamm, R., 1977. Postmortem breakdown of ATP and glycogen in ground muscle: A review. Meat Science 1, 15–39.

Henckel, P., Karlsson, A., Jensen, M.T., Oksbjerg, N., Søholm Petersen, J., 2002. Metabolic conditions in Porcinelongissimus muscle immediately pre-slaughter and its influence on peri- and postmortem energy metabolism. Meat Science 62 (2), 145–155.

Hocquette, J.F., Ortigues-Marty, I., Pethick, D., Herpin, P., Fernandez, X., 1998. Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. Livestock Production Science 56, 115–143.

Immonen, K., 2000. Bovine muscle glycogen concentration in relation to diet, slaughter and ultimate beef quality. Doctoral Thesis, University of Helsinki, EKT Series no 1203, 90 pp. Available at: https://helda.helsinki.fi/bitstream/handle/10138/20879/bovinemu.pdf?sequence=2 (accessed 15.08.13).

Immonen, K., Puolanne, E., 2000. Variation of residual glycogen-glucose concentration at ultimate pH values below 5.75. Meat Science 55, 279–283.

Johnson, L.N., Barford, D., 1990. Glycogen phosphorylase. Minireview. The Journal of Biological Chemistry 265, 2409–2412.

Meléndez-Hevia, E., Waddelm, T.G., Shelton, E.D., 1993. Optimization of molecular design in the evolution of metabolism: the glycogen molecule. Biochemical Journal 295, 477–483.

Scheffler, T.L., Park, S., Gerard, D.E., 2011. Lessons to learn about post-mortem metabolism using the AMPKγ3^{R200Q} mutation in pig. Meat Science 89, 244–250.

Tarrant, P.V., 1988. Animal behaviour and environment in the dark-cutting condition. In: Fabiansson, S.U., Shorthose, W.H., Warner, R.D. (Eds.), Dark-Cutting in Cattle and Sheep, pp. 8–18. Austratian Meat and Live-stock Research & Development Corporation.

Ylä-Ajos, M., 2006. Glycogen debranching enzyme activity in the muscles of meat producing animals. Doctoral Thesis, University of Helsinki, EKT Series no 1363, 64 pp. Available at: https://helda.helsinki.fi/bitstream/handle/10138/20823/glycogen.pdf?sequence=2 (accessed 15.08.13).

Glycolysis

KO Honikel[†]

Glossary

Adenosine triphosphate (ATP) buffer ATP is the universal fuel for energy-consuming or energy-producing reactions in cells. A muscle cell, for example, can accumulate only a limited amount of ATP (5–15 mmol kg^{-1} tissue). The surplus ATP is stored in creatine phosphate (CP) as a buffer of energy, which, in a reaction step, is released in the form of a phosphate group and transferred to adenosine diphosphate (ADP): ADP + CP = ATP + creatine.

Catabolism The use of energy-rich compounds provided by food constituents, like carbohydrates, fat, and proteins, to keep up the steady state of metabolic pathways, the active transport of compounds through membranes, and to permit the movement of muscles in animal tissues.

Conditioning After death of an animal, the bloodstream stops and neither provides oxygen or nutrients to the cells nor removes reaction end products from the cells. Within a few hours postmortem, the energy metabolism of the muscle necessary for movement ends and the muscle gets stiff. Then rigor mortis occurs. The time span from death to rigor mortis is called conditioning. After this time, the real aging of the muscle starts.

Glycogenolysis The breakdown of glycogen to glucose-1-phosphate and further on via glycolysis.

Glycolysis (from glucose, an older general term for glucose + lysis degradation) The metabolic pathway that converts glucose ($C_6H_{12}O_6$) into pyruvate ($CH_3COCOO^- + H^+$), and ATP.

Hypoxanthine A purine base formed postmortem by splitting of ribose from inosine.

Inosine Formed postmortem from inosine monophosphate by dephosphorylation.

Inosine monophosphate Formed postmortem from adenosine monophosphate by deamination.

Anabolism versus Catabolism

Plants use energy from the sunlight to convert oxidized products, such as water (H_2O) and carbon dioxide (CO_2), into reduced products, such as carbohydrates. In the overall reaction [I], plants create glucose, releasing free oxygen into the atmosphere.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6(\text{glucose}) + 6O_2 \qquad [\text{I}]$$

From the monocarbohydrates other compounds are formed, such as polysaccharides and fatty acids in their storable esterified form (triacylglycerols). In summary, the sunlight is used in plants to build up energy-conserving compounds in an anabolic way.

Animals use the energy-rich compounds of plants directly or indirectly through the food chain by breaking them down in a catabolic pathway. Thus, carbohydrates are broken down to pyruvate, which is further metabolized to acetyl-CoA when it meets the catabolic process of fatty acid breakdown in the subsequent citric acid cycle, at the end of which CO_2 and reduction equivalents (XH) are formed that again react with oxygen to form water.

Simultaneously, adenosine triphosphate (ATP) is formed in the oxidative phosphorylation pathway as a unique fuel for most of the energy-converting processes in the body (reaction [II]). Approximately 36 ATP molecules are built up per carbohydrate unit, such as glucose (reaction [II], $\gamma \approx 36$; ADP, adenosine diphosphate; P_i, inorganic phosphate).

$$\gamma ADP + \gamma P_i + 4XH + O_2 \rightarrow 2H_2O + \gamma ATP + 4X \qquad [\text{II}]$$

In living animals, oxygen is available to cells via the bloodstream so that catabolic processes can occur. The blood also transports the energy-rich compounds, such as glucose, to the cells forming the polysaccharide glycogen, a polymer of glucose, in surplus. After the breakdown of glycogen, the blood transports CO_2 and other degradation products to the liver or to the organs of excretion, such as the lungs and kidneys.

Uptake of energy-rich compounds helps in maintaining the organized steady state of reactions in cells, which is required to preserve the living state; moreover, these reactions produce heat to keep the body temperature constant. The surplus energy, as mentioned above, is stored in muscle cells as polysaccharide glycogen and creatine phosphate (CP) or in fat cells as triacylglycerols. When energy is required, these stores of CP, glycogen, and triacylglycerols are broken down, so that their energy can be used for the production of ATP.

In addition to the four chemical elements H, C, O, and P in the nutrients mentioned so far, nitrogen is also needed for the formation of proteins, which in muscles are the most recognizable parts with their filamental and fibrous structure in the muscle fibers. Protein formation requires energy, but protein degradation is used for energy production only at times of starvation.

In slaughter animals that provide muscle for meat, a surplus of energy is usually available. Muscle growth and fat depot formation is the purpose of animal production. Large muscles (requiring protein) and reduced fat depot growth are the aims of modern animal feeding.

At the time of harvest, all well-fed and unstressed animals contain some fat depots, have a reservoir of CP and glycogen, and exhibit a constant and high ATP concentration in their muscle cells.

[†]Deceased.

Catabolism Postmortem

During slaughter, the blood flow stops when the heart stops beating. Oxygen- and energy-rich compounds, such as glucose, are no longer transported to cells and metabolic products are not removed. The tissues sooner or later lose their steady metabolism that supports life. Following this, the well-ordered cellular structures begin to disintegrate.

Nerve cells cease to function within 15–30 min after death. Fat cells break down triacylglycerols to fatty acids very slowly, but postmortem the free fatty acids cannot be used for the formation of other energy-rich compounds, such as ATP, because the two carbon/hydrogen units ($-CH_2-CH_2-$) required for acetyl-CoA ($CH_3-CO-S-CoA$) need oxygen, which is no more available postmortem. Hence, fats remain relatively unchanged in meat.

Muscle cells can still use glycogen as an energy source, but, because of the lack of oxygen, the citric acid cycle and the oxidative phosphorylation pathway no longer function.

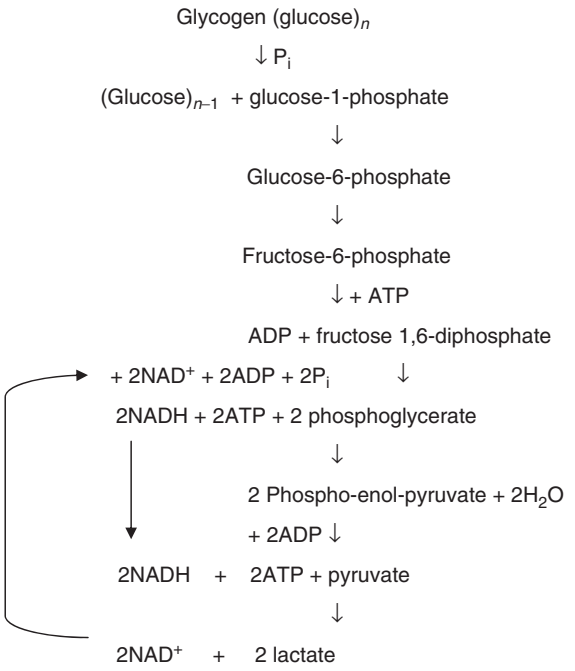
The pyruvate is not decarboxylated (splitting off CO_2) to the acetyl group (CH_3-CO). It is reduced to lactate (Figure 1). This is the endpoint of the anaerobic breakdown of glycogen in muscles postmortem.

The sequence of the breakdown of glycogen to lactate, in equilibrium with lactic acid, can be divided into three steps.

1. Muscle glycogen exists in two forms: low molecular weight proglycogen and high molecular weight macroglycogen molecules. The glycogen molecules consist of branched glucose chains (branching occurs every 4–6 glucose units). *In vivo*, most of the enzymes needed in glycogen metabolism are bound to glycogen. The degradation of glycogen to glucose units is catalyzed by the enzymes glycogen phosphorylase (GP) and glycogen debranching enzyme (GDE). The GDE breaks down the branching points of glycogen. There exist differences in GDE activity between fast twitch glycolytic muscles and slow twitch oxidative muscles. Differences in GDE activity among animal species and among different muscles of an animal also exist.

Glycogen is broken down postmortem via anaerobic pathways to lactate and results in acidification of muscles and has a great influence on meat quality. In pigs and cattle, higher GDE and GP activity exists in the fast twitch glycolytic muscles than in slow twitch oxidative muscles of the same animal. Thus, the high activity of these enzymes enables, for example, a faster rate of glycogenolysis (faster pH fall) in glycolytic M. longissimus dorsi than that in oxidative M. masseter. The relative ratios of GP and GDE activity were higher in fast twitch glycolytic muscles than in slow twitch oxidative muscles of all studied animals.

Chilling significantly decreases GDE activity and below 15 °C porcine GDE is almost inactive. Thus, the activity of GDE does not block rapid glycogenolysis with its fast pH decline when the temperature is high. This may be important in pale, soft, and exudative (PSE) meat, where the pH decreases rapidly at high temperatures, but rapid cooling could limit the activity of GDE and thus glycogenolysis. The effect of pH on GDE activity is only minor at the range normally found in postmortem muscles (pH 7.4 to 5.0). Thus, GDE is not the main factor determining the rate or the extent of postmortem glycogenolysis.



$$(Glucose)_n + ATP + 4ADP \rightarrow (glucose)_{n-1} + 2\ lactate + ADP + H_2O + 4ATP$$

Figure 1 The glycolytic pathway in muscles postmortem by the enzyme phosphorylase.

Both GDE and GP are needed for the complete degradation of glycogen. The activities of these glycogen-degrading enzymes are higher in porcine muscles than in bovine muscles. Some glycogen always remains in bovine muscles after the postmortem reaction sequence. The breaking of a glucose unit provides chemical energy or heat. GDE breaks off only a glucose unit; muscle GP forms a new chemical bond between the glucose unit and an inorganic phosphate.

$$(\text{Glucose})_n + P_i \rightarrow (\text{glucose})_{n-1} + \text{glucose-1-P} \qquad [III]$$

Energy of the glucose–glucose bond is transferred to the glucose–phosphate bond in glucose-1-P (glucose-1-phosphate). After further steps of conversion to glucose-6-P and fructose-6-P, a second phosphorylation with ATP occurs to fructose 1,6-diphosphate (Figure 1).

2. Splitting into C_3 units and energy conversion: the C_6 sugar with two phosphates, fructose 1,6-diphosphate, is split into two C_3-monophosphate units. With this splitting, energy is again released and two ATP molecules from the phosphorylation of two ADP are formed per unit of C_6-sugar.
3. Formation of lactate: finally, lactate is the endpoint of anaerobic glycolysis. This step is necessary to keep up redox equilibrium (NAD^+/NADH), where NAD, nicotinamide–adenine dinucleotide. In the breakdown from glucose to lactate, hydrogen ions are set free and the pH of muscles falls. The overall chemical reaction is shown at the bottom of Figure 1. Starting from glycogen, stochiometrically three ATP molecules are formed per glucose unit by the action of enzyme phosphorylase. If glucose, the monocarbohydrate, is used in the first step of glycolysis, it would consume one additional ATP molecule.

$$\text{Glucose} + \text{ATP} \rightarrow \text{glucose-6-P} + \text{ADP} \qquad [IV]$$

This would lead, at the endpoint of anaerobic glycolysis, to only two ATP molecules per glucose unit.

$$\text{Glucose} + 2P_i + 2\text{ADP} \rightarrow 2 \text{ lactic acid} + 2\text{ATP} \qquad [V]$$

Muscles postmortem use glycogen and GP preferentially and not glucose in anaerobic glycolysis. The free glucose in muscle cells remains unused postmortem.

With these sequences of reactions postmortem, ending in the formation of lactate/lactic acid and a fall of pH from ~7 to 5.5, glycogen is used to convert the energy in glycogen to ATP in order to maintain the necessary steady-state concentrations of ATP of 4–10 mmol kg^{-1} muscle until approximately a half of the glycogen has disappeared (pH ~ 6.3). Depending on muscle type and glycogen concentration in unstressed animals, the final pH ranges from 5.4 to 6.0 (mean ~ 5.5–5.6).

However, before glycolysis starts postmortem, CP keeps up the ATP concentration according to the following reaction.

$$\text{CP} + \text{ADP} \rightarrow \text{ATP} + \text{Creatine} \qquad [VI]$$

CP thus acts as an 'ATP buffer' in muscle cells; it amounts to 20–30 mmol kg^{-1} in resting muscles.

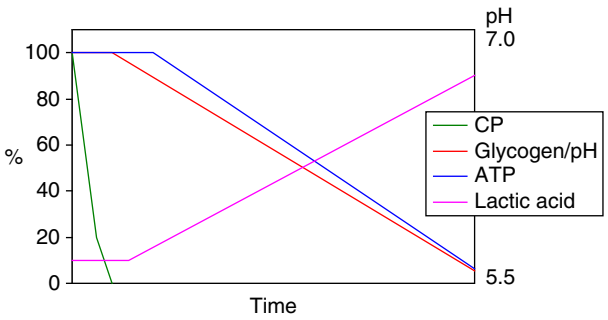


Figure 2 General postmortem changes in muscles. There are variations in concentrations and timescales among muscles and species. The concentrations at death are set to 100%.

Table 1 Timing of glycolysis in various muscles and species

Species	*Muscle*		*Time to reach pH 5.5–5.7 (h)*
Pork	Longissimus dorsi	Normal	6
		Pale, soft, and exudative	1
	Adductor	Normal	8
Chicken	Pectoralis		1.5
Beef	Longissimus dorsi		18
	Adductor		22
	Sternomandibularis		25
Lamb	Longissimus dorsi		16

Three stages of chemical change are observed during postmortem (Figure 2).

1. A period of a few minutes to 30 min after slaughter when no pH fall occurs. The ATP consumption is buffered by CP (reaction [VI]).
2. Fall of pH (from ~7.0) and lactic acid production, with initially constant ATP concentrations (1–3 h).
3. Exhaustion of ATP owing to the lack of glycogen and inactivation of glycolytic enzymes at 1–30 h after death. At the end, when no ATP is available any longer, the pH is ~5.5.

Time Course of Glycolysis

The rate of glycolysis varies among species and among muscles in a carcass. Glycolysis occurs faster in pork and poultry muscles than in beef and lamb (Table 1). In pork muscles, which are prone to PSE meat, the end of glycolysis occurs in 1 h or less (Figure 3). The basis for the various rates of glycolysis is the ATP consumption in muscle cells. Many processes need ATP as fuel: contraction, membrane transport, metabolic processes, and so on. As muscles and species, and with postmortem treatment (chilling rate and electrical stimulation), vary with regard to ATP consumption, the velocity of glycolysis varies accordingly.

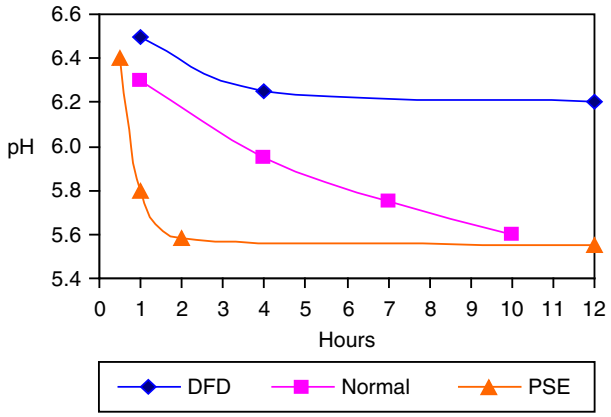


Figure 3 Postmortem changes in PSE, normal, and dark, firm, and dry (DFD) pork muscles.

Structural Changes Postmortem

ATP maintains the integrity of cellular structures, but its energy is also used for contraction – the mechanical work of muscles. The complexity of the involvement of ATP in muscle contraction is described in this article.

If ATP is exhausted, the fibrillar structures change with the formation of permanent bonds across the fibers. The muscle becomes stiff and enters into rigor mortis.

Consequences for Water Retention and Tenderness

With the fall in pH, the surface electric charge of proteins changes in the direction of equal numbers of positive and negative charges (the isoelectric point). As positive and negative charges attract each other, the water-filled myofibrillar structures shrink in volume. Immobilized water or movement-restricted water is squeezed out into the sarcoplasm, where it is much less immobilized. It is kept inside muscle cells primarily by the cellular membranes.

With the destruction of cellular membranes by enzymic (internal) or mechanical action (cutting and mincing), the 'bound' water of living muscle cells within intact cell walls is reduced and water is lost through the action of forces of gravity, pressure, and vacuum. The water-holding capacity (WHC) of meat is reduced by internal forces exerted by pH or shrinkage and disintegration of membranes as well as by external forces.

With the formation of cross-links between filaments, the meat becomes tough. The onset of rigor mortis marks the most rigid interaction in muscle and its toughest state. The rigid state is reduced by enzymic action during the aging period.

Consequences for Shelf Life and Flavor

With the fall of pH to 5.5, growth of a number of spoilage organisms is greatly slowed and meat is generally protected. Lactic acid additionally has a pleasant flavor. Furthermore, ATP that is converted to ADP is further degraded to adenosine monophosphate and from there to inosine monophosphate (IMP). IMP again has a desirable flavor. A long period of storage produces hypoxanthine from IMP via inosine. Hypoxanthine has a bitter flavor. During long storage, more and more free amino acids, such as glutamic acid, are formed from degraded proteins and influence flavor in a positive manner.

Abnormal Postmortem Changes

A serious problem for final meat quality can be created by either a very fast or an incomplete pH fall postmortem. A very fast pH fall (Figure 3) at the prevailing body temperature and above ~40–42 °C, which occurs primarily in stress-susceptible pigs, leads to PSE meat. These characteristics occur as a result of the early membrane leakage and protein denaturation, causing shrinkage of fibers.

An incomplete pH fall (e.g., not lower than 6.2, Figure 3) leads to advanced spoilage due to the high pH (less lactic acid). The reduced formation of lactic acid is due to antemortem stress that has already depleted glycogen stores. This meat is flat in taste because there is less lactic acid. It also converts IMP to hypoxanthine faster. The meat is called DFD meat in general or dark cutting beef (DCB) as less pH-induced shrinkage (higher WHC and firmer structure) leads to a darker color (no denaturation and higher oxygen binding).

Conditioning and Aging

In the period of anaerobic glycolysis during the depletion of available and usable energy compounds, the pH falls to ~5.5. This stage of chemical and structural change is called conditioning and is very responsive to outside factors in the conversion of muscle to meat.

Temperature, time, cutting, and carcass suspension influence the final quality characteristics. After the final pH is reached, the meat aging period occurs.

See also: Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Chemical Composition; Water-Holding Capacity. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycogen; Rigor Mortis, Cold, and Rigor Shortening. Modeling in Meat Science: Meat Quality. Tenderness Measurement

Further Reading

Aberle, E.D., Forrest, J.C., Gerrard, D.E., *et al.*, 2001. Principles of Meat Science, fourth ed. Dubuque, IA: Kendall/Hunt.

Addis, P.B., 1986. Poultry muscle as food. In: Bechtel, P.J. (Ed.), Muscle as Food. Orlando, FL: Academic Press, pp. 371–404.

Bendall, J.R., 1973. Post mortem changes in muscle. In: Bourne, G.H. (Ed.), The Structure and Function of Muscle, vol. 2. New York, NY: Academic Press, pp. 243–309.

Bendall, J.R., 1979. Relation between muscle pH and important biochemical parameters during post mortem changes in mammalian muscles. Meat Science 3, 143–157.

Greaser, M.L., 1986. Conversion of muscle to meat. In: Bechtel, J.P. (Ed.), Muscle as Food. Orlando, FL: Academic Press, pp. 37–102.
Hamm, R., 1977. Postmortem breakdown of ATP and glycogen in ground meat. Meat Science 1, 15.
Joo, S.T., Kauffman, R.G., Kim, B.C., Park, B.G., 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to color and water-holding capacity in porcine longissimus muscle. Meat Science 52, 291–297.
Kristensen, L., Purslow, P.P., 2001. The effect of aging on the water-holding capacity of pork: Role of cytoskeletal proteins. Meat Science 58, 17–23.
Lawrie, R.A., 1998. Lawrie's Meat Science, sixth ed. Cambridge: Woodhead Publishing.
Offer, G., Knight, P., Jeacocke, R., *et al*., 1989. The structural basis of water holding, appearance and toughness of meat and meat products. Food Microstructure 8, 151–170.
Pearson, A.M., Young, R.B., 1989. Muscle and Meat Biochemistry. San Diego, CA: Academic Press.
Purslow, P.P., Schäfer, A., Kristensen, L., *et al*., 2001. Water-holding of pork: Understanding the mechanisms. In: Proceedings of the 54th Annual Reciprocal Meat Conference, pp. 134–142. Savoy, IL: American Meat Science Association.
Secrist, J.L., Huffman, D.L., Cordray, J.C., *et al*., 1982. Restructured meat products. In: Franklin, K.R., Cross, H.R. (Eds.), Proceedings of the International Symposium on Meat Science and Technology, Lincoln, Nebraska, pp. 222–313. Chicago, IL: National Livestock and Meat Board.
Warner, R.D., Kauffman, R.G., Greaser, M.L., 1997. Muscle protein changes postmortem in relation to pork quality traits. Meat Science 45, 339–352.
Ylä-Ajos, M., 2006. Glycogen debranching enzyme activity in the muscles of meat producing animals. PhD Thesis, University of Helsinki.

Rigor Mortis, Cold, and Rigor Shortening

KO Honikel[†]

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Glossary

Actin Thin filaments of striated muscle – involved in contraction.

Actomyosin The structure formed when actin and myosin are irreversibly bound during rigor.

Contraction This is an event when the muscle cell contracts on a stimulus and then relaxes.

Contracture This is a prolonged event from which there is usually no relaxation, for example, contracture during cold shortening or rigor shortening.

Cytoskeletal proteins A group of structural proteins not involved in contraction but involved in tenderization.

Ion pump Nerve impulses and muscle contraction function by the exchange of ions through intramuscular lipid bilayer membranes by ATP-driven ion pumps.

Myosin Thick filaments of striated muscle – involved in contraction.

Rigor Occurs when a single muscle fiber runs out of ATP. Rigor mortis occurs when all fibers run out of ATP after the death of an animal. When either of these occurs the actin and myosin cross bridges become bound together causing progressive stiffness.

Sarcoplasmic reticulum The sarcoplasmic reticulum (SR) (formally sarcotubular system) is a network of intracellular tubular bilayer membrane surrounding myofibrils and invaginations of the cell membrane (T tubules). Following a nerve impulse, Ca^{2+} ions from the SR (10^{-4} M) released into the myofibrillar space ($<10^{-7}$ M) initiate a contraction of the sarcomeres. During relaxation, Ca^{2+} ions are pumped back into the SR.

Troponin and tropomyosin These are control proteins on the actin filaments that bind calcium to cause muscle contraction.

Introduction

The main constituent parts of lean meat aside from water (73–75%) and proteins (21–22%) are lipids and lipoids of cellular and subcellular membranes, amounting to approximately 1–2%. Various inorganic ions (1%), vitamins, DNA, and many other precursor compounds such as amino acids and peptides from degraded proteins exist in minor amounts. In muscle of a living animal, the polysaccharide glycogen (0.7–1%) is also present.

Muscle is composed of fiber bundles (Figure 1) that are surrounded by a collagen network, the perimysium. Bundles are an aggregation of fibers; the latter represent the muscle cells. The fibers are between 20 and 100 μm in diameter and vary in length from a few millimeters to several centimetres. The cells are surrounded by a connective tissue sheath, the endomysium, and the cell membrane, the sarcolemma. Within the cell, approximately a thousand myofibrils are arranged in the direction parallel to the long axis of the cell. The myofibrils have a diameter of 1–2 μm and run the whole length of the muscle fiber. The fibrils are composed of thick and thin filaments that are well ordered in repeating units, the so-called sarcomeres (Figure 2).

The filaments are constructed of various myofibrillar proteins. Thick filaments consist mainly of myosin, a protein with a molecular weight of approximately 520 kDa and amounting to approximately 45% of the myofibrillar proteins. Thin filaments are composed of actin (20%), troponin, and tropomyosin (5% each).

Numerous other cytoskeletal proteins are involved in the sarcomere structure. One of these proteins, with 10% of the total myofibrillar weight, is titin. Titin is a high-molecular weight protein (3.7 million Da) of a longitudinal structure running parallel to the thick and thin filaments, which titin seems to arrange in their ordered state.

Contraction takes place when thick and thin filaments slide into each other, thus shortening the length of a sarcomere, which is 2–2.5 μm long in the resting state of a muscle (Figure 2). One sarcomere can shorten by approximately 0.7 μm. It is followed by relaxation.

The filaments, as well as other subcellular structures such as sarcoplasmic reticulum (SR), mitochondria, and lysosomes, are imbedded in the fluid of the sarcoplasm. The sarcoplasm contains dissolved proteins, salts, and other low-molecular weight compounds. It is believed that approximately 20% of the water of the cell is in the sarcoplasm; the major part is located in the myofibrillar space between and within the filaments.

Muscles serve the purpose of movement. Muscles in a living organism contract in response to a nerve stimulus that is transmitted into the muscle cell via t-tubules. Within the muscle cell, the contraction is caused by Ca^{2+} ions that increase to as high as 10^{-4} mol l^{-1}, and are released from the SR and bind to the troponin of the actin filament. Contraction – relaxation is a balance between Ca^{2+}released from the SR during a stimulus to bind to troponin on the actin filament (to initiate the contraction) and then reuptake of Ca^{2+}into the SR (relaxation). In the relaxed state, the Ca^{2+} concentration around myofilaments is approximately 10^{-7} mol l^{-1} or lower. Contraction uses adenosine triphosphate (ATP) as fuel. In muscles after death, before the onset of rigor mortis at a time

[†]Deceased.

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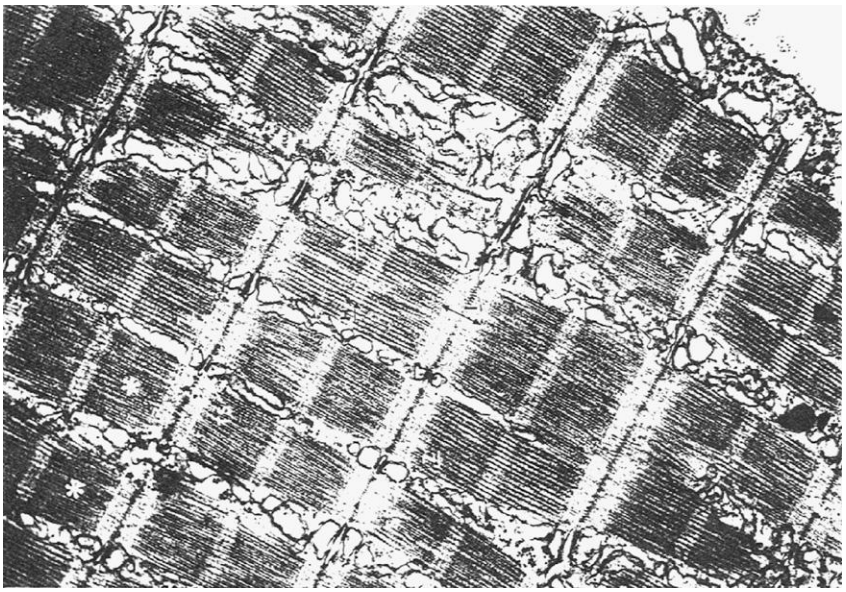

Figure 1 Electron-micrograph of a cross-striated muscle.

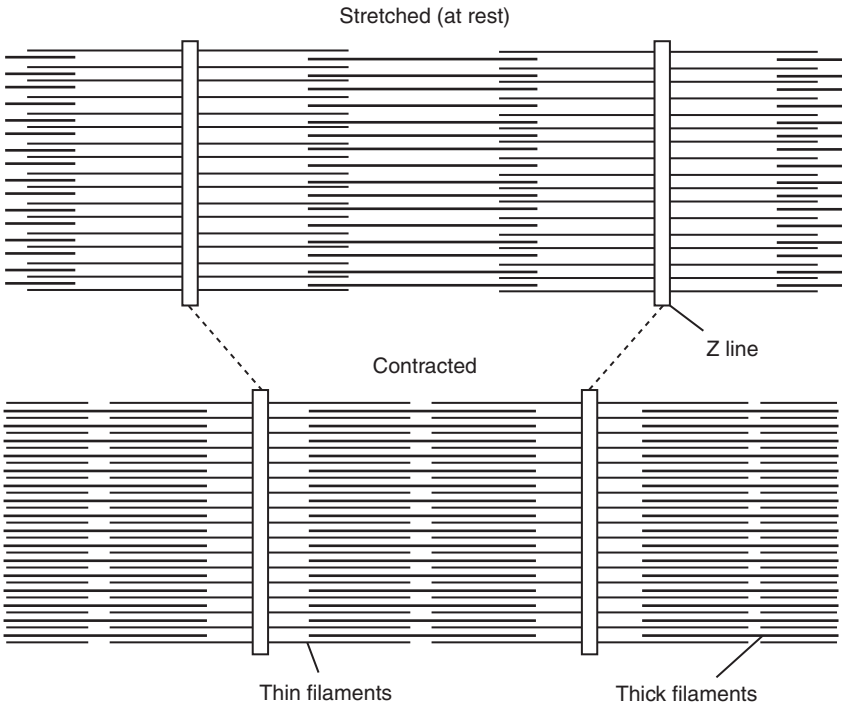

Figure 2 Schematic diagram of sarcomere structure at rest and contracted. Z-line and Z-disks are synonyms.

when ATP is still present in sufficient concentration, the myofibrils can contract if Ca^{2+} ions are released from the SR. This happens if muscles are chilled rapidly or very slowly (Figure 3). In both cases, more Ca^{2+} ions will be released and faster from the SR than the ATP-driven Ca^{2+} pump is pumping them back into the SR.

As mentioned above, on contraction the basic units of the fibrils (the sarcomeres) shorten by sliding of the thick and thin filaments into each other. As this occurs, the myofibrillar space decreases and a part of the water in the fibrils must be translocated into the sarcoplasmic space. As a contraction usually lasts only a short time in a living animal, the exchange of water from one substructure to another is rather short term and reversible. In contrast, after continuous shortening has occurred, as may be the case post mortem, the displacement of water from the myofilaments permanently increases the amount of sarcoplasmic water. This water is then no longer immobilized in and between the filaments. The effect of displacement is

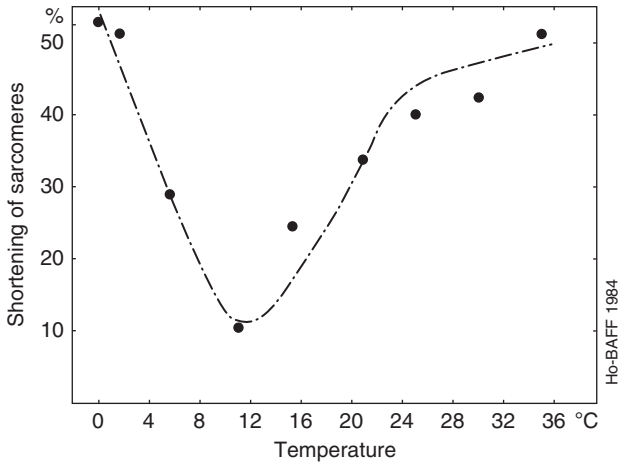

Figure 3 Influence of temperature on sarcomere shortening in the prerigor state; original sarcomere length (0% shortening) was 1.9 μm; m. longissimus dorsi of pork.

further enhanced after death as the pH falls from 7.0 to a value of approximately 5.5, causing shrinkage of myofilaments, but the still-intact cellular membrane that keeps the water as intracellular water for some time becomes permeable and water leaks out, appearing as drip at the surface.

Relaxed State of Muscles

In cross-striated muscles, from which the meat one eats comes, the filaments and fibrils are subdivided into sarcomeres limited by the *Z*-disks (Figures 1 and 2). These are the basic contractile units of the muscles. The sarcomeres are able to contract in the presence of ATP. As well as being used for contraction and the Ca^{2+} ion pump, ATP also keeps the thick and thin filaments separated (Figure 4) after the end of contraction (end of nerve impulse).

Thus, ATP has three functions in muscle contraction:

- It releases the myosin head from its position on the actin filament.
- By hydrolysis to adenosine diphosphate (ADP) plus inorganic phosphate, it releases chemical energy, which is converted to mechanical energy by 'spanning,' i.e., backward movement of the myosin head.
- At the end of a nerve impulse, the ATP-driven calcium pump transports Ca^{2+} ions into the SR, the disappearance of Ca^{2+} ions around troponin causing a positional change of the troponin/tropomyosin system and thus sterically inhibiting the binding of myosin to actin. The relaxed state of muscle is reached.

Contraction

Figure 4 shows that the cycle of contraction requires ATP as fuel to 'span' the myosin head. Contraction also requires Ca^{2+} ions, which bind to troponin to make the actin surface open for myosin binding. The 'spanned' head of myosin releases like the spring in a mouse trap after binding to actin, its mechanical energy moving the myosin head forward in relation to actin. ATP again releases the bound myosin from actin allowing relaxation.

Rigor Mortis

With the continuous production of ATP from ADP+phosphate via the anaerobic breakdown of glycogen to lactic acid/lactate, the pH falls. Owing to the pH fall, its influence on the various enzymes in the glycogenolysis pathway, and the diminishing concentration of glycogen, the resynthesis of ATP slows down. Below a certain concentration of ATP in the muscle cell the release of myosin heads from actin no longer takes place. Myosin and actin remain bound together to actomyosin (Figure 4(c)). Additionally, with the low ATP concentration the ATP-driven ion pump cannot cope with the release of Ca^{2+} ions from the SR. The onset of rigor mortis starts. Both events:

1. the binding of ATP to the myosin head (Figure 4(a)) ends and
2. the efficiency of the ion pump of the SR membrane is strongly reduced.

The time, post mortem at which these events occur is dependent on initial glycogen concentration, the temperature, and the pH as shown in Table 1 for the latter two factors in comparison to porcine and bovine muscles. The higher the temperature, the time of onset of rigor occurs earlier due to a faster turnover rate and at a higher pH value. The onset of rigor mortis is an event that eventually encompasses the whole muscle, but it occurs sequentially as each individual fiber runs out of ATP (termed rigor) depending on glycogen content in a cell. Therefore, not all possible actin–myosin cross-bridges in the muscle as a whole form at the same time. Owing to the differences between the cells the muscle can contract. The rigor contracture of a muscle in a carcass remains in the range of 10–15% shortening, whereas, as shown in Figure 3, the sarcomere length of a small excised piece of muscle produces a tension and gradually contracts much more – up to 50% of its resting length at higher temperatures.

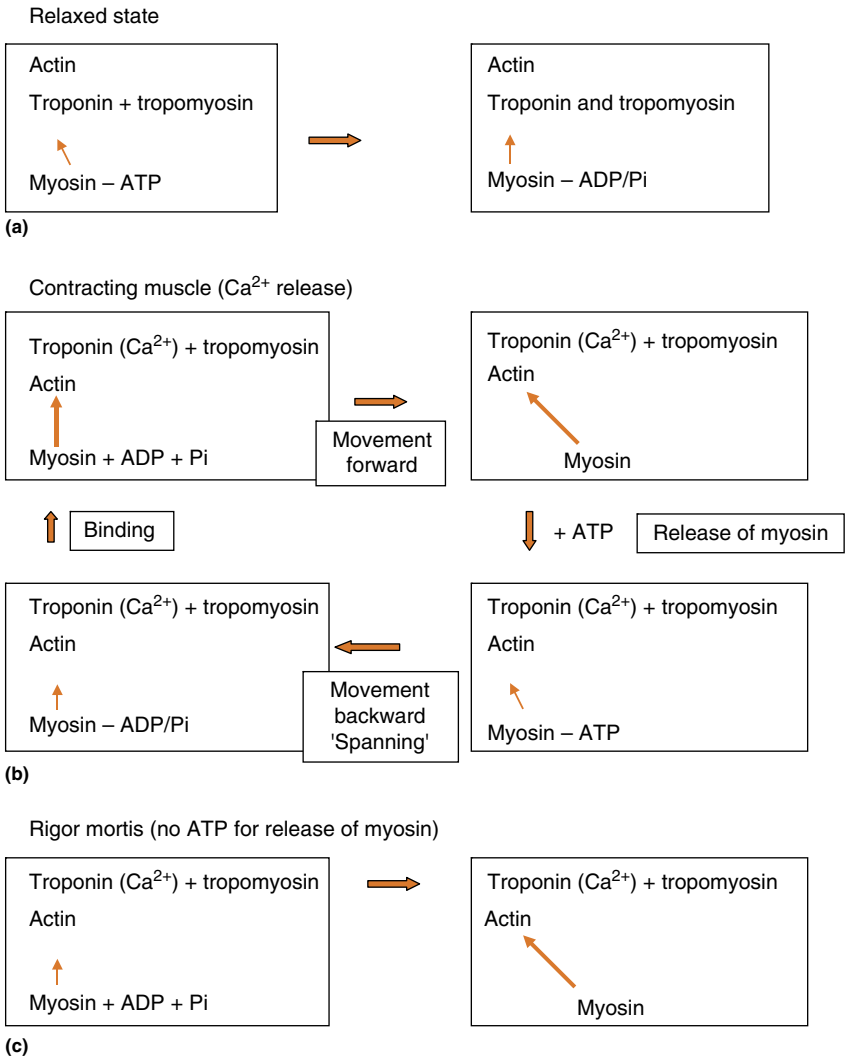

Figure 4 Schematic of the contraction cycle, relaxed state, and rigor muscle. The placing of troponin and tropomyosin, the regulating proteins, between myosin and actin symbolizes steric hindrance of the two regulatory proteins.

Table 1 Time and pH of onset of rigor mortis in two animal species at preset temperatures in m. sternomandibularis

Effect of holding temperature on rigor mortis

Species	*37 °C*				*20 °C*			
	1 h pH	*Onset of rigor mortis*		*Ultimate pH*	*1 h pH*	*Onset of rigor mortis*		*Ultimate pH*
		Time (h)	*pH*			*Time*	*pH*	
Bovine	6.85	3	6.05	5.5	6.8	9	5.85	5.5
Porcine	6.6	1.5	5.6	5.55	6.7	5	6.0	5.6

Rigor shortening takes place at the end of post mortem glycogenolysis and occurs at all temperatures. In contrast, the shortening at low temperatures called cold shortening (see Section Influence of temperature) takes places in the middle of glycogenolysis in the prerigor state. In theory, cold shortening could be reversed by enhancing the muscle temperature as enough ATP exists. Rigor shortening, the onset of rigor, and the rigor mortis itself cannot be reversed, only delayed, as they all occur due to the lack of ATP.

There are three abnormal types of rigor mortis:

1. If a muscle is frozen fast in the prerigor state, the forming of ice crystals prevents a cold shortening-like contraction. If such a frozen muscle is thawed fast or slow partially by ice

crystals disintegrated subcellular membranes like the SR release Ca^{2+} ions and an extreme contracture (<50% of the resting length of sarcomeres) occurs called thaw contracture. The final status is called thaw rigor. Not only are SR membranes disintegrated by the ice crystals but also the cellular membranes are not fully intact, and thus an extreme high and fast drip loss is observed.

2. The opposite sequence of events occurs in PSE muscles of pigs. The fast glycogen breakdown in PSE-prone muscles (pH in 45 min post mortem <5.8) is caused by a high ATP turnover by futile cycling of intermediates during the glycolytic pathway and/or the futile intention of pumping Ca^{2+} ions back via the leaky SR membrane. The latter is disintegrated by high temperatures >38 °C. The lack of ATP throughout the muscle leads to a fast onset of rigor mortis and full rigor within a short time. There is no or little rigor shortening in PSE muscles.
3. In electrically stimulated bovine muscles the pH falls within the stimulation period of a few minutes up to 0.6 pH units. During stimulation the temperature increases slightly to approximately 39 °C. This means that at pH values of approximately 6.5 and lower temperatures >37 °C prevail. The onset of rigor mortis in these stimulated muscles occurs at higher pH values (see Table 1) than in nonstimulated carcasses that are chilled during the prerigor pH fall. Thus, the onset of rigor and the development of full rigor occur fast and the rigor shortening is limited. The time within the rigor-shortening area of Figure 5 is rather short.

After full rigor mortis has occurred the binding of the myosin head to actin remains. Aging does not release the actomyosin complex. Other myofibrillar structures are involved in aging and tenderizing the meat.

It should be mentioned in this context that the addition of diphosphate (not mono- or triphosphate) can separate the myosin head and actin. However, a contraction cannot occur. But due to the release of actomyosin the myofibrillar structure can swell with the addition of water and salt. This reversal of the rigor mortis is used in sausage manufacturing for enhancing the water-holding capacity of meat batters. The pH-induced shrinkage can be overcome.

Influence of Temperature

It is well known that with lower temperatures the movement of molecules becomes slower and chemical reactions take place at reduced rates. Membrane transport is also retarded at lower temperatures. In a muscle, the membrane transport of Ca^{2+} out of and back into the SR is essential for switching muscle contraction on and off. Membranes are lipid bilayers that function in warm-blooded organisms at their normal body temperature. Lowering the temperature reduces their functionality. The membrane of the SR keeps Ca^{2+} ions at a concentration of 10^{-3}–10^{-4} mol l^{-1} inside the SR. At the myofibre level, the Ca^{2+} concentrations in the relaxed state are approximately 10^{-7} mol l^{-1}.

With a Ca^{2+} gradient of a thousand to ten thousand times concentration difference, the membrane is not totally impermeable. There is thus a small Ca^{2+} efflux into the myofibrillar space. With falling temperatures, the ATP-driven Ca^{2+} pump acting from the myofibrillar space to the SR becomes slower and the resulting efflux gradually increases. In bovine muscles at approximately 15 °C, the efflux and pump-driven influx are equal. Below 10 °C, the efflux is distinctly higher than that pumping into the SR. This means that as the Ca^{2+} concentration around the filaments increases, Ca^{2+} ions bind to troponin and, owing to the presence of ATP, a contracture occurs (Figures 3 and 4). The lower the temperature, the higher the Ca^{2+} concentration at the myofibres becomes and the more intense the contracture will be. Because of its occurrence at cold temperatures, this phenomenon is called 'cold shortening.' The degree of cold shortening is dependent on temperature but also on muscle and species. Darker muscles with more myoglobin also contain more mitochondria and are often the so-called slow-switch muscles with less SR than fast-switch lighter

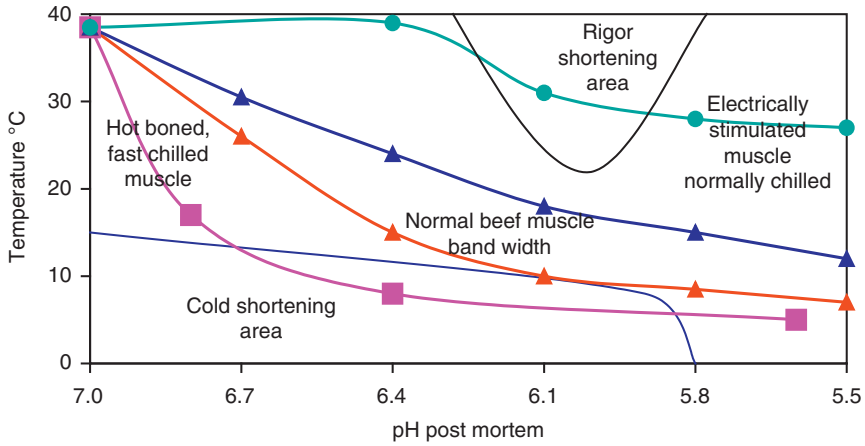

Figure 5 pH–Temperature relationship of a beef carcass chilling (band width due to different depth and metabolism of muscles and chilling regime) and the cold-shortening and rigor-shortening areas. For electrically stimulated muscles with inadequate stimulation, some rigor shortening can occur. With adequate stimulation, not only is rigor shortening insignificant but other advantages of stimulation such as faster and more complete tenderization emerge.

muscles. Mitochondria also contain Ca^{2+} ions, which are released in the anaerobic state. The presence of less SR means less effective Ca^{2+} pumping. Thus, the often darker slow-switch beef muscles start to contract below 15 °C and the lighter pork m. longissimus dorsi (fast-twitch muscle) below 7 °C.

Influence of Adenosine Triphosphate

As described in the article on glycolysis, the state of life is a metabolic equilibrium that keeps the ATP concentration in a muscle constant. An unstressed meat animal contains approximately 4–10 mmol ATP per kg of muscle at the moment of slaughter, depending on animal species and muscle type. The subsequent anaerobic glycogenolysis keeps the ATP concentration constant for some time. In addition, adenylate kinase converts the ADP that is formed back to ATP plus adenosine mono phosphate (AMP) as in eqn [1].

$$2ADP \rightarrow ATP + AMP \quad [1]$$

Postmortem, at a glycogen concentration of approximately 50% of its original value, the ATP concentration starts to fall, depending on the temperature dependence of the glycolytic enzymes as the meat is chilled. The pH falls in parallel. Below a certain ATP concentration, at temperatures below 15 °C and with falling pH, the Ca^{2+} pump in the SR membrane is unable to remove the Ca^{2+} efflux from the SR. Pumps are proteins and they are affected by pH and temperature. Whereas at 18 °C and pH 6.0 the SR Ca^{2+} pump is still effective, inactivation occurs at temperatures as high as 25 °C and below pH 6.0. A pH value of 6.0 or lower means lower ATP concentrations, but a contracture is still possible. Shortly afterwards, however, the ATP becomes exhausted and rigor mortis occurs.

Cold shortening and rigor shortening both occur for a similar reason – an increase in Ca^{2+} ions on the myofibres. Cold shortening is a prolonged contracture that occurs due to inefficiency of the Ca^{2+} pump in the SR membrane – Ca^{2+} leaks out and thus the fibers contract to produce a tension. Cold shortening commences as soon as the temperature falls below a certain value (15 °C in unfixed beef muscles, 7 °C in unfixed pork muscles, and close to 2 °C in poultry). Rigor shortening occurs gradually as each fiber contracts sequentially and late in glycgenolysis – it is completed at rigor mortis. As rigor shortening is greatest at high temperatures, a proper chilling regime prevents rigor shortening in a normal glycolysing muscle. Cold shortening can be prevented by moderate chilling or acceleration of glycolysis, example, by electrical stimulation.

Figure 5 shows a general scheme of the temperature–pH postmortem pH relationship for unstimulated beef carcasses. Normal chilling does not cause either cold shortening or rigor shortening in the temperature band between the cold-shortening and rigor-shortening areas. However, for electrically stimulated muscles, it could be expected that as the pH falls it could pass through a rigor-shortening area, and become tougher than expected, unless it was chilled rather fast. However, this does not happen in situations where it is used commercially and good electrical stimulation is used (sufficient current and duration). The paradox arises because of the sequential nature of rigor occurring in individual muscle fibers – those individual muscle fibers that enter rigor immediately or soon after electrical stimulation are protected from any further shortening. However, in some examples with relatively ineffective electrical stimulation, situations can arise where some rigor shortening could occur. Hot-boned and fast-chilled muscles may enter the cold-shortening area. Shortening in general, however it arises, is not good for the tenderness or the water-holding capacity of meat.

Influence on Tenderness

In an unshortened muscle, the sarcomeres are between 1.8 and 2.0 μm long. Overstretched muscles, such as the fillet in a carcass, can be up to 2.5 μm. With the onset of rigor mortis, because of the enhancement of Ca^{2+} concentration and the pH-driven shrinkage of filaments, the sarcomere length is reduced to approximately 1.6–1.8 μm. This represents approximately 10–15% shortening. Cold-shortened and rigor-shortened muscle cells are reduced by up to 50%. Even after aging shortened muscle exhibits enhanced shear forces (Figure 6). The parallelism between Figure 3 and Figure 6 is evident.

Influence on Water-Holding Capacity

As already noted, the water in the muscle cells (~75% by weight) is bound primarily within the filamental structure. The mobility of this water is retarded and it is referred to as immobilized water. Furthermore, the cellular membrane with its lipid bilayers keeps the water inside the cells. Even after changes within the myofibrillar structures when water is moved to the sarcoplasm it stays for some time within the intact cell wall.

With falling pH, postmortem and/or shortening of sarcomeres, the fibrillar structure shrinks and water moves to the sarcoplasm. This water is fairly freely mobile, but as mentioned above it is initially kept inside the cell. With ongoing aging, however, the water passes through the membrane into the extracellular space collecting between the cells. Whereas in muscle of a live animal at approximately pH 7 the filaments take up most of the muscle space, with postmortem shrinkage

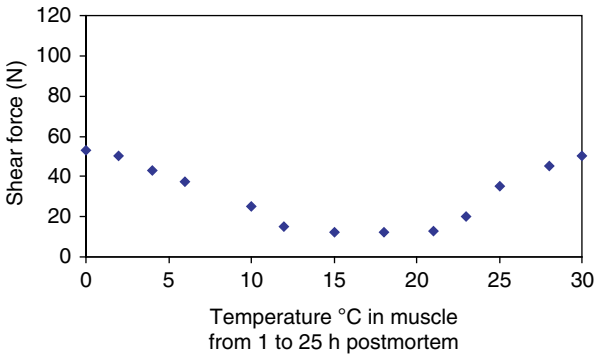

Figure 6 Shear force (N) in beef m. longissimus dorsi muscle pieces kept unrestrained at the temperatures indicated from 1–25 h postmortem; subsequent ageing was at 5 °C for 2 weeks.

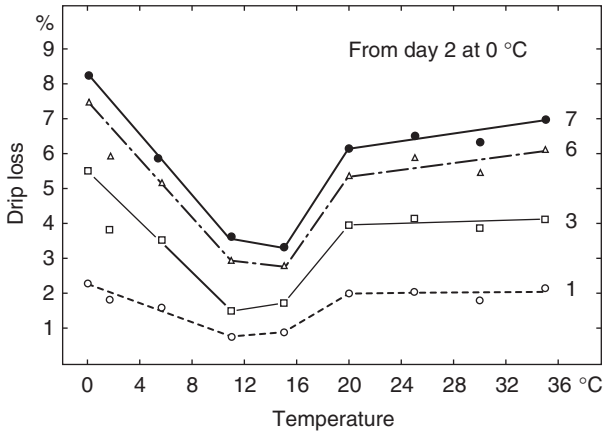

Figure 7 Relationship between temperature of incubation during the first 24 h post mortem of m. cleidomastoideus and drip loss of muscle cubes (about 30 g) after storage for 1, 3, 6, and 7 days postmortem. From day 2 the samples were stored at 0 °C.

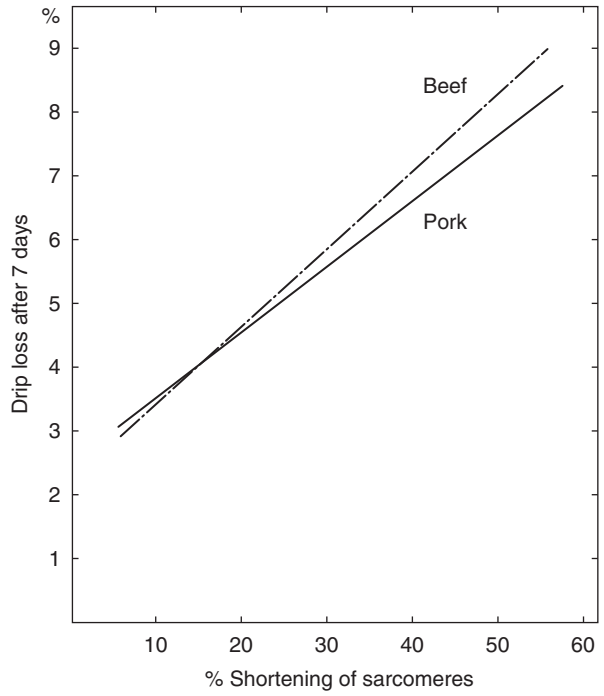

Figure 8 Relationship of cold shortening and rigor shortening on drip loss at 7 days post mortem. At 1–24 h the muscles were kept between 0 °C and 32 °C. All muscles were stored at 2 °C from day 1 to 7 in beef and pork: m. longissimus dorsi.

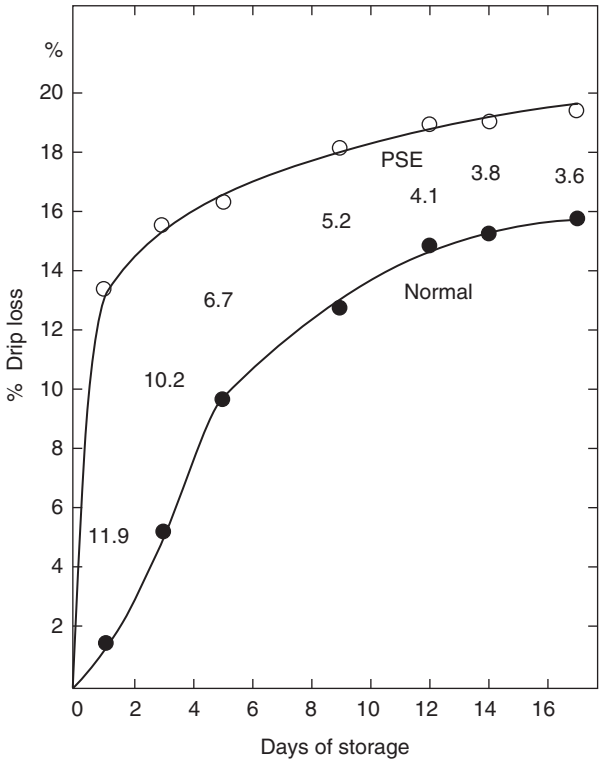

Figure 9 Drip loss from the m. longissimus dorsi of a pig carcass stored at 0 °C; the same muscle was taken for both experiments with $pH_1 = 5.8$; PSE meat was produced by slow chilling (25 °C air up to 120 min postmortem); normal meat by rapid chilling (1 h postmortem in a 20 °C water bath); the figures between the curves represent the difference in drip.

of the fibers the intracellular sarcoplasmic space increases and the extracellular space fills with water. In a muscle at approximately pH 7, more than 95% of the water is within cells; some days postmortem, approximately 15% is in the extracellular space. From these capillary-like channels, the water appears as drip at the surface of the meat. All kinds of meat undergoing a pH fall from approximately 7.0 to 5.5 lose drip water. Drip loss commences at 1 day post mortem following rigor mortis and increases with time of storage (Figure 7). DFD (dark, firm, dry) meat with a high ultimate pH (>6.2) loses less drip.

If in addition to the pH-induced shrinkage a contraction occurs as shown in Figure 2, as mentioned above, the space of the contracted sarcomeres is further reduced. This means that even more water is forced from the fibrillar structures and consequently enhances drip during storage.

In cold-shortened and rigor-shortened muscle, the drip loss increases (Figure 7). This is evident in Figure 8. In beef and pork, the shortening has a linear relationship to drip loss at day 7.

In addition to pH-induced fiber shrinkage, the cellular membrane plays a role in drip loss. In muscle prone to the PSE (pale, soft, exudative) condition, the high temperatures shortly after slaughter (40–42 °C) and the already low pH lead to early membrane destruction. In PSE muscles, drip loss occurs with virtually no time lag (Figure 9). The strongest increase occurs in the first 2 days. This seems to be a minor issue in beef and lamb.

In cold-shortened and rigor-shortened muscles there is a close relationship between shortening on the one hand and shear force and drip loss on the other, as can be seen by comparison of Figures 3, 6, 7, and 8. Rigor mortis is unavoidable, but shortening can be avoided.

See also: Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: pH Measurement; Water-Holding Capacity. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycogen; Glycolysis. Electrical Stimulation. Muscle Fiber Types and Meat Quality

Further Reading

Bendall, J.R., 1975. Cold contracture and ATP-turnover in red and white musculature of the pig, post-mortem. Journal of the Science of Food and Agriculture 26, 57–71.

Bendall, J.R., Restall, D.J., 1983. The cooking of single myofibres, small myofibre bundles, and muscle strips of beef *M. psoas* and *M. sternomandibularis* of varying heating rates and temperatures. Meat Science 8, 93–117.

Davey, C.L., Gilbert, K.V., 1976. Thaw contracture and disappearance of adenosine triphosphate in frozen lamb. Journal of the Science of Food and Agriculture 27, 1085–1092.

Fennema, O.R., 1977. Water and protein hydration. In: Whitaker, J.R., Tannenbaum, S.R. (Eds.), Food Proteins. Westport, CT: AVI, pp. 50–90.

Hamm, R., 1960. Biochemistry of meat hydration. Advances in Food Research 10, 355–463.

Hamm, R., 1986. Functional properties of the myofibrillar system and their measurement. In: Bechtel, P.J. (Ed.), Muscle as Food. New York: Academic Press, pp. 135–199.

Honikel, K.O., 1987. Influence of chilling on meat quality attributes of fast glycolysing pork muscles. In: Tarrant, P.V., Eikelenboom, G., Monin, G. (Eds.), Evaluation and Control of Meat Quality in Pigs. Dordrecht: Martinus Nijhoff, pp. 273–283.

Honikel, K.O., Kim, C.J., 1985. Über die Ursachen der Entstehung von PSE-Schweinefleisch. Fleischwirtschaft 65, 1125–1131.

Honikel, K.O., Kim, C.J., Hamm, R., Roncales, P., 1986. Sarcomere shortening and its influence on drip loss. Meat Science 16, 267–282.

Honikel, K.O., Roncales, P., Hamm, R., 1983. The influence of temperature on shortening and rigor onset in beef muscle. Meat Science 8, 221–241.

Jeacocke, R.E., 1984. The kinetics of rigor onset in beef muscles fibres. Meat Science 11, 237–251.

Penny, J.F., 1977. The effect of temperature on drip, denaturation and extracellular space of pork longissimus dorsi muscle. Journal of the Science of Food and Agriculture 28, 329–338.

Purslow, P.P., Schäfer, A., Kristensen, L. *et al.*, 2001. Water-holding of pork: understanding the mechanisms. In: Proceedings of the 54th Annual Reciprocal Meat Conference, pp. 134–141. Indianapolis, IN. American Meat Science Association, Savoy, IL.

Riedel, L., 1961. Zum Problem des gebundenen Wassers im Fleisch. Kältetechnik 13, 122–128.

Robson, R.M., Huiatt, T.W., 1983. Roles of cytoskeletal proteins, desmin, titin and nebulin in muscle. In: Proceedings of the 36th Annual Reciprocal Meat Conference, Knoxville, IN, pp. 116–124. American Meat Science Association, Savoy, IL.

Relevant Websites

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter10/animation__breakdown_of_atp_and_cross-bridge_movement_during_muscle_contraction.html
Human Anotomy.

http://people.eku.edu/ritchisong/301notes3.htm
Human Physiology.

http://www.banyantree.org/jsale/actinmyosin/index.html
Jeff Sale.

http://www.teachpe.com/anatomy/sliding_filament.php
Teach PE.

Slaughter-Line Operation and Pig Meat Quality

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Glossary

Blood splash The presence of blood in the muscle leading to increasing meat trimming.
Carcass dressing Different operations leading to smooth skin surface.
Lairage Animal handling facilities at abattoirs, including pens, waterpoints, and shower.
On farm handling Operation of gathering the animals before loading them in the truck for transportation to the abattoir.
Stunning A method to render animal unconscious.

Introduction

The pig slaughtering process includes a number of operations, each of which has an effect on meat quality: transportation, lairage, driving the pigs to stunning, stunning, exsanguination, dressing, and chilling the carcasses. Over the past few decades, huge progress has been made with respect to mechanization and hygiene. However, in developed countries, meat processors, consumers, and authorities alike have all continuously increased their quality requirements, while at the same time, legislation aimed at protecting animals has been considerably strengthened. These trends have sparked abundant research, which has resulted in recommendations designed to optimize meat and carcass quality plus animal welfare.

Stress and Meat Quality

Many of the major traits governing pork quality, including technological and sensory qualities, are affected by animal conditions at slaughter. The slaughter process subjects the animals to a number of aversive situations, i.e., situations they perceive as aggressive, which induce psychological and physical stress. Reactions include various physiological and metabolic changes, the most important being increased blood 'stress hormones' (catecholamines and corticoids) and increased body temperature. High body temperature at death accelerates postmortem metabolism, thus favoring the occurrence of pale, soft, and exudative (PSE) meat. Adrenaline (one of the catecholamines) lowers muscle glycogen levels, which can increase meat pH and tends to prevent PSE. Preslaughter stress thus has contrasting effects on pork quality. Prolonging animal exposure to aversive conditions before slaughter will tend to increase meat pH, which improves meat quality, as long as the pH stays under a threshold pH value corresponding to dry, firm, and dark (DFD) meat. Intense stress applied shortly before or during slaughter tends to accelerate muscle metabolism and increase muscle temperature, giving rise to accelerated postmortem muscle acidification which favors PSE meat.

Beyond physical quality, the concept of ethical quality is becoming increasingly paramount for consumers. Ethical quality is related to production systems that are more or less environmentally friendly and socially acceptable. Animal welfare is one of the main components of ethical slaughter conditions.

Transport from the Farm to the Abattoir

In industrialized countries, most animals are transported from the farm to a specialized preslaughter facility called lairage. The abattoir will generally anticipate a lairage time to serve as a buffer between the animal's arrival and slaughter, to ensure a smooth and consistent workflow rate.

On-Farm Handling and Loading

The transfer of pigs from the farm to the abattoir is difficult for both workers and the animals. The animals are suddenly confronted with a situation that is new to them: the change of environment, mixing with unknown animals, noise, etc. Furthermore, pigs have to be fasted before transportation, because slaughtering pigs while they have a full digestive tract makes evisceration more difficult: full stomachs often get pierced, so their content can contaminate the carcass. Moreover, fasting has two important positive effects: it reduces mortality during transport and tends to increase meat pH by reducing muscle glycogen content. Fasting pigs for more than 24 h would result in significant carcass weight loss. To avoid such a negative effect, it is sometimes recommended that the last feed should be given 18–24 h before slaughter. However, there is no general consensus on the ideal duration of fasting before slaughter, and Danish statutory requirements, for example, state that pigs must be fed and watered if they are kept more than 12 h in lairage in order to meet animal welfare requirements.

On the farm, a loading area is recommended. If the premises are not correctly designed, driving animals to trucks can be prove difficult and lead to rough handling by the personnel. Brutal handling must be avoided, not only for ethical but also economic reasons. In fact, beyond the potentially harmful effects on meat quality, rough handling can result in carcass defects such as bruising, scratches, and lacerations that can ultimately justify rejection of carcass cuts, especially for dry-curing ham. Use of electric goads must be avoided as far as

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possible. Blows with rubber tubes and, even worse, blows with metal or wooden objects must be absolutely banned. A well designed loading area: (1) allows solely those animals sorted for slaughter to be fasted, thus avoiding negative effects on the growth of the remaining pigs; (2) facilitates loading of the pigs into trucks, thereby diminishing the stress factors leading to carcass and meat quality defects; and (3) decreases the sanitary risk tied to allowing transport staff to enter the farm. Loading the pigs is made easier by the use of elevator floors. If ramps are used for access to trucks, their slope should be less than 20°.

The pigs should be led from the fattening pens into the loading area in small groups, preferably 4–6 h after their last meal. If possible, mixing animals from different fattening pens should be avoided during the waiting period and throughout transport so as not to disturb the established social hierarchy, which would result in fighting and aggressive activity. Fighting causes skin damage and bruising, as well as muscle glycogen loss that can result in DFD meat. Manual or automatic showering is desirable, especially during hot weather, in order to calm and refresh the pigs. The pigs should have access to water, as dehydration is a source of decrease in carcass weight and is particularly a risk when the temperature is high.

Transportation

Trucks used for transport must be covered, effectively ventilated and equipped with nonskid floors to prevent the animals slipping and falling, which can result in bruising, dislocations, and even fractures. To avoid high levels of mortality, the temperature should not exceed 30 °C inside the vehicles to keep within the pigs' thermal neutrality range, although this requirement is difficult to meet in many countries. Where high temperatures are unavoidable, showering can help. Loading density is important from both economic and animal welfare perspectives. When density is increased, transport cost decreases, whereas aggressiveness and consequently fighting, skin damage, and rectal prolapses increase with density. The loading density recommended in the European Union is 235 kg m^{-2} for animals of 90–100 kg body weight. In fact density should be adapted according to ambient temperature, and should be decreased when temperature increases. In Canada, the recommended densities are as follows: 294 kg m^{-2} at a temperature lower than 16 °C, 263 kg m^{-2} between 16 and 23 °C, and 244 kg m^{-2} above 23 °C. There is continued debate over the effects of loading density on meat quality traits. For short journeys, the effect is minor, but dependent on genetic type. For long journeys (more than 36 h), there are some reports that meat pH is increased.

Transport duration and driving conditions have effects on meat quality. The effects of short transportation are minor if conditions are correct (smooth driving, efficient ventilation, and loading density not too high). Frequency of PSE meat decreases and frequency of DFD meat increases with transport time, as a consequence of the muscle glycogenolysis activated by increased blood adrenaline, physical activity and fatigue. There is little experimental data on the effects of long transport times. Feeding pigs during long transport reduces body weight loss but has little effect on meat pH. European Union guidelines recommend that when transport duration is exceeds 24 h, the pigs should be unloaded, fed, and rested for 24 h. However, some specialists consider that unloading and reloading is undesirable as it sparks muscle activity and excitation, making it preferable to feed and rest the pigs inside the vehicle.

The recommendations for unloading the pigs on arrival at the abattoir are much the same as those given above for loading at the farm. The unloading area should be well illuminated, the ramps and the off-loading area should be flat or have a positive slope, the corridors should be wide enough to allow several pigs to walk abreast, be straight or have a long radius of curvature, and open into pens that can be divided by mobile partitions or inner doors. Brutal handling should again be prevented, and as far as possible, pigs originating from different fattening pens should not be allowed to mix.

Lairage at the Abattoir

Pigs must spend a minimum time in lairage in the abattoir so as to minimize meat quality defects. Slaughter immediately after transport can result in an increase in the frequency of PSE meat (Table 1). This is partly attributed to the increase in body temperature that occurs during transport, which can be reduced during resting in the lairage. Lairage at the abattoir should not have to take more than a few hours if the recommendations given above for loading and transportation are followed. In fact, lairage provides the opportunity for further aggressive interactions that activate muscle glycogenolysis and can ultimately lead to DFD meat. The optimal lairage time is 2–4 h. It is consequently necessary to provide a lairage capacity of at least six times the number of pigs killed per hour. It is advisable to shower the pigs during lairage if the ambient temperature is higher than 15 °C. This accelerates the return of body temperature to normal resting values, and generally calms the animals. Showering is carried out using fixed and preferably programmable facilities allowing the showering period to be broken down into stages; for instance, pigs in lairage for 2 h seem to prefer several short showers rather than one long continuous shower.

Table 1 Frequency of PSE and DFD meat in relation to lairage period. PSE was evaluated during cutting the day after slaughter. DFD is defined as a pH value in at least one muscle >6.5 or pH value in two or three muscles >6.1 on the day after slaughter

Time in lairage (h)	*Number of pigs*	*PSE (%)*	*DFD (%)*	*Total defects (%)*
–0	175	13.1	3.4	14.5
–2	174	7.5	10.3	17.8
–4	177	4.0	6.2	10.2
–24	81	2.5	7.4	9.9

Source: Adapted from Nielsen, N.J., 1981. Porcine Stress and Meat Quality – Causes and Possible Solutions to the Problems. Proceedings of EEC congress, pp. 287–297. Bruxelles.

Slaughtering

Stunning and Exsanguination

Driving pigs to stunning constitutes a stress factor with potentially marked effects on meat quality, as it occurs immediately before slaughter. The path from lairage area to stunning must allow several pigs to walk abreast in order to respect their herd instinct and avoid unnecessary fear and excitement. It must be well illuminated and, if possible, be straight with solid walls. In most cases, the pigs are led in single file to a restraining conveyor which introduces them into the stunning machine. Time spent in the conveyor should be as short as possible, as it is a cause of excitement. In the most modern stunning facilities, restraining conveyors are replaced by straddle conveyors that support animals under the sternum and belly and are less aversive for the pigs. In some carbon dioxide gas stunning facilities, pigs are allowed to enter the machine in small groups (five to six animals). It has been reported that group stunning considerably reduces excitement and consequently PSE frequency, whereas other studies have found no effect on occurrence of PSE but higher skin lacerations.

Stunning of meat animals is mandatory in industrialized countries. This obligation aims to:

- Reduce animal suffering.
- Make the work easier and thus allow higher workflow rates.
- Improve worker safety.

Modern abattoirs rely on two main stunning methods: electrical and gas stunning (e.g., CO_2). Electrical stunning is most widespread, but gas stunning is making headway. Electrical stunning can be carried out with a low-voltage (70–90 V) electrical current, which needs a 10–15 s application for efficient immobilization. However, it does not guarantee complete insensitivity, making it unacceptable from an animal welfare standpoint. Higher voltages (250–270 V) can reduce application times down to1 s or less. High-voltage stunning is preferred on slaughter lines with high workflow rates, but it entails full automation of the stunning system (restraining, electrode positioning, application of current, and animal ejection) to ensure staff safety. If the electrical current is applied through the head only, most pigs survive and may recover consciousness if exsanguination is delayed. Current can be applied head-to-back or head-and-heart to allow flows through the heart, which gives rise to cardiac fibrillation leading to irreversible cardiac arrest. In the most perfected automated systems, optimal positioning (ear-to-ear) of the main electrodes is obtained by video-image control.

Gas stunning is achieved by immersing the pigs in an air–carbon dioxide mixture (generally 65–80% carbon dioxide) until they lose consciousness. This stunning method has been criticized as less humane than electrical stunning due to the long time required for loss of consciousness, i.e., several seconds for the gas stunning. The real level of discomfort generated by gas stunning has been a matter of much debate, and needs to be fully elucidated. However, gas stunning also presents some advantage from the animal welfare point of view: it enables the pigs to be handled in groups, and avoids the risk of misplacement of the stunning electrodes.

Electrical stunning can generate violent muscle contractions and convulsions, which generate blood spots, blood splashes and even fractures, often associated with extended hemorrhaging, which requires trimming consequently resulting in financial losses. Shoulder fractures are frequent when pigs are electrically stunned while standing, and their prevalence is considerably reduced by using a restraining device. Vertebral fractures are observed when applying current head-to-back. In this case, there is relatively little hemorrhage due to the cardiac arrest, which stops blood circulation. Blood splashes are reportedly less with high voltages, and also occur less frequently when using straddle conveyors, in which pigs are not pressed on their sides as with retraining conveyors (Table 2). To minimize blood splashes, the time interval between stunning and exsanguinations should be kept as short as possible, preferably at less than 10 s. This is made possible by horizontal bleeding, where the pig is ejected from the stunning machine directly onto the bleeding table and shackling and hanging take place after, rather than before, bleeding. Horizontal bleeding also yields better meat quality, as the pigs struggle less, thus reducing the rate of postmortem muscle acidification. Moreover, with vertical bleeding, the muscles of the leg by which the pig is suspended are subject to strong constraints and show a faster pH drop than the muscles in the free leg. Vertical bleeding is, however, preferable in terms of the bacteriological quality of the blood, provided that sticking is done with a hollow knife.

Fractures and hemorrhages are relatively rare with gas stunning (Table 2), which is another factor explaining why more and more slaughter plants are implementing this method.

Carcass Dressing

In most countries, the first step in carcass dressing is dehairing. The most common carcass dressing procedure involves

Table 2 Frequency of blood spots in deboned pig loins according to stunning method quality

Stunning conditions	*♯ Abattoirs*	*Class 1 (%)*	*Class 2 (%)*	*Class 3 (%)*
High voltage, high rate, restraining conveyor	5	51.6–54.5	29.0–42.0	4.0–18.8
High voltage, medium rate, restraining conveyor	4	38.0–61.5	27.2–48.0	1.9–23.3
Medium voltage, high rate, straddle conveyor	2	70.5–90.7	9.3–27.4	0–2.1
Carbon dioxide stunning	1	85.0	14.5	0.5

Note: Class 1: absence of blood spots; Class 2: superficial blood spots visible; and Class 3: blood spot needing trimming.

Source: Reproduced from Griot, B., Boulard, J., Chevillon, P., Kerisit, R., 2000. Réduire les points de sang dans la viande de porc: des restrainers à bande pour le bien-être et la qualité de la viande. Viande et Produits Carnés 21, 91–97.

Table 3 Effect of chilling regime on pork

Chilling regime[a]	*Cycle time (h)*			
	Nominal	*Drip*	*Paleness*	*Toughness*
−40 °C, 1 m s^{-1} for 80 min, then conventional	24	Unchanged	Unchanged	Increased
−20 °C, <0.5 m s^{-1} for 2 h, then conventional	24	Unchanged	Decreased	Unchanged
−20 °C, <0.5 m s^{-1} for 3 h, then conventional	24	Unchanged	Decreased	Unchanged
2 °C, 1.5 deceasing to 0.4 m s^{-1}, 99% RH	24	Unchanged	Unchanged	Unchanged
4 °C, 0.3 m s^{-1} with spray	24	Unchanged	Unchanged	Unchanged
−30 °C, 1 m s^{-1} for 4 h	4	Increased	Unchanged	Increased

[a]Conventional: 4 °C, 0.5 ms^{-1}, 90% RH.

Source: Reproduced from Tarrant, P.V., 1989. The effects of handling, transport, slaughter and chilling on meat quality and yield in pigs. Irish Journal of Food Science and Technology 13, 79–107.

successive scalding, dehairing, singeing, and polishing operations. Dehairing can be performed by singeing and scratching without previous scalding, but it results in appearance defects such as burst skin and a less smooth skin surface. Two processes can be used for scalding, which is carried out at a temperature of 60–65 °C: immersing the carcass in hot water for a few minutes, or steam-scalding. Even though the skin surface is submitted to high temperatures, there is no noticeable increase in deep muscle temperature during dehairing, and consequently this operation has little effect on meat quality other than cooking at the sticking wound and possibly entry of bacteria into the sticking wound. In some East-European and Far-East countries, where the pig leather industry is active, pigs are dehided. This method (dehiding) is better from both microbiological and meat quality perspectives. Chilling is more efficient, resulting in a darker color and lower drip. The economic interest in dehiding depends on whether there are markets for the hide to yield good value. A disadvantage of dehiding is that it is not compatible with dry ham production.

Chilling

Carcasses are chilled in order to increase the shelf-life of the meat before further processing or consumption. Chilling not only prevents growth of the microorganisms present on the carcasses after slaughter, but also has marked favorable effects on cutting yield, as it reduces postmortem muscle acidification rate and can thus improve water-holding capacity and color intensity. However, these effects generally remain little (Table 3) due to the rapidity of postmortem biochemical changes in pig muscle: in most cases, carcass temperature cannot be dropped rapidly enough to prevent protein denaturation. Fast chilling is particularly unable to prevent PSE in carcasses from halothane-sensitive pigs or from pigs that were heavily stressed at slaughter. Furthermore, fast chilling can result in increased meat toughness due to cold shortening. This effect is more marked when the postmortem pH drop is slow. Electrical stimulation has been proposed as a way to avoid cold-toughening, but research carried out in Denmark concluded that stimulation is not economically attractive as it generally leads to a deterioration in meat quality, i.e., increased drip loss and higher frequency of PSE meat. However the electrical stimulation can be used if it is associated with the appropriate chilling method.

See also: By-Products: Edible, for Human Consumption. Chemical and Physical Characteristics of Meat: Protein Functionality. Conversion of Muscle to Meat: Glycolysis. Cooking of Meat: Cooking of Meat. Electrical Stimulation. Functional Foods. Human Nutrition: Macronutrients in Meat; Micronutrients in Meat. Nutrient Claims on Packaging. Preslaughter Handling: Preslaughter Handling. Stunning: CO_2 and Other Gases; Electrical Stunning

Further Reading

Backstrom, L., Kauffman, R., 1995. The porcine stress syndrome – a review of genetics, environmental-factors, and animal well-being implications. Agri-Practice 16, 24–30.

Barton Gade, P., 1996. Meat quality. In: Teverner, M.R., Dunkin, A.C. (Eds.), World Animal Science – Pig Production. Amsterdam: Elsevier, pp. 17–39.

Casteels, M., Vanoeckel, M.J., Boschaerts, L., Spincemaille, G., Boucque, C., 1995. The relationship between carcass, meat and eating quality of 3 pig genotypes. Meat Science 40, 253–269.

Channon, H., Payne, A.M., Warner, R.D., 2000. Halothane genotype, pre slaughter handling and stunning method all influence pork quality. Meat Science 56, 291–299.

Faucitano, L., 1998. Preslaughter stressors effects on pork: A review. Journal of Muscle Foods 9, 293–303.

Fernandez, X., Tornberg, E., 1991. A review of the causes of variation in muscle glycogen content and ultimate pH in pigs. Journal of Muscle Foods 2, 209–235.

Gregory, N.G., Grandin, T., 1998. Animal Welfare and Meat Science. Oxford: CAB International

Hambrecht, E., Eissen, J.J., Nooijen, R.I.J., *et al.*, 2004. Preslaughter stress and muscle energy largely determine pork quality at two commercial processing plants. Journal of Animal Science 82, 1401–1409.

Taylor, A.A., Tantikov, M.Z., 1992. Effect of different electrical stimulation and chilling treatments on pork quality. Meat Science 31, 381–395.

Troeger, K., 1991. Slaughtering – animal protection and meat quality – current practice-what need to be done. Fleischwirtschaft 71, 298–302.

Van der Wal, P.G., 1978. Chemical and physiological aspects of pig stunning in relation to meat quality. A review. Meat Science 2, 19–30.

Warriss, P.D., Brown, S.N., Nute, G.R., *et al.*, 1995. Potential interactions between the effects of preslaughter stress and postmortem electrical-stimulation of the carcasses on meat quality in pigs. Meat Science 41, 55–68.

Relevant Websites

www.inspection.gc.ca
Canadian Food Inspection Agency.

https://www.gov.uk/animal-welfare
Department for Environment, Food & Rural Affairs.

COOKING OF MEAT

Contents

Cooking of Meat

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Glossary

Actin One of the main myofibrillar proteins of meat.
Denaturation A change in the tertiary structure of proteins.
Maillard reactions A cascade of chemical reactions taking place when meat is heated. These are responsible for the fried, roasted flavor of meat.
Myoglobin The pigment protein of meat.
Myosin One of the main myofibrillar proteins of meat.

Introduction

With the exception of specially dried and fermented products, meat is generally cooked before consumption. Biochemical and physical changes occur during the heating process and these changes affect microbiological quality and sensory characteristics. Furthermore, the heating of meat results in better digestibility and, to some extent, in a change of the nutritive value.

The method of cooking is one of the major factors that affects the eating quality of meat. Meat is cooked using different media for heat transfer, such as dry heat methods (roasting, broiling, or panfrying), moist heat methods (boiling or braising), or microwave cooking (electromagnetic energy). Sometimes a combination of dry and moist heat methods is used. The cooking method chosen should be appropriate to the type of meat, the amount of connective tissue, and the shape and size of the meat. In studies and trials, it must be clear whether the cooking method is to be used for experimental purposes or for common consumer practice. For experimental purposes, the method must be standardized and controlled and must not overshadow the effects of the treatment. If it is to be used by consumers, the method should result in a good eating quality.

Changes in Meat during Heating

During cooking, the core temperature of the meat increases from approximately 0 °C to as much as 85 °C. The surface temperature of the meat can be very high – up to 300 °C – depending on the cooking method. The increase in temperature results in a tremendous change in both the structure and the water distribution in the meat. Water is lost as cooking loss, fat melts and drips out, and the texture and flavor change (Figure 1).

The cooking loss starts to develop around 40 °C. In meat with low pH (below 5.4 for pork), cooking loss begins as low as around 30 °C. The rate of development of cooking loss is greatest between 50 and 70 °C, after which it falls again.

These changes in cooking loss can be explained by the changes in the meat structure. In a nuclear magnetic resonance study, a shift in the water populations was seen at 46 °C: the water within the myofibrils diminished, whereas the water in the intermyofibrillar space increased. The sarcoplasmic proteins, α-actinin and myosin, begin to denature in the temperature interval from 40 to 50 °C, and a transverse shrinkage of the myofibrils begins at 45 °C. This can explain the change in water distribution and the initiation of the cooking loss. In the temperature interval from 50 to 60 °C, where the rate of the cooking loss is largest, sarcomere length decreases and

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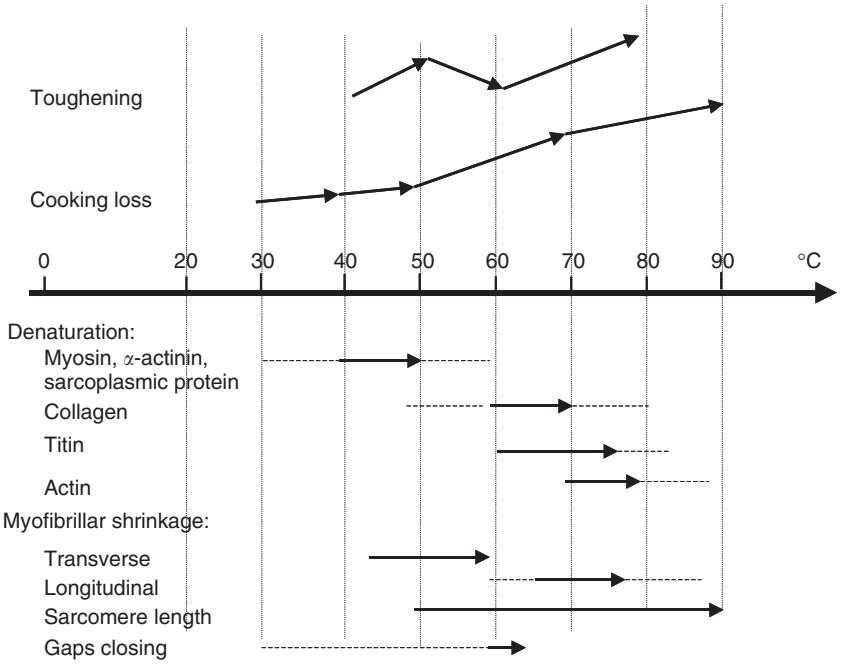

Figure 1 Changes in meat during heating.

collagen starts to denature. At 60 °C, the gaps between the fibers are closed and a parallel shrinkage of the myofibrils begins. This might be due to water being expelled from the meat matrix with a high concentration of proteins. The decreasing sarcomere length and the shrinkage of the myofibrils continue with further heating, and it is not understood why the rate of cooking loss decreases.

Myoglobin begins to denature around 60 °C. The denaturation of myoglobin is responsible for the change in color from a raw meat appearance to a cooked meat appearance. The maximum precipitation of myoglobin pigment occurs in the range 60–67 °C. The heat-induced color change of the meat also depends on the oxidative status of the myoglobin before cooking.

In general, the denaturation of myosin and actin results in toughening of the meat. In a model system, the toughness of a whole muscle increases up to 50 °C, followed by a decrease to approximately 60 °C; with further temperature rise, the meat becomes tougher again. The first rise in toughness can be explained by denaturation of myosin. The decrease in toughness between 50 and 60 °C is most likely due to a partial denaturation and shrinkage of the collagen fibers in the intramuscular connective tissue. From 60 °C, the cytoskeletal protein titin begins to denature and later, at 70–80 °C, actin also denatures, which can explain the second increase in toughening. At high temperatures, above 75 °C, the collagen reaches a soluble gelatinized state. In muscles rich in connective tissue, softening of the texture is, therefore, seen at very high core temperatures. Collagen can also be solubilized at a lower temperature when given enough time and can be aided by moist heat cooking.

The Heating Process in Meat

The rate of heating in meat depends on the coefficient of conductivity in meat and the surface temperature of meat. The surface temperature of meat is affected by the temperature of the heating source (e.g., oven temperature) as well as the air circulation and the relative humidity. Increasing air circulation improves heat conduction and increases the evaporation from the surface of meat, whereas high humidity improves heat conduction but reduces evaporation. The heat absorbed in meat causes a temperature rise by heat conduction through meat from the surface to the center. The rate of temperature rise in meat is different at different depths of a meat cut (roast). Cooking to a certain core temperature in the center of roasts produces meat with layers of different doneness, depending on the heating method and temperature. A low heating temperature (<100 °C) yields a more homogeneous appearance and less distinct layers of doneness compared with traditional oven roasting at 160–200 °C. Figure 2 indicates two different temperature profiles: the low heating temperature gives a slow temperature rise in meat compared with the higher heating temperature.

Main Cooking Methods

Conventional cooking methods use conduction, convection, and radiation as media for heat transfer, whereas unconventional methods use energy supplied in other forms, such as microwaves. Three main factors differ among the various cooking techniques: the temperature at the surface of meat, the temperature profile through meat, and the method of heat transfer (contact, air, water, steam, or microwaves). Cooking of meat often involves more than one type of heat transfer. When selecting a cooking method, attention should be paid to the type of meat involved. Muscles with a high content of connective tissue normally yield less tender meat; accordingly, a moist heating method, such as braising or

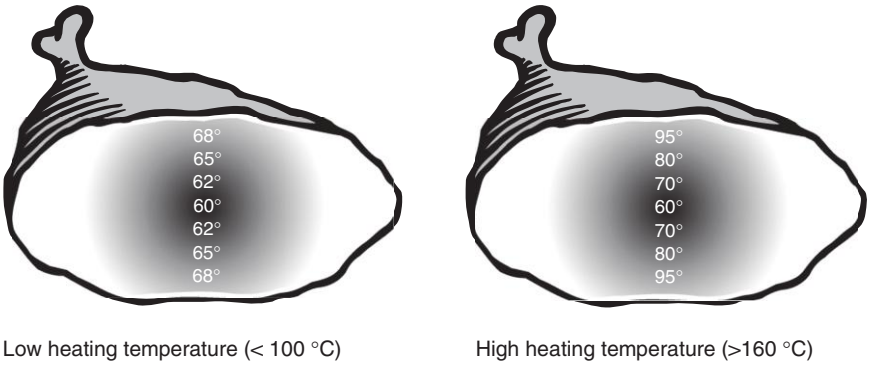

Figure 2 Illustration of temperature profiles in roasts cooked at low and high temperatures.

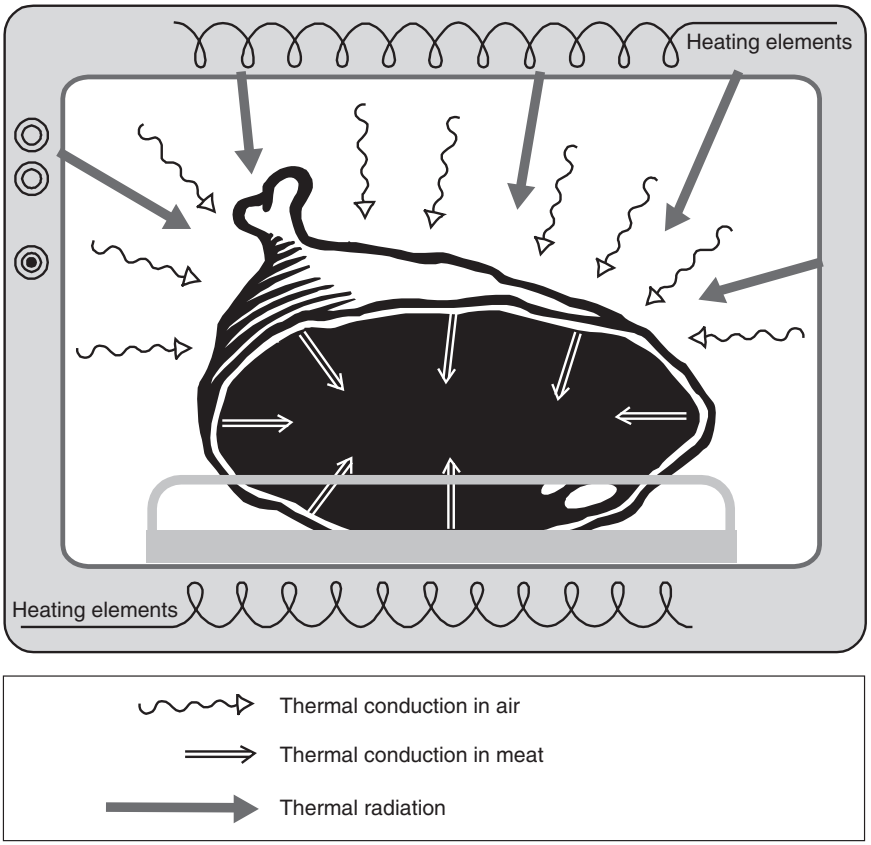

Figure 3 Illustration of heat transfer to a roast when cooked in an oven.

cooking in liquid (stewing or boiling) or cooking at a low temperature for a long time, is recommended. Dry heat methods, such as roasting, broiling, and panfrying, are used for cooking muscles with a low content of connective tissue.

Roasting

In roasting, transfer of heat is accomplished by a combination of conduction, convection, and radiation (Figure 3). The heat is transmitted to meat by normal or forced air convection (high-velocity air) in a closed oven, often preheated. Large meat pieces are placed on a rack in a roasting pan for even circulation of heat around meat. Normally, meat is not covered and is not turned during cooking. The rate of heat transfer in an oven depends on temperature and air velocity; a forced air convection oven produces a faster temperature rise than a conventional oven. Oven roasting can apply the temperature in two different ways: a constant oven cooking temperature at approximately 150–160 °C or roasting at a high temperature of up to 250 °C followed by a lower temperature of approximately 150 °C until the required core temperature is obtained. The use of the constant oven temperature of 150–160 °C throughout the cooking period results in lower cooking loss compared with a high starting temperature. The constant temperature method also ensures that the surface of meat becomes brown due to the Maillard reaction; this method is recommended for tender meat

with a low content of connective tissue. If the cooking condition is changed to a combination of convection and steam, the method can be used for less tender meat.

Broiling

Broiling is a dry heat method in which meat is cooked using direct radiant heat. The heat source may be an oven broiler, an electric broiler, or an outdoor grill, with the meat placed either above or below the heat source. The heat radiates from one direction, so meat must be turned during cooking. The method is used for cuts with a low content of connective tissue, such as steaks, chops, and patties. The cooking time is short.

Low-Temperature Cooking

Low-temperature cooking uses a constant oven cooking temperature below 100 °C, resulting in a very slow heating profile. The meat is placed in the oven as for the roasting method. Compared with normal oven roasting at 150–160 °C, the cooking time is two to three times longer, and because of the low temperature, minimal Maillard reactions occur on the surface and the appearance is like that of boiled meat. Prebrowning is, therefore, recommended to improve appearance. This method is useful for meat with a high content of connective tissue or for cuts of meat containing different muscles that differ in collagen content. 'Prime rib' is prepared by cooking at a low temperature for more than 8 h. The method can be combined with steam and is often used by the catering sector and for institutional food service.

Pan Broiling/Panfrying

Pan broiling and panfrying are methods by which small, thin cuts, such as chops, steaks, or patties, are cooked by direct heat conduction. Heat is transmitted by contact between the pan and the meat. Meat is placed in a preheated, uncovered frying pan and cooked with or without added fat. The meat should be turned frequently. The cooking time is relatively short because of the high frying temperature, and the meat surface becomes brown because of the Maillard reaction. This quick method is not recommended for meat with high connective tissue content because the tenderizing effect of converting collagen into gelatin cannot be accomplished. This is a method commonly used by consumers.

Braising/Casseroling

These are moist cooking methods, especially used for meat with a high content of connective tissue where maximum tenderization is required. The meat is often prebrowned and then placed in a covered pan to which some liquid, often a small amount of water, is added. The meat is cooked slowly in the moist atmosphere, and the maximum temperature reached is not higher than 100 °C.

Boiling/Stewing/Water Bath

These are methods of cooking in liquid and therefore constitute moist cooking methods like braising. The meat is placed in a pan and covered with water. Normally, the meat is not prebrowned. As the heat exchange medium is water, the maximum temperature reached is not higher than 100 °C. The method is used for meat with low as well as high content of connective tissue. If cooking is prolonged, meat with high connective tissue content will become tender because the collagen dissolves. Meat reaches the final endpoint temperature faster when cooked in water than when cooked in air of equal temperature, because the specific heat of water is higher than that of air. For experimental and research purposes, cooking in a regulated water bath is often used. Small meat samples are sealed in plastic bags and immersed in the water bath with a set temperature below 100 °C.

Microwave Cooking

Microwave cooking is a very popular method, especially for defrosting and reheating precooked meat. The principle of microwave cooking is conversion of electromagnetic energy into thermal energy within meat. During cooking, microwave energy is absorbed by rotation of water molecules and translation of ionic components in meat; the water content and the dissolved ion content are, therefore, important factors. In practice, meat is placed in a container suitable for microwave cooking, covered with a film wrap or a suitable lid, and then cooked in the microwave oven. Cooking time depends on the cooking rate, i.e., the power output (watts). Total cooking time can be decreased by one-third to one-half of that in conventional cooking in an oven. Weight of meat mass, shape, composition, and temperature before cooking are factors that influence the duration of microwave cooking. A problem with microwave cooking is that the surface of the meat does not brown because no Maillard reactions occur owing to the relatively low meat surface temperature and the low temperature of the surrounding air. If the microwave oven is supplemented with another heat source, such as convection, browning of the surface occurs. Other methods used for browning of the surface include the use of a special browning dish or a special metallic film that is responsible for some of the microwave energy being absorbed and converted into heat. Another problem with microwave cooking is uneven heating; for this reason the method is not recommended for meat with a high content of connective tissue, because the tenderizing effect of converting collagen into gelatin is not achievable within the short cooking time. Compared with conventional cooking methods, microwave cooking often results in a greater cooking loss and decreased tenderness, but this depends on the microwave setting (power output).

Sous Vide

The sous vide method for preparation of meat was introduced in the 1970s. It is used for industrial production of meat or meals for the food service industry or for consumers through retail sale. The meat is vacuum packed and cooked at a temperature < 100 °C using precisely controlled heating and then rapidly cooled. The meat is reheated after a period of chilled storage. The advantage of using vacuum packaging before cooking is the prevention of evaporative losses during cooking

and elimination of oxidation during storage. Owing to the low temperature, the sous vide method has a positive effect on tenderness compared with conventional oven cooking and results in an easily controlled and uniform doneness of meat. Sous vide cooking reduces vegetative bacteria to a safe level in a combination of time and temperature. Extended cooking time increases collagen solubility.

Other Cooking Methods

Belt Grill Cooking

With belt grilling, meat is cooked by conduction on both sides simultaneously. Heat transmission is affected by contact between meat and heat source. Meat is conveyed between preheated plates or belts without added fat and does not have to be turned. The cooking time is relatively short owing to the high temperature.

Clamshell Grilling

A clamshell grill is a cheaper domestic cooker using the same principle as a belt grill cooker.

Temperature Control and Timetable

When cooking meat, it is important to control the core temperature or the endpoint temperature to achieve the ideal degree of doneness. A timetable is only a guideline for an approximate cooking time. Variation in cooking time depends on cooking method, cooking equipment, size, and shape of the meat cut as well as fat and bone content. Boneless meat cooks more quickly than meat with bones. It is important that control of the core temperature in the meat is precise. This means inserting the thermocouple of the thermometer into the geometric center of the meat without touching either bone or fat. With low-temperature cooking, it is easier to measure the internal temperature exactly because of the slow rise of temperature rise in meat (Figure 2).

Resting Period after Cooking

When the meat has been cooked and removed from the heating medium, the core temperature continues to increase, because the cooking process goes on inside meat. The final endpoint temperature after the resting period depends on the cooking method, the cooking temperature, and whether or not the meat is covered with foil. In general, the higher the temperature of the heating medium, the larger is the residual heat and the higher is the final temperature after resting. Postcooking temperature rise is greater for microwave cooking. The resting period is often 15–30 min. The reason for recommending a resting period, as described in many cookery books, is that the juice in the meat can redistribute and the meat remains juicy after being cut into slices. However, a recent study has reported that pork and beef remain juicy even when being cut into slices immediately after cooking.

Effects of Cooking Methods on the Eating Quality of Meat

It is well known that different cooking methods, core temperatures, and types of muscles result in different eating qualities. Three main factors differ depending on cooking method: the temperature at the surface of meat, the temperature profile through meat, and the method of heat transfer. The temperature at the surface is important for the color, odor, and flavor of meat. Temperature gradient influences the rate and extent of the changes of protein structure in the meat, whereas the method of heat transfer influences the odor, flavor, and color. In general, optimal tenderness and juiciness and minimum cooking loss in meat are achieved when it is cooked at moderate to low temperatures. With respect to odor and flavor, higher temperatures yield different flavor perceptions compared with low cooking temperatures. Numerous studies have indicated a poorer eating quality in meat with increasing internal temperature.

Effect of Cooking on Color

The color of meat is a combination of the amount of myoglobin and the reflectance of protein. Raw meat has a bright pink or red color that depends on the nature and composition of meat (see Changes in Meat during Heating). Color is influenced by heat treatment (cooking method) and endpoint temperature. Increase in endpoint temperature increases the brown color and decreases the pink color. Dry heat methods, especially panfrying, influence the surface color: a brownish color is achieved compared with the moist heating methods where no browning effect is seen.

Effect of Cooking on Odor and Flavor

Flavor is a combination of taste and aroma. Taste is a sensation related to the tongue, whereas aroma is a sensation of volatile compounds related to the epithelia of the nose. Flavor comprises a combination of nonvolatile and volatile compounds. Odor and flavor of raw meat are bland, weak, and blood like. When meat is heated, several odors and flavors are produced through heat-induced changes in amino acids, carbohydrates, and fat. Many types of heat-induced reactions result in the production of meat flavors. Amino acids and reducing sugars react when heated above 110 °C; this thermally induced reaction is called the Maillard reaction and is important in developing meat flavor. The Maillard reaction is influenced by the method of heat transfer. Dry heat methods, especially panfrying, increase the amount of Maillard reaction and moist heat methods prevent the reactions from taking place. A similar effect is achieved using roasting bags when cooking meat in an oven. In addition to the Maillard reaction, lipid degradation products are responsible for developing meat flavor during heating. The lipid degradation reactions take place at a much lower temperature than the Maillard reaction and the flavoring compounds can, therefore, be produced not only on the surface of meat but throughout meat. Lipid-derived flavor compounds are very important for the meaty

flavor and are said to be responsible for the species-specific flavor.

Effect of Cooking on Texture

The texture of meat determines the feeling in the mouth perceived during chewing. When meat is introduced into the mouth, the structure is intact. During chewing, the meat structure is broken down and soaked in saliva until it is in a state suitable for swallowing. The attributes related to texture can be divided into three groups:

- Attributes relating to the breaking-down process (hardness, tenderness, etc.)
- Attributes relating to the structure during chewing (fibrousness, crumbliness, and elasticity)
- Attributes relating to the end of chewing (chewing time and chewing rest).

In general, these attributes are highly correlated, but they can, to some extent, also vary independently between cuts, depending on the cooking method.

Changes in the texture of meat during cooking are due to heat-induced structural changes combined with enzymatic breakdown of proteins. The effect of the time/temperature factor and the core temperature depends on the composition of meat. The M. biceps femoris (BF, outer hind leg muscle) has a high content of connective tissue. It gains more in tenderness when heated slowly than the M. longissimus dorsi (LD, loin), which is low in connective tissue (Figure 4).

The effect of core temperature on tenderness depends both on the meat cut (content of connective tissue) and the heating rate. In BF, the tenderness decreases when the core temperature increases from 65 to 75 °C (Figure 5). As meat is slowly heated further up to 80 °C, connective tissue begins to soften and gelatinize and BF becomes more tender. LD, however, becomes less tender when the core temperature increases from 60 to 80 °C, probably owing to the denaturation of actin and myofibrillar toughening. Heating at low temperatures in an oven increases the overall tenderness of the meat compared with the use of medium and high oven temperatures (Figure 6). The effect is largest at 60 °C core temperature and decreases up to 80 °C core temperature. In selecting the optimal core temperature during cooking, attention must be paid to both the muscle and the cooking method. Cooking at temperatures below 100 °C for a long time increases tenderness of meat, but too low temperature does not increase tenderness. Cooking at 58 °C resulted in more tender meat compared with that at 53 °C for

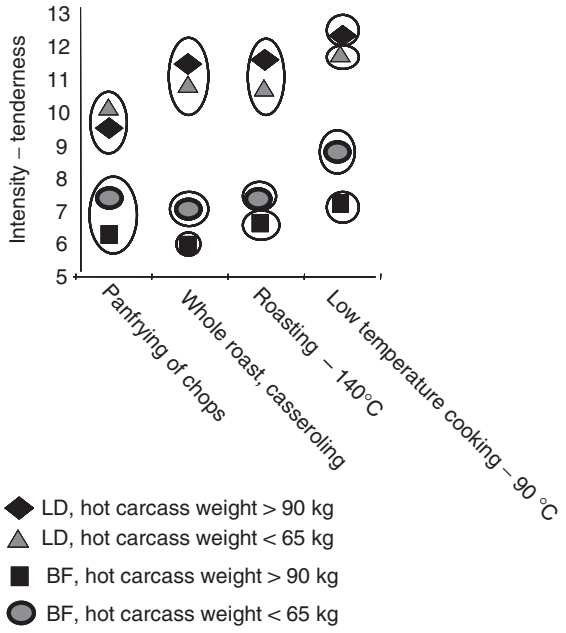

Figure 4 The effect of cooking method on the tenderness of a pork muscle with a high content of connective tissue compared with one with low content of connective tissue. Different symbols enclosed in the same circle indicate significant differences ($p < .05$). LD, M. longissimus dorsi; BF, M. biceps femoris. Tenderness is expressed in a continuous line scale from 0 to 15 (high intensity). Data plotted from Bejerholm, C., Aaslyng, M.D., 2003. The influence of cooking technique and core temperature on results of sensory analysis of pork – Depending on raw meat quality. Food Quality and Preference 15, 19–30.

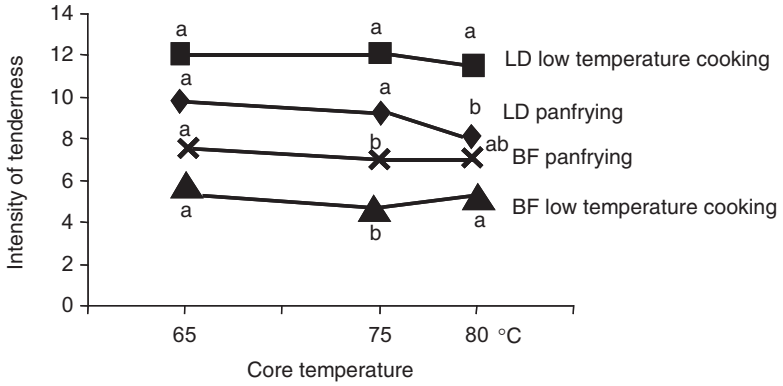

Figure 5 The effect of core temperature on pork tenderness, depending on muscle and cooking procedure. Different letters on the same line indicate significant differences ($p < .05$). LD: M. longissimus dorsi, BF: M. biceps femoris. Tenderness is expressed on a 9-point scale from 0 (extremely tough) to 9 (extremely tender). Modified with permission from Bejerholm, C., Aaslyng, M.D., 2003. The influence of cooking technique and core temperature on results of sensory analysis of pork – Depending on raw meat quality. Food Quality and Preference 15, 19–30.

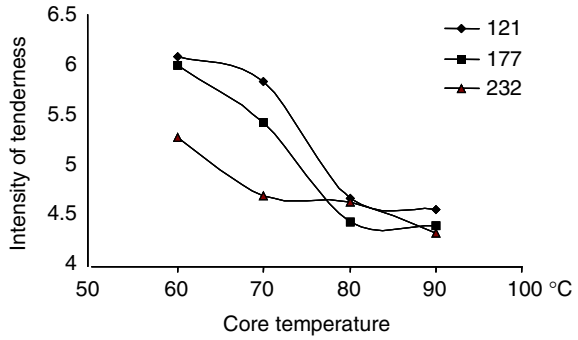

Figure 6 The effect of core temperature on the tenderness of beef M. longissimus dorsi, depending on oven temperature. Tenderness is expressed on a 9-point scale from 0 (extremely tough) to 9 (extremely tender). Data plotted from Cross, H.R., Stansfield, M.S., Koch, E.J., 1976. Beef palatability as affected by cooking rate and final internal temperature. Journal of Animal Science 43 (1), 114–121.

both beef and pork, but meat at both temperatures was very tender for chicken. No difference was seen in tenderness between 58 and 63 °C cooking temperature.

The effect of core temperature on juiciness depends more on the cooking method than on the amount of connective tissue in meat. Cooking loss is generally larger when roasting than when panfrying because of the longer cooking time. Increasing the oven temperature results in less juicy meat at the same core temperature. At 65 °C core temperature, there is no difference between roasting and panfrying. With increasing core temperature, the decrease in juiciness is faster when meat is cooked in an oven compared with panfrying, irrespective of the cut.

Effect of Cooking on Weight Loss

Cooking loss increases as core temperature increases (see Changes in Meat during Heating; Figure 1). The actual amount of cooking loss depends on the cooking method and the amount of connective tissue in meat. Cooking methods with a very short cooking time, such as panfrying, result in a lower cooking loss than conventional oven cooking methods at the same core temperature. The correlation between cooking time and cooking loss is not linear, as cooking loss is determined by a combination of cooking time and heating rate. Low-temperature cooking results in lower cooking loss compared with cooking at conventional temperatures. Compared with broiling, roasting results in a lower cooking loss owing to the gentler heating. Cooking loss also differs among cuts. Cuts with a high amount of connective tissue have a higher cooking loss than those with a lower amount of connective tissue. The higher the core temperature, the smaller the difference is between muscle types. Moreover, at 80 °C or more, only minor differences exist among cooking methods and cuts with different amounts of connective tissue.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment; Palatability. Cooking of Meat: Flavor Development; Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor. Human Nutrition: Macronutrients in Meat; Micronutrients in Meat. Muscle Fiber Types and Meat Quality. Sensory and Meat Quality, Optimization of. Sensory Assessment of Meat

Further Reading

Anonymous, 1995. Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat. Centennial, CO: American Meat Science Association and National Live Stock and Meat Board (The National Cattlemen's Beef Association).

Bejerholm, C., Aaslyng, M.D., 2003. The influence of cooking technique and core temperature on results of sensory analysis of pork – Depending on raw meat quality. Food Quality and Preference 15, 19–30.

Christensen, L., Ertbjerg, P., Aaslyng, M.D., Christensen, M., 2011. Effect of prolonged heat treatment from 48 °C to 63 °C on toughness, cooking loss and color of pork. Meat Science 88, 280–285.

Christensen, L., Gunvig, A., Tørngren, M.A., *et al.*, 2011. Sensory characteristics of meat cooked for prolonged times at low temperature. Meat Science 90, 485–489.

Cross, H.R., Stansfield, M.S., Koch, E.J., 1976. Beef palatability as affected by cooking rate and final internal temperature. Journal of Animal Science 43 (1), 114–121.

Ghazala, S., 1998. Sensory and nutritional aspects of sous vide processed foods. In: Ghazala, S. (Ed.), Sous Vide and Cook-chill Processing for the Food Industry. Gaithersburg: Aspen Publishers, pp. 57–88.

Kropf, D., Bowers, J., 1997. Heat-induced changes in meat. In: Bowers, J. (Ed.), Food Theory and Applications, second ed. New York, NY: Macmillan Publishing Co, pp. 615–641.

Laakkonen, E., 1973. Factors affecting tenderness during heating of meat. Advances in Food Research 20, 257–314.

Lawrence, T.E., King, D.A., Obuz, E., Yancey, E.J., Dikeman, M.E., 2000. Evaluation of electric belt grill, forced-air convection oven and electric broiler cookery methods for beef tenderness research. Meat Science 58, 230–246.

Lawrie, R.A., 1998. The eating quality of meat. In: Lawrie, R.A. (Ed.), Lawrie's Meat Science, sixth ed. Cambridge: Woodhead Publishing, pp. 212–257.

Martens, H., Stabursvik, E., Martens, M., 1982. Texture and colour changes in meat during cooking related to thermal denaturation of muscle protein. Journal of Texture Studies 13, 291–309.

Obuz, E., Dikeman, M.E., Grobbel, J.P., Stephens, J.W., Loughin, T.M., 2003a. Endpoint temperature, cooking method and marbling degree have different effects on Warner–Bratzler shear force of beef longissimus lumborum, biceps femoris, and deep pectoralis muscles. In: Proceedings of the 49th International Congress of Meat Science and Technology, pp. 171–172. Campinas, Brazil: Instituto de Tecnologia de Alime.

Obuz, E., Dikeman, M.E., Loughin, T.M., 2003b. Effects of cooking method, reheating, holding time and holding temperature on beef longissimus lumborum and biceps femoris tenderness. Meat Science 65, 841–851.

Resurreccion, A.V.A., 1994. Cookery of muscle foods. In: Kinsman, D.M., Kotula, A. W., Breidenstein, B.C. (Eds.), Muscle Foods: Meat, Poultry and Seafood Technology. London: Chapman and Hall, pp. 406–429.

Seideman, S.C., Durland, P.R., 1984. The effect of cookery on muscle proteins and meat palatability: A review. Journal of Food Quality 6, 291–314.

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Flavor Development

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Introduction

Flavor is an important sensory characteristic that contributes to the overall palatability of muscle foods. Qualitatively and quantitatively, the flavors associated with cooked meats have proven to be extremely difficult to characterize for both the sensory analyst and the flavor chemist. This is because the sensory impression of meat flavor is influenced not only by compounds contributing to the sensation of taste but more importantly also by those stimulating the olfactory organ. To complicate matters, there is no one single compound or class of compounds that are accountable for meat flavor. Numerous constituents resulting from a myriad of reactions during thermal processing of meat are responsible for the flavor one associates with each animal species. Ultimately, it is the chemical composition of fresh meat that gives the basis for development of a desirable aroma during thermal processing.

The Precursors of Meat Flavor

The meat matrix is very complex: its macroconstituents include water, proteins, and lipids, whereas its microconstituents include vitamins (notably B vitamins), peptides, sugars (e.g., ribose), nucleotides, and their metabolites. Raw meat itself does not have much flavor: it has a slight serum-like odor and a blood-like taste. During postmortem aging, hydrolytic activity generates a reservoir of precursor molecules, reactive flavor chemicals, and intermediates, which during thermal processing react/degrade to give the desirable taste tactile properties and aroma characteristics associated with finished meat products. For the generation of meat flavor to occur, meat and meat products must be heated/cooked/thermally processed. Thermal processing induces a complex network of chemical reactions among the nonvolatile components of lean and fatty tissues present in meat and yields a heterogeneous system containing many volatile compounds with odoriferous properties, smaller nonvolatile compounds with taste properties, as well as flavor potentiators and synergists. The type of thermal processing employed, such as grilling, roasting, broiling, stewing, boiling, or smoking, and the final internal temperature of the product contribute significantly to the formation and stability of both volatile and nonvolatile compounds and are, therefore, related, at least to some extent, to the differences in the overall meat flavor sensation.

The present working theory is that aroma volatiles are derived from nonvolatile precursors in the lean and fatty tissues of meat during cooking. These precursors can be further subdivided into water- and lipid-soluble components. Basically, meat aroma is the composite sensation of low molecular weight products of the Maillard reaction (i.e., a nonenzymatic browning reaction between amino groups, such as those associated with amino acids, and with a carbonyl group of a reducing sugar) as well as the thermal melting and oxidative degradation of lipids. Lipids would also more likely participate in the Maillard reaction, as the carbonyls produced from lipid oxidation can replace the carbonyls on reducing sugars. These oxidation products can produce desirable flavors such as those in fried foods, but some lipid oxidation reactions often result in negative flavors. A detailed discussion on the role of the Maillard reaction, as it relates to meat flavor development, is discussed in another article.

A delicate balance exists between the aroma volatiles generated from Maillard and lipid reactions as well as their interaction with one another and molecular oxygen. It is this balance that dictates whether the desirable meaty/savory, fatty flavor or warmed-over or off-flavors will result. Although the odor threshold values (OTVs) of sulfur- and nitrogen-containing heterocyclic compounds, which contribute to meaty/savory notes, are lower than those of lipid-derived volatiles, changes in meat flavor are the result of a dynamic process. With time, the levels of lipid-derived compounds increase and eventually overwhelm the desirable meaty notes of products, even though many of these compounds' OTVs are several degrees of magnitude greater than those of the heterocyclic compounds. Such changes to the perception of meat flavor make its characterization difficult.

Makeup of Meat Flavor: Taste and Odor

Flavor is a wide sweeping term that encompasses not only the taste and aroma associated with cooked meat but also the intangibles like texture, mouthfeel, and temperature sensation. The human tongue has millions of gustatory receptors in which one uses to identify taste. There are only five recognized classes of taste: sweet, sour, salty, bitter, and umami (i.e., a Japanese term for a fifth basic taste that is triggered by some amino acids, peptides, and their salts, notably monosodium glutamate). Umami is difficult to translate, but some equivalent English terms are savory, essence, pungent, deliciousness, and meaty. However, it is the volatile constituents of meat that account for the dominant sensory phenomenon that one refers to as flavor.

To date, more than 1000 compounds have been identified in the aroma profiles of cooked meat products. The volatiles are a combination of low molecular weight products from the degradation of amino acids and lipids via oxidation as well as further reaction products from these aforementioned degradation compounds. The resulting products include a broad array of compounds from varying classes of chemicals: these

include hydrocarbons, aldehydes, alcohols, ketones, carboxylic acids, ethers, esters, lactones, and *S*-, *N*-, and *O*-containing heterocyclic compounds. Some examples are provided in Figure 1.

There is a simplified theory: it states that the basic flavor of cooked meat is similar to all species, that is, flavors derived from protein breakdown (e.g., Maillard reaction) and from the formation of heterocyclic compounds such as pyrazines, pyrroles, thiazoles, oxazolines, thiolanes, thiophenes, and furans are similar. The species-specific flavors come from the lipid constituents of meat. It is the melting and/or oxidation of lipid constituents that contributes toward the species-specific flavors identified in meat products. It is now known that lipid oxidation products interact with Maillard reaction products to generate new flavors associated with cooked meat. So, the foregoing theory is an oversimplification of what actually happens in terms of meat flavor generation; nevertheless, it provides a good basis for discussion.

Sources of Heat-Induced Meat Flavor

The Decomposition of Individual Substances

The degradation of mono- and oligosaccharides to yield volatile compounds involves temperatures greater than those generally associated with the cooking of meat. Nevertheless, some decomposition of simple sugars to furanones and furfurals can occur during cooking/heating/thermal processing of meat. Amino acids tend to be more stable and unlikely to undergo pyrolysis. Only along the surface of grilled or roasted meat, where localized dehydration allows the temperature to

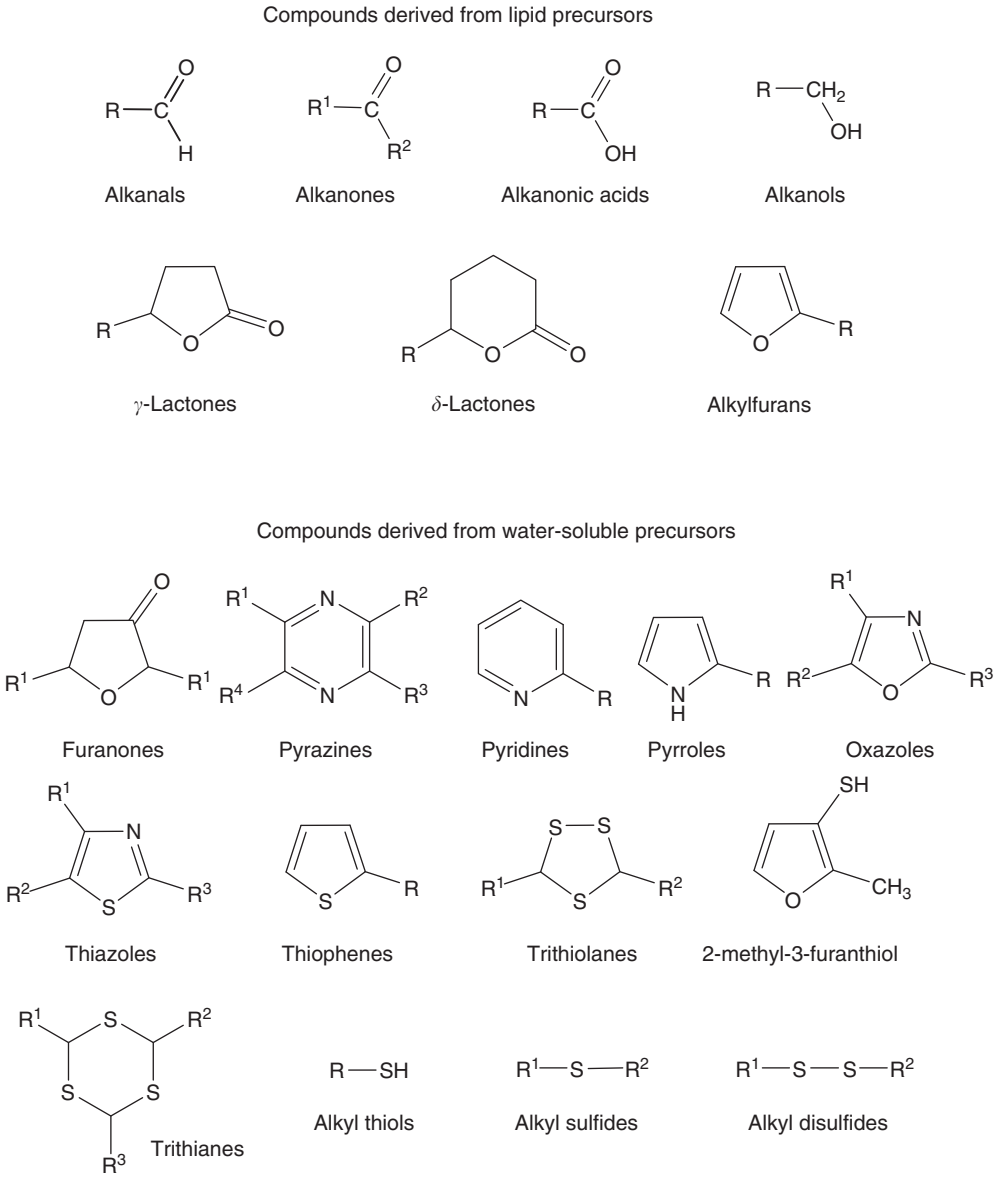

Figure 1 Some classes of volatile compounds generated during the cooking of meat. Reprinted from Mottram, D.S., 1998. Flavor formation in meat and meat products: A review. Food Chemistry 62, 415–424.

rise significantly above the boiling point of water, does pyrolysis of amino acids take place, and this results in decarboxylation and deamination reactions. Breakdown of protein into oligopeptides, peptides, and free amino acids is also important because these protein subunits, monomers, or their salts are taste active but contribute very little to the aroma of cooked meat. During heat processing, free amino acids in meat, like cysteine, react with reducing sugars (products of glycolysis) via the Maillard reaction to generate aroma volatiles. Products of one reaction often become precursors for another. One can begin to appreciate why the chemistry underlying meat flavor is a complex topic.

Thiamine (i.e., vitamin B_1) is an important microconstituent in the meat matrix that significantly impacts meat flavor development. It is a sulfur-containing compound with a thiazole and pyrimidine ring system. When meat is thermally processed, the vitamin degrades and potent aromas are generated, of which some have been described as meaty. Thiamine's breakdown products include thiophenes, thiazoles, furans, furanthiols, hydrogen sulfide, and bicyclic heterocyclic compounds. An important degradation product from thiamine is 5-hydroxy-3-mercapto-2-pentanone: this very reactive compound is the intermediate for a number of thiols, including 2-methyl-4,5-dihydro-3-furanthiol and 2-methyl-3-furanthiol as well as the mercaptoketones.

The breakdown of lipid constituents and their subsequent oxidation, as it relates to cooked meat flavor, is described in a separate section below.

Maillard Reaction/Advanced Glycation Endproducts

Although a description of the Maillard reaction, as it relates to meat and meat products, is presented in detail in another article, a brief overview of the reaction is provided for the sake of completeness. Free amino groups of amino acids or peptides in meat can react with reducing sugars in the presence of heat. They undergo a series of complex nonenzymatic browning reactions known simply as the Maillard reaction. Maillard reaction products are sometimes intermediates for further reactions and in other cases, end products. There is confusion at times in the terminology with advanced glycation endproducts (AGE), which are the result of a chain of reactions after an initial glycation reaction. The Maillard reaction, which does not require the very high temperatures associated with sugar caramelization and protein pyrolysis, is one of the most important routes for the generation of flavors in cooked foods. Its products include high molecular weight brown-colored compounds, known as melanoidins, and volatile aroma compounds. The first step of the Maillard reaction in a complex series of interactions involves the formation of an *N*-substituted glycosylamine via the addition of the carbonyl group of the open-chain form of a reducing sugar with a primary amino group of an amino acid, peptide, or protein. The *N*-glycosylamine rearranges itself, forming an Amadori compound, which can degrade further, thus generating compounds such as furfurals, furanones, and dicarbonyls. These compounds may themselves make some contribution to meat flavor but are more important as substrates in the generation of other aroma volatiles. For example, they can interact with reactive compounds such as amines, amino acids, hydrogen sulfide, thiols, ammonia, acetaldehyde, and other aldehydes. These further reactions lead to many important classes of meat flavor compounds such as pyrazines, oxazoles, thiophenes, thiazoles, and other heterocyclic compounds. As will be outlined below, sulfur compounds derived from the interaction of cysteine and ribose seem to be particularly important for the characteristic aroma of meat. Although the Maillard reaction can take place in aqueous solution, it occurs much more readily at low moisture levels; hence, in meat, flavor compounds produced by the Maillard reaction tend to form on the surface of the product where some dehydration has occurred.

Lipid Oxidation

In the early 1960s, two researchers suggested that meat aroma, derived from water-soluble precursors of lean tissue, was similar in all cooked meat and that the characteristic species differences were due to the contribution of volatiles derived from the lipid fraction. It was postulated that lipids provide volatile compounds that give the characteristic flavors of different species and that elimination of the lipid-derived flavors should reveal the true-to-nature flavor of meat itself. Fat influences flavor by producing organoleptically significant quantities of carbonyl compounds (i.e., aldehydes and ketones) as a result of oxidation from unsaturated fatty acids and by acting as a depot of fat-soluble compounds that volatilize on thermal processing. The spectrum of secondary products of lipid oxidation will depend, of course, on the fatty acid composition of adipose and intramuscular tissues, which vary from one species to another and may be influenced by diet. For example, the deposited lipids of monogastric animals, essentially pigs and poultry, can be influenced by the fatty acid patterns in the animals' diet. With the meat industry edging further into the functional foods sector, researchers have been attempting to increase omega-3 fatty acid levels in the meat from monogastrics. However, quality challenges of these functional meats in the form of oxidation/off-flavors exist in the cooked products, even with the supplementation of vitamin E to the diet.

One of the main functions of thermal processing is to generate aroma and flavor precursors from lipids, many of which possess intense odors, as well as to allow intimate mixing of fat- and water-soluble compounds. Yet, lipids alone are not responsible for the species-characteristic aromas. A study reported that the addition of pork backfat to either beef or pork lean meat resulted in a substantial increase in hexanal levels after thermal processing of the preparations but only small changes in most other volatiles. The lack of a relationship between aroma constituents and subcutaneous fat levels suggested that the triacylglycerols of adipose tissue may not be the main precursors for volatiles; instead, intramuscular triacylglycerols and structural phospholipids were deemed to be important.

Interaction between Lipid Oxidation and Maillard Reaction Products

Sensory panels and consumer studies have also failed to find a direct relationship between the flavor of lean meat and the

quantity of fat on the carcass; thus, giving credence to early meat flavor research that meatiness was associated with the water-soluble flavor precursors, whereas species characteristics were derived from the lipids. The aroma of cooked meat is dominated by lipid-derived volatiles and such volatiles are expected to have some effect on meaty flavor.

Triacylglycerols and structural phospholipids have been carefully studied in relation to meat flavor development. In a nutshell, phospholipids play a key role during the thermal generation of meat aroma. In a study where inter- and intramuscular triacylglycerols were extracted from lean meat samples before cooking, the aroma of the thermally processed products indicated that removal of these fats had little effect on the meaty aroma and the volatile compounds were formed on heating of the samples; in fact, one could not differentiate the aroma profile from that of untreated material. This was not the case, however, when phospholipids were also extracted from the muscle tissue. Elimination of phospholipids along with the triacylglycerols resulted in a marked loss in the perceived meatiness of the overall aroma, and the odor descriptor changed from that of meaty to roast or biscuit like. Another significant observation was that removal of phospholipids from the meat before thermal processing brought about a significant increase in certain volatile heterocyclic compounds, notably alkyl pyrazines. As the primary source of these compounds in cooked meat comes via the Maillard reaction, it appeared that interaction of phospholipids or their degradation products in the Maillard reaction is important for the characteristic aroma of cooked meat. In a model system study containing cysteine and ribose, the interaction of phospholipids in the Maillard reaction of these two substrates not only increased the meaty aroma of the reaction mixture but also increased the number of compounds possessing meaty notes. In other words, research has suggested that the interaction between phospholipids and the Maillard reaction can affect meat flavor in three ways: (1) lipid–Maillard reaction products can have their own aroma characteristics; (2) compounds with low OTVs derived from lipid oxidation, such as unsaturated aldehydes, can react with Maillard intermediates and thereby reduce their contribution to rancid and other odors (e.g., green note); and (3) important Maillard intermediates, such as ammonia and hydrogen sulfide, can react with lipid-derived volatiles, thereby reducing their availability for the formation of cooked flavors.

Species Effect

The chemistry of compounds bringing about distinctive species-related flavors of ruminant meats remains obscure. The characteristic flavor of different meat species is generally believed to be derived from their respective lipid profiles. The interaction of lipid constituents with other meat components (e.g., Maillard reaction products, as outlined above) is most likely involved. Numerous reports have shown that the chemical nature of many flavor volatiles of meat from different species is similar qualitatively but different quantitatively. Lamb, mutton, and goat meat are, however, exceptions. A number of volatile, medium-chain fatty acids (C_5–C_{12}), including some methyl-branched chain homologs, have been identified in cooked sheep and goat meats. These saturated fatty acids have not been reported in other meats and were associated with the characteristic 'sweaty' flavor of cooked sheep meat that results in its low consumer acceptance in many countries. In particular, 4-methyloctanoic, 4-ethyloctanoic, and 4-methylnonanoic acids are considered to be primarily responsible for this off-flavor. It has also been reported that mutton aromas contain a higher concentration of 3,5-dimethyl-1,2,4-trithiolane and 2,4,6-trimethylperhydro-1,3,5-dithiazine (thialdine) as compared with those of other species. Additional sulfur-containing compounds were present at notable concentrations and this was attributed to the high content of sulfurous amino acids in mutton as compared with those of beef and pork. Similarly, a marked concentration of alkyl-substituted heterocycles and alkylphenols was noted in mutton volatiles.

Other Effects

Antemortem factors such as the breed, sex, nutritional status, and age of the animal; preharvest stress/handling conditions; and postmortem factors like muscle type, myoglobin content, pH, and thermal processing conditions (i.e., moist heat, dry heat, microwave, convection oven, and final endpoint cook temperature) of the meat products, including the type and duration of storage, all contribute to and affect the flavor of meat. Another important parameter in relation to meat flavor is the diet that animals are fed. For instance, ruminants either graze on grass or are fed hay and silage diets. For those animals feeding on pastoral lands with its great biodiversity, the diets are often switched over to cereal or grain-based ones a number of weeks before harvest, as a means to improve the flavor characteristics of the resultant meat. Skatole (i.e., 3-methylindole) is a natural product that animals ingest from pastoral lands. In sheep meat, skatole in combination with the branched-chain fatty acids discussed above can impart objectionable flavors to the meat products derived from these animals. The effect of skatole is less noticeable in cattle, but the consumption of grass also causes animals to accumulate larger concentrations of linoleic and α-linolenic acids and their derivates (e.g., conjugated linoleic acid). Consequently, any detectable pastoral flavor in meat products tends to result from oxidation products of α-linolenic acid.

As described in the warmed-over flavor (WOF) article, researchers are supplementing the feed of domesticated species with dietary antioxidants like vitamin E. Supplementation in the form of α-tocopheryl acetate to the diet of monogastric animals before harvest can, in some cases, improve the flavor of finished products by minimizing the potential for WOF development. This effect has been seen in a number of studies where the basal diets of hogs have been supplemented with increasing levels of α-tocopheryl acetate. A progressive increase in the concentration of α-tocopherol has been found in the muscle tissue, mitochondria, and microsomes of hogs. α-Tocopherol migrates into muscle cell membranes, where it lies adjacent to highly oxidizable phospholipids; this localization makes it a particularly effective antioxidant. Sensory studies of meat products have confirmed that vitamin E supplementation can prolong flavor freshness of cooked products

and retard WOF development. As aforementioned, the addition of α-tocopheryl acetate to omega-3 fortified diets (e.g., in the form of flaxseed) of monogastrics does not always sufficiently curb oxidation of polyunsaturated fatty acids.

Desirable Meaty Aromas of Cooked Meat

In studying meat aroma, two realities exist: first, a means in which to 'trap' meat flavor volatiles and second, a way to separate and identify the odor impact compounds. Analyzing the volatile organic compounds that impact meat flavor is not an easy task. As noted above, more than 1000 compounds have been identified in the volatile constituents of cooked red meats and poultry by gas chromatographic–mass spectrometric techniques. Obtaining useful information from the analysis of meat volatiles, however, can be even more challenging than the isolation/identification steps themselves. The critical question becomes, "What is the relative sensory significance of these thermally derived volatiles?" The answer is not totally clear, but many volatiles are relatively unimportant. For example, aliphatic and aromatic hydrocarbons, saturated alcohols, carboxylic acids, esters, ethers, and carbonyl compounds (i.e., aldehydes and ketones) are probably not the main contributors to desirable meaty flavor. Rather, lactones; acyclic sulfur-containing compounds (i.e., mercaptans and sulfides); nonaromatic heterocyclic compounds containing sulfur, nitrogen, or oxygen (e.g., hydrofuranoids); and aromatic heterocyclic compounds containing sulfur, nitrogen, or oxygen (e.g., pyrazines and thiophenes) possessed characteristic meaty flavor notes. Whether a compound is a key aroma impact substance depends on both its concentration and OTV. Gas chromatography–olfactometry (GC–O) coupled with aroma extraction dilution analysis has assisted with identifying key aroma impact compounds in cooked meat.

The importance of sulfur-containing heterocyclic compounds in the volatiles of cooked meats should not be underestimated. Although these compounds are present in very low concentrations, their parts-per-billion OTVs make them potent aroma compounds. As an example, 2-methyl-3-(methylthio) furan was identified in cooked beef and found to have an OTV of 0.05 $\mu g\,kg^{-1}$ and a meaty aroma at levels below 1 $\mu g\,kg^{-1}$. The interest in such compounds stems partly from research into developing simulated meat flavorings with desirable meaty aroma characteristics for use in processed food products. Dr. Donald Mottram and his research group in the United Kingdom have carried out extensive work on characterizing the importance of heterocycles in desirable meaty aromas.

The breakdown of cysteine (e.g., via hydrolysis or Strecker degradation) and thiamine in meat gives hydrogen sulfide, which is critical in the formation of *S*-containing heterocycles. Hydrogen sulfide can react with dicarbonyls, furanones, and furfurals – most likely generated from inosine 5′-monophosphate, ribose 5-phosphate, and ribose via the Maillard reaction – to yield thiol and mercaptoketone derivatives. These derivatives can undergo oxidation and result in quite a number of symmetrical and unsymmetrical disulfides; some of these are depicted in Figure 2. Various odor descriptors from GC–O studies have been employed to characterize some of these compounds and include the following: cooked meat, meaty, brothy, pungent, fried onion, sulfury, fatty, nutty, and roasted. Heterocyclic compounds with one, two, or three sulfur atoms in five and six-membered rings are much more prevalent in boiled than in roasted meats. The meaty character of some sulfur-containing compounds depends on the position of the thiol group and the degree of unsaturation. For instance, furans and thiophenes with a thiol group in the 3-position and their related disulfides have been reported to possess strong meat-like aromas with very low OTVs, whereas those with the thiol in the 2-position were characterized as being burnt and sulfurous. One researcher noted that the best meat-like aroma is produced when there is a methyl group adjacent to the thiol moiety and the ring contains at least one double bond. A complication in the analysis of such compounds arises from the fact that the aroma of these sulfur volatiles, which is pleasant at the levels found in meat, becomes objectionable at higher concentrations. Therefore, when assessing the flavor quality of muscle foods, both qualitative and quantitative aspects of volatiles must be considered.

Flavor of Nitrite-Cured Meat

Nitrites and nitrates are unique ingredients found in processed cured meat products. Nitrite plays a multifunctional role in the meat matrix: it is responsible for the development of the characteristic color associated with cured meat; a distinct flavor that distinguishes the flavor of cured ham from cooked, uncured pork, and this may be related to the antioxidative capacity it imparts; and in combination with sodium chloride, it suppresses the outgrowth and production of toxin from the anaerobic bacterium, *Clostridium botulinum*.

The mechanism by which nitrite imparts a characteristic cured flavor to thermally processed meat and meat products (i.e., a flavor that distinguishes cooked ham from pork) is unclear. Nevertheless, cured meat flavor is probably a composite sensation derived from contributions of many odoriferous compounds. Research into cured meat flavor has been divided into two main areas, namely, the sensory evaluation of flavor imparted to meat by nitrite and the qualitative and quantitative identification of volatile and nonvolatile components responsible for it, but caution must be exercised. A compound-by-compound search of meat flavor volatiles might misidentify the true nature of cured meat flavor, because a mixture of two or more odors can produce an aroma that is perceived as qualitatively distinct from the odors of their components.

Nitrite's role in the development of thermally derived cured meat flavor involves its antioxidative activity, which retards the breakdown of unsaturated fatty acids and the formation of secondary oxidation products. Numerous researchers have attempted to identify the volatile compounds generated during the thermal processing of cured meat. The results indicate that all compounds identified were also contributors to the aroma of uncured cooked meat. Some important research in the 1960s involved the examination of the volatile constituents isolated from uncured and cured hams by gas chromatography. Qualitatively, the volatile compounds of cured ham were similar to uncured hams but were quantitatively

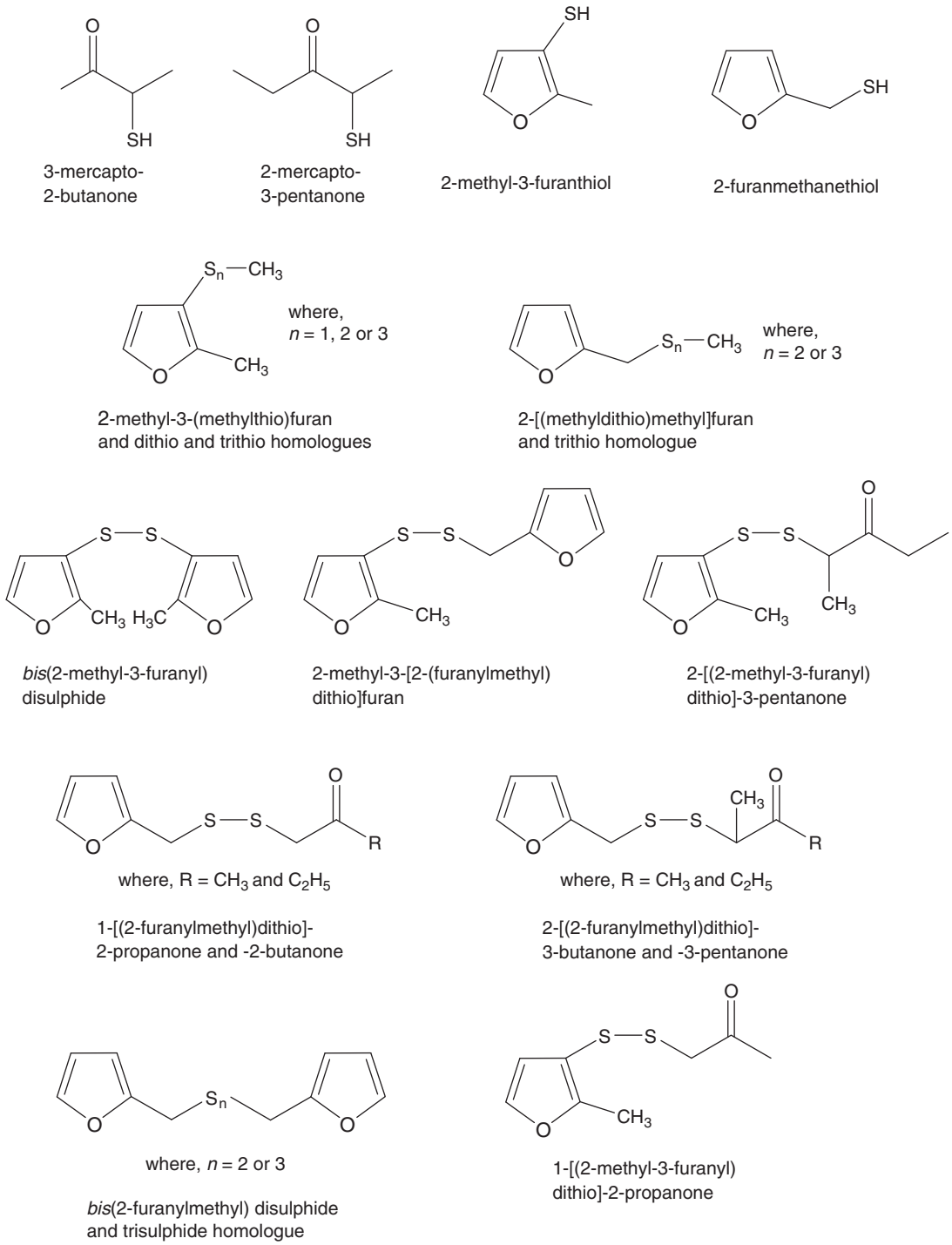

Figure 2 Some thiols and sulfides detected in the headspace volatiles of heated meat systems. Adapted from Mottram, D.S., Madruga, M.S., 1994. Important sulfur-containing aroma volatiles in meat. In: Mussinan, C.J., Keelan, M.E. (Eds.), Sulfur Compounds in Foods. ACS Symposium Series, vol. 564. Washington, DC: American Chemical Society, pp. 180–187. Copyright (1994) American Chemical Society.

different. Hexanal and pentanal were present in appreciable amounts in the volatiles of uncured but were barely detectable in the volatiles of cured ham. It was suggested that the absence of these aldehydes and those of higher molecular weight aldehydes was responsible for the flavor differences between cured and uncured hams. It was also noted that the volatiles, after passage through a solution of 2,4-dinitrophenylhydrazine (2,4-DNPH), had the characteristic cured-ham aroma, regardless of whether cured or uncured hams were used. Cured and uncured chicken and beef volatiles, after stripping their carbonyl compounds by passage through 2,4-DNPH solutions, also possessed an aroma similar to that of

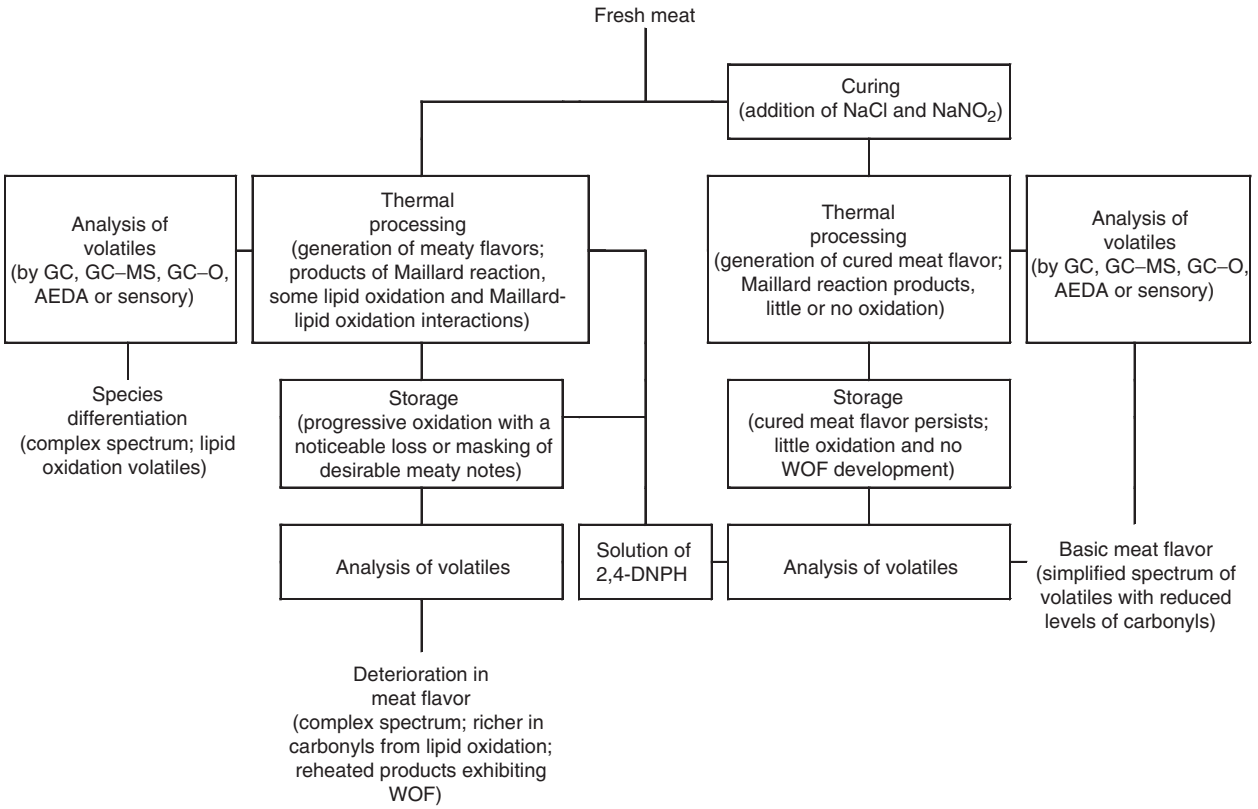

Figure 3 Consequence of thermal processing, nitrite curing, and storage on meat flavor. AEDA, aroma extraction dilution analysis; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; GC–O, gas chromatography–olfactometry; WOF, warmed-over flavor; 2,4-DNPH, 2,4-dinitrophenylhydrazine. Adapted from Shahidi, F., 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In: St. Angelo, A.J. (Ed.), Lipid Oxidation in Food. ACS Symposium Series, vol. 500. Washington, DC: American Chemical Society, pp. 161–182. Copyright (1992) American Chemical Society.

cured ham. A conclusion reached was that treating meat with nitrite does not seem to contribute any new volatile compounds to the flavor of cooked meats, with the exception of nitrogen oxides that are not present in cooked uncured meat. Therefore, it was postulated that cured-ham aroma represents the basic flavor of meat derived from precursors other than triacylglycerols and that the aromas of various types of cooked meat depend on the spectrum of carbonyl compounds derived by lipid oxidation.

An oversimplistic view attempting to provide a unifying theory on the origin of the thermally generated flavor of meat, species differentiation, and off-flavor development is provided in Figure 3. It postulates that meat acquires its characteristic species-specific flavor on cooking from volatile carbonyl compounds formed by oxidation of its lipid components (i.e., primarily phospholipids) and their reaction products after interaction with Maillard reaction products. Further oxidation during storage of cooked meat results in the deterioration of desirable meaty notes. Curing with nitrite suppresses the formation of oxidation products. Hence, it may be assumed that the flavor of nitrite-cured meats is actually the true-to-nature flavor of meat from different species without being influenced by overtone carbonyls derived from oxidation of their lipid components. The postulate, however, does not easily explain the fact that the intensity of cured meat flavor in bacon has been reported to be proportional to the level of ingoing nitrite levels, whereas the characteristic 'mutton' flavor is persistent regardless of the level of nitrite used in curing of sheepmeat.

See also: Cooking of Meat: Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor

Further Reading

Brewer, M.S., 2009. Irradiation effects on meat flavor: A review. Meat Science 81, 1–14.
Calkins, C.R., Hodgen, J.M., 2007. A fresh look at meat flavor. Meat Science 77, 63–80.
Martins, S.I.F.S., Leussink, A., Rosing, E.A.E., Desciaux, G.A., Boucon, C., 2011. Meat flavor generation in complex Maillard model systems. In: Mottram, D.S., Taylor, A.J. (Eds.), Controlling Maillard Pathways to Generate Flavors. ACS Symposium Series, vol. 1042. Washington, DC: American Chemical Society, pp. 71–84.
Melton, S.L., 1999. Current status of meat flavor. In: Xiong, Y.L., Ho, C.-T., Shahidi, F. (Eds.), Quality Attributes of Muscle Foods. New York: Kluwer Academic/Plenum Publishers, pp. 115–133.
Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62, 415–424.

Mottram, D.S., Elmore, J.S., 2002. Novel sulfur compounds from lipid-Maillard interactions in cooked meat. In: Reineccius, G.A., Reineccius, T.A. (Eds.), Heteroatomic Aroma Compounds. ACS Symposium Series, vol. 826. Washington, DC: American Chemical Society, pp. 101–109.

Mottram, D.S., Madruga, M.S., 1994. Important sulfur-containing aroma volatiles in meat. In: Mussinan, C.J., Keelan, M.E. (Eds.), Sulfur Compounds in Foods. ACS Symposium Series, vol. 564. Washington, DC: American Chemical Society, pp. 180–187.

Pegg, R.B., Shahidi, F., 2000. Nitrite curing of meat. The N-nitrosamine Problem and Nitrite Alternatives. Trumbull, CT: Food & Nutrition Press, Inc.

Shahidi, F., 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In: St. Angelo, A.J. (Ed.), Lipid Oxidation in Food. ACS Symposium Series, vol. 500. Washington, DC: American Chemical Society, pp. 161–182.

Shahidi, F. (Ed.), 1998. Flavor of Meat, Meat Products and Seafoods, second ed. London, UK: Blackie Academic & Professional.

Shahidi, F., Rubin, L.J., D'Souza, L.A., 1986. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. CRC Critical Reviews in Food Science and Nutrition 24, 141–243.

Spanier, A.M., Flores, M., Toldrá, F., *et al.*, 2004. Meat flavor: Contribution of proteins and peptides to the flavor of beef. In: Shahidi, F., Spanier, A.M., Ho, C.-T., Braggins, T. (Eds.), Quality of Fresh and Processed Foods. Advances in Experimental Medicine and Biology, vol. 542. New York: Kluwer Academic/ Plenum Publishers, pp. 33–49.

Vasta, V., Priolo, A., 2006. Ruminant fat volatiles as affected by diet. A review. Meat Science 73, 218–228.

Wang, R., Yang, C., Song, H., 2012. Key meat flavour compounds formation mechanism in a glutathione–xylose Maillard reaction. Food Chemistry 131, 280–285.

Heat Processing Methods

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Glossary

Convection, thermal Mechanism for heat transfer. The process of heat transfer through a liquid or gas by means of circulating currents caused by changes in density.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Pasteurization A form of heat treatment that kills certain vegetative bacteria and spoilage organisms in milk and other foods. Temperatures below 100 °C are used.

Radiation, thermal It is a mechanism for heat transfer. Electromagnetic radiation generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emits thermal radiation.

Refrigeration It is defined as the process of removing heat from any substance to (1) render the substance colder, for example, reduce temperature, (2) change its state, for example, water to ice, (3) maintain its state, for example, preserving foods, storing ice.

Introduction

Cooking is the most common heat treatment applied to meat; its primary aim is to cause structural and chemical changes that will make the meat more palatable. Industrially, the aim is more often to pasteurize the meat, for example, kill vegetative pathogens and spoilage organisms and to extend the safe shelf life of the product, with the consumer completing the cooking process at home. Sterilization extends the shelf life even further by killing all of the microorganisms present, including the spores.

The organoleptic changes that are caused by heat treatment (doneness, flavor, firmness, consistency, and cured-meat color development) are time–temperature-dependent processes. The basic effect of the heat treatment is the coagulation of meat proteins. Between 70 and 80 °C, the majority of meat proteins are completely coagulated; these structural changes of proteins are responsible for the characteristic firmness of heat-treated meat products. Products containing connective tissue become tenderer owing to solubilization of the collagen (gelling), such as cuts of meat. Frankfurters have an elastic firmness, and on reheating before consumption become even firmer. Products such as meat paste that are in a liquid state before heating change, becoming more viscous and attain a 'spreadable' consistency.

There are also a number of other heat treatments used during slaughtering operations for both red and poultry meat production. Poultry and pork carcasses are scalded, and pork carcasses singed. Surface heat decontamination processes have also been developed for meat.

Sterilization

Commercial sterilization is intended to produce an ambient stable product with a long shelf life by destroying both microbial and enzyme activity. The severity of the heat treatment produces substantial changes in the nutritional and sensory qualities of the meat. 'Commercial' sterilization implies that there is a very low probability of the survival of microorganisms that are injurious to human health. It does not, however, mean that there are no microorganisms left in the food.

Although the pH of meat is generally slightly acidic, it is considered a low acid food (pH higher than 4.5) in danger of supporting *Clostridium botulinum* and so must be given a severe enough treatment to inactivate spores of this organism. Although these spores are not as resistant as the spores of some other *Clostridium* and *Bacillus* types, *C. botulinum* is capable of producing lethal toxins, sometimes without swelling the container or obvious alteration of the appearance of the product. Because this organism presents a public health risk, recommended heat treatments must have a large safety margin.

The severity of heat processes for canned meat products is measured in terms of F_0 values, which means that the product received a heat treatment with the same inactivating effect as exposure for one minute at 121 °C. For example, one minute at 121 °C gives the same amount of inactivation of spores as 4 min at 115 °C or 13 min at 110 °C, for example, all those processes have the same F_0 value. The F_0 value for the majority of canned meat products ranges between 1 and 10. Meat processed in large cans requires longer processing times to allow for heat penetration; consequently, closer to the surface F_0 values can be between 20 and 25.

Traditional canning is a nonsteady state heat transfer process in which a container is heated, held at a given temperature for a given time, and then cooled. The whole heating/holding/cooling cycle contributes to the sterilization. The vessels used for sterilization are commonly called retorts and the process retorting. Heat can be provided by three methods, each being more suitable for different containers.

Traditionally canned meats, such as corned beef, are processed by steam inside a pressure vessel. Latent heat is transferred to the food when the saturated steam condenses on the outside of the container. Air must be removed from the retort to prevent it forming a noncondensable area around the can and preventing the steam condensing on the surface. After sterilization the cans are usually cooled with water. An overpressure is used to prevent strain on the can seams (pressure

cooling). When the food has cooled to below 100 °C, the overpressure of air is removed and cooling continues to approximately 40 °C. At this temperature, moisture on the can dries to prevent surface corrosion, and allow label adhesives set more rapidly.

Meats and meat products in glass containers or flexible pouches are processed under hot water with an overpressure of air. Processing meat products in glass containers is slower than in cans or pouches because the glass has a lower thermal conductivity and has to be thicker to provide adequate strength. There is also a higher risk of thermal shock during processing glass containers. Although the plastics used for pouches have a low thermal conductivity, the thinness of the plastic and the smaller cross-section of the container means that processing is often faster than for cans. The flexible nature of the pouches and their use for liquid or semiliquid meat products, such as sauces and chili con carne, can cause a problem. Vertical packs promote better circulation of hot water in the retort, but frames are needed to prevent the pouches from bulging at the bottom, which would increase the thickness of the pouch and hence decrease the rate of heat penetration and increase the process time. Processing the pouches horizontally ensures that the thickness is constant across the pouch. Alternatively, the packs can be circulated through an agitated system where the motion of the water stirs the packs, ensuring mixing of the contents and good heat transfer, provided the system has been properly designed.

An alternative to steam or hot water is direct flame heating. Flame sterilization involves heating cans by passing them over a gas flame. This method is extremely fast because the flame is at temperatures in excess of 1500 °C and internal temperatures can reach 116 °C in a few minutes. The cans are closed under a very high vacuum then pass through a four-stage process: the cans are first preheated in steam; the cans then pass over a gas flame while agitated to stir the contents; the cans are held for the required holding time; then the cans are cooled. The entire operation of preheat, process to 130 °C, hold and cool can take only 12 min. However, the process is limited to low viscosity liquids or solids, such as cubed beef or ham. The internal pressure in the can during processing is very high (275 kPa at 130 °C), and this may strain the can seams and limits the process to small cans.

These processes can be batch or continuous. Continuous retorts permit close control over the processing conditions; gradual changes in the pressure inside the food container can be made and therefore less stress is placed on the container than with batch equipment. However, they are less flexible than batch systems, and in practice, are used for the production of high-volume products where there is no requirement to regularly change the container size or processing conditions.

Cooking (Pasteurization)

Many commercial pasteurization processes differ little from those used in the catering and domestic environments. Often, with the exception of meat products that are to be eaten cold (i.e., ready-to-eat products), the heat treatment is carried out to pasteurize (kill the vegetative pathogenic and spoilage microorganisms) and not necessarily cook them, because they will often be heated by the consumer before ingestion. Pasteurized products require chilled or frozen storage to prevent proliferation of any microorganisms not killed in the pasteurization process. The time to chill the product from cook temperatures is often as important as the internal cooked temperature attained.

The microbiological criteria for pasteurized meats is often based on *Listeria monocytogenes* (considered the most dangerous heat-tolerant pathogen in many chilled products), with a recommendation to cook to 70 °C for 2 min, or equivalent. *C. botulinum* is viewed as a potential hazard in vacuum-packed products and requires a much more severe treatment, 10 min at 90 °C, or equivalent.

Textbooks on cooking use a wide variety of terms to describe cooking (Table 1). Conventional commercial cooking systems for meat joints and products are based on roasting/baking, boiling, or frying methods. Microwaves are commonly used in reheating, and some cooking processes and ohmic heating has been advocated for some products.

Surface heat transfer coefficients with foodstuffs in boiling and frying operations are much higher than in hot air ovens (Figure 1). However, in unpressurised systems water temperatures are below 100 °C and the temperature difference between the food and cooking environment can be much less than in a hot air oven operating at 200–360 °C. Condensing steam results in high surface heat transfer coefficients but is only suitable for a small range of products.

Hot Air

The majority of cooked meat and many individual meat products are roasted or baked in hot air ovens. These ovens

Table 1 Common cooking methods/terms

Boiling	Cooking of foods in a liquid, usually stock or water
Steaming	Cooking in moist heat where the water exists as a vapor, generally above 100 °C
Stewing	Slow cooking in a small quantity of water, stock, or sauce in which the food is always cut up and the food and cooking liquid are served together
Roasting	The subjection of food to the action of heat in an oven, or while it is roastingon a spit; in both cases, fat is used as a basting agent
Braising	A combination of roasting and stewing in a pan with a tight-fitting lid
Baking	Same as roasting, except no fat is used
Grilling/broiling	Cooking with radiated heat
Shallow frying	Cooking the food in hot shallow fat in a pan
Deep frying	Cooking the food by completely submerging it in hot fat
Microwaving	A colloquial term for microwave cooking

commonly consist of either a compartment with shelves for the product or a long tunnel through which the product is transported on a conveyor belt. The heat transfer medium is air, which is sometimes mixed with steam. The heat transfer mechanism is either natural convection or forced convection by means of fans. The heating rate is controlled by the air velocity over the product, the air temperature, the condensation of steam, and the thermal properties of the product. Belt speed can be varied to achieve the required residence time. To some extent, heat is also transferred via radiation from the walls, shelves, and elements (Table 2).

Forced convection tunnel ovens may be divided into different sections with different temperature zones if required as in the baking of meat pies. Typical air temperatures range from 150 to 250 °C and heat transfer coefficients between 20 and 90 $Wm^{-2}K^{-1}$. However, in cooking operations for large joints, low temperatures (75–90 °C) and high humidities are used to minimize weight loss. Long cooking times (up to 16 h) are required in large joints such as beef topsides or hams used for sliced products.

Smokehouse cooking, often used for bacon smoking, is a specialist form of hot air cooking.

Steam

Steam cooking is heating in saturated air, at atmospheric pressure this is at 100 °C, at lower pressures the temperature is below 100 °C, whereas at high pressures the temperature is above 100 °C. Latent heat is given up as the steam condenses at the meat surface, leading to higher heat transfers than are possible with air. However, unless the process is pressurized the surface temperature is restricted to 100 °C, thus browning and other reactions associated with roast meat do not occur. This is not a problem with products that are traditionally boiled, such as gammons. Otherwise steam cooking can be combined with a second-stage radiant or hot air cooking stage to impart the characteristic roast appearance and flavor.

Direct injection of steam can be used for heating meat stews, soups, and similar products.

Steam at pressures greater than atmospheric is generally associated with retorting (canning) but it is also employed in the production of pasteurized soups, stews, etc. Higher temperatures lead to faster processing times and can lead to the retention of nutrients that are damaged by long processing.

Hot Water

Open top vessels and closed pressurized vessels are used for the cooking of meat containing mixtures that make up pie and pasty fillings, curries, stroganoff, chicken chasseur, Chinese and Italian dishes, etc.

An open top vessel is used in many small- and medium-scale operations. The advantage of such systems are low cost, ease of cleaning, and versatility. As they are open topped, they allow components to be conveniently added at different times through the cooking process.

The disadvantages in open top vessels are that considerable temperature stratification occurs, with differences of up to 50 °C. The vessels are also energy inefficient with up to 15% of heat lost to the environment via evaporation from the surface. Cooling is a major problem and often the vessel is emptied into a large bin that is allowed to cool in ambient temperatures or in a refrigerated room. Temperatures as high as 65 °C have been recorded in the center of bins after 16 h of 'cooling' and during this period the product continues to cook with a consequent deterioration in texture and flavor. Spore-forming bacteria will survive these cooking operations and proliferate at temperatures between 10 and 50 °C.

Closed pressurized vessels are water, and sometimes, vacuum-cooled. Numerous designs of vessels are available for a wide field of applications, including atmospheric and super atmospheric pressure operation, with or without agitation with various impeller types such as paddles, turbines, anchors, or propellers, and vessel shapes such as vertical, cylindrical, or hemispherical. Most vessels are of a double-skinned stainless

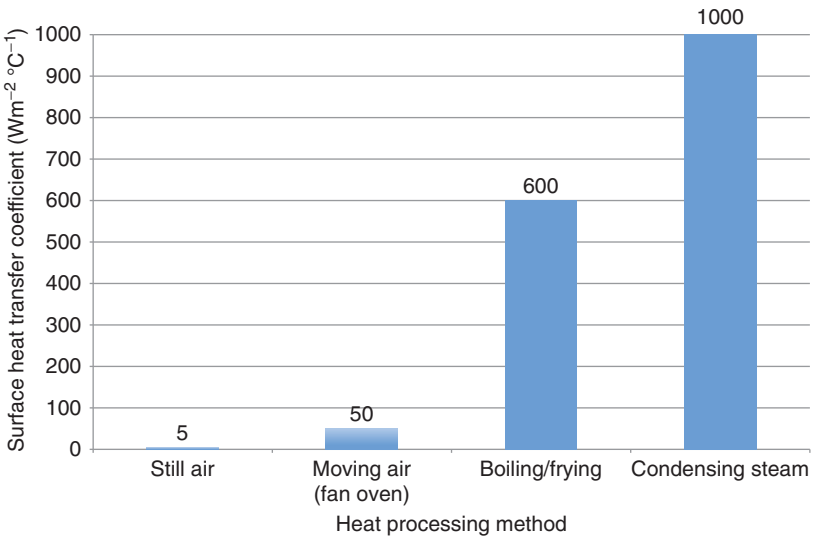

Figure 1 Typical heat transfer coefficients in cooking operations.

Table 2 Radiant heat sources

Type of emitter	*Maximum running temperature (°C)*	*Maximum intensity (kWm^{-2})*	*Maximum process temperature (°C)*	*Radiant heat (%)*	*Convective heat (%)*
Short wavelength					
Heat lamp	2200	10	300	75	25
Quartz tube	2200	80	600	80	20
Medium wavelength					
Quartz tube	950	60	500	55	45
Long wavelength					
Standard element	800	40	500	50	50
Ceramic element	700	40	400	50	50

steel design and are heated via steam in the jacket. Direct steam injection into the product is more efficient and reduces processing time. The design and operation of pressurized systems is dependent on:

- The optimum time–temperature relationship required during cooking and cooling for the range of products processed.
- Overall heat transfer coefficients and their variation with temperature, agitator speed and design, dimensions of vessel, and product characteristics.
- Optimum agitator design for each product.

Hot water may also be used to cook meats in plastic bags or metal moulds, like hams. Heat transfer is much greater than in air systems and the barriers used prevent weight loss. The products can be cooked in water baths, either batch or continuous, or using hot water sprays.

Hot Fat or Oil Frying

Many coated meat products such as rissoles, Kiev's, breaded chicken portions, etc., are deep fat fried. In deep fat frying, heat is transferred via convection from the oil to the product. The heat transfer coefficient has been found to vary during the process. As water from the product is evaporated, turbulence occurs in the fat causing an increased heat transfer rate. The temperature of the oil is usually between 160 and 180 °C, depending on the product being fried. The size of the deep fat fryer may differ from small batch oil baths to large continuous frying baths. In the case of a continuous system, a conveyor belt, often combined with a pushing paddle arrangement, transports the product through the bath.

Fat used for frying foods has to fulfill the following demands:

- A melting point below 37 °C in order not to cause an unpleasant feeling in the mouth.
- A neutral flavor.
- Withstand frying temperatures for long periods without foaming due to polymerization and oxidation.
- A high smoking temperature.

Heated fats and oils rapidly become oxidized and off-flavors can readily be picked up by the meat being fried. It is usual practice to replace part of the oil at the end of each production cycle.

Radiant

Radiant heating (grilling) primarily involves the infrared portion of the electromagnetic spectrum. Radiant heat transfer is very high and high surface temperatures can be attained, with rapid onset of browning reactions and charring, although not all the heat transfer is radiant (Table 2). For this reason its use is restricted to thin pieces of meat, or in combination with other heating systems (such as conventional air heating, or after steam cooking) to brown the surface of larger products. Electrical elements or flames can be used as the heat source (Table 2). In some cases, flames are used directly to char the surface to impart a barbeque appearance/flavor.

Extrusion

Extrusion cooking cannot be applied to conventional meat products but can be used to produce new and novel products. Basic extrusion cookers consist of a screw rotating within a barrel. Meat and other ingredients are carried through the barrel by the screw through a constriction toward the end of the barrel and are combined through mechanical work and heat into a viscous dough. The dough leaves through a die, with accompanying pressure release, cooling, and moisture loss. Heating can be applied directly to the barrel or via steam injection into the ingredients. A significant amount of heating is also provided by the mechanical energy input used to drive the extruder screw; this can account to 50–100% of the total energy input.

Dielectric

Dielectric heating is a generic term that includes both microwave and radio frequency heating. It is important to recognize that microwaves and radio frequency radiation are a form of energy, not a form of heat, and are only turned into heat when they interact with a material. When they are intercepted by dielectric materials such as food, they interact with the dielectric material, giving up energy, which results in a temperature increase within the material. There are two main mechanisms in which heat is produced in dielectric materials: ionic polarization and dipole rotation. The major heating mechanism in microwave heating is dipole rotation, in which polar molecules (water being the most common polar material in foods) are rotated by the alternating microwave field

resulting in frictional heat generation. Ionic conductivity is more important during radio frequency heating and occurs when ions in solution move in response to an electric field; the ions are accelerated and collide with each other converting kinetic energy into heat.

In many countries, microwave-heating systems are restricted to two frequency bands close to 896 or 2450 MHz. The designs can be separated into resonant cavity systems or waveguide systems. The resonant cavity system is essentially an oven with a conveyor belt passing through it. Absorbent end loads or radio frequency chokes, or both, prevent microwave energy emission from the oven above established limits. The waveguide system is made from a standard waveguide folded back and forth on itself with a slot running through, normal to the fold, through which the conveyor transports the food product.

The main reasons for considering the use of microwaves are to accelerate the process, improve quality, reduce costs, and increase yield.

Uses in the meat industry of microwave technology have included the tempering of frozen meat blocks, precooking of chicken portions and sliced bacon, burger cooking, and frankfurter processing. Radio frequency has found few meat-based applications, but combination hot air/radio frequency baking ovens have been developed and used for cooking meat pies and pastries.

Ohmic Heating

The ohmic heating effect occurs when an electrical current is passed through an electrically conducting product. The idea has been around the last century but within past 20 years new and improved materials and designs have led to commercial systems for continuous flow ohmic heating becoming available. The main interest in this technology is from food manufacturers who wish to aseptically process particulate foods.

Conventional methods of heating particulate foods rely on heating of the liquid phase to transfer heat to the solid phase, which necessitates the overprocessing of the liquid phase to ensure that the center of each solid particle receives sufficient heat treatment. This results in reduced quality due to the destruction of flavors and nutrients and mechanical damage to the outside of the particulate. The advantage of ohmic heating in this respect is that liquid and particulate are heated virtually simultaneously without large temperature gradients being produced. The product is heated by internal generation but does not suffer from the temperature nonuniformity commonly associated with microwave heating.

The applicability of ohmic heating is dependent on the product being electrically conductive, which is the case with most food preparations as they contain a percentage of free water with dissolved ionic salts.

The equipment consists of a column of electrodes with tubular spacers in between, mounted in a near vertical position with the product flow upward. The column is configured so that each heating section has the same electrical impedance; hence the interconnecting tubes increase in length as the product electrical conductivity increases with progressively increasing temperature as it is pumped up the column. Control parameters include the inlet temperature, mass flow rate, outlet temperature, back pressure, and electrical power. Product temperatures of 90–95 °C can be obtained at a pressure of two bar and 120–140 °C at four bar. Systems range from small 5 kW laboratory systems capable of a throughput of 50 kg h^{-1} to a 600 kW model capable of 6 tons h^{-1}.

Thermal Surface Decontamination Processes

There is often no terminal step (such as cooking) to eliminate pathogenic organisms from most of red and white meat until it reaches the consumer. The consumer is relied upon to adequately cook the meat sufficiently to kill any bacteria injurious to health before ingestion. A number of thermal intervention processes have been applied to red meat and poultry carcasses to reduce surface microbial contamination without changing the intrinsic nature of the raw meat.

Hot Water

Hot water can be applied as a spray, deluge, or by immersion at temperatures of between 60 and 90 °C. Sprays can be applied manually or preferably via automated spraying cabinets. Deluge systems employ sheets of water and are reported to be more effective than sprays at covering the surface of a carcass. Immersion is effective but difficult to engineer for large carcasses, such as beef carcasses.

Hot-water treatments are often reported to initially impart a slight 'milky' or 'ghostly' appearance to the surface of the carcass; this diminishes on cooling and is virtually undetectable after 24 h storage in chill.

Steam

Steam at 100 °C has a substantially higher heat capacity than the same amount of water at that temperature. If steam is allowed to condense onto the surface of meat then it has the ability to rapidly raise the surface temperature of the meat. One very attractive feature of condensing steam is its ability to penetrate cavities and condense on any cold surface. Commercial steam cabinet decontamination systems for beef carcasses are in use in the USA. High-temperature pressurized steam systems and low-pressure vacuum steam systems have been investigated for treating poultry carcasses and cuts of meat at temperatures above and below 100 °C, respectively, but the batch nature of such processes has restricted their development, at present.

Scalding

Pork and poultry carcasses are both subjected to a scalding operation during processing. The carcasses are treated with hot water or steam to loosen the hair or feather in the follicle to aid their removal. The time and temperature of the heat treatment are primarily determined by the need for efficient removal of the bristles or feathers by the dehairer/defeatherer. Too low a temperature and the hair/feathers will not be loosened and too high a temperature and the skin will be

cooked and the hair/feathers difficult to remove. The simplest equipment consists of a tank into which the carcass is lowered by a hoist. The water is heated by oil, gas, electricity, or an open steam pipe. Alternatively, vertical cabinets utilizing hot-water sprays or steam can be used. Temperatures between 58 and 62 are normally used for 5–6 min for pig carcasses, whereas temperatures of 50–51 °C for 3.5 min are employed for 'soft' scalded chicken carcasses destined for chilling, or 56–58 °C for 2–2.5 min for 'hard' scalded carcasses destined for freezing.

Singeing

Residual hair left on a pig carcass after the scalding/dehairing process is burnt by singeing. The carcass is subjected to an exposed high temperature flame either with a hand-held gas torch or in automated systems that transport the pig into a furnace running at 1000 °C for as little as 6 s. During singeing, any water remaining on the surface of the carcass and in the outer layers of the skin evaporates. The heat denatures the collagen fibers in the epidermis and the skin shrinks. Any charred hair and surface dirt are removed in a final polishing/scrapping operation.

See also: Canning. Chemical and Physical Characteristics of Meat: Chemical Composition; Palatability. Cooking of Meat: Flavor Development; Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor. Extrusion Technology. Microbiological Safety of Meat: *Clostridium botulinum* and Botulism; *Listeria monocytogenes*. Processing Equipment: Smoking and Cooking Equipment

Further Reading

Anon, 2006. CFA Best Practice Guidelines for the Production of Chilled Foods, fourth ed. London: Chilled Food Association.
Blackburn, C.d.W., 2009. Foodborne Pathogens. Cambridge, UK: Woodhead Publishing Ltd.
Fellows, P.J., 2009. Food Processing Technology: Principles and Practice. Cambridge, UK: Woodhead Publishing limited.
Gould, G.W., 1989. Mechanism of Action of Food Preservation Procedures. Barking, UK: Elsevier Science Publishers Ltd.
Høyem, T., Kvåle, O., 1977. Physical, Chemical, and Biological Changes in Food Caused by Thermal Processing. London: Applied Science Publishers Limited.
Hui, Y.H., 2012. Handbook of Meat and Meat Processing, second ed. Boca Raton, US: CRC Press.
James, C., James, S.J., 1997. Meat Decontamination – The State of the Art. Bristol, UK: FRPERC, University of Bristol.
Knipe, C.L., Rust, R.E., 2009. Thermal Processing of Ready-to-Eat Meat Products. New York: Wiley-Blackwell.
Lawrie, R.A., Ledward, D.A., 2006. Lawrie's Meat Science. Cambridge, UK: Woodhead Publishing limited ISBN 1 84569 159 8.
Sandeep, K.P., 2011. Thermal Processing of Foods: Control and Automation. New York: Wiley-Blackwell.
Simpson, R., 2009. Engineering Aspects of Thermal Food Processing. Boca Raton, FL: CRC Press.
Sun, D.W., 2012. Thermal Food Processing: New Technologies and Quality Issues, second ed. Boca Raton, FL: CRC Press.
Toldrá, F., 2010. Handbook of Meat Processing. Ames, IA: Wiley-Blackwell.

Relevant Websites

http://www.ecff.net/
European Chilled Food Federation.
http://www.fao.org/
Food and Agriculture Organization of the United Nations.
http://www.chilledfood.org/
UK Chilled Food Association.

Maillard Reaction and Browning

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Glossary

Heterocyclic compounds The cyclic compounds generated during Maillard reaction that contain nitrogen, oxygen, or sulfur atoms or their combination in the ring.
Lipid oxidation aldehydes The aldehydes generated from oxidation of lipids, including phospholipids.
Maillard browning The nonenzymatic Maillard reaction that leads to the formation of brown colors.
Maillard reaction A nonenzymatic reaction occurring on heat processing of meat between reducing sugars and amino group of free amino acids, peptides, and proteins.
Meat flavor Is affected by both volatile compounds responsible for the aroma of products and the nonvolatiles responsible for taste effects.

Introduction

Flavor is an important aspect of food quality and in the case of cooked meats, it determines their overall acceptability. Raw meat has little or no aroma and only a blood-like taste. The flavor of meat is thermally derived, and each type of cooked meat has a characteristic flavor based on the animal, presence of other ingredients, and the type of heat processing (i.e., roasting, grilling, and stewing) employed. The nonvolatile taste active compounds and the volatile aroma constituents generated from meat during thermal processing contribute mainly to the specific cooked meat flavor. Other sensations such as mouth feel, texture, and juiciness also affect the overall flavor attributes of cooked meat. Yet, it is the flavor volatiles of cooked meat that determine the product's aroma and have a profound effect on sensory acceptability even before the meat product is consumed.

Both water-soluble components (e.g., amino acids, peptides, carbohydrates, nucleotides, thiamine) and lipids in raw meat contribute to the development of meat flavor. The main reactions that occur during cooking and generate aroma volatiles are the Maillard reaction (i.e., a nonenzymatic browning reaction between amino acids and reducing sugars), the breakdown and oxidation of lipid constituents, and the degradation of vitamins, particularly thiamine. Intermediary products from these primary reactions can function as precursors and react with other degradation products of meat, depending on the cooking conditions employed, to form a large number of volatiles responsible for the characteristic flavor of cooked meat. To date, more than a thousand volatile compounds have been identified from cooked meat systems; Figure 1 illustrates some important classes of volatile compounds that have been identified.

The occurrence of the Maillard reaction is very important when meat is cooked, because it generates a large number of compounds that contribute to meat flavor. Most flavor compounds of cooked meats with roasted, boiled, and meaty notes are generated via the Maillard reaction and are generally *N*-, *S*-, *O*-heterocyclics. The Maillard reaction is also associated with brown color formation, and for this reason is often referred to as the 'browning' or 'nonenzymatic browning' reaction. The brown pigments, known as melanoidins, contain variable amounts of nitrogen and have differing molecular weights and solubilities in water. The dark brown color on the surface of roasted meats is a key factor in consumers' acceptance of these products. Several other changes in the characteristics of cooked meat may also result from Maillard-type reactions and include the production of bioactive compounds with beneficial (i.e., compounds with antioxidant properties) or toxic (e.g., imidazoles) effects, a loss of nutritional quality (especially of proteins), and modification to the product's texture.

The Maillard Reaction

In 1912 at the University of Nancy, France, Louis Camille Maillard first observed the generation of different odors and the formation of brown colored pigments after heating amino acids in the presence of various sugars. The term 'Maillard reaction' was thereafter used to describe the complex series of chemical reactions between carbonyl compounds, especially those of reducing sugars, and primary or secondary amino groups in foods. Browning in most foods is a combined result of the Maillard reaction and caramelization and depends on the product formulation and processing conditions. The ingredients present affect the formation of Maillard reaction products (MRP). In this connection, protein hydrolysates obtained by enzymatic hydrolysis of mechanically deboned chicken meat and the resultant MRPs produced at 90 °C and 100 °C possessed good antioxidant activity and also positively affected the texture and sensory properties of Cantonese sausages. In another study, honey-lysine MRP had an antioxidant effect in linoleic acid emulsion and when honey was added to turkey meat, it enhanced antioxidant properties of the meat as reflected in its thiobarbituric acid reactive substances values. Hence, ingredients present can affect the flavor quality of cooked muscle food products.

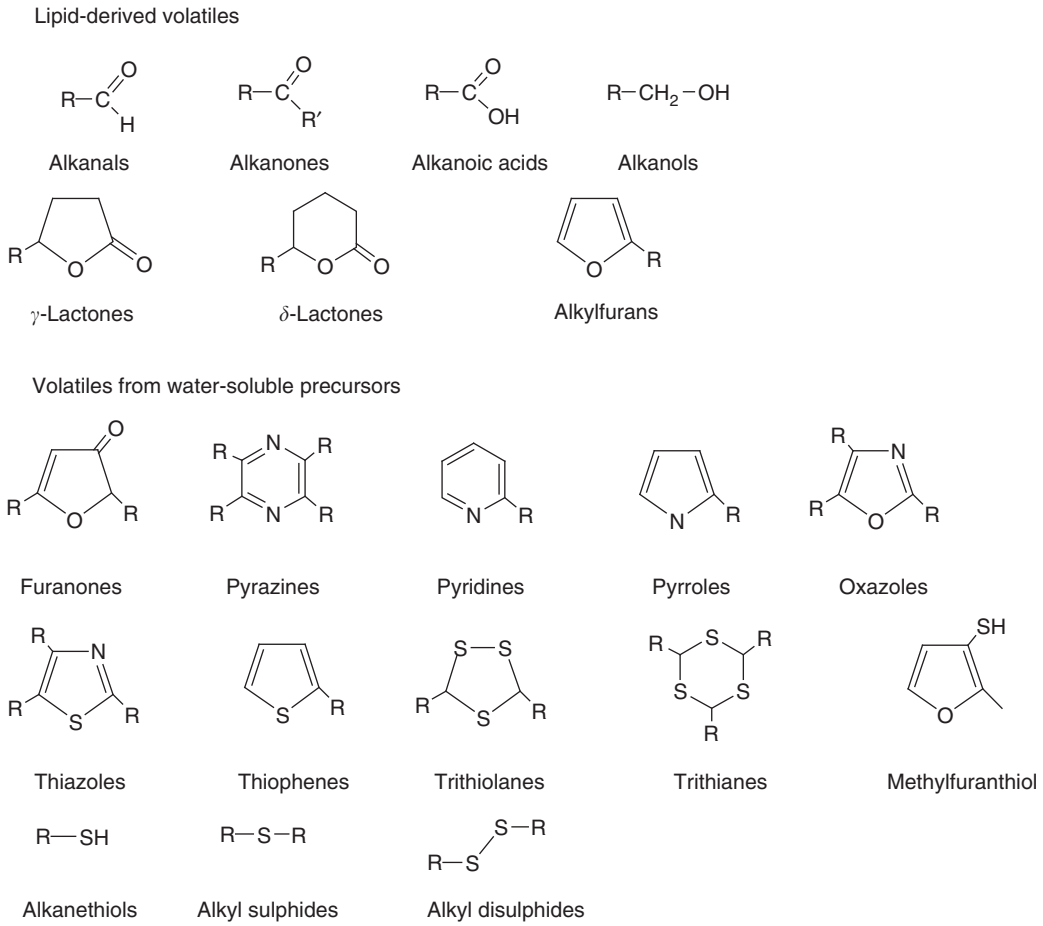

Figure 1 Some classes of volatile compounds produced during the cooking of meat. Reproduced from Shahidi, F., Rubin, L.J., D'Souza, L.A., 1986. Meat flavour volatiles: A review of the composition, techniques, analyses and sensory evaluation. CRC Critical Reviews in Food Science and Nutrition 24, 141–243 and Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62, 415–424.

The actual products formed from the Maillard reaction in biological systems depend on the temperature and time (duration) of cooking, water activity/moisture content, pH, as well as the nature and concentration of the reactants involved. An increase in the formation of brown-colored pigments (i.e., melanoidins) and low-molecular weight flavor compounds in cooked foods significantly correlated with higher cooking temperatures. The optimum rate for the Maillard reaction occurs at a water activity of 0.65–0.75. In other words, the Maillard reaction proceeds more readily at low moisture levels, and the flavor compounds generated are associated mostly with the exterior areas of meat, which have been dehydrated during cooking.

Early Stages of the Maillard Reaction

The initial stages of the Maillard reaction have been well studied. The mechanism proposed by Hodge (1953) the so-called Hodge-scheme to describe the initial stages of the reaction still provides the basis for our understanding of this reaction. The beginning reactions are depicted in Figure 2. Some researchers have suggested that the cyclic pyranose and furanose conformations of sugars are more likely to be involved in the reactions, as they are most abundant in aqueous solution.

To initiate the Maillard reaction, a carbonyl group from the open chain of an aldose sugar reacts reversibly with an amino group of an amino acid, peptide, protein, or other compound possessing a primary or a secondary amino moiety, followed by water elimination leading to an intermediate imine, which cyclizes to produce a glycosylamine (i.e., *N*-glycoside). The Schiff base formed from a hexose and an amino compound may not necessarily cyclize to the glycosylamine and rearrange to form the key intermediate, an 1,2-enaminol, in this primary step of the Maillard reaction. The 1,2-enaminol has three possibilities in which to further react. One possibility is that, it undergoes Amadori rearrangement to yield 1-amino-1-deoxyketose (i.e., an Amadori compound). At higher pH, however, 2,3-enolization is favored and β-elimination of the amine affords a 1-deoxydicarbonyl compound (i.e., 1-deoxyosone). The reaction between ketosugars (e.g., fructose) and amines follows a similar sequence of reactions to form an *N*-ketosylamine, which undergoes a Heyns rearrangement to give a 2-amino-2-deoxyaldose. A second possibility is that, at lower pH, 1,2-enolization of the Amadori product occurs, and the

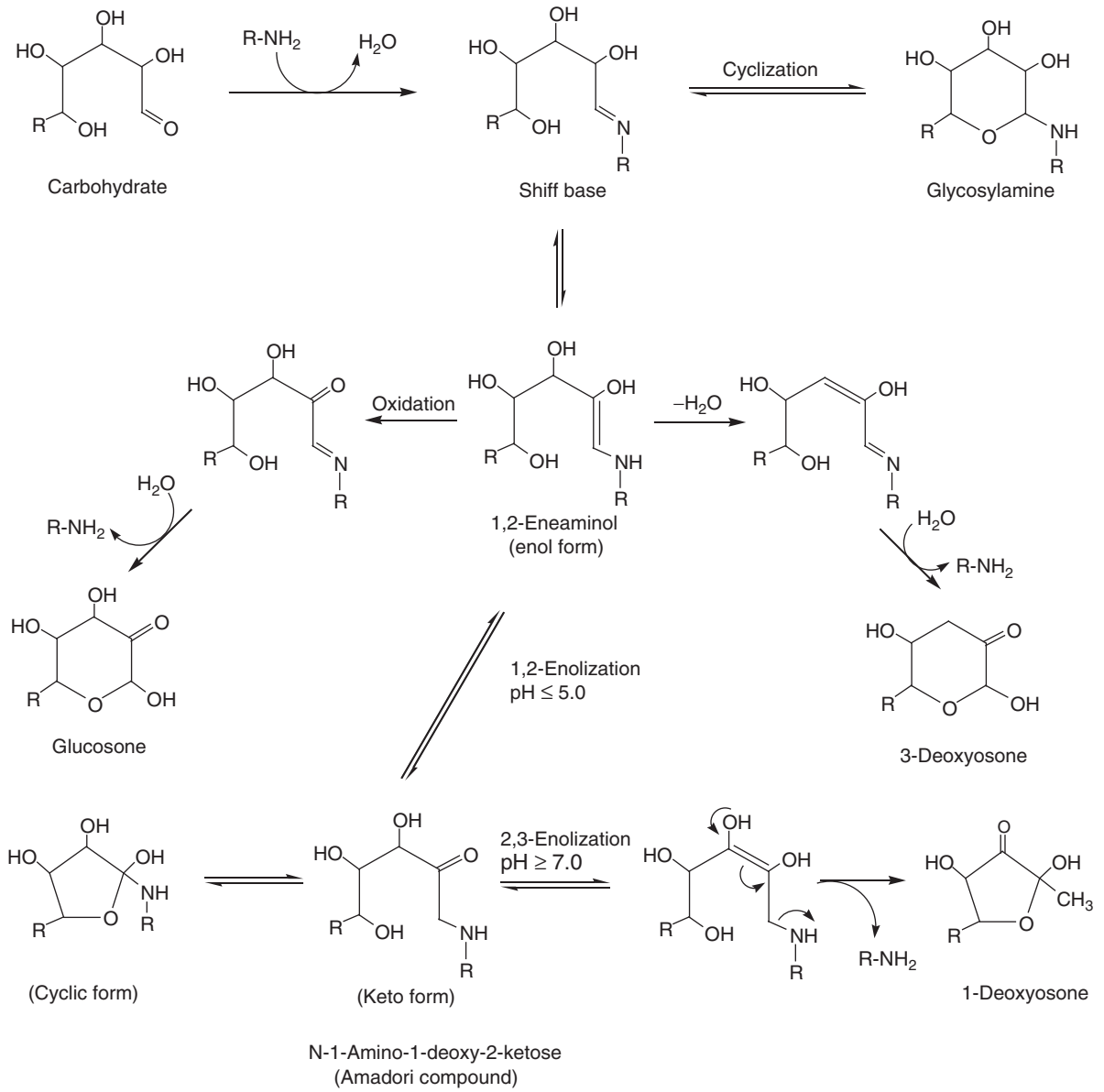

Figure 2 Primary reaction pathways of the Maillard reaction. Modified from Schieberle, P., Hofmann, T., 2002. New results on the formation of important Maillard aroma compounds. In: Swift, K.A.D. (Ed.), Advances in Flavour and Fragrances: From the Sensation to the Synthesis. Cambridge, UK: Special publications of the Royal Society of Chemistry, pp. 163–177, with permission from RSC.

1,2-enaminol formed can lose a molecule of water to yield 3-deoxyosone after hydrolysis of the intermediary α-oxoimine (Figure 2). Thirdly, oxidation of the 1,2-enaminol may take place generating an α-oxoimine, which after hydrolysis forms a hexosone.

The reaction intermediates so formed are thermally unstable and can degrade to a number of flavor compounds (i.e., *N*-, *S*-, *O*-heterocyclics) by the so-called advanced Maillard reaction during further heat treatment. The types of reaction that can take place during the degradation of formed reductones and dehydroreductones include dehydration reactions while maintaining the carbohydrate skeleton, retroaldol reactions leading to fission products, aldol-type reactions of generated fission products, substitution of oxygen-containing compounds by nitrogen and sulfur atoms, redox reactions, and Strecker reactions. Some of the reaction pathways leading to the formation of key intermediary flavor compounds in meat are illustrated in Figures 3 and 4. These compounds can react with other Maillard reaction degradation products as well as with other constituents of the meat matrix (e.g., lipid oxidation products) to form characteristic flavor compounds in cooked meats.

Dehydration and cyclization of 1-deoxyosone lead to the formation of 4-hydroxy-5-methyl-3(2*H*)-furanone from pentoses and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) from hexoses. Maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one), 5-hydroxy-5,6-dihydromaltol, isomaltol [1-(3-hydroxy-2-furanyl)-ethanone], and cyclotene are other important dehydration

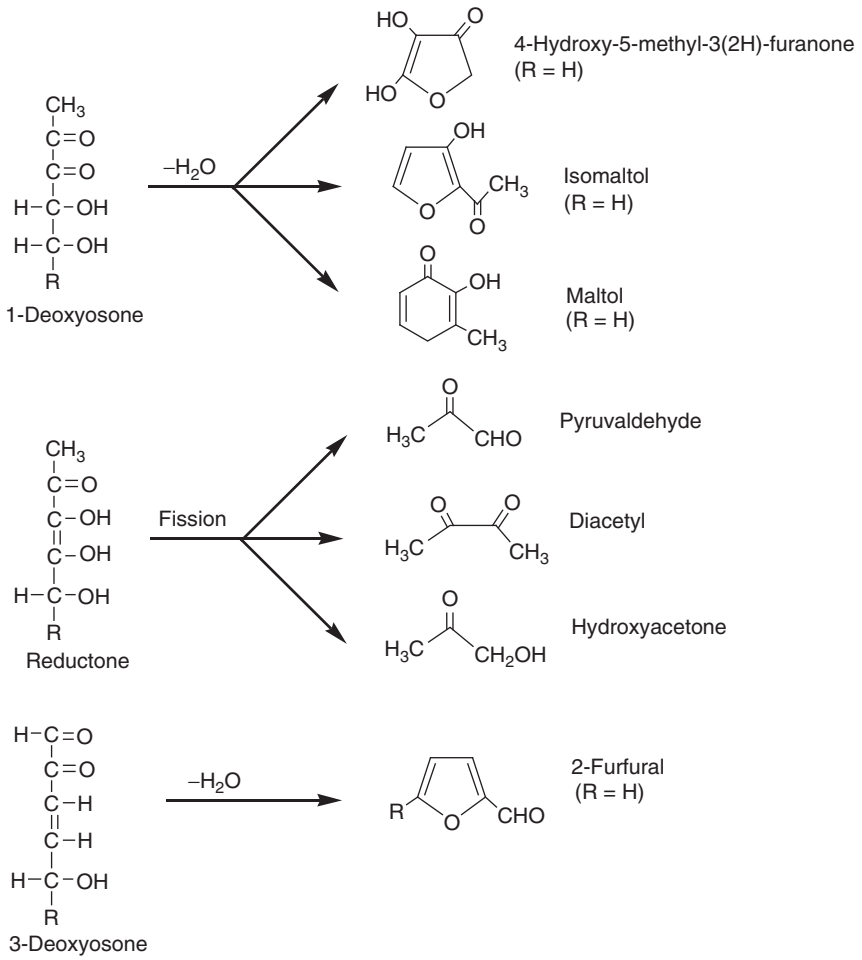

Figure 3 Degradation of Maillard reaction intermediates to form important meat flavor precursors. Reproduced from Bailey, M.E., 1998. Maillard reactions and meat flavour development. In: Shahidi, F. (Ed.), Flavour of Meat, Meat Products and Seafoods. London: Blackie Academic and Professional, pp. 267–289, with permission from Blackie Academic.

products of hexoses (Figure 4). Cyclotene can also be formed by condensation of hydroxyacetone. Degradation of 3-deoxyosones produces 5-hydroxymethyl-2-furfural from hexoses and 2-furfural from pentoses. These furfural derivatives react readily with ammonia and hydrogen sulfide to give many heterocyclic flavor compounds in cooked meats.

Retro-aldol (fission) reactions of 1-deoxyreductone (i.e., an equilibrium product of 1-deoxyosone) in a basic medium (pH > 5) can lead to the formation of very reactive carbonyl compounds such as pyruvaldehyde, diacetyl, dihydroxyacetone, glyoxal and hydroxyacetal, and acetic acid. These compounds participate in Strecker reactions to produce meat flavor compounds.

Strecker Reaction

The reaction between amino acids and α-dicarbonyl compounds (e.g., deoxyosones), which occur as intermediary or end products of other decomposition reactions of the Maillard reaction (e.g., diacetyl, pyruvaldehyde, hydroxyacetone), is one of the most important interactions relating to meat flavor generation. This reaction, termed the Strecker reaction, leads to the formation of an aldehyde, often called a Strecker aldehyde, which contains one less carbon atom than the original amino acid and carbon dioxide; meanwhile, the dicarbonyl, originating from saccharides, is converted into an α-aminoketone or aminoalcohol (Figure 5).

Aminoketones are important intermediates in the formation of several classes of heterocyclic compounds such as pyrazines, oxazoles, and thiazoles; all of which are powerful aroma constituents (Figure 1). If the amino acid is cysteine, the Strecker reaction leads to the formation of ammonia, hydrogen sulfide, and acetaldehyde; these three compounds are very important intermediates in the formation of different classes of flavor compounds in cooked meat. Sulfur compounds, derived from cysteine and ribose, are particularly significant for the generation of aroma notes characteristic of cooked meat.

Later Stages of the Maillard Reaction

As aforementioned, a number of oxygenated sugar degradation products are formed during the initial stages of the Maillard reaction in meat and undergo further interactions at the elevated temperatures associated with cooking. The

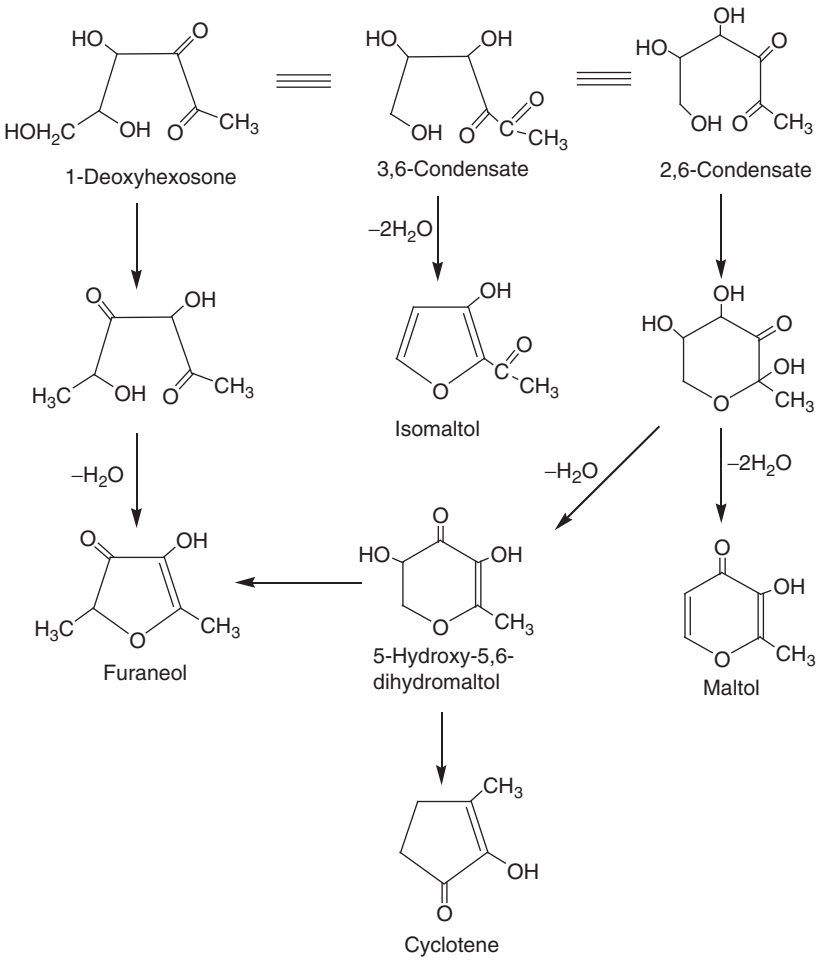

Figure 4 Formation of specific meat flavor intermediates by cyclization and dehydration of 1-deoxyhexosones. Reproduced from Bailey, M.E., 1998. Maillard reactions and meat flavour development. In: Shahidi, F. (Ed.), Flavour of Meat, Meat Products and Seafoods. London: Blackie Academic and Professional, pp. 267–289, with permission from Blackie Academic.

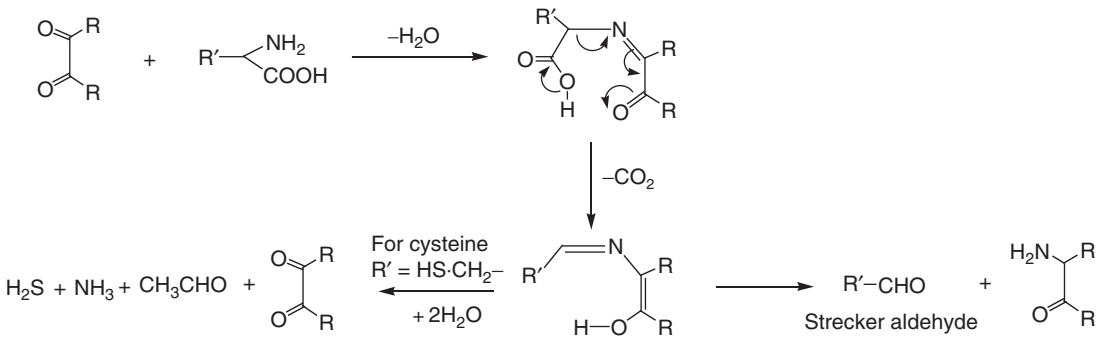

Figure 5 Strecker reaction of amino acids. Reproduced from Mottram, D.S., 1994. Flavor compounds formed during the Maillard reaction. In: Parliament, T.H., Morello, M.J., McGorrin, R.J. (Eds.), Thermally Generated Flavours. Maillard, Microwave and Extrusion Processes. ACS Symposium Series 543. Washington, DC: American Chemical Society, pp. 104–126, with permission from ACS.

Strecker reaction also leads to the production of many reactive compounds such as Strecker aldehydes, ammonia, and hydrogen sulfide. Many of these possess aroma or taste notes, as already commented upon, but they are also crucial intermediates for further flavor-forming reactions in meat during later stages of the Maillard reaction.

Meat Flavor Compounds from Maillard Reaction

Most of the flavor compounds generated via the Maillard reaction are *N*-, *S*-, *O*-heterocyclics and other sulfur-containing compounds that give roasted, boiled, and meaty aromas to cooked meat. These compounds contribute significantly to the

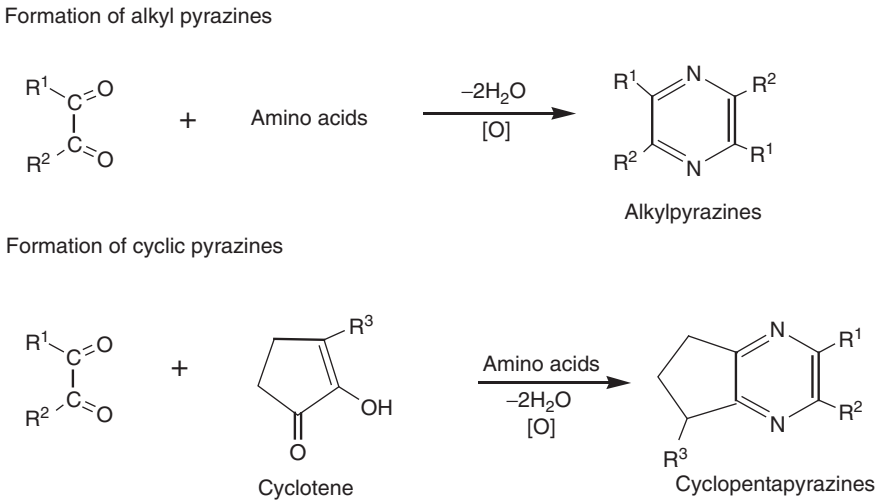

Figure 6 Reaction pathways proposed for the formation of pyrazines. Reproduced from Bailey, M.E., 1998. Maillard reactions and meat flavour development. In: Shahidi, F. (Ed.), Flavour of Meat, Meat Products and Seafoods. London: Blackie Academic and Professional, pp. 267–289, with permission from Blackie Academic.

overall aroma profile of cooked meat; they include furans, furanones, pyrazines, pyrroles, thiophenes, thiazoles (thiazolines), imidazoles, pyridines, oxazoles, cyclic ethylene sulfides, alkyl sulfides, and disulfides (Figure 1).

Oxygen-Containing Compounds

Early stages of the Maillard reaction produce oxygenated furans and pyrans, such as furfural, 5-methylfurfural, 2-acetylfuran, maltol, isomaltol, and furanones like 4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol), and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol) (Figures 3 and 4). Usually, molecules having a planar enol-carbonyl structure in a cyclic dicarbonyl compound originate from sugars and elicit a caramel-like aroma. Most of the reaction products (e.g., maltol, ethylmaltol, dihydromaltol, 4-hydroxy-5-methyl-3(2*H*)-furanone, and norfuraneol) containing this structural element contribute to the 'caramel-like' odor of cooked meats. As previously mentioned, these compounds are also important intermediates in the formation of other *N*- and *S*-containing meat flavor volatiles during thermal processing. For example, sugar degradation products like maltol, isomaltol, 4-hydroxy-5-methyl-3(2*H*)-furanone, furaneol, and cyclotene can exchange oxygen in the ring with nitrogen and sulfur to produce other flavor compounds.

Nitrogen-Containing Compounds

Pyrazines

Pyrazines have been found in all meat species following cooking and constitute a major class of volatiles formed via the Maillard reaction. The nature and quantity of pyrazines generated during thermal processing are a function of the reaction conditions employed, such as moisture content, pH, temperature, and the duration (time) of cooking. Several mechanisms have been proposed for pyrazine formation by the Maillard reaction: one important route is the condensation of α-dicarbonyl compounds formed from the Strecker reaction with amino compounds to give alkylpyrazines (Figure 6).

Two other classes of compounds, mainly bicyclic products, 6,7-dihydro-5(*H*)-cyclopentapyrazines and pyrrolopyrazines, have also been reported in meat volatiles. Cyclopentapyrazines can be formed from the condensation of cyclic ketones such as cyclotene (Figure 6). The alkylpyrazines generally have nutty and roasted aromas, whereas cyclopentapyrazines have roasted, grilled, and species-related flavor notes of roasted meat. The pyrrolopyrazines have only been reported for meat. Recent evidence indicates that 3-deoxyglucosone is a chief precursor for pyrazine formation by retro-aldolization and 2,4-scission to yield pyruvaldehyde. Pyruvaldehyde is involved in the Strecker reaction to give dimethylpyrazine.

Formation of 48 pyrazines from beef, 36 from pork, and 16 from lamb have been documented. These are extremely important constituents of meat cooked at high temperatures and contribute mainly to the flavor of roasted meat. Pyrazines account for 77% of the total volatiles found in well-done grilled pork.

Oxazoles and oxazolines

Several oxazoles have been identified in cooked meats, and these possess green and vegetable-like aroma characteristics. The compound 2,4,5-trimethyl-3-oxazoline, with a woody, musty, and green note, has also been detected in boiled beef. However, contribution of oxazoles and oxazolines to the overall aroma of meat is not as significant as that of sulfur-containing compounds such as thiazoles and thiazolines, which possess closely related chemical structures.

Sulfur-Containing Compounds

Sulfur compounds, both aliphatic and heterocyclic, are among the most important volatiles formed during meat processing. Most occur at low concentrations, but their very low odor thresholds make them potent aroma compounds giving sulfurous, onion-like, and meaty aromas to cooked meat

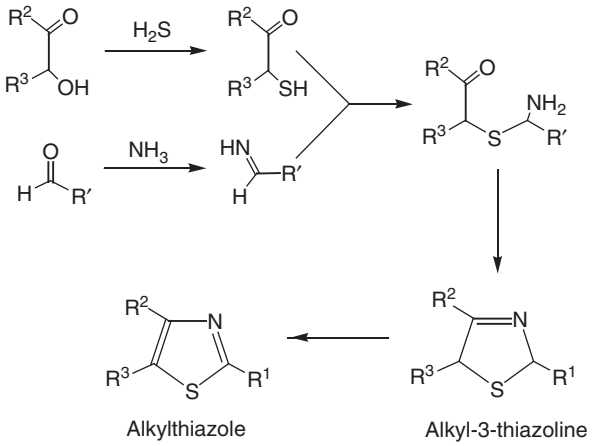

Figure 7 Route for the formation of thiazolines and thiazoles in the Maillard reaction from the reaction of hydroxyketones and aldehydes with ammonia and hydrogen sulfide. Reproduced from Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62, 415–424.

products. Hydrogen sulfide, produced from cysteine by hydrolysis or via the Strecker reaction, is an essential precursor in the formation of many sulfur-containing aroma compounds in meat during thermal processing.

The cooking method employed has a significant effect on the generation of sulfurous compounds. For example, more aliphatic thiols, sulfides, and disulfides have been reported in boiled beef compared to those of roast beef. Heterocyclic compounds with 1, 2, or 3 sulfur atoms in their 5- and 6-membered rings (e.g., thiophenes, trithilanes, trithianes) are formed in greater amounts in boiled meat than in roasted meat.

Thiazoles and thiazolines

These compounds are important constituents of roasted or fried meat, and their content increases with higher cooking temperatures. Most thiazoles present in meat are alkyl substituted, and their aroma depends on the nature and the number of alkyl moieties attached. A number of the di- and tri-alkyl derivatives formed in meat have been reported to possess roasted and meaty aroma characteristics. Some acetyl-substituted thiazoles and thiazolines have also been found in cooked meats.

One possible route for the formation of thiazoles and thiazolines is via the Maillard reaction, and this involves the action of ammonia and hydrogen sulfide in the presence of α-carbonyls, dicarbonyls, or hydroxyketones (Figure 7) derived from the Strecker reaction of amino acids in heated foods. However, lipid-derived aldehydes can also participate in this reaction during cooking and, in fact, long-chain trialkylthiazoles have been identified in the aromagrams of roast beef and fried chicken.

Thiophenes

Perhaps the most important flavor compounds arising from the Maillard reaction are the thiophenes and furans with methyl or sulfur groups at 1, 2, or 5 positions. These compounds give desirable 'meaty' aroma to cooked meat. Furans and thiophenes with a thiol group at the 3-position also possess a strong meaty-like aroma and have very low odor threshold values. There are a number of possible routes for the formation of thiophenes, involving the reaction of a sulfur compound derived from sulfur-containing amino acids (e.g., cysteine, cystine, methionine) or thiamine, with intermediary sugar degradation products from the Maillard reaction, such as deoxyosones. Another pathway has also been proposed for the formation of long-chain 2-alkylthiophenes from reactions involving hydrogen sulfide and the 2,4-alkadienals derived from the degradation of lipids.

Polysulfur heterocyclics

A number of polysulfur heterocyclics have been found in meat, and the formation of these compounds is very important for the desirable meaty aroma in cooked meats. Seventy-eight compounds having meat-like aromas have been reported, of which 65 are heterocyclic sulfur compounds and seven are sulfur-containing aliphatic compounds. The remaining six are nonsulfur-containing heterocyclics. The mechanisms of formation of a number of these cyclic-sulfur compounds in the aroma of cooked beef via the Maillard reaction or thermal degradation of thiamine have been reported.

Acetaldehyde, formed by the Strecker reaction of alanine during thermal processing, and other aldehydes can react with precursors such as hydrogen sulfide, ammonia, and methanethiol to yield a large number of heterocyclic and straight chain polysulfur compounds in meat (Figure 8). Under oxidative conditions, dialkyltrithiolanes are formed from *bis*-(1-mercaptoethyl)sulfide, whereas at low pH trialkyltrithianes are produced (Figure 8). At elevated temperatures, *bis*-(1-mercaptoethyl)sulfide isomerizes to trisulfides and leads to the formation of di- and tetrasulfides. Dithiazines and thiadiazines are generated in the presence of ammonia (Figure 8). Thus, the formation of these compounds in cooked meats depends on the reaction conditions (i.e., acidity and temperature of thermal processing) and the types of Strecker reaction products available for each of the reactions to proceed.

Sulfur compounds from furan-like components

As aforementioned, furans with a thiol group in the 3-position, and related disulfides, possess strong meat-like aromas with very low odor threshold values. The presence of 2-methyl-3-(methylthio)-furan and 2-methyl-3-(methyldithio)-furan in cooked beef has been reported; these compounds have odor thresholds of 50- and 10 parts-per-billion, respectively. In addition, 2-methyl-3-furanthiol and the corresponding disulfide, *bis*-(2-methyl-3-furanyl)disulfide (odor threshold of 0.02 ng kg^{-1}), have been identified as major contributors to the meaty aroma of cooked beef. In meat, 4-hydroxy-5-methyl-3(2*H*)-furanone, formed via dephosphorylation and dehydration of ribose phosphate (from ribonucleotides), reacts with hydrogen sulfide to yield the above mentioned meat flavor character impact compounds (Figure 9). Some other furan-like sulfur compounds (Figure 10), apart from those described above, have also been identified in cooked meats.

Oxidation of thiols formed via interaction of hydrogen sulfide with dicarbonyls, furanones, and furfurals (i.e., prominent products of the Maillard reaction) may provide other routes in the formation of furan sulfides and disulfides.

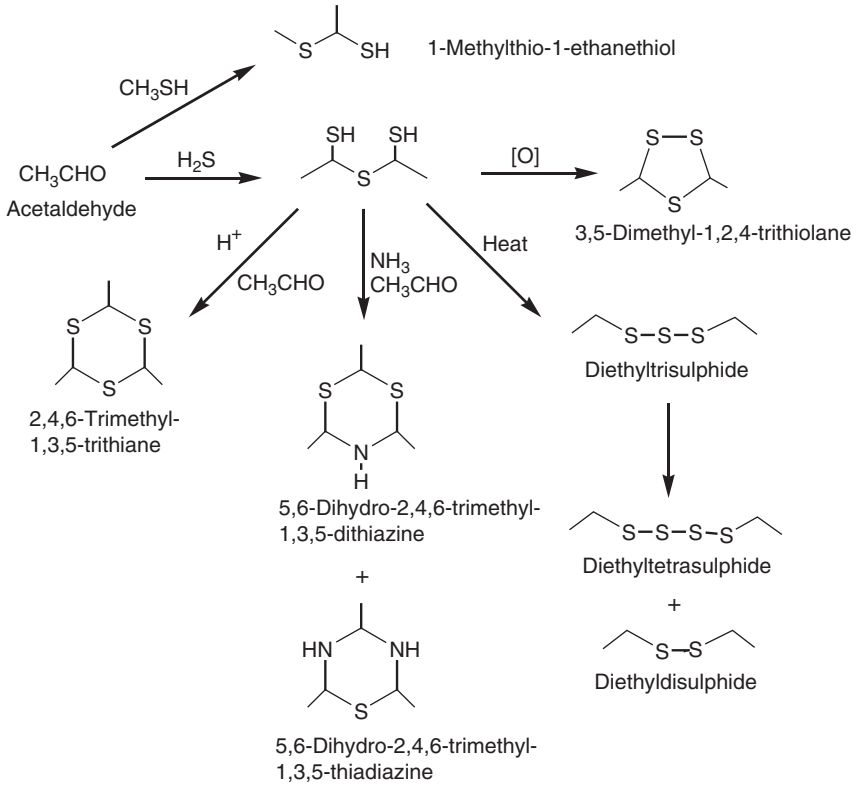

Figure 8 Formation of some sulfur-containing aroma compounds from reaction of acetaldehyde, hydrogen sulfide, ammonia, and methanethiol. Modified from Mottram, D.S., 1994. Flavor compounds formed during the Maillard reaction. In: Parliament, T.H., Morello, M.J., McGorrin, R.J. (Eds.), Thermally Generated Flavours. Maillard, Microwave and Extrusion Processes. ACS Symposium Series 543. Washington, DC: American Chemical Society, pp. 104–126, with permission from ACS.

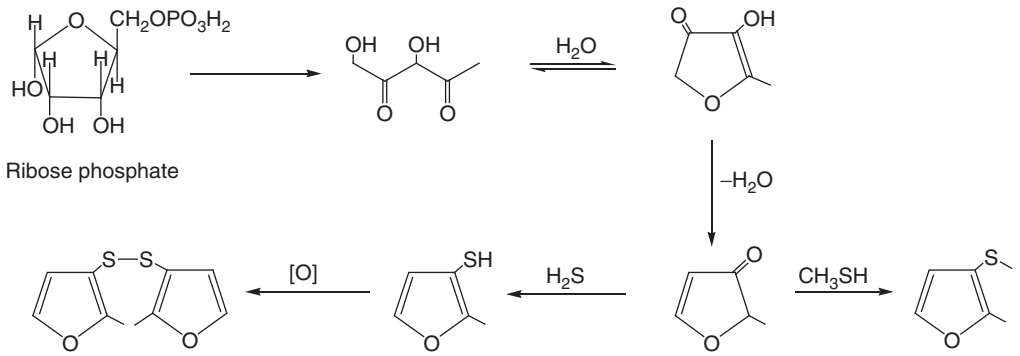

Figure 9 Route for the formation of 2-methyl-3-furanthiol, *bis*-(2-methyl-3-furanyl) disulfide, and 2-methyl-3-(methylthio)-furan from ribose phosphate. Reproduced from Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62, 415–424.

Cyclotene (2-hydroxy-3-methylcyclopent-2-enone), another product of the Maillard reaction, formed from 5-hydroxy-5,6-dihydromaltol (Figure 4) and from the condensation of hydroxyacetone, can react with ammonia and hydrogen sulfide to generate volatile compounds with meaty aromas (e.g., 1,2,4-trithiolane, 5-trithiane, and 1,2,4,6-tetrathiepane) and a roasted beefy note (e.g., 2-methylcyclopentanone and 3-methylcyclopentanone). Isomaltol is also an important precursor of the heterocyclic compounds related to cooked meat flavor.

Meat Flavor Compounds from Lipid–Maillard Interactions

Both saturated and unsaturated aldehydes formed via autoxidation of lipids are among the major contributors to the volatile profile of cooked meats. At the same time, these aldehydes can participate in the Maillard reaction at both the initial and later stages during thermal processing of meat. Volatile compounds such as pyridines, pyrazines, thiophenes, thiazoles, and oxazoles with alkyl substituents are formed.

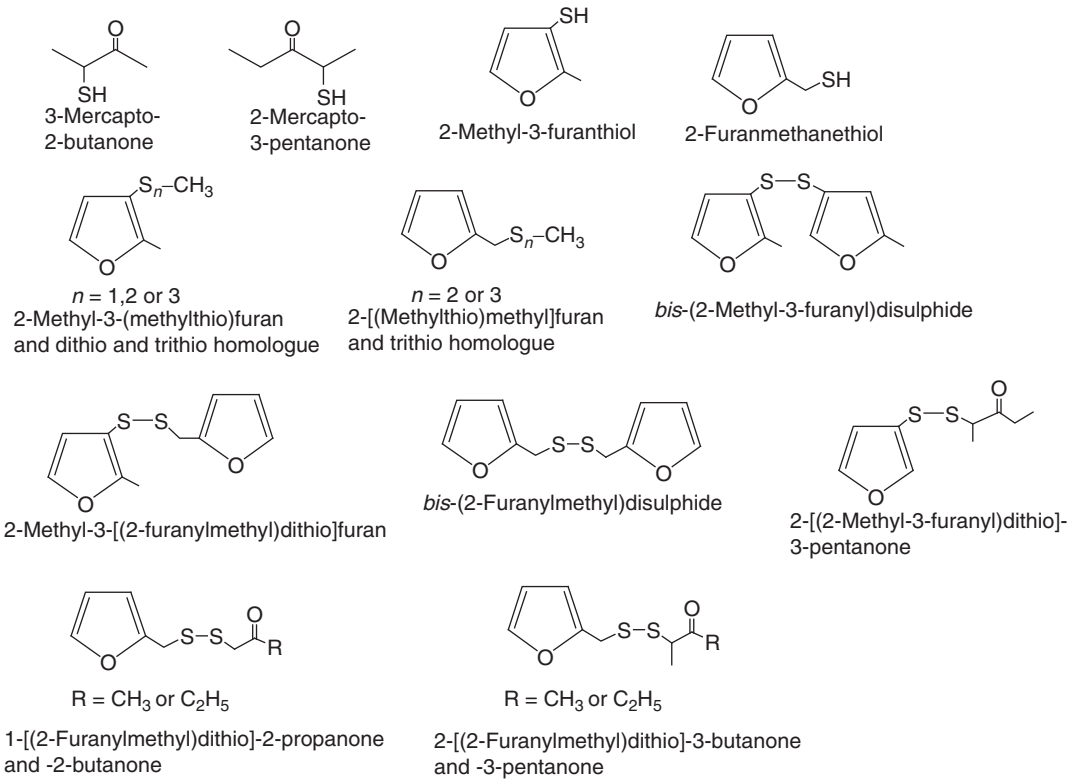

Figure 10 Some thiols, sulfides, and disulfides found in the volatiles of cooked meat. Reproduced from MacLeod, G., Ames, J.M., 1986. 2-Methyl-3-(methylthio)furan: A meat character impact aroma compound identified from cooked beef. Chemistry and Industry (London) 50, 175–176; Gasser, U., Grosch, W., 1988. Identification of volatile flavor compounds with high aroma values from cooked beef. Zeitschrift fuer Lebensmittel Untersuchung und Forschung 186, 489–494; Farmer, L.J., Patterson, R.L.S., 1991. Compounds contributing to meat flavour. Food Chemistry 40, 201–205; Madruga, M.S., 1994. Studies on some factors affecting meat flavour formation. Ph.D Thesis, The University of Reading, UK; and Madruga, M.S., Mottram, D.S., 1995. The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. Journal of the Science of Food and Agriculture 68, 305–310.

Figure 11 Alkyl-3-thiazolines and alkylthiazoles found in cooked beef and lamb. Reproduced from Mottram, D.S., Elmore, J.S., 2002. Novel sulfur compounds from lipid–Maillard interactions in cooked meat. In: Reineccius, G.A., Reineccius, T.A. (Eds.), Heteroatomic Aroma Compounds. ACS Symposium Series 826. Washington, DC: American Chemical Society, pp. 93–101, with permission from ACS.

A number of thiazoles with C_4–C_8 *n*-alkyl substituents in the 2-position have been reported in roast beef and fried chicken. Several other alkylthiazoles with longer 2-alkyl chains of C_{13}–C_{15} have been identified in the volatiles of heated beef and chicken, with the highest concentrations reported in beef heart muscle. Recently, a series of alkylthiazoles and alkyl-3-thiazolines were isolated from the volatiles of cooked beef and lamb (Figure 11); the amounts formed were greater in pressure-cooked meat than in grilled meat. Furthermore, the number and concentration of these compounds were much higher in meat from animals fed a linseed and fish oil diet than in the control diet devoid of them. Lipid-derived aldehydes can participate in the formation of long-chain 2-alkylthiazoles in heated meats. Reactions of aldehydes, hydroxyketones, ammonia, and hydrogen sulfide with one another, as illustrated in Figure 7, have already been discussed.

The compound 2-pentylpyridine has been identified in all major meat species; a likely route of its formation is via the reaction of *E,E*-2,4-decadienal with ammonia (Figure 12). Similar types of reactions between 2,4-alkadienals and hydrogen sulfide may be responsible for the formation of 2-alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans (Figure 12).

Butyl- and pentyl-substituted pyrazines have been identified in cooked meats. The probable mechanism for their formation is via the reaction of pentanal or hexanal with a dihydropyrazine, formed by the condensation of two aminoketone molecules (Figure 13). Pentanal and hexanal, which are breakdown products of linoleic acid (18:2*n*6), also appear to be involved in the formation of 5-butyl-3-methyl-1,2,4-trithiolane and its 5-pentyl homologue. Both trithiolanes have been isolated from the volatiles of fried chicken and pork.

Volatile compounds derived from lipid-Maillard interactions of meat possess weak odor intensities (i.e., weak fatty, fried, and garlic-like notes) and high odor thresholds;

consequently, they may not contribute directly to the aroma of cooked meat. However, interactions between lipid degradation and MRP during thermal processing may modify the aroma compounds generated via the Maillard reaction and by lipid degradation and, therefore, have an indirect impact on the aroma profile of cooked meat. In particular, phospholipids and their degradation products inhibit reactions involved in the formation of heterocyclic aroma compounds via the Maillard reaction. Thus, the generation of sulfur-containing heterocyclics during thermal processing of meat may be curbed by this inhibition. This interaction may help to maintain the level of key sulfur compounds at their optimum concentrations in cooked products.

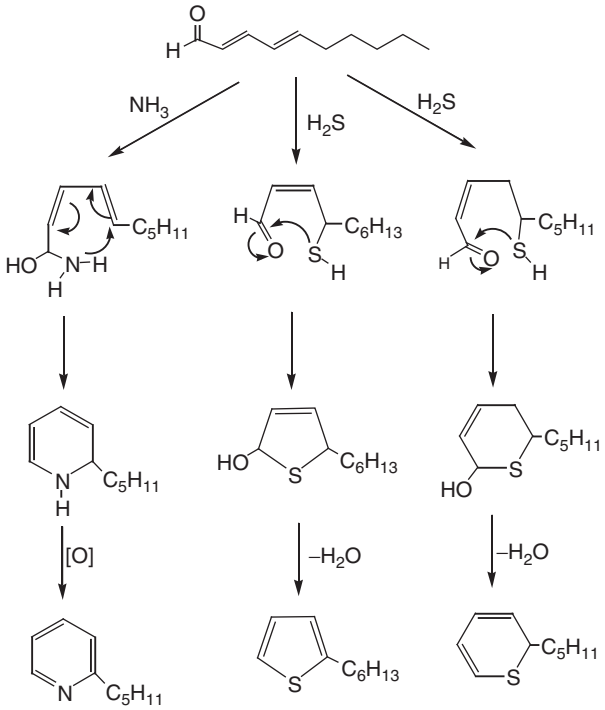

Figure 12 Formation of 2-pentylpyridine, 2-hexylthiophene, and 2-pentyl-(2*H*)-thiapyran from 2,4-decadienal, ammonia, and hydrogen sulfide. Reproduced from Farmer, L.J., Mottram, D.S., 1990. Interaction of lipids in the Maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. Journal of the Science of Food and Agriculture 53, 505–525.

Maillard Browning

Besides aroma, the brown color formation due to the Maillard reaction (i.e., nonenzymatic browning) is a key factor in the overall acceptability of thermally processed foods such as roasted meat and coffee. Little is known, however, about the chromophores responsible and the reaction mechanisms leading to the formation of these brown color compounds from carbohydrates and amino acids. From studying reducing sugar/amino acid reaction mixtures, the chromophores identified as 'browning agents' can be divided into two classes: low-molecular weight colored compounds and high-molecular weight melanoidins. Model experiments indicate that condensation reactions between methylene-active intermediates (e.g., 4-hydroxy-5-methyl-3(2*H*)-furanone) and reactive carbonyl compounds (e.g., furan-2-aldehyde, acetaldehyde, acetone, pyrrolaldehydes, or 2-oxopropanal) generated during the Maillard reaction are the dominant reaction in nonenzymatic browning and aid in the formation of low-molecular weight colored compounds (Figure 14).

Besides the classical reaction pathway proposed by Hodge (Figure 2), model experiments have shown another reaction pathway leading to the formation of color in very early stages of the Maillard reaction and before Amadori rearrangement. The proposed mechanism involves cleavage of the sugar molecule with the production of glycolaldehyde imine; this product dimerizes and oxidizes to form a 1,4-dialkylpyrazinium radical cation (Figure 15) as an important intermediate. Early stages of browning in foods and beverages may be a result of this phenomenon.

Although the exact structures of high-molecular weight melanoidins have not been elucidated, it has been found that such compounds can be generated during thermal processing

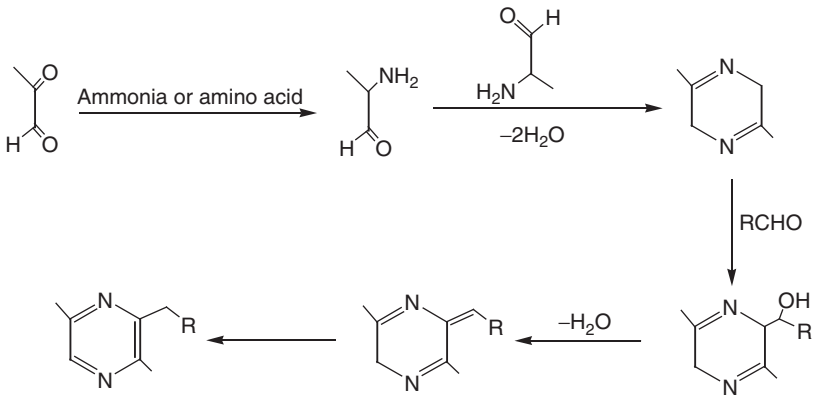

Figure 13 Route to alkyldimethylpyrazines from the interaction of lipid-derived aldehydes with the Maillard reaction. Reproduced from Ho, C.T., Carlin, J.T., Huang, T.C., 1987. Flavour development in deep-fat fried foods. In: Martens, M., Dalen, G.A., Russwurm, H. (Eds.), Flavour Science and Technology. Chichester: Wiley, pp. 35–42.

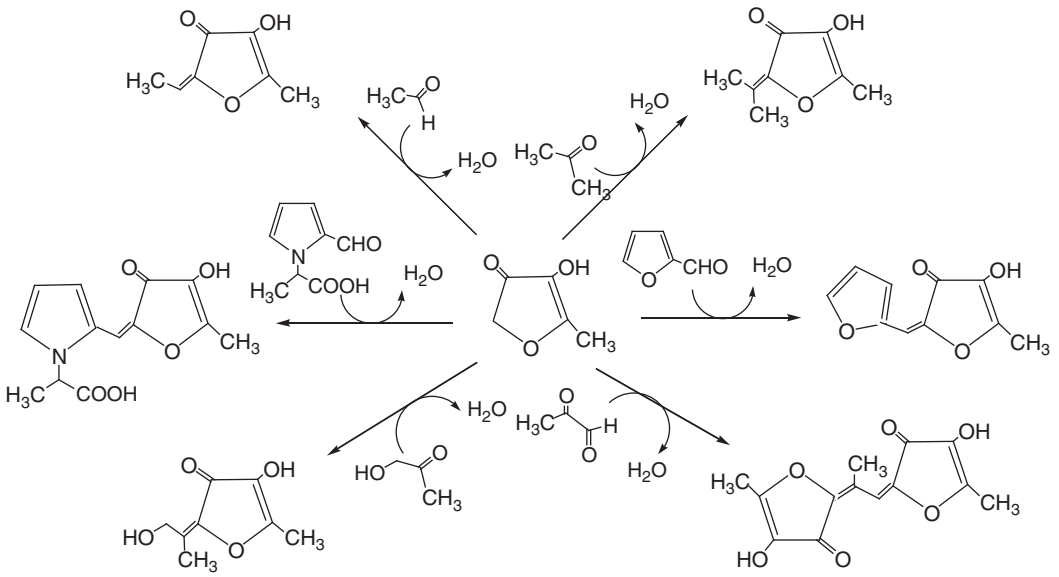

Figure 14 Formation of colored condensation products from carbohydrate-derived carbonyls and 4-hydroxy-5-methyl-3(2*H*)-furanone (norfuraneol). Reproduced from Hofmann, T., Frank, O., Heuberger, S., 2001. The color activity concept: An emerging technique to characterize key chromophores formed by non-enzymic browning reactions. In: Ames, J.M., Hofmann, T. (Eds.), Chemistry and Physiology of Selected Food Colorants. ACS Symposium Series 775. Washington, DC: American Chemical Society, pp. 168–179, with permission from ACS.

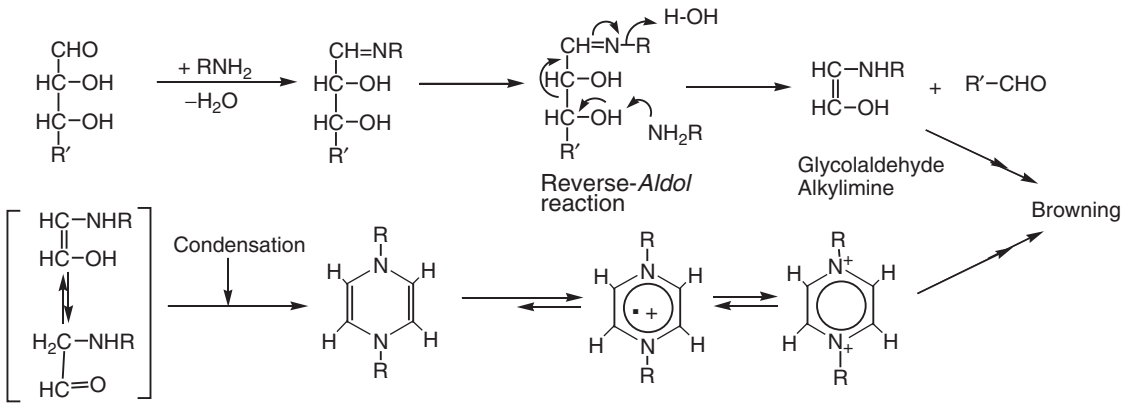

Figure 15 A possible pathway for formation of the free radical product and browning in the reaction of sugar with amino compound. Reproduced from Namiki, M., Hayashi, T., 1981. Formation of novel free radical products in an early stage of Maillard reaction. In Eriksson, C. (Ed.), Maillard Reactions in Food. Chemical, Physiological and Technological Aspects. Oxford, UK: International Union of Food Science & Technology, Pergamon Press, pp. 81–91.

of food by a cross-linking reaction between low-molecular weight MRP and high-molecular weight noncolored proteins. Lysine and arginine residues of protein help bind the carbohydrate-derived compounds to the protein backbone and produce the chromophoric substructures of melanoidins (Figure 16). Thus, proteins seem to act as noncolored skeletons of food melanoidins, to which different chromophoric substructures might be covalently bound through reactive side chains. Meat contains a fairly high protein content (e.g., 20–25% in lean muscle tissue) and the carbohydrate-induced oligomerization and browning of proteins may be involved in the formation of melanoidins during thermal processing of meat. This leads, at least to some extent, to the characteristic dark brown color associated with roasted meat.

Summary

The aroma of cooked meats is thermally derived, and the volatile compounds originate from both lipid- and water-soluble precursors. The Maillard reaction occurs during cooking of meat and is mainly responsible for the formation of a large number of heterocyclic compounds present in the volatile fraction of meat; these are responsible for the savory, roasted, and boiled flavors associated with cooked meat. Reducing sugars and amino acids of meat, notably ribose and cysteine, respectively, are important precursors for these reactions. Initial stages of the Maillard reaction lead to the formation of α-dicarbonyls such as 1-deoxyosones, 3-deoxyosones, and 1-deoxyreductones through Amadori and Heyns intermediates.

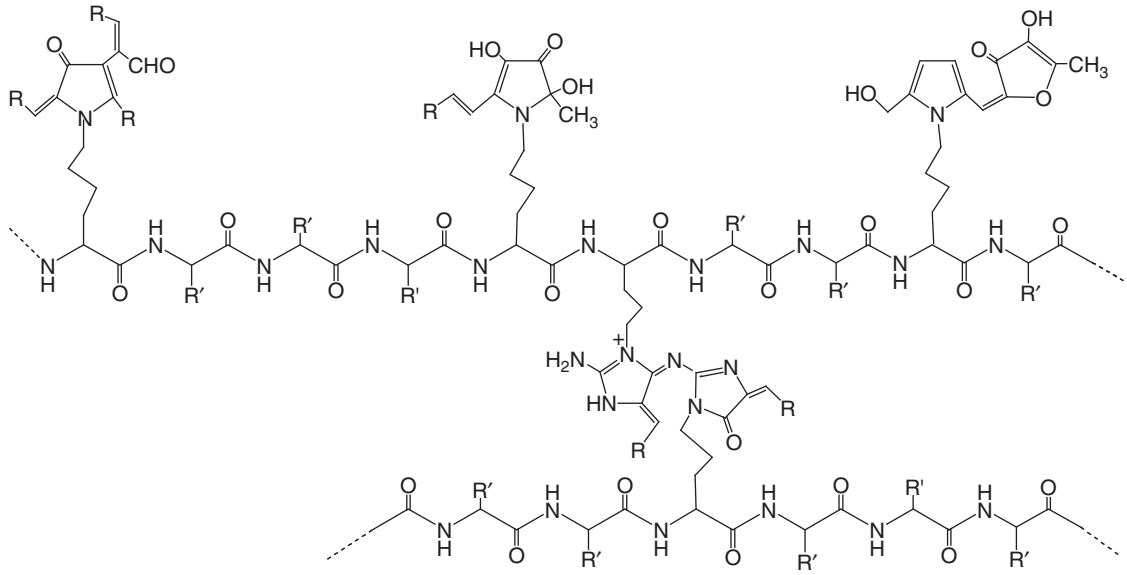

Figure 16 Chromophoric substructures involved in melanoidin formation. Reproduced from Hofmann, T., 2001. Structure, colour and formation of low- and high-molecular weight products formed by food-related Maillard-type reactions. In: Ames, J.M., Hofmann, T. (Eds.), Chemistry and Physiology of Selected Food Colorants. ACS Symposium Series 775. Washington, DC: American Chemical Society, pp. 134–151, with permission from ACS.

The breakdown of these compounds yields key intermediates such as furfurals, furanones, and other dicarbonyl compounds. Other related reactions (e.g., the Strecker reaction) lead to the formation of simple compounds such as aldehydes, ammonia, and hydrogen sulfide, which are precursors for further reactions. Many different interactions of these compounds subsequently result in the formation of important classes of aroma compounds like furans, furanones, pyrazines, pyrroles, thiophenes, thiazoles (thiazolines), imidazoles, pyridines, oxazoles, cyclic ethylene sulfides, alkylsulfides, and disulfides. Of these compounds, aromatic nitrogen derivatives such as pyrazines, *N*-, *S*-heterocyclics like thiazoles, 2-methyl-3-thio (or sulfide)-furans, 2-methyl-5-thio (or sulfide)-thiophenes, sulfur-substituted tetrahydrofuranones, and nonaromatic ring sulfur derivatives with two or more sulfur groups are particularly important for the meaty-roasted flavor of cooked meats.

Other constituents of the meat matrix, such as lipids, can react with products of the Maillard reaction. Lipid-derived aldehydes participate in Maillard reactions via reactions with hydrogen sulfide and ammonia to produce new volatile compounds. Lipid degradation products from phospholipids are particularly important and appear to control or limit the generation of sulfur compounds during thermal processing of meat. This process may be involved in the modification of flavor profiles of cooked meats and would depend on the type of meat (i.e., lean meat with less fat, meat with more unsaturated fatty acids) and reaction parameters (i.e., cooking method, temperature, duration of cooking) used.

Owing to the complexity of the nonvolatile MRP, very little is known about the compounds responsible for the typical brown color on the exterior areas of products. According to the model experiments, both high- and low-molecular weight colored compounds generated during thermal processing contribute to the brown color on the surface of cooked meats.

See also: Cooking of Meat: Flavor Development; Physics and Chemistry; Warmed-Over Flavor

Further Reading

Adams, A., deKimpe, N., 2006. Chemistry of 2-acetyl-1-pyroline, 6-acetyl-1,2,3,4-tetrahydropyridine, 2-acetyl-2-thiazoline and 5-acetyl-2,3-dihyho—4 H—thiazine: Extraordinary flavour compounds. Chemical Reviews 106, 2299–2319.

Antony, S.M., Han, I.Y., Rieck, J.R., Dawson, P.L., 2002. Antioxidative effect of Maillard reaction products added to turkey meat during heating by addition of honey. Journal of Food Science 67, 1720–1724.

Bailey, M.E., 1983. The Maillard reaction and meat flavour. In: Waller, G.R., Feather, M.S. (Eds.), The Maillard Reaction in Foods and Nutrition. ACS Symposium Series, 215. Washington, DC: American Chemical Society, pp. 169–184.

Bailey, M.E., 1998. Maillard reactions and meat flavour development. In: Shahidi, F. (Ed.), Flavour of Meat, Meat Products and Seafoods. London: Blackie Academic and Professional, pp. 267–289.

Cadwallader, K.R., Shahidi, F., 2001. Identification of potent odorants in seal bubbler oil by direct thermal desorption-gas chromatography-olfactometry. In: Shahidi, F., Finley, J.W. (Eds.), Omega-3 Fatty Acids. Chemistry, Nutrition and Health Effects. ACS Symposium Series, 788. Washington, DC: American Chemical Society, pp. 221–235.

Farmer, L.J., Mottram, D.S., 1990. Interaction of lipids in the Maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. Journal of the Science of Food and Agriculture 53, 505–525.

Farmer, L.J., Patterson, R.L.S., 1991. Compounds contributing to meat flavour. Food Chemistry 40, 201–205.

Ho, C.-T., 1996. Thermal generation of Maillard aromas. In: Ikan, R. (Ed.), The Maillard Reaction. Consequences for the Chemical and Life Sciences. New York: John Wiley & Sons, pp. 27–53.

Hodge, J.E., 1953. Chemistry of browning reactions in model systems. Journal of Agricultural and Food Chemistry 1, 928–943.

Hofmann, T., 2001. Structure, colour and formation of low- and high-molecular weight products formed by food-related Maillard-type reactions. In: Ames, J.M., Hofmann, T. (Eds.), Chemistry and Physiology of Selected Food Colorants. ACS

Symposium Series, 775. Washington, DC: American Chemical Society, pp. 134–151.
Hofmann, T., Frank, T., Heuberger, S., 2001. The colour activity concept: An emerging technique to characterize key chromophores by non-enzymatic browning reactions. In: Ames, J.M., Hofmann, T. (Eds.), Chemistry and Physiology of Selected Food Colorants. ACS Symposium Series, 775. Washington, DC: American Chemical Society, pp. 168–179.
Maillard, L.C., 1912. Action of amino acids on sugars. Formation of melanoidins in a methodical way. Compte Rendus 154, 66–68.
Mottram, D.S., 1994. Flavor compounds formed during the Maillard reaction. In: Parliment, T.H., Morello, M.J., McGorrin, R.J. (Eds.), Thermally Generated Flavours. Maillard, Microwave and Extrusion Processes. ACS Symposium Series, 543. Washington, DC: American Chemical Society, pp. 104–126.
Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62, 415–424.
Mottram, D.S., Elmore, J.S., 2002. Novel sulphur compounds from lipid-Maillard interactions in cooked meat. In: Reineccius, G.A., Reineccius, T.A. (Eds.), Heteroatomic Aroma Compounds. ACS Symposium Series, 826. Washington, DC: American Chemical Society, pp. 93–101.
Romero, M.V., Ho, C.T., 2007. Maillard reaction in flavour generation. In: Nolle, H. L.M.L. (Ed.), Handbook of Meat, Poultry and Seafood Quality. Oxford, UK: Blackwell Publishing, pp. 259–274.
Schieberle, P., Hofmann, T., 2002. New results on the formation of important Maillard aroma compounds. In: Swift, K.A.D. (Ed.), Advances in Flavour and Fragrances: From the Sensation to the Synthesis. Cambridge, UK: Special publications of the Royal Society of Chemistry, pp. 163–177.
Shahidi, F., 1998. Flavour of Meat, Meat Products and Seafoods, second ed. London, UK: Blackie Academic and Professional.
Shahidi, F., Rubin, L.J., D'Souza, L.A., 1986. Meat flavour volatiles: A review of the composition, techniques, analyses and sensory evaluation. CRC Critical Reviews in Food Science and Nutrition 24, 141–243.
Sun, W., Zhao, M., Cui, C., Zhao, Q., Yang, B., 2010. Effect of Maillard reaction products derived from the hydrolysates of mechanically deboned chicken residue on antioxidant, textural and sensory properties of Cantonese sausages. Meat Science 86, 276–282.

Physics and Chemistry

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Introduction

Meat is a complex structure composed of muscle fibers, extracellular matrix, lipids, and water. The meat proteins are the main constituents that form the structure of the meat product. During heating they undergo significant structural changes and therefore the quality of the meat, mainly influenced by the meat structure, also changes significantly. Among the sarcoplasmic meat proteins there are globular proteins and enzymes. A typical example of globular proteins is myoglobin responsible for meat color. At increased temperatures the hydrophobic side chains of the compact globular proteins, in the aqueous environment, undergo expansion and partial unfolding, followed by association of unfolded proteins. The large degree of protein association decreases their solubility and precipitates are formed. If, however, the three-dimensional network is formed, a gel sets. These gels bind the water and are solid-like in their mechanical behavior. The fibrous proteins (actin, myosin, titin, collagen) have a lot of hydrogen bonds and show electrostatic interactions that keep the molecules in register in the large building blocks, which in turn are broken during heating. The fibrous proteins contract on cooking in contrast to the globular proteins, which expand. Physicochemical processes that occur in the meat tissue during heating, causing significant changes in the spatial arrangement of the meat proteins, affect the final physicochemical and sensory properties of the heated meat.

The method of heating is important too. For the same raw material, to achieve equivalent denaturation of proteins, more energy is needed during fast (40 °C min^{-1}) than during slow (5 °C min^{-1}) heating. Different heating rates dictate the rate of enzymatic and chemical reactions in the meat. They influence the conformation of the meat proteins, enzyme activity, solubility, and hydration and lead to thermal and hydrolytic rupture of peptide bonds, thermal degradation, and derivatization of amino acid residues, cross-linking, oxidation, and formation of sensory-active compounds. Most of those reactions can be reflected in the meat as either desirable or detrimental changes in color, flavor, juiciness, rheological properties, and enzyme activity, depending on the various combinations. These processes are affected by the temperature and time of heating, pH, oxidizing compounds, antioxidants, radicals, and other reactive constituents, especially reducing saccharides.

The susceptibility of the meat proteins to thermal denaturation depends on their structure, predominantly on the number of cross-links, but also on the simultaneous action of other denaturing agents. Salt bridges, side chain hydrogen bonds, and a large proportion of residues in α-helical conformation increase the thermal stability of proteins. The stabilizing effect is related to heating temperature. On the one hand, thermal changes cause a decrease of solubility due to aggregation of the myofibrillar proteins, but on the other hand, they lead to an increase in solubility as a result of the degradation of tertiary protein structures of intramuscular collagen. Further effects of heating are gel formation (in most types of sausages and meat products), hydrolytic changes, alteration in the rate of proteolysis, and modification of the nutritive value.

Chemical Changes in Meat Protein Systems

During heating of meat, conformational changes (heat denaturation) of the protein systems occur. These changes take place at a particular temperature, called the denaturation temperature. The next step in the structural changes during heating of meat are the protein–protein interactions resulting in loss of solubility and aggregation.

Sarcoplasmic Proteins (30% of Total Meat Proteins)

Most of the sarcoplasmic proteins denature between 40 and 67 °C, but their heat aggregation may extend up to 90 °C. The aggregated sarcoplasmic proteins can form a gel between the structural meat elements in such a way that they have a role in the texture of the heated meat. This fraction of proteins also includes enzymes. Some of them have a tenderizing effect. During heating of the beef muscles at a temperature below 60 °C for a long time (heating rate of 0.1 °C min^{-1}), the collagenases remain active in the meat, and tenderizing effect is achieved after 6 h, whereas they are inactived at faster heating at the end temperature of 70–80 °C.

Myofibrillar Proteins (65% of Total Meat Proteins)

Heating of these proteins to a temperature of 65 °C causes a progressive increase of the surface hydrophobicity, whereas at higher temperatures it decreases again. This suggests that a part of the hydrophobic residues participates in protein–protein interactions leading to formation of aggregate network. According to differential scanning calorimetry measurements, α-actinin is the most labile and becomes insoluble at 50 °C; myosin and actomyosin denature between 54 and 58 °C; actin between 80 and 83 °C; tropomyosin and troponin at above 80 °C; and titin from pork and beef at 78.4 °C and 75.6 °C, respectively.

The heat denaturation of myofibrillar proteins in solution usually results in a gel formation. It is caused by the fact that especially myosin forms gels at a very low concentration (0.5% w/w), compared to sarcoplasmic proteins (3% w/w). For purified myosin, the firmest gels are reached at 45 °C and pH

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5.5 or at 60 °C and pH 6. Ionic strength and pH are important factors determining monomeric or filament structure of the myosin. At ionic strengths above 0.3 and at neutral pH, the myosin molecules are dispersed as monomers, forming a coarse network with large pores. At lower ionic strength the myosin molecules are assembled in filaments, and give a firmer gel. On heating, the gel formation of purified myosin occurs in two steps, in two separate temperature regions. The first part of the reaction (aggregation of the globular heads of myosin) occurs between 30 and 50 °C. The second stage (above 50 °C), connected with the structural changes in the helix structure of the myosin tails, leads to a network formation, where hydrophobic groups interact with each other. For the chicken-derived salt soluble myofibrillar proteins (SSP) heated in 0.6 M NaCl at pH 6, protein unfolding is observed at 30–32 °C, protein–protein association at 36–40 °C, and gelation at 45–50 °C. A lower degree of aggregation, better water-holding, and greater softness characterize the breast SSP gels, whereas a higher degree of aggregation and more hardness characterize the leg SSP gels.

Connective Tissue Proteins (5% of Total Meat Proteins)

The thermal denaturation of the meat collagen occurs in two steps. The first stage of the reaction is its shrinkage observed in the range of 53–65 °C. It involves the breakage of hydrogen bonds loosing up the fibrillar structure followed by the contraction of the collagen molecule up to one-quarter of its resting length. The second stage (gelatinization), running at approximately 70–80 °C, is connected with the breaking of heat-unstable intermolecular bonds. The degree of collagen shrinkage increases with the quantity of heat-stable (mature) links. In young animals the epimysium contains primarily heat-labile cross-links, the perimysium a mixture of heat-labile and heat-stable, and the endomysium of heat-stable cross-links. With increasing animal age, the amount of the heat-stable cross-links in the meat increases. Their higher levels lead to a development of greater tension in the connective tissue during heating. The shrinkage temperature of epimysial collagen is usually higher than that of other connective-tissue membranes in the muscle. The observations made by scanning electron microscopy (SEM) indicate that after heating of bovine sternomandibularis muscles at the temperature of 60 °C and 80 °C for 1 h, the epimysium does not show large changes, whereas the perimysial and endomysial collagen become granular at 60 °C and start to gelatinize at 80 °C. There are also differences in solubilization between different types of collagens. The highest thermal stability occurs in collagen of the endomysium, due to the large contribution of disulfide bonds in type IV collagen. The gelatinization of the intramuscular collagen depends also on the time of postmortem ageing of the meat, that probably results from changes in proteoglycans. In the 12-day-aged bovine semitendinosus (ST) muscles solubility of collagen is twice as high as in that aged for 5 days. During heating of the 5 day-aged bovine ST muscles by two methods in the range of temperatures between 50 and 100 °C, most of the soluble collagen is found at 70 °C during retorting and at 80 °C throughout roasting. During roasting of ST, when the temperature increases up to 90 °C, quantity of soluble collagen decreases in the meat aged for 5 days remaining at the same level in the 12 day-aged meat. Therefore, differences in the thermal collagen solubility of the intramuscular connective tissue are a consequence of differences in the proportion of the collagen types, the level of heat-stable cross-linking, and the level of glucosaminoglycans in the structure, as well as the time of postmortem ageing and the method of heating.

Heating also causes changes in pH, reducing activity, ion-binding properties, and enzyme activity. Slight upward change of pH (approximately 0.3 units) results from exposure of reactive groups of histidine. Increased reducing activity develops due to unfolding of the protein chains and exposition of sulphydryl groups. Conformational changes in proteins cause their ability to bind various ions, such as Mg^{2+} and Ca^{2+}.

Severe heating of the proteinaceous foods leads to a development of color and flavor compounds due to Maillard reactions and to thermal degradation of methionine and cysteine residues as well as other low-molecular weight compounds.

The meat fat melts during heating. Solubilization of collageneous connective tissue provides channels through which melted fat may diffuse, as a component of thermal leak.

Water-Holding Capacity

The raw meat contains 69–75% of water. Heating induces structural changes, which cause a decrease in water-holding capacity (WHC) of the meat. As the internal meat temperature increases, the WHC of meat decreases due to thermal denaturation of the meat proteins, especially myosin, which plays a significant role in water binding. During heating of meat, depending on the method, the amount of water decreases to 65% at internal temperature of 70 °C and to 60% at 90 °C. The water retention in the heated meat influences the quantity of the other basic constituents. The loss of water during heating of meat results from both evaporation and exudates. The fluid is drained by gravity from the cut surface of the meat, if the viscosity of the exudate is low enough and the capillary forces do not retain it. The loss of fluid arises predominantly from the longitudinal channels through the meat between the fiber bundles. In the raw muscle most of the water (80%) is held within the myofibrils. There are only changes in the water distribution, if the myofibrils change in volume. The fibers and fiber bundles shrink when their constituents (myofibrils) shrink, giving rise to the two extracellular fluid compartments around fibers and fiber bundles. The transverse shrinkage to the fiber axis, occuring mainly at 40–60 °C, widens the gap between the fibers and endomysium. At 60–70 °C the connective tissue network and the muscle fibers cooperatively shrink longitudinally. This shrinkage causes the highest increase in water losses during heating. For the samples of heated meat, the amount of water around fiber bundles increases up to 50 °C, in comparison with the raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles. Above 50 °C, these widened gaps diminish up to 70 °C, probably mainly due to the shrinkage of the connective tissue. The increase in extracellular space from 70 to 90 °C may be connected with a swelling of the

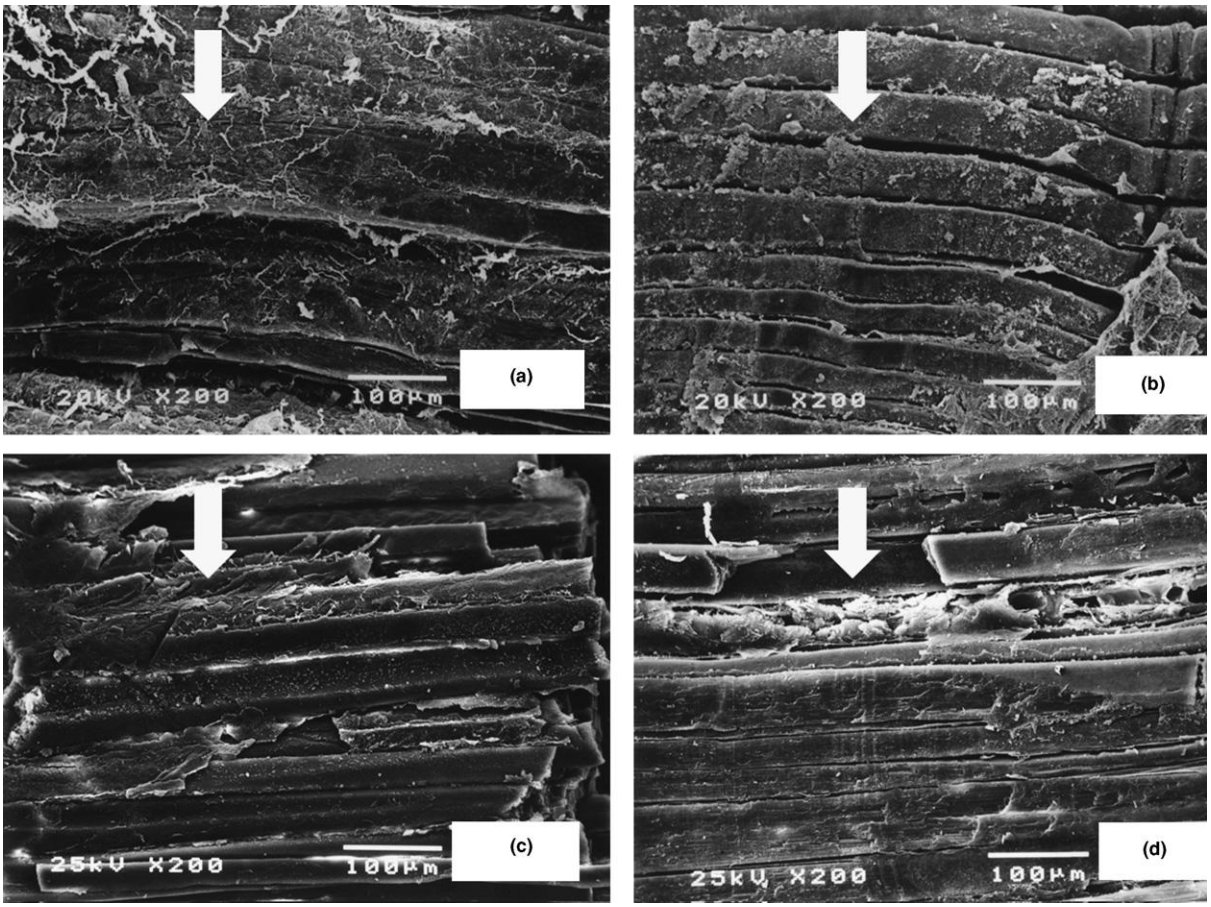

Figure 1 Connective tissue changes on heating. SEM micrographs of perimysium and endomysium from bull ST muscle: after ageing for 5 days at 4 °C and roasted to 70 °C (a) and to 90 °C (b); after ageing for 12 days at 4 °C and roasted to 70 °C (c) and to 90 °C (d). Reproduced from Palka, K., 2003. The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Science 64, 191–198.

perimysium and solubilization of the intramuscular collagen, which occur at this range of temperature.

The extent of the loss of the fluid depends on the WHC of the tissue and the degree of its marbling. The highly marbled meat shrinks less during heating and remains juicier than the lower marbled meat. The subcutaneous fat also reduces moisture losses during dry heating (roasting).

The structural origin of water-holding in the whole meat and in the highly comminuted products is different. In the first, the crucial factor is the shrinkage or swelling of the myofibrils, and in the comminuted meat products, the ability of the meat proteins to form different types of gels. The comminution of meat with salt addition leads to solubilization of the meat proteins, which exists as a protein gel after heat treatment. The higher amounts of the soluble myofibrillar proteins create a dense protein network that holds more water.

Effects of Heating on Meat Microstructure

When the meat proteins are exposed to heating, they first lose their tertiary structure and undergo several changes in configuration. In general, thermal denaturation leads to a loss in protein solubility. These chemical changes are also associated with changes in the physical character of the meat tissues. Elastin, however, is not susceptible to effects of heat. The transverse shrinkage to the fiber axis occurs at 40–60 °C, which widens the gap already present at rigor between the fibers and their surrounding endomysium. There is a controversy regarding these observations. Some authors found no changes in the cross-sectional area on cooking of the neck muscle, whereas others found that the transverse shrinkage of both fibers and fiber bundles of bovine psoas major muscle starts at approximately 40 °C. There is also a disagreement between the results presented in the literature with regard to the temperature, in which the longitudinal shrinkage of the fiber starts. Some observations indicate that fibers do not shorten below 60 °C, and the others, that both sarcomere shortening and fiber shortening usually begin at temperatures of 40–50 °C. The divergence in the results may be due to the large biological diversity within a muscle as well as between different muscles. At 60–70 °C the connective tissue network and the muscle fibers shrink. This is mainly based on the fact that the perimysial collagen shrinks at approximately 64 °C.

In the bovine ST muscles aged for 5 or 12 days and roasted to internal temperatures in the range of 50–90 °C and then

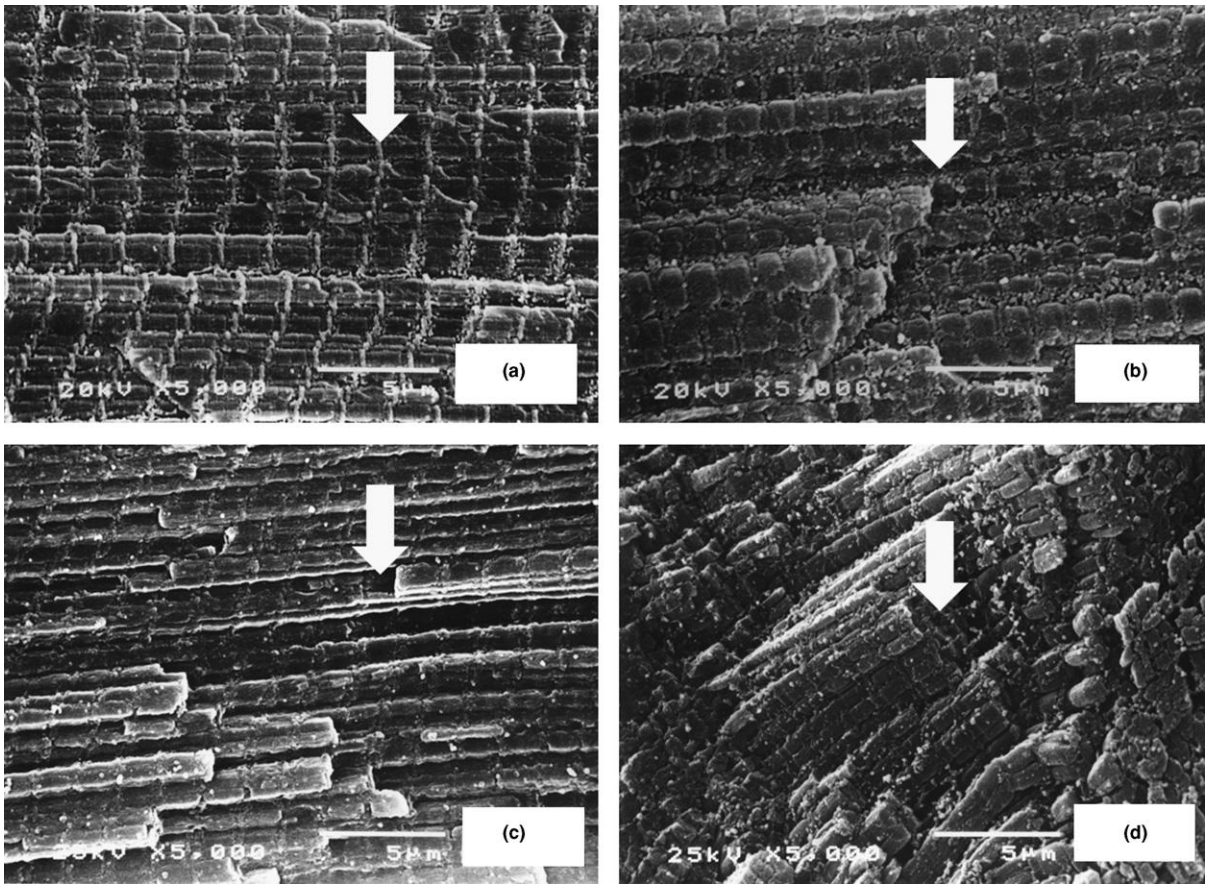

Figure 2 Myofibrillar changes on heating. SEM micrographs of myofibrils from bull ST muscle: after ageing for 5 days at 4 °C and roasted to 70 °C (a) and to 90 °C (b); after ageing for 12 days at 4 °C and roasted to 70 °C (c) and to 90 °C (d). Reproduced from Palka, K., 2003. The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Science 64, 191–198.

visualized using SEM, no significant structural changes are seen at the internal temperature of 50 °C. However, in the range between 60 and 90 °C, significant changes occur both in the myofibrils and in the intramuscular connective tissue, and this is further affected by the degree of postmortem ageing. The changes in the connective-tissue structure of perimysium and endomysium during roasting of the 5-day-aged bull ST muscles to 70 °C are shown in Figure 1(a) and to 90 °C in Figure 1(b), for the 12 day-aged muscle, the changes are shown in Figure 1(c) and (d), respectively. The granulation of perimysium and the cracks of endomysium tubes are observed in 5 day-aged meat roasted to an internal temperature of 80–90 °C (Figure 1(b)), however, in 12 day-aged meat after roasting to 60–70 °C (Figure 1(c)). The changes in the myofibrillar structure during roasting of the 5-day-aged bull ST muscles to 70 °C are shown in Figure 2(a) and to 90 °C in Figure 2(b), whereas for the 12 day-aged muscle in Figure 2(c) and (d). In the 5 day-aged samples the disintegration of the myofibrillar structure starts at 70 °C (Figure 2(a)) and is considerable at 90 °C (Figure 2(b)). In the 12 day-aged meat roasted to 70 °C (Figure 2(c)), the degree of structural destruction is similar to that of 5 day-aged meat roasted to 90 °C (Figure 2(b)). At 90 °C complete disintegration of the myofibrillar structure of 12 day-aged meat is observed (Figure 2(d)).

As the endpoint temperature increases from 50 to 60 °C, there is a significant decrease in the fiber diameter. As the heating temperature is raised, the sarcomere length decreases, the effects being greater in the aged meat. The larger structural changes observed during roasting of the more aged meat may be a consequence of the changes during ageing in both the cytoskeletal proteins and the intramuscular connective tissue, leading to a weakening of the transversal and longitudinal integrity of the muscle fibers. In general, the microstructural changes are considerably less in the meat heated after 5-day ageing in comparison with the meat heated after 12-day ageing.

There is a high negative correlation ($r=-0.97$) between changes in the sarcomere length and the cooking losses during heating of the bovine ST at the temperature range of 50–120 °C (Figure 3).

Texture and Tenderness of Heated Meat

The rheological properties of meat result from changes in proteins, with the texture of the meat being affected mainly by the quantity and cross-linking of collagen; the morphological structure of the meat tissues; the biochemical state of the

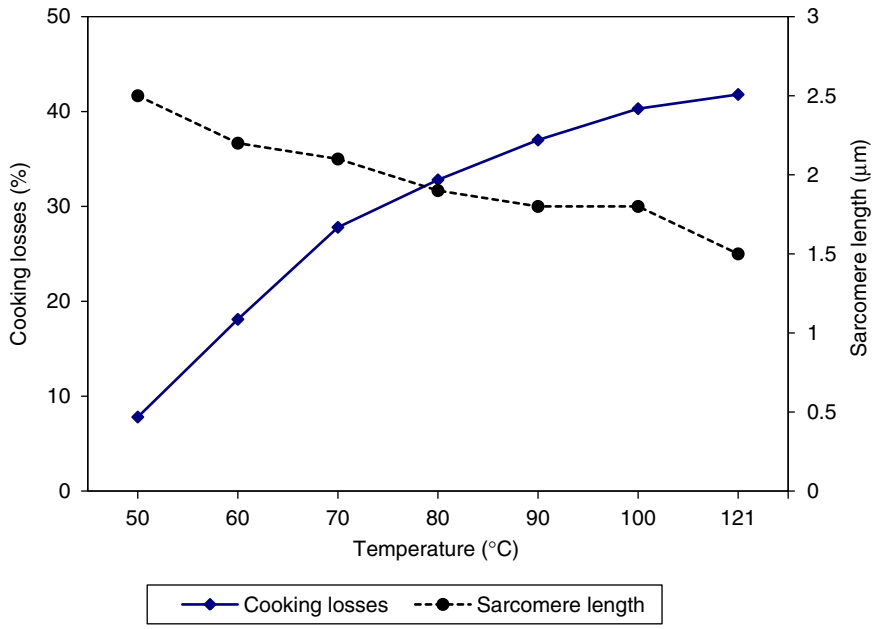

Figure 3 Effect of heating temperature on cooking losses (–) and sarcomere length (– – –) of beef ST muscle samples retorted after 5 days ageing at 4 °C. Reproduced from Palka, K., Daun, H., 1999. Changes in texture, cooking losses, and myofibrillar structure of bovine M. semitendinosus during heating. Meat Science 51, 237–243.

muscle pre- and postrigor; and the mechanical disintegration of the muscle structure.

The hardening of the myofibrillar structure and the gelatinization of the intramuscular collagen depend on the extent of the postmortem changes (ageing) related to the time-temperature regime. Generally, hardening of meat is observed throughout the heating. The first increase of meat hardness that occurs after heating in the range of 40–65 °C is mainly due to sarcoplasmic and actomyosin complex protein denaturation. A contribution of intramuscular connective tissue to the changes of toughness is relatively small, although heat-induced shrinkage of endomysium occurs. This is because the endomysium is an amorphous, nonfibrous sheet. However, some authors observed an increase in tenderness of the meat heated up to approximately 50 °C. The reason for this is probably the fact that the applied stress during mastication is reduced by viscous flow in the fluid-filled channels in between fibers and fiber bundles. The viscous flow then becomes lower as the elasticity of the meat increases in that temperature region. At above 65 °C elasticity acts adversely and impairs the tenderness, Warner–Brazler (WB) shear force increases significantly. The further hardening that occurs at 65–75 °C is connected with the drastic shrinking of the perimysium and continuation of the myofibrillar component shrinking. In the range of 75–80 °C, there is further shrinkage and dehydration of the actomyosin component. The collagen fibers begin to granulate, which can result in crispness of the perimysium. At this time the combining effects of the myofibrillar component and the perimysium are observed, and the increase in WB shear force becomes lower, compared with the second phase. At higher temperatures (80–90 °C), the overall influence of thermal-induced changes in the intramuscular connective tissue is a tenderizing effect, whereas the changes in myofibrillar proteins result in a toughening effect.

Meat hardness depends on the fiber size and the degree of sarcomere shortening during heating to 70 °C through tension caused by collagen fibers (mainly endomysium) shortening. This is also influenced by many of the differences in the histological structure and the amount of the collagen fibers type III and type I as well as differences in the collagen cross-linking. For example, mechanical resistance of the perimysium at the interface with the endomysium mostly affects hardness of the heated meat, whereas endomysium shrinkage may result in a tightening of the structure and squeezing out of intramuscular water. Prolonged heating of meat (4–6 h) at relatively low temperatures (50–60 °C) improves tenderness because of enzyme activity up until 60 °C.

Drip loss ranging from 20% to 40% of the original weight and shrinkage also has an effect on rheological properties. Meat with a high pH has lower cooking losses and is more tender after heating.

For the bovine ST and psoas major muscles at the same stage of ageing, boiled (100 °C), roasted (170 °C), or fried (160 °C) to the end temperature of 75 °C, WB shear force values are the highest for boiled, middle for roasted, and the lowest for fried muscles indicating that the method of heating is also important.

The sensory-evaluated toughness of the whole bovine biceps femoris muscle decreases drastically in the range of temperature from 55 to 60 °C, thereafter increases again up to 80 °C. For the comminuted meat products from the same muscle, the hardness increases over the whole temperature range and is significantly lower than the toughness of the whole meat at heating temperatures below 60 °C. It means that the spatial arrangement of the fibers is most important for the textural properties of the meat and the comminuted meat products.

See also: Chemical and Physical Characteristics of Meat: Chemical Composition; Palatability. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Glycolysis. Cooking of Meat: Cooking of Meat; Flavor Development; Heat Processing Methods; Maillard Reaction and Browning; Warmed-Over Flavor

Further Reading

Bailey, A.J., Light, N.D., 1989. Connective Tissue in Meat and Meat Products. London: Elsevier.

Kołczak, T., Krzysztoforski, K., Palka, K., 2008. Effect of post-mortem ageing, method of heating and reheating on collagen solubility, shear force and texture parameters of bovine muscles. Polish Journal of Food and Nutrition Sciences 58 (1), 27–32.

Kołczak, T., Pospiech, E., Palka, K., Łącki, J., 2003. Changes of myofibrillar and centrifugal drip proteins and shear force of psoas major and minor and semitendinosus muscles from calves, heifers and cows during post-mortem ageing. Meat Science 64, 69–75.

Laakkonen, E., 1973. Factors affecting tenderness during heating of meat. Advances in Food Research 20, 257–323.

Lawrie, R.A., 1998. Meat Science. Oxford: Pergamon Press.

Li, C.B., Zhou, G.H., Xu, X.L., 2010. Dynamical changes of beef intramuscular connective tissue and muscle fiber during heating and their effects on beef shear force. Food Bioprocess Technology 3, 521–527.

Nishimura, T., Hattori, A., Takahashi, K., 1996. Arrangement and identification of proteoglycans in basement membrane and intramuscular connective tissue of bovine semitendinosus muscle. Acta Anatomica 155, 257–265.

Offer, G., Knight, P., 1988. The structural basis of water-holding in meat. In: Lawrie, R. (Ed.) Developments in Meat Science, vol. 4. London: Elsevier, pp. 63–243.

Offer, G., Knight, P., Jeacocke, R., *et al*., 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. Food Microstructure 8, 151–170.

Palka, K., Daun, H., 1999. Changes in texture, cooking losses, and myofibrillar structure of bovine M. semitendinosus during heating. Meat Science 51, 237–243.

Palka, K., 2003. The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. Meat Science 64, 191–198.

Pospiech, E., Greaser, M.L., Mikołajczak, B., Chiang, W., Krzywdzinska, M., 2002. Thermal properties of titin from porcine and bovine muscles. Meat Science 62 (2), 187–192.

Purslow, P.P., 2002. The structure and functional significance of variations in the connective tissue within muscle. Comparative Biochemistry and Physiology Part A 133, 947–966.

Sikorski, Z.E., 2007. Proteins. In: Sikorski, Z.E. (Ed.), Chemical and Functional Properties of Food Components. New York: CRC Press, pp. 155–160.

Tornberg, E., 2005. Effects of heat on meat proteins – Implications on structure and quality of meat products. Meat Science 70, 493–508.

Warmed-Over Flavor

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Glossary

Chelators Organic chemicals that form two or more coordination bonds with a central metal ion. Heterocyclic rings are formed with the central metal atom as part of the ring.

Gas chromatography–mass spectrometry An analytical method that combines the features of gas–liquid chromatography and mass spectrometry to identify different substances within a test sample.

Heme compound An iron compound of protoporphyrin, which constitutes the pigment portion or protein-free part of the hemoglobin molecule and is responsible for its oxygen-carrying properties.

Maillard reaction products Any of hundreds of different compounds created as a result of the Maillard reaction (a process of nonenzymatic browning resulting from a chemical reaction between an amino acid and a reducing sugar). Many of the products are of interest for their flavor attributes, and also for their possible antioxidative properties.

Pyrolysis The thermochemical decomposition of organic material.

Singlet oxygen The electronically excited state of molecular oxygen, which is less stable than the more typical ground-state triplet oxygen.

Umami A strong meaty taste imparted by glutamate and certain other amino acids. It is often considered to be one of the basic taste sensations along with sweet, sour, bitter, and salty.

Introduction

It has been more than 50 years since the term 'warmed-over flavor' (WOF) was first coined in reference to a notable deterioration in the quality of cooked meat products following chilled storage followed by reheating. Even though still most frequently linked to chill-stored cooked meat, the term today is somewhat of a misnomer, as it may apply to deterioration of meat flavor in a variety of different contexts, including raw meat, meat served on warmers, and meat in frozen storage. The onset of the distinctive off-flavor was originally attributed solely to lipid oxidation, but it is now understood that protein oxidation is of importance as well. Meats that are cooked before storage are most susceptible to the development of noticeable off-flavors, because heat treatment accelerates the oxidative processes (the distinctive off-flavor can become readily apparent within just a few hours of thermal processing). Products develop an undesirable stale flavor, and at the same time desirable meaty flavor notes are lost. The stale off-flavors so formed have often been described as 'cardboard like,' 'painty,' and 'rancid'; they are considered most noticeable when refrigerated cooked meat products are reheated. The demand for precooked, ready-to-eat meat products in the marketplace and in fast-food franchises continues to grow, thereby expanding the concern of consumer exposure to WOF. However, a greater awareness of the process, along with improved methods of control, ensures the loss in quality of meat products due to the development of WOF, which occurs to a much lesser extent today than it did 50 years ago.

When the concern of WOF is raised, it is often in relation to comminuted products, such as meat loaves, chicken nuggets, and precooked burgers. Thus, it is generally accepted that any process involving disruption of the integrity of muscle tissue (such as cooking, grinding, mechanical deboning, massaging, or restructuring) enhances the development of WOF. Warmed-over flavor is also a big concern for the hotel/restaurant/institution service where processed convenience entrées or prepackaged cooked meat products are served. Prolonged holding of products containing meat at high temperatures (such as in a steam table) can produce undesirable flavors. Freezing can delay the onset of WOF development, but it does not prevent it. Stale or off-flavor notes, such as 'ice box,' 'rancid,' and 'freezer burn,' have been used to describe the phenomenon. The flavor compounds responsible for the off-flavor in stored fresh meat are qualitatively the same as in previously cooked meat, but the compounds occur at different concentrations and therefore create a somewhat different aroma profile. Because WOF development is a dynamic process of flavor change due principally to a cascade of oxidative events, an understanding of the mechanism(s) and prevention of its occurrence in meat and meat products are important to the food scientist.

Warmed-Over Flavor as a Consequence of Lipid Oxidation

WOF development is attributable mainly to lipid oxidation. Meat lipids are made up of intermuscular and intramuscular adipose tissues, and they contain both saturated and unsaturated fatty acids. The membrane lipids (i.e., the phospholipids) tend to possess the lion's share of the polyunsaturated fatty acids (PUFAs), and these are most prone to oxidation. Membranes are disrupted when meat is ground, chopped, or cooked, thereby releasing cell contents and exposing PUFAs to oxidative stress. Consequently, the process of WOF development can begin within hours of cooking the meat product, as compared with several days for the development of lipid oxidation in uncooked meat. Research has shown that subcutaneous fat from meat can produce approximately 50 volatile compounds during WOF development,

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whereas intramuscular lipids can generate more than 200. There seems to be no question that oxidation of phospholipids is the primary source of off-flavor notes generated during WOF development.

Oxidation of the unsaturated C_{18} fatty acids found in meat (namely oleic, linoleic, and α-linolenic acids) produces low molecular weight aldehydes (C_3–C_{12}), which are believed to be partly responsible for the development of WOF and rancidity in cooked meats during storage. Hence, it can be reasonably hypothesized that meats containing higher levels of PUFAs should be more susceptible to oxidation. This is indeed the case as certain muscle tissue exhibits more of a propensity toward the development of WOF, with a key correlating factor being the degree of unsaturation in the tissue's fatty acids. Fish is most at risk followed by poultry, pork, beef, and lamb.

The initial stage in the oxidation of unsaturated fatty acids involves the formation of lipid free radicals – potent species that react with oxygen and propagate the process via a chain reaction. This phenomenon, commonly referred to as autoxidation, can be triggered by singlet oxygen, metal ions, heme compounds, UV light, and certain enzymes. Thermal processing results in changes to protein and lipid constituents of meat and destroys some of the natural reducing capabilities found in muscle tissue, which help to combat oxygen stress and free radical generation. During autoxidation, primary products of lipid oxidation (known as hydroperoxides) are formed. Lipid hydroperoxides are odorless and tasteless but quite unstable. Their degradation leads to the formation of a large number of secondary oxidation products, such as aldehydes, acids, alkanes, alkenes, ketones, alcohols, esters, epoxy compounds, and polymers. Aldehydic scission products of hydroperoxides, such as pentanal, hexanal, and *E,E*-2,4-decadienal, are of key interest, as these short-chain aldehydes give rise to the off-flavors known as 'warmed-over' and are very potent volatiles even at concentrations in the parts-per-billion range. When refrigerated cooked meats are warmed up/reheated, these active off-flavor compounds volatilize and the unpleasant WOF notes become more noticeable. Hexanal, which is the main oxidation breakdown product of linoleic acid, is sometimes monitored as an indicator of meat lipid oxidation.

Conflicting views exist concerning the role of heme and nonheme iron as it relates to lipid oxidation during cooking of meats and the subsequent formation of WOF in stored products. Some researchers have reported that heat treatment of meats releases ferrous iron from heme-containing compounds (such as myoglobin), which then acts as a primary catalyst in oxidative processes resulting in WOF. However, several investigations have suggested that the intact heme iron is in fact a stronger prooxidant in muscle tissue than iron that has been released. Data from heme-containing model system studies suggest that changes in protein structure occur during heating, which expose the heme cavity to the surrounding lipid hydroperoxides and thereby increase the prooxidative activity of the catalytic heme group. In other words, it is asserted that it is the heme compounds themselves and not free iron(II) or iron(III) which have considerable prooxidative activity at the concentrations relevant to meat.

Addition of sodium chloride is mandatory in meat formulation for flavor and functionality, but the chloride ions promote iron-catalyzed oxidation of unsaturated lipid constituents and therefore its use facilitates oxidation. Other trace metal ions, such as copper, that can be introduced to meat via water and processing equipment are also promoters of oxidation. These trace metal ions can react directly with lipids in oxidation reactions by reducing the energy of activation necessary for free radical formation or serving to catalyze the decomposition of formed lipid hydroperoxides.

Warmed-Over Flavor as a Consequence of Protein Oxidation

It was initially believed that lipid oxidation was the sole cause of WOF development, but in the late 1980s several researchers produced strong evidence that protein degradation reactions were also involved in WOF development. Flavor chemists have been quite interested in the chemical instability of sulfur-containing constituents in meat (i.e., sulfhydryl–disulfide interchanges in proteins and the degradation of sulfur-containing heteroatomic compounds) because breakdown of these compounds is believed to lead to a reduction in desirable meaty flavor notes. In an effort to better describe the complex series of chemical reactions that contribute to an overall increase in off-flavor notes and a loss in desirable meaty ones, the term 'meat flavor deterioration' (MFD) was proposed as an alternative to WOF. Although MFD may be a better expression for what is actually occurring, the term 'warmed-over flavor' and the WOF acronym are still routinely used in the scientific literature.

Although the intensity of the undesirable sensory notes has been positively correlated with the content of carbonyl compounds formed via lipid oxidation reactions, the decrease in flavor intensity of desirable notes can be attributable to both lipid oxidation and protein oxidation. Protein oxidation might decrease the concentration of those volatiles that contribute to desirable meaty flavor, whereas the off-flavors produced by lipid oxidation might 'mask' the perception of such desirable compounds. The reduction or disappearance in the sensory perception of 'meatiness' due to changes to heteroatomic compounds is probably important during the early days of storage when lipid oxidation-derived odors and flavors are not as concentrated, and flavor masking probably becomes the main factor during the later days of storage when WOF is more fully developed.

Another contributing factor to the dull flavor impact associated with WOF could be the loss of peptides to enzymatic degradation. Research has revealed that some meat enzymes remain active even after thermal processing and subsequent refrigeration of the product, including those that might break down peptides that are capable of stimulating the taste receptors to give a note of 'umami' (a Japanese term for a fifth basic taste that is triggered by some amino acids, translating roughly to 'savory' or 'meaty').

Sensory and Chemical Analysis in Relation to Warmed-Over Flavor

The contribution of sensory analysis toward the development of descriptors, definitions, and references to describe the

Table 1 Sensory descriptive terms with definitions developed for the evaluation of warmed-over flavor in ground chicken

Term	*Definition*	*Reference*	*Rating*[a]
Odors	*Aromatics associated with*		
Chicken brothy	Chicken broth	Chicken broth	12
Fishy	Cooked fish	Freshly cooked tilapia	10
Sulfury	Boiled egg yolk	Boiled egg yolk	5
Musty	Wet cardboard	Wet cardboard	4
Rancid	Oxidized oil	Oxidized flax seed oil	None[b]
Tastes	*Taste associated with*		
Sweet	Sucrose	5% sucrose	5
Sour	Citric acid	0.08% citric acid	5
Salty	Sodium chloride	0.5% sodium chloride	5
Bitter	Caffeine	0.05% caffeine	5
Umami	Monosodium glutamate	0.1% monosodium glutamate	7.5
Flavors	*Flavor associated with*		
Metallic/serumy	Blood or rare meat	Rare beef (top sirloin)	3
Cooked chicken	Cooked chicken breast	Boiled chicken breast	9
Fatty	Rendered chicken fat	Rendered chicken skin	8
Fishy	Cooked white fish	Freshly cooked tilapia	11
Rancid	Rancid/oxidized oil	Oxidized flax seed oil	6
Appearance			
Surface color	Color of the outer surface of the sample	Boiled chicken breast	1
		Rare beef (top sirloin)	14

[a]Ratings assigned according to a 15-point scale; a larger number signifies a greater degree of intensity for the associated trait within the reference sample.
[b]A rating was not determined for the reference sample of the 'rancid' odor term.
Source: Reproduced from Brannan, R.G., 2009. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. Meat Science 81, 589–595.

phenomenon of WOF in cooked meat products has come a long way in the last 50 years. Today, well defined sensory descriptive vocabularies for WOF have been prepared that allow trained panelists to accurately track the development of WOF with time. An example lexicon of flavor descriptors used for ground chicken is presented in Table 1. Such a vocabulary can be used by panelists to describe perceived sensory characteristics in a sample set – producing a perceptual map of the variations in a sample type. Sensory analysis can be employed alone or in combination with chemical/instrumental data to help explain and elucidate underlying sensory and chemical relationships. Data from the mid-1980s indicate that the sensory perception of WOF was similar across meat patties of beef, pork, chicken, and turkey, but the intensity of its occurrence varied among the samples. Some specific data on beef showed that the intensity of fresh cooked beef notes is strong immediately after cooking, but over time, cardboard notes develop and then disappear. At about the same time, there is a marked reduction in fresh beefy notes, and then oxidized notes begin to become apparent. For samples that had been stored for 3–7 days, oxidized/rancid/painty notes were dominant.

There are a variety of chemical methods to semiquantify WOF development in meat and meat products, including the measurement of changes in conjugated dienes and carbonyl values as well as the more recent employment of electronic noses and gas chromatography. Malon(di)aldehyde is a relatively minor product of autoxidation of PUFAs in muscle tissue, but its presence and concentration in meat products is commonly monitored as a marker of lipid oxidation by the classical 2-thiobarbituric acid (TBA) test. The TBA test involves the reaction of malonaldehyde in oxidized foods with the TBA reagent under acidic conditions; a pink adduct forms with a distinctive absorption maximum at 532 nm. The TBA test was once believed to be specific for malonaldehyde, but this is not so. In fact, the TBA method has been criticized for lacking specificity and adequate sensitivity toward the dialdehyde. Owing to the uncertainty about the exact identity of compounds that can react with the TBA reagent, the ambiguous term '2-thiobarbituric acid-reactive substances' (TBARS) is now commonly employed in lieu of TBA number or value. Nevertheless, determining the content of TBARS (i.e., often reported as mg malonaldehyde equivalents per kg meat) appears to be a useful indicator of meat quality deterioration. Recent studies have shown that TBARS are highly correlated with many of the sensory terms related to WOF. However, the importance of hexanal, a dominant volatile oxidation product of linoleic acid, as an indicator of the sensory perception of WOF development has been questioned. Hexanal has a characteristic 'tallowy' or 'green leafy' aroma, but this odor term was not strongly perceived as being associated with WOF odor terms (e.g., linseed oil like and cardboard like) during vocabulary development. Results from gas chromatography–mass spectrometry analyses have indicated that oxidation compounds, such as pentanal, 2-pentylfuran, octanal, nonanal, 1-octen-3-ol, and hexanal, covaried with the sensory descriptor green.

Preventive Strategies

It can be important to food scientists to understand how to control or limit WOF development in meat and meat products and to know what arsenal of countermeasures are available.

By examining the causes of off-flavor development, strategies can be designed to limit its occurrence. Curtailing the detrimental effect of WOF requires the inhibition of lipid oxidation, protein oxidation, the consumer's ability to detect the resulting reduction in sensory quality, or some combination thereof. Although much of the methods to diminish WOF have historically focused on the inhibition of lipid oxidation, it is important to note that these procedures have largely been considered effective measures against protein oxidation as well. The most recent evidence now shows that protein oxidation can, in fact, be inhibited very effectively by strategies that include the incorporation of primary antioxidants. Many find the most effective means of controlling WOF to be a comprehensive strategy that utilizes a combination of the approaches described below.

Meat Quality and Handling

The choice of meat is an important factor in the occurrence of lipid oxidation. Fish and other muscle tissues containing high levels of PUFAs exhibit more of a propensity toward off-flavor development. Chicken has less of a tendency to develop oxidized flavors than turkey due to the higher level of the antioxidant vitamin E in chicken; nevertheless, the problem will be worse in chicken thigh meat than in white meat, as the darker meat contains more lipids and heme iron. Fresh meat used in product formulations shows less of a tendency to develop WOF than older meat, as older meat will have more time to undergo enzymatic degradation processes, which generate autocatalytic compounds that can propagate oxidation. Endogenous antioxidant enzymes, such as catalase, glutathione peroxidase, and superoxide dismutase, continue to function postmortem at curbing lipid oxidation in uncooked muscle foods. However, their efficacy in this regard diminishes with increasing age of the meat. Ensuring that high quality meat is used in product formulations is critical.

In addition to quality meat selection, some steps can be taken at the operator level to limit WOF. Incorporating antioxidants into the product and reducing the time from cooking to plate are among the most effective and common means food service operators can utilize to minimize WOF. WOF can also be curbed by avoiding the use of steam tables and warming lights to hold products at elevated temperatures for long periods of time. Many fast-food franchises make an effort to ensure that their burger products stay under warming lights for relatively short periods so they do not lose that fresh-grilled flavor.

The actual cooking method employed for precooked products can also influence the extent of WOF. One might assume that grilling of meat would exaggerate the potential for WOF on account of the high temperatures to which lipid and protein constituents are subjected, but this is not so. In fact, thermal processes that employ very high temperatures, like grilling, seem to inhibit WOF development through the formation of antioxidant Maillard browning intermediates. Details of how this occurs have been discussed in a previous article. Similarly, conditions that favor browning, such as addition of glucose or smoke intermediates, can help to retard or inhibit WOF development.

Primary Antioxidants: Synthetic and Natural

Food technologists attempt to reduce the problem of WOF in meat products by adding food-grade antioxidants or ingredients that impart antioxidant properties. Primary antioxidants extend the induction period and delay the onset of fatty acid oxidation by acting as free radical acceptors or hydrogen atom donors. Such antioxidants trap free radicals directly and delay the free radical chain reaction in a concentration-dependent manner.

Synthetic compounds such as *tert*-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate are commonly employed antioxidants by the food industry. Their usage levels, however, are strictly regulated, and in recent years consumers have demonstrated reluctance to consume such compounds. Fortunately, there are a wide variety of natural alternatives that can also offer protection against oxidation in the form of food ingredients. The most common natural antioxidants utilized to fight WOF are vitamin E, extracts from rosemary and sage, and carotenoids such as β-carotene and lycopene. In addition, many other spices, fruits, and vegetables contain constituents with antioxidant properties that can provide benefits to a meat formulation. Reducing exposure of these antioxidants to oxygen and light enhances their effectiveness in minimizing WOF development.

The addition of spice and herb extracts to meat products has become a popular means of incorporating natural antioxidants and represents a 'consumer-friendly' option. Rosemary, oregano, and sage extracts have been of common use in recent years, oftentimes in combination with tocopherols and/or erythorbate. Rosemary in particular appears to be the most effective, as it contains a number of antioxidant compounds called diterpenes (including the highly active and prevalent carnosic acid). Because carnosic acid and rosmanol (another antioxidant constituent of rosemary) are odorless, manufacturers can develop spice-based ingredients with reduced flavor impact and increased protection against oxidation and the subsequent WOF development. Spice companies continue to develop new odorless extracts possessing antioxidant activity for addition to meat systems. Yeast extracts and its derived products also exhibit antioxidant properties due to the presence of glutathione, Maillard reaction products, and sulfur-containing amino acids. Papers that cite the benefits of new food ingredients against WOF appear frequently in the scientific literature, many of which describe the employment of novel plant extracts.

Dietary Antioxidants

Typically, antioxidants are added to meat products during formulation, but scientists have demonstrated the potential to increase the antioxidant capacity of muscle tissue before the animal being harvested. One such approach is to supplement the feed of domesticated species with dietary antioxidants. A number of studies with hogs and poultry have shown that supplementation of feed with vitamin E (typically as α-tocopheryl acetate) can minimize the potential for eventual WOF development. Additions to supplementation levels have been found to result in progressive increases in the

concentration of α-tocopherol in the resulting muscle tissue, mitochondria, and microsomes. α-Tocopherol migrates into muscle cell membranes, where it lies adjacent to highly oxidizable phospholipids; this localization makes α-tocopherol a particularly effective antioxidant. Sensory studies of meat products have shown that vitamin E supplementation can prolong flavor freshness, inhibit WOF development, and positively influence tenderness and juiciness.

Secondary Antioxidants

Secondary antioxidants inhibit oxidation by indirect means, such as scavenging oxygen or binding prooxidative compounds, and provide another method of reducing oxidation in meat products – most commonly via the incorporation of chelators. A chelator or sequestrant is a food additive that reacts with trace metal ions in foods and forms tightly bound complexes, thereby inhibiting the metal ion's catalytic action on lipid constituents. Typical chelators added to processed meat products are alkaline phosphates. Not only do alkaline phosphates improve the functionality of the meat product in question (via water-binding capacity, chewiness, and other textural attributes), but also they have the ability to complex with or 'chelate' free iron ions in the meat matrix. Furthermore, sodium tripolyphosphate, tetrasodium pyrophosphate, and sodium hexametaphosphate have the capability to complex with iron ions that are released from the heme moiety of myoglobin during thermal processing. The level of added phosphates must be controlled because additions of 0.5% or more tend to leave metallic and bitter tastes in the product. Citric, ascorbic, and ethylenediaminetetraacetic acids are additional common food-grade additives that help to stabilize metal ions by reducing their capability to act as oxidants. Ascorbic acid, its sodium salt (sodium ascorbate), and its isomer (erythorbate) also function synergistically with other antioxidants and added polyphosphates to give protection to meats against oxidative degradation.

Nitrites and Nitrates

Nitrites and nitrates are additives to meat products that perform multiple functional roles in the meat matrix, one of them being to retard oxidation and WOF development. The mechanism by which nitrites prevent oxidation within meat (and subsequently WOF development) is still a matter of discussion. Four different mechanisms have been proposed for the antioxidative effect of nitrite in meats: (1) formation of a stable complex between heme pigments and nitrite, thereby preventing the release of iron ions from the porphyrin molecule; (2) stabilization of unsaturated lipids within tissue membranes against oxidation; (3) chelation of metal ions; and (4) formation of antioxidative nitroso and nitrosyl compounds.

Natural and Liquid Smoke

Smoking is another popular meat preservation technique. Like nitrites, constituents of smoke (specifically, phenolic compounds) impart antioxidant properties to meat and meat products. Additionally, incomplete combustion of gases during natural smoke generation from the pyrolysis of hardwood can result in the formation of various nitrogen oxides, which can function in the curing process as nitrite does. Research has shown that certain smoke flavorings can reduce the occurrence of WOF and extend shelf life when added to fresh, precooked, and processed meats. Formulators can add liquid smoke to their products directly by atomizing, dipping, drenching, spraying, or injecting. Smoke ingredients with strong flavors can help to mask the perception of WOF, whereas flavorless smoke fractions can be employed at low levels in marination systems to reduce the development of WOF. In some cases, the incorporation of smoke flavors has reduced lipid oxidation by 20–30%. The flavors also contain certain carbonyls that react with amino groups of meat proteins to inhibit WOF production.

Packaging

Packaging is a physical means to reduce off-flavor development in meat and meat products. Because light and the presence of oxygen can accelerate oxidation, eliminating their exposure through packaging technologies will help. Vacuum packaging controls oxygen interactions at the meat surface, and thereby minimizes oxidation. This technique, coupled with nitrogen flushing or modified atmosphere packaging (e.g., 70% N_2 and 30% CO_2) techniques, can give substantial shelf life to finished products. Other packaging technologies, such as oxygen scavengers, can be added to the package as stand-alone units or be incorporated into the packaging film. Another approach employed by the packaging industry is the use of films that act as oxygen barriers. Edible films containing natural antioxidants have been examined as a means to control WOF development in cooked meat products, but the release and delivery of the actives require further elucidation. WOF development was recently inhibited by whey-based edible coatings of sausages, with the speculated mechanism being chelation by present carboxymethyl cellulose. In some cases, it is more effective to mix the antioxidant directly into the meat formulation, but this does not work for whole-muscle and some restructured meat products.

Flavor Masking

Flavor masking or other methods using flavors that modify the perception of off-notes can be effective tools in the fight against oxidative off-flavors in meat products. An example to which has already been eluded is that of smoking. Addition of complementary (i.e., savory) flavors from herbs and spices can not only inhibit lipid oxidation but also potentiate meat flavors, which might otherwise fade during processing and refrigerated storage. Beer flavoring is another ingredient that helps to reduce the flavor problems that occur during the reheating of meats. Beer flavoring's anti-WOF effect might be a function of the typical yeast notes found in the brew, as yeast-based flavors can improve and enhance flavors, mask bitterness, increase aroma, and also provide some protection against oxidation.

Masking agents that have been developed to cover other forms of off-flavors, such as metallic notes in high-intensity

sweeteners or beany notes in soybean products, have also been proven effective in masking WOF. Good masking agents will not have much flavor on their own when employed at low levels, but they are often coprocessed with compounds that impart desirable flavors (such as meaty flavor notes). Because the flavor can also mask certain desirable meaty notes if used at too high of a concentration, product testing is necessary to develop the best application level.

Another option is that of simply disguising the oxidative notes. For example, the highly flavored systems, such as those found in spicy Mexican or East Indian seasonings, can distract the consumer from any off-notes, because the sensations of heat and tanginess dominate the consumers' palate. Heavy spicing of meat products is common practice in countries where refrigerated storage is an issue.

In developing a flavor system designed to combat WOF, it is important to consider the overall desired flavor profile of the finished product. Both temperature and hold time during the cooking process influence the overall taste. To achieve an optimal flavor, it is generally recommended that spices and flavoring agents be incorporated at a slightly higher level than would be needed for immediate consumption. Once an appropriate flavor system is developed, it is incorporated into the product by means of a mix-in seasoning, rub, or marinade, depending on the type of product formulated. In a whole-muscle product, marination is recommended to enhance protection and minimize the possibility of off-notes developing within the internal area of the product. As the product's surface is more prone to oxidation than the interior of the meat, even topical application would still certainly help to minimize the formation of WOF notes.

See also: Cooking of Meat: Flavor Development; Heat Processing Methods; Maillard Reaction and Browning; Physics and Chemistry. Cutting and Boning: Traditional. Packaging: Modified and Controlled Atmosphere; Technology and Films; Vacuum. Smoking: Liquid Smoke (Smoke Condensate) Application

Further Reading

Bauer, F., 2007. Fleisch und Fleischerzeugnisse – Oxidative Veränderungen bei Erhitzung und Fermentation. Die Ernährung 31, 505–509.

Brannan, R.G., 2009. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. Meat Science 81, 589–595.

Clausen, I., Jakobsen, M., Ertbjerg, P., Madsen, N.T., 2009. Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef. Packaging Technology and Science 22, 85–96.

Grün, I.U., Ahn, J., Clarke, A.D., Lorenzen, C.L., 2006. Reducing oxidation of meat. Food Technology 60 (1), 37–43.

Jayathilakan, K., Sharma, G.K., Radhakrishna, K., Bawa, A.S., 2007. Antioxidant potential of synthetic and natural antioxidants and its effect on warmed-over-flavour in different species of meat. Food Chemistry 105, 908–916.

Jittrepotch, N., Ushio, H., Ohshima, T., 2006. Effects of EDTA and a combined use of nitrite and ascorbate on lipid oxidation in cooked Japanese sardine (Sardinops melanostictus) during refrigerated storage. Food Chemistry 99, 70–82.

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., 2011. Protein oxidation in muscle foods: A review. Molecular Nutrition & Food Research 55, 83–95.

O'Sullivan, M.G., Byrne, D.V., Jensen, M.T., Andersen, H.J., Vestergaard, J., 2003. A comparison of warved-over flavour in pork by sensory analysis, BC/MS and the electronic nose. Meat Science 65, 1125–1138.

Pegg, R.B., Shahidi, F., 2000. Nitrite Curing of Meat: The N-Nitrosamine Problem and Nitrite Alternatives. Trumbull, CT: Food & Nutrition Press, Inc.

Ross, C.F., Smith, D.M., 2006. Use of volatiles as indicators of lipid oxidation in muscle foods. Comprehensive Reviews in Food Science and Food Safety 5, 18–25.

Sárraga, C., Carreras, I., García Regueiro, J.A., Guàrdia, M.D., Guerrero, L., 2007. Effects of α-tocopheryl acetate and β-carotene dietary supplementation on the antioxidant enzymes, TBARS and sensory attributes of turkey meat. British Poultry Science 47, 700–707.

St. Angelo, A.J., Bailey, M.E. (Eds.), 1987. Warmed-Over Flavor of Meat. Orlando, FL: Academic Press, Inc.

Tikk, K., Haugen, J.-E., Andersen, H.J., Aaslyng, M.D., 2008. Monitoring of warmed-over flavour in pork using the electronic nose – Correlation to sensory attributes and secondary lipid oxidation products. Meat Science 80, 1254–1263.

Tims, M.J., Watts, B.M., 1958. Protection of cooked meats with phosphates. Food Technology 12, 240–243.

CURING

Contents

Brine Curing of Meat

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Glossary

Brine Salt solution possibly together with nitrite and other agents.
Pickle curing Curing by dipping in brine.
Salt meter Measures salt content of the brine or product in solution.
Sea salt Salt from sea water.
Warmed-over flavor Off-flavor note in reheated cooked meat, especially poultry meat.

Introduction

Curing is one of the oldest meat preservation processes known to man. Treating of meat with a solution of salt (sodium chloride), or packing the meat in dry salt, preserved the meat. Salt helped in preventing microbial spoilage and other deteriorative processes occurring in meat and fish for a considerable period through a decrease in water activity. The ancient Sumerians around 3000 BC were the first to make use of salt for meat and fish preservation. Dead Sea salt was used in ancient Palestine as early as 1600 BC. The Chinese and Greeks also used rock salt to preserve meat and are credited with passing this practice on to the Romans, who included pickled meats in their diet.

As the use of salt from sea, desert, and rocks in preservation of meat spread, it was found that only certain types of salt helped in developing a desirable pink color and a special flavor in cured meat. Investigations in the nineteenth century revealed that sodium nitrate, present as an impurity in these salts, was the precursor responsible for developing the characteristic color and flavor in cured meat. Further, it was reported that nitrite, which was produced by microbial reduction of nitrate, was responsible for the curing effect. From subsequent experiments, it was proposed that the reaction of hemoproteins with nitric oxide (NO) derived from nitrite was the chemical basis for the color of cured meats. On the basis of these findings, the United States Department of Agriculture (USDA) regulated the use of sodium nitrite in meat curing in 1925.

Present-day meat curing practice involves the intentional addition of sodium nitrite and salt to meat. Ascorbates or erythorbates are usually incorporated as cure accelerators. Other additives, such as sweeteners, phosphates/polyphosphates, seasonings (e.g., spices and herbs), smoke, and other nonmeat extenders, may be included in the curing mixture to impart characteristic properties to the end product. Curing methods can be divided into three main categories, namely, dry, direct addition, and wet (brine) curing. Dry curing is the oldest traditional technique in which the curing ingredients are rubbed onto the surface of the meat. For direct addition, curing ingredients are added to the meat during mixing or chopping of the meat product. In the brine curing process, the curing ingredients are dissolved in water to form a pickle or brine, which is introduced to or injected into the meat.

Most of the processed meats available in North America today are cured in order to impart a desirable color, flavor, and texture plus a long shelf life to the end product. In addition, nitrite, together with sodium chloride, inhibits the formation of deadly neurotoxin by *Clostridium botulinum*. However, formation of *N*-nitrosamines from the reaction of nitrite with free amino acids and amines in some cured meat products, under certain heat processing conditions (e.g., high temperatures

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associated with frying of bacon), or in the stomach of the consumer is a particular concern. Thus, attempts have been made to lower the amount of nitrite used or to find alternatives to it in meat curing. Research in the latter area has concentrated on formulating multicomponent alternatives, as it was recognized that a single compound could not duplicate the multifunctional properties of nitrite.

The Chemistry of Meat Curing

Although meat curing was originally used for preserving meat when it was plentiful, for use in times of scarcity, the need for preserving meat by curing alone has greatly diminished with the advent of sophisticated refrigeration and packaging techniques. Thus, the primary aim of present-day meat curing practices is to create flavor and appearance variations in food and to inhibit the outgrowth of *C. botulinum* spores. Another factor that has come to the forefront in modern curing practices is the increase of product yield and juiciness by incorporation of curing solutions. This can be achieved by using phosphates in the brine to increase the water-binding capacity of the meat by massaging and controlling other processing factors, such as smoking and cooking time, humidity, and the type of casing used.

Cured Meat Color

NO is derived from the added nitrite (or nitrate) in the curing formula. It helps in fixation of cured meat color by stabilizing the muscle pigment myoglobin, [MbFe(II)], through a reversible chemical bond formation. Nitrite is the conjugate base of nitrous acid (HNO_2). In an acidic environment, equilibrium is established between the ionized salt and the unionized nitrous acid, depending on the pH of the solution ($pK_a = 3.4$) (eqn [1]).

$$HNO_2 \rightleftharpoons H^+ + NO_2^- \qquad [1]$$

The concentration of HNO_2 in cured meat is very low (0.1–1.0%) at the usual pH values of meat (i.e., 5.5–6.5). Thus, the main reactive species in meat systems is dinitrogen trioxide (N_2O_3) (eqn [2]).

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \qquad [2]$$

In the presence of reducing agents (HRd), such as ascorbic acid or ascorbate, and endogenous reducing groups or compounds in meat tissue, such as cysteine, reduced nicotinamide adenine dinucleotide, cytochromes, and quinones, NO is formed from N_2O_3, as shown in eqns [3] and [4].

$$N_2O_3 + HRd \equiv RdNO + HNO_2 \qquad [3]$$

$$RdNO \equiv Rd + NO \qquad [4]$$

The NO molecule has the ability to form very stable complexes with metal ions, such as iron. Thus, NO reacts with meat pigments to form a red-colored nitrosylmyoglobin [MbFe(II)NO]. The oxidized pigment metmyoglobin [MbFe (III)] is formed by the oxidation of myoglobin after the addition of nitrite to the meat. MbFe(III) can then react with NO to form an intermediate pigment, nitrosylmetmyoglobin (Figure 1). Autoreduction of nitrosylmetmyoglobin pigment by the endogenous and exogenous reductants in the postmortem muscle tissue forms nitrosylmyoglobin.

If MbFe(II) is present in meat after nitrite addition of nitrite, it can also react with NO to form MbFe(II)NO. However, formation of MbFe(II) NO in conventional curing occurs by the action of nitrite, as described in Figure 1.

During thermal processing of cured meats, the globin moiety of nitrosylmyoglobin denatures and separates from the iron atom and surrounds the heme moiety to form nitrosylprotoheme or the cooked cured-meat pigment with a characteristic pink color. Figure 2 illustrates the formation of cooked cured-meat pigment and its possible side reactions during the curing process and subsequent storage.

Salt (either sodium or potassium chloride) in the curing mixture accelerates the curing reaction owing to the formation of nitrosyl chloride (NOCl), which is a more powerful nitrosating species than N_2O_3 (eqn [5]).

$$HNO_2 + H^+ + Cl^- \rightleftharpoons NOCl + H_2O \qquad [5]$$

A lower pH accelerates the formation of nitrosating species (N_2O_3 and NOCl). For this reason, acidulants (e.g., glucano-δ-lactone) are sometimes added to the formulation in order to accelerate the curing process.

The cooked cured-meat pigment (i.e., nitrosylhemochrome) is quite stable to heat. However, the presence of light and oxygen may cause discoloration of cured meats. Proper packaging systems can be used to minimize product's exposure to light (e.g., translucent films) and oxygen (e.g., vacuum packaging). Furthermore, mixing, massaging, and stuffing of cured meats must be performed under vacuum to exclude oxygen.

Cured Meat Flavor

The characteristic flavor of cured meats is also due to the action of nitrite in the curing mixture. However, the chemical changes that are responsible for this flavor formation are not yet fully understood. The antioxidative role of nitrite in retarding the breakdown of unsaturated fatty acids and the formation of secondary lipid oxidation products may be the main processes involved in modifying the volatile profile of cooked cured meats by suppressing the formation of oxidation products, thus allowing the unique flavor associated with cured products to be revealed.

In the pickle curing process, halotolerant bacteria, such as *Vibrio* spp., have also been shown to affect the flavor volatiles formed. Volatile compounds, such as 2-methylbutanal and 3-methylbutanal, have been identified in meats cured with cover brines. These two compounds can react with hydrogen sulfide, ammonia, and ammonium sulfide in meat to form 3,5-diisobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-triisobutyl-4H-1,3,5-dithiazine, both of which are claimed to have cured meat aroma.

Owing to the amount of salt used in most curing processes, salt plays a vital role in determining overall flavor of cured meats. In addition, smoking and added seasonings and sugar (especially in fried bacon) also participate in determining the characteristic flavor of different cured meat products.

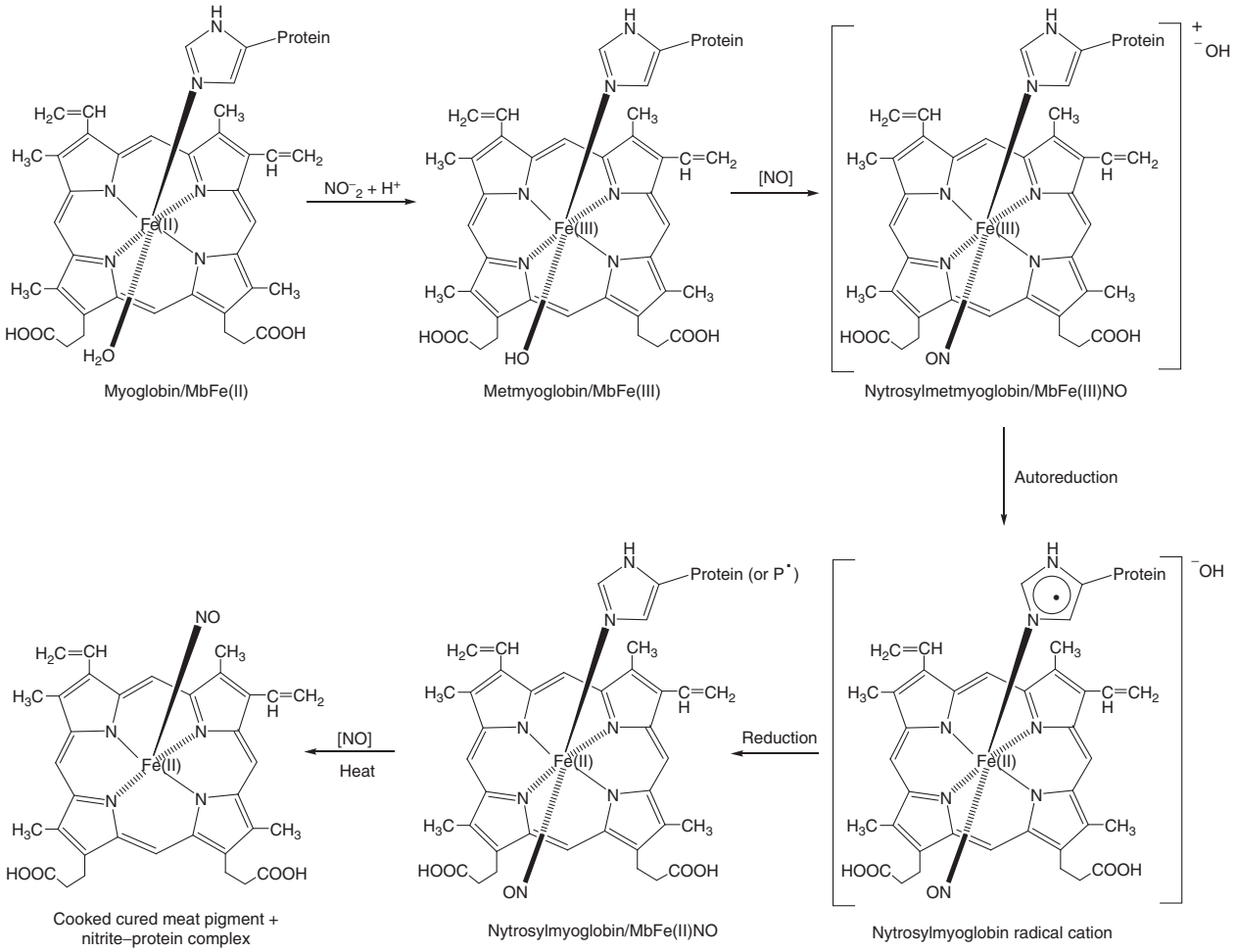

Figure 1 A new mechanism for the meat curing process. Reproduced from Killday, K.B., Tempesta, M.S., Bailey, M.E., Metral, C.J., 1988. Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the meat curing reaction. Journal of Agricultural and Food Chemistry 36, 909–914.

Curing Ingredients and Their Role in Cured Meats

Salt (Sodium Chloride/Potassium Chloride)

Salt is the main ingredient used in all curing mixtures and it is used for the purpose of developing flavor and for solubilizing proteins that are important for emulsion stability of comminuted and restructured meat products. Salt also helps in controlling microbial action in cured meats by lowering the water activity. Sodium chloride is the salt most commonly used in brine solutions, and its usage level varies with the type of product, being 1–2% in sausages, 2–3% in hams, 1.2–1.8% in bacon, and 2–4% in jerkies. Approximately 0.4–0.7% of potassium chloride on a finished-weight basis is used in low-sodium meat products, but it may impart bitter and metallic flavor if used at $>0.75\%$.

Sodium Nitrite/Sodium Nitrate

Sodium nitrite (or nitrate) is the most important cure additive responsible for the typical color and flavor associated with cooked cured meats. It also provides oxidative stability to meat by preventing lipid oxidation and helps in controlling the development of warmed-over flavor in cooked, stored meats. Nitrite also serves as a vital bacteriostatic agent for control of the outgrowth of *C. botulinum*, particularly under conditions of product mishandling. However, addition of sodium nitrite to meat and meat products is highly regulated owing to the possible risk of formation of *N*-nitrosamine.

In Canada, maximum allowable limit for the use of sodium nitrite, potassium nitrite, or their combinations in preserved meat and meat products (e.g., hams, loins, shoulders, cooked sausages, and corned beef) is 200 ppm (20 g per 100 kg; equivalent to 0.32 oz nitrite per 100 lbs raw batch). However, the industry has taken steps to reduce the level of nitrite used in such products to 120–180 ppm. In pumped bacon, in-going nitrite levels usually do not exceed 120 ppm (i.e., 0.19 oz nitrite per 100 lbs meat) owing to the possible risk of *N*-nitrosamine formation. These regulated levels are based on the amounts used in the product formulation before any cooking, smoking, or fermentation and are usually added as a cure salt, such as Prague powder.

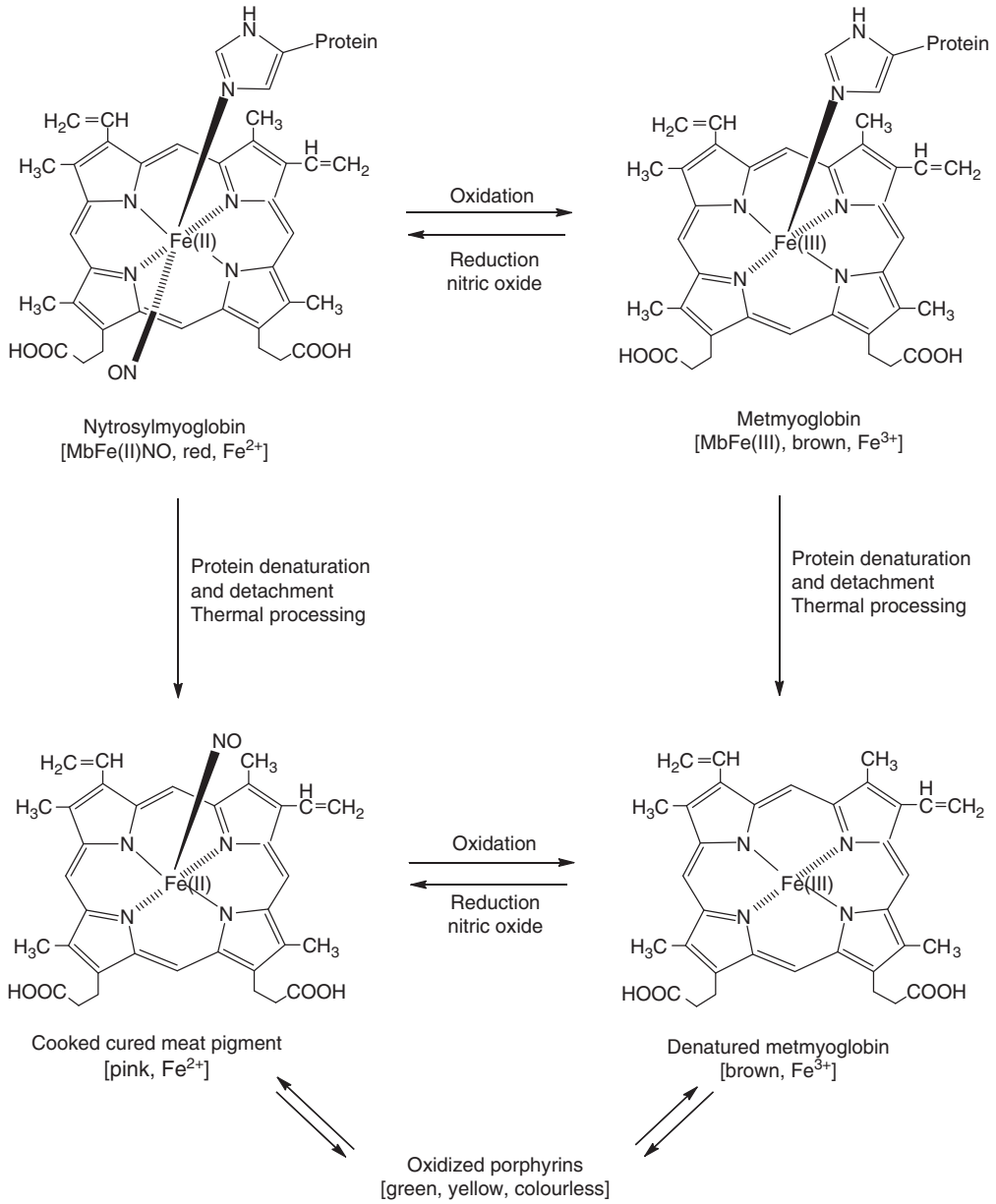

Figure 2 Some of the possible curing reactions that result from the addition of nitrite to meat. Reproduced from Bard, J., Townsend, W.E., 1978. Cured meats: Meat curing. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products, second ed. Westport: Food and Nutrition Press, pp. 452–470.

In the US, the Food Safety and Inspection Service (FSIS) regulations permit the use of sodium or potassium nitrite in all products except bacon at the following levels: 2 lb in 100 gallons of pickle at 10% pump or 200 ppm; 1 oz for each 100 lb of meat (60 g in 100 kg) in dry cure; and 0.25 oz per 100 lb meat or 156 ppm maximum in comminuted and (or) meat by-products. For immersion-cured and dry-cured bacon, in-going nitrite level limits according to FSIS are 120 and 200 ppm, respectively. Residual nitrite levels in the finished pumped bacon cannot exceed 40 ppm.

Use of sodium or potassium nitrate as a curing agent is limited to some specialty products that require a long cure, such as dry-cured country ham and dry or semidry sausages. For such specialty products produced in Canada, a maximum of 200 ppm of nitrate may be used in addition to the 200 ppm of nitrite. In the US, FSIS regulations permit the use of 3.5 oz of nitrate in 100 lb of meat (215 g per 100 kg) in dry-cured country ham, 700 ppm nitrate in pickle cure, and 2.75 oz of nitrate in 100 lb (170 g per 100 kg) chopped meat and (or) meat by-product.

Ascorbates/Erythorbates

Ascorbic acid, isoascorbic (erythorbic) acid, and their respective salts are widely used in ham, bacon, and corned beef

processing. Ascorbates are used in the curing process primarily to help improve and maintain the color (i.e., nitrosylhemochrome pigment) of cured meats. The primary function of ascorbic acid may be in reducing metmyoglobin to myoglobin, thus accelerating the overall curing reaction. Under suitable conditions, ascorbic acid also helps in the production of NO from nitrite or its derivatives in the curing mixture. Besides their role in color development, ascorbates and erythorbates have been shown to block the formation of carcinogenic *N*-nitrosamines in cooked cured meats (particularly in bacon).

The USDA FSIS regulations permit the addition of 547 ppm of ascorbic or erythorbic acid, or the molar equivalent of their sodium salts, per 100 lb of chopped meat. For pumping pickle, the level is 75 oz of ascorbic acid or 87.5 oz of sodium ascorbate per 100 gallons (i.e., 469 ppm ascorbic acid or 547 ppm sodium ascorbate) when the pickle is to be used at a level of 10% of green weight (weight of raw meat used for curing before employing any treatments, such as pumping, tumbling, or cooking).

Sweeteners

In addition to salt, nitrite, and nitrate, sugar is commonly used in the curing mixture. Sweeteners, such as table sugar (sucrose), brown sugar, dextrose, glucose solids, corn syrup solids, and lactose, can be added at different levels mainly to impart flavor and moderate the harshness of salt in certain products. Use of honey or maple syrup in small amounts during curing results in special flavor and aroma in cooked meats. Addition of reducing sugars (e.g., glucose solids and dextrose) to the brine also helps in browning reactions during thermal processing to produce a desirable color and a caramel flavor in some products, such as bacon. However, in some instances, the browning reaction may become too pronounced and could result in burned flavors and dark colors (e.g., rapid darkening of bacon on frying). Different levels of sugars, in the range 1–2%, are added to the brine during various commercial operations in order to lower the water activity of meat during curing and hence provide some preservative action in cured meats. When nitrates are used as curing agents, sugar enhances the growth of microorganisms that reduce nitrate to nitrite, the first step in the curing process.

Phosphates/Polyphosphates

Phosphates and polyphosphates are used primarily to increase the water-holding capacity of cured meat products. Alkaline phosphates increase the pH of the meat and also help in solubilizing muscle proteins in order to impart the water retention action. In addition to increased water binding (i.e., increase in product yield), phosphates improve the cured meat flavor by retention of natural juices and by reduction of oxidative rancidity and warmed-over flavor in reheated meats by chelation of prooxidant metal ions. They also help to improve retention of the cured meat color.

Phosphates that have been approved by the USDA for use in brine solutions include sodium acid pyrophosphate, monosodium phosphate, sodium hexametaphosphate, disodium phosphate, sodium tripolyphosphate, and sodium pyrophosphate as well as mono- and dipotassium phosphate, potassium tripolyphosphate, and potassium pyrophosphate. These phosphates may be added to the pickle for ham, bacon, pork shoulders, picnics, Boston butts, boneless butts, and pork loins in the USA and Canada. Use of acidic and alkaline phosphates and blends of phosphates is restricted to 5.0% in the pickle and 0.5% (usually used at 0.3%) in the finished product. In addition, use of sodium hydroxide in combination with phosphate, in a ratio not to exceed 1 part sodium hydroxide to 4 parts of phosphate, was approved by the USDA for meat formulations where a higher pH is desirable and feasible. However, care must be exercised in the way sodium hydroxide is used.

Tripolyphosphates and their combinations with hexametaphosphates are the most widely used phosphates for cured meat cuts, as they provide the proper pH, good solubility, calcium compatibility, and a high degree of protein-modifying effect. In some preparations, sodium acid pyrophosphate may be added to bacon and hams at a level of up to 0.5% to decrease pH and to accelerate cure development. However, acid phosphates are not typically used in sausage formulations, as a rapid pH decline can cause emulsion breakdown.

Seasonings

Different types and levels of seasonings are used in curing mixtures by meat processors to impart unique flavors and appearance to meat products. These include spices and their extracts, herbs, hydrolyzed plant and vegetable proteins, and autolyzed yeast. The most common flavorings used in brine preparation are extracts from pepper, cloves, allspice, and cinnamon. Garlic and onion flavorings may also be added. An aqueous smoke solution is sometimes introduced into the curing pickle to provide a smoked flavor. In addition to flavoring properties, certain spices and herbs used as seasonings act as antioxidants by reducing the rate of oxidative rancidity development in cured meats.

Brine Curing Process

Two fundamental procedures are used in meat curing: dry curing and brine curing. Although dry curing is the oldest method, brine curing has also been practiced for many years for the preservation of meat. In fact, a book dating from the reign of Augustus (63 BC to AD 14) contains directions for preservation of cooked meat in a brine solution containing water, mustard, vinegar, rock salt, and honey. The brine curing process uses the same ingredients as used in dry curing except that the cure mixture is dissolved in water to form a brine or pickle. In some cases, prepared brine may be used as a source of salt. Although the early application of curing used brines with high salt concentrations, mild cures with considerably lower salt concentrations are used in meat curing today due to the development of refrigeration techniques and owing to the trend toward reducing sodium consumption by health-conscious consumers. Present-day curing practices sometimes use a combination of dry curing and brine curing methods to produce certain specialty products.

Brine Preparation

A pickle cure may include: (1) water and salt (plain/salt pickle), (2) water, salt, and nitrite or nitrate, or (3) water, salt, nitrite or nitrate, and sugar (sweet pickle). Other ingredients, such as smoke, seasonings, ascorbates, and phosphates, may also be added to enhance or improve flavor and to speed up the curing process and increase product yield.

The amount of green weight to be pumped and the equipment design (e.g., the size of the curing tank; the rate of processing; the capability of weighing and measuring; and the quantity of pickle retained in the injector reservoir) are important factors that should be considered in determining the amount of brine to be prepared. Brine should also be formulated in the near-exact amount to prevent pickle being left over, as the age of the brine is very critical to the nitrite level. Appropriate and accurate scales should be used in measuring and checking weights of cure ingredients. All measurements of solid ingredients used to prepare the brine should be by weight. Liquids, such as water, however, can be measured by volume. If ice is used in the preparation of brine, its weight must be used in all calculations. The order of mixing the curing ingredients to permit complete dissolution and to reduce the nitrite and ascorbate depletion during pickle preparation would be: (1) water; (2) phosphates; (3) ascorbate; (4) salt, sugar, and flavorings; and (5) nitrite.

In most commercial curing operations, temperature of the curing room is held at 2–5 °C (36–40 °F) to retard bacterial growth during brine preparation and application and until salt penetration is complete. Experimental evidence indicates that a temperature near 0 °C is the optimal condition for the curing process and for storage of the curing pickles. Meat to be cured and water used in brine preparation should be cold enough (i.e., near 0 °C) to maintain the temperature of the brine and brine-treated meat close to 0 °C. At higher temperatures, microbial growth is accelerated. In addition, keeping the brine solution at temperatures above 15.6 °C for a long period in the air may result in oxidation of NO to the red nitrogen dioxide (NO_2). Agitation may also hasten the reaction with oxygen. This process can reduce the extent of MbFe(II)NO production and hence the extent of color and flavor formation in cured meats. Thus, proper refrigeration and close monitoring of the temperature of the brine is necessary.

In brine curing, brine preparation should be closely monitored and good sanitation maintained throughout the process. A salometer (a ballasted glass vacuum tube graduated in 'degrees') is used for testing the strength (density) or salinity of the pickle. If brine needs to be stored overnight, it must be kept cold and analyzed for nitrite and ascorbate before its use the next day. Curing tanks should be emptied and cleaned at least once a week to prevent growth of halophilic microorganisms.

Techniques of Brine Curing

Pickle curing (immersion curing)

In this process, meat products are immersed in brine until the cure ingredients penetrate the entire piece of meat. Sweet pickle with a salometer reading of 75–85° can be used for home curing; Table 1 lists the amounts of basic ingredients necessary to make such pickles. The pickle curing method can be used for thick and thin cuts of meat and stainless-steel or selected plastic containers are used to store the meat during curing in order to avoid corrosion problems.

Table 1 Sweet pickle formulations

Salt (kg)	*Sugar (kg)*	*Sodium nitrite (g)*	*Cold water (l)*	*Degree of pickle by Salometer at 40 °F*
4.5	1.4	7.5	15.1	95
4.1	1.4	7.5	15.1	90
4.5	1.4	7.5	18.9	85
3.6	1.4	7.5	15.1	85
3.6	1.4	7.5	18.9	75
2.7	1.4	7.5	15.1	70
3.2	1.4	7.5	18.9	65
2.7	1.4	7.5	18.9	60

Source: Data calculated based on Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., Jones, K.W., 1994. Meat curing and smoking. In: The Meat We Eat. Danville, CA: Interscience Publishers Inc., pp. 727–772.

Curing times prescribed for the different strengths of pickles listed in Table 1 are: 85° pickle, 9 days per inch; 75° pickle, 11 days per inch; and 60° pickle, 13 days per inch. Meat cuts with a higher thickness should be placed at the bottom of the curing barrel and the lighter ones placed on the top. Sufficiently cold (i.e., temperature close to 0 °C) pickle (usually 33 l per 100 kg (4 gallons per 100 lb) of closely packed meat and 37–42 l per 100 kg (4.5–5 gallons per 100 lb) of loosely packed meat) should be poured to cover the meat when the hold-down plate of the tank is weighted down. The thickness of each meat layer should be recorded in order to determine the date when the layers are to be taken out. It is desirable to overhaul (repack/move and turn) meat once or twice during the curing period in order to permit the pickle to reach all parts of the meat.

The rate of diffusion of the curing ingredients depends on the size of the cut, the amount of fat covering, and the temperature during curing. As meat cuts used for curing can vary in size and ability to absorb brine, all parts of the meat may not absorb the same amount of cure. This is one disadvantage in using this process. Also, as brine penetration into meat cuts for the brine curing method is a relatively slow process and takes a fairly long time, spoilage can develop with large cuts of meat before curing is complete. Cure accelerators, such as ascorbates, are not used in brine solutions employed for pickle curing because the process is a fairly slow. However, alkaline phosphates can be used in pickle curing to retain the moisture in the meat. At present, this method is mostly used for curing of small meat items, such as tongues, corned beef, and hocks.

Pickle injection

Owing to the problem associated with pickle curing (i.e., brine soaking), curing ingredients are now more commonly injected into the meat parts in order to obtain a rapid and uniform distribution of the cure throughout the tissue. Several techniques are used for this purpose, such as arterial pumping, stitch pumping, spray pumping, and multineedle injection.

Arterial Pumping

For meat cuts where the vascular system remains relatively intact (e.g., hams and tongues), brine solution can be introduced into meat through the arterial system. Pickle is pumped into the femoral artery on the inside butt end of the ham by means of a needle connected to a pump at a pressure of 275–345 kPa (40–50 lbs per square inch). The strength of the brine (i.e., salt concentration) used in this process is usually approximately 65–80° salometers. Most commercial processes use a brine solution of 65° salometer and phosphates are also incorporated within regulatory levels to help water retention and to increase yield. Nitrite, instead of nitrate, is used in this method to obtain a level of 156 ppm nitrite in the injected product. Sugar is also incorporated into the brine at a prescribed level. Normally, arterial pumping adds 8–10% of brine by weight to the final product. It is advised to allow at least 24 h of refrigerated storage to permit not only equilibration of the cure but also fixation of the cured meat color. This is necessary because the vascular system is not uniform throughout the meat. However, this process is fairly slow, because pumping of brine has to be carried out carefully and gently in order to avoid bursting of arteries. It also requires a high labor input and careful handling during slaughter, cutting, and subsequent handling of meat cuts to ensure that the arteries remain intact and are not damaged. Uncured spots can develop in cured meat products, such as hams, due to damaged arteries.

Stitch and Spray Pumping

Stitch pumping involves introduction of the brine into various parts of the meat tissues using a single-orifice needle. The spray pumping method is a variation of stitch pumping that uses a needle having several openings along the length of the needle to allow for a more uniform distribution of the pickle. Curing time is greatly reduced by this process as the curing ingredients diffuse from inside as well as outside of the cut. Also, the salt is introduced to the center of the ham cut before spoilage has a chance to take place.

The brine strength varies depending on the amount of pickle to be pumped into the meat, the desired intensity of salty flavor, and the storage conditions. In normal commercial operations, approximately 65° salometer brine with 150 ppm nitrite and adequate alkaline phosphate is used for injection at approximately 10% by weight. The injections are usually made at several sites, as close together as possible. However, uniform distribution of the cure is greatly dependent on the operator and, with bone-in meat parts (e.g., bone-in ham), it is difficult to distribute the cure uniformly around bones. Thus, stitched meat cuts should be held under refrigeration, perhaps in a cover pickle, for 5–7 days to allow uniform distribution of the cure.

Multineedle Injection

This method is widely used in the industry for curing bacon and pork cuts, both bone-in and boneless. This process is very rapid, continuous, and cost effective and reduces the number of workers involved during the curing process. The principle is quite similar to the stitch pumping method but uses 100–250 stainless-steel needles at uniform distances for injection of the brine. A series of offset needles are used in most commercially available machines and, on activation, pickle is pumped until the desired weight is obtained. This process helps to achieve a rapid and uniform distribution of the cure. The quantity of brine injected can be controlled by adjusting belt speed and number of strokes per minute. Independently balanced and functioning needles permit brine injection to follow the configuration of the meat cuts more closely.

The pumping pressure must also be carefully controlled to avoid muscle tissue damage, loss of cure retention, and formation of open pockets of pickle in the meat. In addition, all air should be removed from the pumping system, or vacuum pumping should be used, to avoid incorporation of air into the product. Needles must be periodically checked and cleaned to insure uniform pumping and to avoid bacterial contamination. However, if microbes are present on the surface of a meat cut, they can pass into the interior of the meat during injection. More importantly, as this is a continuous operation (injection of several meat cuts per cycle), contamination can occur from one piece of meat to another. Thus, good sanitation practices are necessary during this process. Some processors recirculate the brine during multiple injections, and this can also result in recontamination. Fresh brine should be used for each batch of meat cuts when using this method.

Massaging and Tumbling

Physical processes, such as massaging and tumbling, are used for brine-treated meat cuts to draw out water-soluble proteins (mainly actomyosin) to the meat surface and enhance the overall water-binding capacity when the exuded proteins gel on heating. Simultaneously, massaging and tumbling result in an increase in the internal tissue temperature that increases penetration and distribution of the brine. These mechanical treatments have been shown to shorten the curing period to 24 h, partly by aiding the distribution of the curing salts. Vacuum tumblers are used to overcome problems of tissue softening and incorporation of air and thus foaming of the protein matrix that has been brought to the surface during tumbling. Some tumblers have brine injection needles built into the vacuum chamber to allow simultaneous injection and mechanical action.

Smoking, Cooking, and Drying of Brine-Cured Meats

Smoke, generally produced by slow combustion of sawdust from hardwood (consisting of approximately 40–60% cellulose, 20–30% hemicellulose, and 20–30% lignin), inhibits bacterial growth and lipid oxidation and imparts flavor to cured meat. Many cured meat products are smoked in order to achieve these objectives. The cooking step is important for cured meats for fixation of the characteristic cured meat color (i.e., formation of nitrosylhemochrome pigment) and flavor. Cooking and smoking are often carried out simultaneously. Either steam or gas can be used for cooking. The cooking and smoking cycle must be carefully controlled to obtain the desired color, flavor, yield, and destruction of microorganisms in the brine-treated meats. The temperature and time of cooking,

as well as the humidity, are the most important parameters to be controlled. The final temperature depends on the product and is expressed as the internal temperature achieved for the finished product. The USDA regulations require that the fully cooked meat products attain a minimum temperature of 64 °C. However, drying, one of the earliest forms of meat preservation, remains in use for the production of dry or semidry fermented sausages.

Critical Control Factors during the Brine Curing Process

During meat curing, the composition of the curing mixture (such as the amount of nitrite, ascorbate, and phosphates) and the processing conditions (such as curing time, order of mixing of curing ingredients, and temperature during curing) should be controlled to achieve the desired color and flavor in the cured meats. It must also be considered that the intensity of the cured meat color depends on the availability of meat pigments (i.e., myoglobin) to form nitrosylmyoglobin during curing. A more intense cured color will thus be achieved for corned beef, containing more heme pigments than that for cured ham, when the same amount of nitrite is added.

Nitrite in the brine exerts an antimicrobial effect and retards the formation of *C. botulinum* toxin. Salt also helps in controlling microbial growth in cured meats by lowering the water activity. Combination of the practices used commercially for production of safe cured meat include addition of sodium nitrite at an initial concentration of 75–150 ppm with a residual concentration of 20 ppm or more, a sodium chloride concentration of 1.5–2.0% (both on a product basis), heating of the product to approximately 71 °C, and maintenance of a good sanitation throughout the curing process in order to minimize bacterial contamination. In addition, cured meats must be stored at temperatures below 10 °C.

Water used for the preparation of brine should be potable and free from bacteria, as bacteria can interfere with the curing reactions. The use of chlorinated water can adversely affect cured meat flavor. Food-grade salt should be used for brine preparation to insure a good flavor and color. Trace impurities in salt, such as copper, iron, and chromium, can catalyze oxidative reactions in cured meat products. Phosphates added to the brine help by chelating metal ions in the brine and reduce their catalytic activity in flavor deterioration. During brine preparation, phosphates should be dissolved in water before the addition of salt, as the salt can reduce their solubility. If the level of phosphates in the brine is too high, or if the salt concentration is too high, phosphates may precipitate, hence reducing their effectiveness. Thus, the proportions of phosphates and salt added to the brine should be properly controlled. Another problem in using phosphates is the formation of disodium phosphate crystals on the surface of the cured products owing to the loss of moisture during the processing and storage of cooked cured meat products. This problem can be overcome by reducing the level of phosphates in the brine, maintaining adequate humidity during processing, and using proper packaging systems to reduce moisture losses during subsequent storage. Owing to the corrosive nature of phosphates, another critical point with phosphates is the use of stainless-steel or plastic equipment and containers for brine processing of meat.

Levels of nitrite used in cured meats should be within the regulatory limits in order to reduce possible risk of *N*-nitrosamine formation. Formation of compounds such as *N*-nitrosopyrrolidine has been found to occur in bacon when frying at high temperatures. Thus, a maximum/minimum level of 120 ppm sodium nitrite is used for bacon curing. Bacon should also contain the maximum permitted level (547 ppm) of ascorbate or erythorbate in order to reduce *N*-nitrosamine formation. Use of buffered curing premixes containing nitrite, seasonings, and other flavorings is no longer permitted due to the risk of *N*-nitrosamine formation. Nitrite and nitrate are packaged separately from flavorings and seasonings in commercial curing mixes, and these separately packaged ingredients are not to be combined until just before use. Nitrite and nitrate must be uniformly mixed with other cure ingredients in order to avoid unexpected problems due to the presence of toxic levels of ingredients in any product batches.

Sodium Reduction in Brined Products

High sodium intake has been associated with increased risk of hypertension. To reduce sodium intake, there are three ways to lower the content of sodium chloride in processed meats. The first is to partially replace sodium chloride with potassium chloride; it is the most commonly used process to date. However, potassium chloride has a slightly bitter taste and other substances may have to be added in order to mask this unwanted taste. In this connection, lysine hydrochloride may be used at approximately 1% in such products. Second, flavor enhancers may be added to the meat, although their combination with salt has proven to provide a salty taste. Third, the physical structure of sodium chloride can be changed so that a lower concentration of it can still provide the same salty taste. This method is still being studied.

Nitrite Reduction

Nitrite renders multiple effects in cured meats by preventing oxidation and allowing the true flavor of meat to reveal itself. It also produces a desirable color in cured meat products and inhibits microbial growth. Because of its efficiency, nitrite is very difficult to replace; therefore, the common practice is to reduce its level and add other substances to mimic its properties.

To inhibit lipid oxidation, antioxidants, such as spices like rosemary extracts, are added. Many of these antioxidants also have multiple benefits, such as antimicrobial effects. However, simply changing the food packaging and preparation procedures to reduce the meat's exposure to light and oxygen can help to reduce the lipid oxidation and production of free radicals, hence retaining the desirable flavor and color of the meat.

To mimic the multiple preservative effects of nitrite, different ingredients with varying effects must be added. Ingredients with naturally high nitrate content are useful preservatives, as nitrate can be reduced to nitrite with naturally occurring nitrate-reducing bacteria. Such ingredients include vegetables, such as celery and spices. Use of celery juice in production of the so-called nitrite-free meat products has

taken place, but products may contain higher levels of residual nitrite than their nitrite-cured counterparts. Another way to preserve meat without nitrite is to add antimicrobials, including spices, herbs, and their oils. However, such antimicrobials usually only inhibit the growth pathogens and food-spoiling organisms in one way, whereas nitrite inhibits their growth in multiple ways. For this reason, different types of antimicrobials are needed to duplicate the effect of nitrite.

Summary

Although the origin of meat curing is lost in antiquity, the empirical observation that salting can preserve meat was made several thousand years ago. The occurrence of nitrate impurities in rock salt was found to be responsible for color formation in cured products. Present-day meat curing practices use a mixture of salt (sodium and/or potassium chloride); nitrite (or nitrate in some specialty products); sugar; cure accelerators, such as ascorbates or erythorbates; curing adjuncts, such as phosphates; seasonings; and other nonmeat ingredients in regulated levels.

Dry curing, direct addition, and brine curing are the three fundamental procedures used in meat curing. Brine curing uses the same ingredients as in dry curing and direct addition (perhaps at different levels to achieve adequate curing), but the ingredients are dissolved in pure water in order to produce a pickle that can be used in pickle curing or injected into the meat cuts. Methods for brine injection can be arterial, stitch, or multineedle injection. Each of these methods has its own advantages and disadvantages. Most industrial curing processes use the multineedle injection method because it facilitates rapid curing and faster production rate (i.e., number of meat cuts pumped per minute). However, there are some critical control factors to be considered during brine preparation, injection, maturation, and thermal processing of meat in order to obtain the desired color, flavor, and microbial quality of cooked, cured products. Furthermore, the issue of sodium reduction is a recent interest in preparation of processed meat products.

See also: Cooking of Meat: Flavor Development; Warmed-Over Flavor. Curing: Dry; Production Procedures. Ethnic Meat Products: North America. Processing Equipment: Brine Injectors; Smoking and Cooking Equipment; Tumblers and Massagers. Smoking: Traditional

Further Reading

Armenteros, M., Aristoy, M.-C., Barat, J.M., Toldra, F., 2012. Biochemical and sensory changes in dry-cured ham salted with partial replacement of NaCl by other chloride salts. Meat Science 90, 361–367.

Holland, G., 1983. Curing of ham and smoked meats-the state of art. The National Provisioner, March 5, 6–13.

Pegg, R.B., Shahidi, F., 2000. Nitrite curing of meat. The *N*-Nitrosamine Problem and Nitrite Alternatives. Trumbull: Food and Nutrition Press Inc.

Romans, J.R., Costello, W.J., Carlson, C.W., Greaser, M.L., Jones, K.W., 1994. Meat curing and smoking. In: The Meat We Eat. Danville, CA: Interstate Publishers Inc., pp. 727–772.

Townsend, W.E., Olson, D.G., 1987. Cured meats and cured meat products processing. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products, third ed. Westport: Food and Nutrition Press Inc., pp. 431–456.

Shahidi, F., 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In: St. Angelo, A.J. (Ed.), Lipid Oxidation in Food. Washington, DC: American Chemical Society, pp. 161–182. ACS Symposium Series 500.

Shahidi, F., Rubin, L.J., D'Souza, L.A., 1986. Meat flavour volatiles: A review of the composition, techniques, analyses and sensory evaluation. Critical Reviews in Food Science and Nutrition 24 (2), 141–243.

Skibsted, L.H., 1992. Cured meat products and their oxidative stability. In: Ledward, D.A., Johnston, D.E., Knight, M.K. (Eds.), The Chemistry of Muscle Based Foods. Cambridge, UK: Royal Society of Chemistry, pp. 266–286.

Weiss, J., Gibis, M., Schuh, V., Salminen, H., 2010. Advances in ingredient and processing systems for meat and meat products. Meat Science 86 (1), 196–213.

Dry

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Glossary

Decarboxylases Enzymes able to transform an amino acid into an amine.
Glycolysis Enzymatic breakdown of carbohydrates with the formation of pyruvic acid and lactic acid and the release of energy in the form of ATP.
Lipase Enzyme that catalyzes the release of fatty acids by hydrolysis of triacylglycerols at positions 1 and 3.
Lipolysis Enzymatic breakdown of lipids with the formation of free fatty acids.
Proteases Enzymes that catalyze the release of an amino acid from the amino terminus of a peptide (exopeptidases) or able to hydrolyze myofibrillar proteins to polypeptides (cathepsins and calpains).
Proteolysis Enzymatic breakdown of proteins with the formation of peptides and free amino acids.
Water activity (a_w) Indication of the availability of water in a food and is defined as the ratio of the equilibrium water vapor pressure over the system to the vapor pressure of pure water at the same temperature.

Introduction

The origin of dry cured meats is lost in ancient times and the processing of these products is varied depending on the particular customs and habits of each country. Dry cured ham constitutes one of the main and representative products obtained through dry curing. Consumption of dry cured ham is typical in Mediterranean countries where it is produced in substantial amounts (i.e., more than 30 million pieces per year in Spain). Some of the most well-known products are Spanish Iberian and Serrano hams, French Bayonne ham, Italian Parma and San Danielle hams and Portuguese presunto. Other dry cured hams produced in China are the Jinhua ham, Xuanwei ham and Rugao ham. In some cases, hams are submitted to short processes and smoked like the American Country-style and German Westphalia hams. In the European Union, the most famous hams are protected by designations of origin, being controlled by consortiums that guarantee the authenticity and quality of their products.

Dry curing consists of the application of a dry cure (no water added) containing salt, nitrate and/or nitrite and other agents like sugar and ascorbic or erythorbic acids. The process involves several stages: (1) a salting stage for the penetration of salt into the product by solubilization in the moisture of the meat, (2) a postsalting for salt diffusion and equalization through the entire piece and (3) a drying/ripening stage for water loss and development of numerous biochemical reactions affecting color, texture, and flavor. Dry curing is a traditional process, where the knowledge of the process has been transmitted from generation to generation. However, the need to obtain products of constant high quality, based on reproducible and controlled production processes, has prompted a considerable amount of scientific and technical research over the past 25 years. Today, there is a considerable amount of information available on the biochemical mechanisms involved in the process. Proteolysis and lipolysis constitute two groups of enzymatic reactions directly affecting protein and lipids, respectively, and resulting in important contributions to flavor and texture development. Proteins and lipids constitute the major chemical components of dry cured meat products, and their breakdown products are essential for flavor and texture.

Proteolysis

Proteolysis constitutes one of the most important groups of reactions responsible for degradation of sarcoplasmic and myofibrillar proteins and further hydrolysis of the generated polypeptides and peptides to small peptides and free amino acids. Skeletal muscle contains a wide variety of enzymes able to hydrolyze either internal peptide bonds (cathepsins and calpains) or peptide chains from their ends (tri- and di-peptidylpeptidases and aminopeptidases). Most of the enzymes are located in lysosomes, in the myofibrillar structure or bound to membranes, and have optimal pH near the values typically found in dry cured meats.

Action of Proteases During Dry Curing

The long processing time, several months or even years, allows for an intense action of muscle proteases. In general, these enzymes are quite stable and thus able to act for long periods of time. There are some exceptions like cathepsin D that tends to disappear approximately the sixth month of processing, and calpains which are restricted to the initial 2 weeks of the process owing to their rather poor stability. The full flow chart for the proteolysis during dry curing is shown in Figure 1. Initially, major protein breakdown, mostly due to calpains, is focused on Z-disc proteins, desmin and other major proteins like titin and nebulin. However, calpains are unstable and further protein breakdown, observed during the entire process, is due to cathepsins, especially cathepsins B and L. Polypeptides that are generated are further hydrolyzed to peptides within the range 2700–4500 Da, and most of those are further hydrolyzed to smaller peptides (below 1200 Da),

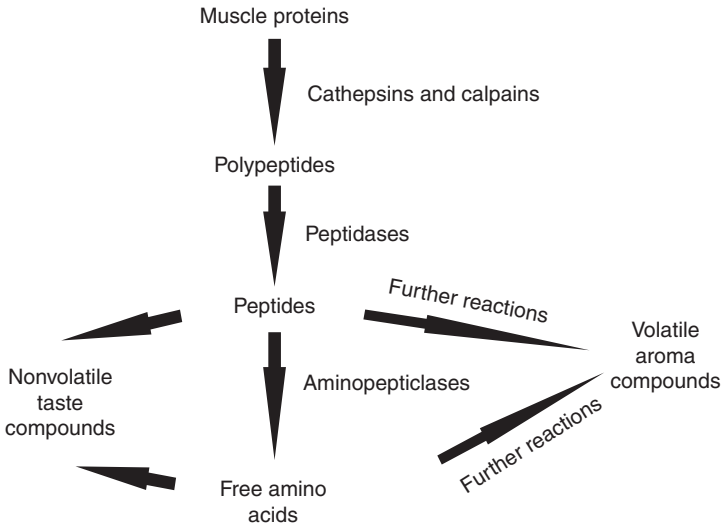

Figure 1 Flow chart showing the major important steps in muscle proteolysis during the processing of dry cured meats. Reprinted from Toldrá, F., 1998. Meat Science 49, s101–s110.

especially tripeptides and dipeptides by tripeptidylpeptidases and dipeptidylpeptidases, respectively. Peptide mappings, analyzed at different stages by reverse-phase HPLC and capillary electrophoresis, have confirmed the generation and/or increase of numerous peptides that have been further fractionated by size and fully sequenced by proteomics techniques. Savory fractions have been shown to be related to peptides within the range 1500–1700 Da. The generation of free amino acids by aminopeptidases constitutes the last step in the proteolysis chain. Some amino acids, mainly glutamic acid, alanine, leucine, lysine, valine and aspartic acid, are abundantly generated reaching amounts as high as 50–350 mg per 100 g of product by the end of the process. Alanyl aminopeptidase is the main exopeptidase involved in this process as it is the major aminopeptidase in muscle and has a wide substrate specificity. However, the generation of basic amino acids, arginine and lysine, is mainly due to aminopeptidase B. In general, the longer the process, higher is the amount of free amino acids generated. The combination of peptides and free amino acids together contribute to the characteristic taste of the product.

Proteolysis is important in dry curing and has many benefits for the final quality of the product although it needs to be controlled as an excess of proteolysis may impair the sensory characteristics and result in poor ratings by sensory panelists and consumers for the following reasons: (1) an excessive accumulation of low molecular weight nitrogen compounds (peptides and free amino acids), enhancing a bitter and metallic taste, (2) the presence of randomly distributed white crystals of tyrosine, making the product less attractive, and (3) excessive breakdown of myofibrillar proteins resulting in an undesirably soft product.

Lipolysis

The process of lipolysis can be considered as a group of enzymatic reactions affecting triacylglycerols and phospholipids.

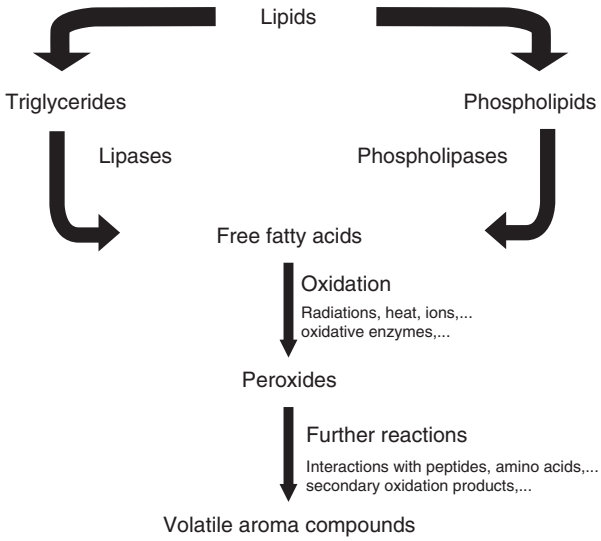

Figure 2 Flow chart showing the major important steps in muscle lipolysis and oxidation to volatile compounds during the processing of dry cured meats. Reprinted from Toldrá, F., 1998. Meat Science 49, s101–s110.

Free fatty acids are released in these reactions and act as precursors of further oxidative reactions leading to volatile aroma compounds as shown in Figure 2. Lysosomal acid lipase and neutral lipase are present in skeletal muscle and are able to hydrolyze tri- and di-acylglycerols at acid and neutral pH, respectively, and generate free fatty acids. Acid phospholipase also generates free fatty acids acting on phospholipids as substrate. Lysosomal acid lipase and acid phospholipase are located in lysosomes and have optimal pH near the pH values usually found in dry cured meats. Furthermore, both enzymes have shown good stability along the full process. The activity of lipases and phospholipases is higher in oxidative muscles than in glycolytic muscles. Acid and neutral esterases are also present in muscle tissue but the generation of short chain free

fatty acids is almost negligible suggesting that these enzymes have a minor role. In addition, esterase activity remains unaffected by the oxidative pattern of the muscle.

Hormone-sensitive lipase and mono-acylglycerol lipase are located in adipose tissue and are active at neutral pH. These lipases find good conditions for activity during dry curing and are responsible for the generation of free fatty acids from triacylglycerols and diacylglycerols and from monoacylglycerols, respectively.

Action of Lipases During Dry Curing

The rate of lipolysis is fast during the first 6 months but then decreases, reaching slower rates toward the end of the process. A great percentage of the generated free fatty acids in muscle occurs as a result of phospholipid hydrolysis, indicating a major role of phospholipases. In fact, the hydrolysis of phospholipids is very important for the final flavor of the product because they release long chain polyunsaturated fatty acids which are very sensitive to oxidation. Fatty acid profiles during the processing of dry cured meats usually reaches a maximum at some point but, before the end of the process, oxidation results in a decrease in long chain polyunsaturated fatty acids. Oxidation is favored by the presence of salt, action of oxidative enzymes like muscle lipoxygenases and cycloxygenases, drying/ripening temperatures, and long time for reactions. The minor amount of lysophospholipids can be explained by the high activity of lysophospholipases in relation to phospholipases. Triacylglycerols are hydrolyzed and also provide a significant amount of free fatty acids although at a lower rate. In this sense, the disappearance of triacylglycerols is correlated with the increase in di- and monoacylglycerols, as products of the hydrolytic action.

Triacylglycerols from adipose tissue also undergo an intense lipolysis during the salting and postsalting stages, with a substantial increase in free fatty acids. Hormone-sensitive lipase that remains active during the full ripening/drying period is the main enzyme responsible for lipolysis in the adipose tissue. Monoacylglycerols are further degraded to glycerol and the respective fatty acid by the mono-acylglycerol lipase. The generation rate, especially in mono and polyunsaturated fatty acids depends on the composition of the feed given to the pigs. This is of extreme importance for oxidative reactions that will generate different volatile compounds, and thus different aromas, depending on the composition in fatty acids.

Nucleotides Degradation During Dry Curing

Nucleotides experience an intensive enzymatic degradation during the dry curing process until almost complete disappearance at approximately 7 months. On the contrary, hypoxanthine and xanthine, as final products of the enzymatic cascade, are generated primarily during the first 7 months of dry curing, and remain for the rest of process. Hypoxanthine, which is generated at more than 15 μmol per gram of dry matter, might be considered as a potential biochemical marker of a minimum time of processing (7 months) in view of its evolution and stability.

Curing Factors Affecting Muscle Enzyme Activity

Curing agents and processing conditions exert different effects on the activity of muscle enzymes. Salt is a basic compound in dry curing processes and exerts a clear and important influence on muscle enzymes. Cathepsins D and H, dipeptidylpeptidases II and III and alanyl aminopeptidase are strongly inhibited by salt whereas calpains, aminopeptidase B, and lysosomal acid lipase are slightly activated, especially at low salt levels. Other curing agents like nitrate and nitrite, glucose, or ascorbic acid exert only a slight effect on the enzyme activity. Conditions such as temperature during drying and ripening are favorable for enzyme activity. However, the slightly acid pH of meat reduces the activity of strictly acid enzymes (i.e., cathepsin D) and neutral/basic enzymes (i.e., calpains, leucine and pyroglutamyl aminopeptidases, neutral lipase and esterase).

Microbial Evolution

Low bacterial counts are usually found inside the hams due to limiting factors like salt concentration, presence of nitrite and progressive water activity reduction. Microorganisms present in the natural flora of ham include *Lactobacillus sakei*, *Lactobacillus curvatus* and *Pediococcus pentosaceus*. They have good exo-proteolytic activity although its contribution to proteolysis is minimal owing to the low counts. *Staphylococcus xylosus* is also naturally present and has an important nitrate reductase activity that contributes to the reduction of nitrate to nitrite. Amines might be generated by decarboxylation of certain free amino acids as a consequence of undesirable bacterial growth having decarboxylase activity although low or negligible levels are usually found. Humidity and temperature must be taken into account, particularly in the air-conditioned rooms, to avoid growth and development of molds, usually *Penicillium*, and sometimes yeasts such as *Candida zeylanoides* and *Debaryomices hansenii*, on the outer surface of the hams.

Sensory Characteristics

Color

The intensity of color depends on the concentration of myoglobin, which varies depending on the type of muscle (myoglobin concentration is higher in muscles with oxidative pattern) and the age of the animal (myoglobin concentration tends to be higher in muscles from older animals). Nitrosomyoglobin is generated through the reaction of nitric oxide with myoglobin when nitrate and/or nitrite have been used, giving hams a typical bright-red cured color. Those hams without added nitrate or nitrite like Parma hams present a pink-red color that is attributed to the Zn protoporphyrin IX complex. Dark colors on the surface of the hams are typical after smoking treatment.

Texture

Proteolysis of key myofibrillar and associated proteins is responsible for tenderization. An intense degradation of the

myofibrillar structure is observed during dry curing. Major structural proteins like titin, nebulin, and troponin T as well as myosin heavy chain and α-actinin are severely proteolyzed. Two clear fragments corresponding to 150 and 85 kDa appear along the processing. Hams produced from pale, soft, exudative (PSE) meats show an absence of these fragments when compared to normal hams and there is a trend toward softer hams. In fact, the application of a texture analysis shows that PSE hams have lower hardness, springiness, cohesiveness, and chewiness values.

Texture of the product depends not only on the extent of myofibrillar protein breakdown but also on other factors like the extent of drying, the degradation of the connective tissue and the content of intramuscular fat which also exerts a positive influence on some texture and appearance traits.

Flavor

Flavor generation is strongly associated with the proteolysis and lipolysis phenomena. Taste is mainly associated with nonvolatile compounds accumulated by the end of the process (see Table 1) and, in fact, the concentration and composition of free amino acids and small peptides at the end of dry curing has been related to specific taste descriptors. For instance, the composition of peptides in savory fractions consisted of glutamic acid, glycine, alanine, valine, proline, histidine, and leucine. However, lysine and tyrosine have been correlated with aged taste whereas glutamic acid, aspartic acid, methionine, phenylalanine, tryptophan, lysine, leucine, and isoleucine have been correlated with the length of the drying and the fully ripened ham taste. The excessive accumulation of tryptophan, tyrosine and phenylalanine, in hams with a high level of proteolysis, is associated with a bitter taste. Nucleotides are strong taste enhancers but their concentration in dry cured meats is very low and in most of the cases below the sensory threshold value, and thus its contribution can be considered almost negligible. Only hypoxanthine, which is generated at concentrations higher than 15 $\mu mol\ g^{-1}$ dry matter, may contribute to bitter taste.

In the case of aroma, nearly 200 volatile compounds, most of them with impact on aroma perception, have so far been reported in dry cured meats. They are representative of most classes of organic compounds such as aldehydes, alcohols, hydrocarbons, pyrazines, ketones, esters, lactones, furans, sulfur and chloride compounds, carboxylic acids, etc. Some of these compounds are generated through oxidation of unsaturated fatty acids resulting from lipolysis, as shown in Figure 2. The generation of free amino acids like pyrazines, sulfide compounds, and branched-chain aldehydes during dry curing constitutes an important source of volatile compounds with important aromatic characteristics. The most representative classes of volatile compounds found in dry cured meats and the main routes for generation are listed in Table 2. The final flavor strongly depends on the specific aromas and odor thresholds for each particular volatile compound. In general, pleasant aroma is correlated with the presence of certain ketones, esters, aromatic hydrocarbons, and pyrazines.

Processing Control

The ability to control this complex system in a dry cured product is very important for economical and quality reasons. There are several ways to control the proteolytic and lipolytic activity in the hams: (1) the genetics and age of pigs exert a clear influence on the final quality of the products. Different enzyme profiles and composition have been detected depending on the specific crossbreed and age. The amount of lipids and marbling, very important for the sensory quality of the final product, also depend on the breed (i.e., the Duroc

Table 1 Major nonvolatile compounds generated or present in dry cured meats and the relative contribution of each to taste

Groups	*Main compounds*	*Contribution to taste*
Peptides	Many tri and dipeptides, carnosine and anserine	High
Free amino acids	Lysine, glutamic acid, aspartic acid, leucine, alanine, arginine, valine, serine, threonine, ...	High
Free fatty acids	18:1 *n*–9, 18:2 *n*–3, 16:0, 18:0, 20:4 *n*–6, 18:3 *n*–6, ...	Low
Nucleosides	Hypoxanthine, xanthine	Low/medium
Inorganic compounds	Sodium chloride	High

Table 2 Major classes of volatile compounds generated in the processing of dry cured meats and the relative contribution of each to final flavor

Groups of volatile compounds	*Main routes for generation*	*Odor threshold*	*Contribution to flavor*
Aliphatic hydrocarbons	Lipids auto oxidation	High	Very little
Aromatic hydrocarbons	Oxidative decomposition of lipids	High	Low
Aliphatic aldehydes	Oxidation of unsaturated fatty acids	Low	High
Branched-chain aldehydes	Strecker degradation of valine, leucine, and isoleucine	Low	High
Alcohols	Oxidative decomposition of lipids	High	Low
Ketones	Lipid oxidation	Low	High
Esters	Interaction of carboxylic acids and alcohols	Low	High
Pyrazines	Maillard reactions	Low	Medium
Sulfide compounds	Strecker degradation of sulfur-containing amino acids	Low	Medium

breed gives more intramuscular fat than many others) and age (i.e., higher lipids content as animal gets older), (2) the feed, especially the lipid composition in fatty acids, strongly affects the composition of pork fat and thus the final aroma of the dry cured meat because many volatile compounds arise from the oxidation of particular unsaturated fatty acids, (3) processing control of important parameters like temperature, relative humidity and air speed in computer-controlled curing rooms are important for drying and subsequent water loss from the product. The reduction in water activity affects enzymatic hydrolysis reactions, like proteolysis and lipolysis, lowering the rate of hydrolysis, and (4) the addition of salt affects muscle enzymes. Based on its proved inhibitory effect on cathepsins, salt may be used to prevent an excessive tenderness when using hams from pigs with high levels of cathepsin activity. The excess of cathepsin activity may be controlled in the raw material through rapid test kits and, in those cases with an excessive activity, the addition of an excess of salt constitutes an effective controlling measure because its inhibitory effect on cathepsins results in a slower protein breakdown.

Salt Reduction

Dietary intake of excessive amounts of sodium may have negative effects on the cardiovascular health of the consumers and following such medical concern, the meat industry has made serious efforts in recent years to reduce salt content in hams. The strategies to reduce the amount of salt used in the processing of dry cured ham consist of a direct reduction in the amount of added salt and/or partial replacement of sodium chloride by other chloride salts like potassium chloride, calcium chloride, and magnesium chloride. However, the percentage of replacement is restricted to less than 40% due to bitterness associated with an excess of potassium or metallic aftertastes associated with the presence of certain levels of calcium and magnesium.

See also: Chemical Analysis: Raw Material Composition Analysis; Standard Methods. Chemical and Physical Characteristics of Meat: Water-Holding Capacity. Curing: Production Procedures. Drying. Ham Production: Dry-Cured Ham. Sausages, Types of: Dry and Semidry. Sensory Assessment of Meat

Further Reading

Armenteros, M., Aristoy, M.C., Barat, J.M., Toldrá, F., 2011. Biochemical and sensory changes in dry-cured ham salted with partial replacement of NaCl by other chloride salts. Meat Science 90, 361–367.

Flores, M., Spanier, A.M., Toldrá, F., 1998. Flavour analysis of dry-cured ham. In: Shahidi, F. (Ed.), Flavor of Meat, Meat Products and Seafoods. London: Blackie Academic & Professional, pp. 320–341.

Hernández-Cázares, A.S., Aristoy, M.C., Toldrá, F., 2011. Nucleotides and their degradation products during processing of dry-cured ham, measured by HPLC and an enzyme sensor. Meat Science 87, 125–129.

Ripollés, S., Campagnol, P.C., Armenteros, M., Aristoy, M.C., Toldrá, F., 2011. Influence of partial replacement of NaCl for KCl, $CaCl_2$ and $MgCl_2$ in lipolysis and lipid oxidation in dry-cured ham. Meat Science 89, 58–64.

Toldrá, F., 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Science 49, s101–s110.

Toldrá, F., 2002. Dry-cured meat products. Trumbull, CT: Food & Nutrition Press.

Toldrá, F., 2006. Dry-cured ham. In: Hui, Y.H., Castell-Pérez, E., Cunha, L.M., *et al.* (Eds.), Handbook of Food Science, Technology and Engineering, vol. 4. Boca Raton, FL: CRC Press, pp. 164-1a–164-11a.

Toldrá, F., 2011. Improving the sensory quality of cured and fermented meat products. In: Kerry, J.P., Kerry, J.F. (Eds.), Processed meats: improving safety, nutrition and quality. Cambridge: Woodhead Publishing, pp. 508–526.

Toldrá, F., Aristoy, M.-C., 1995. Isolation of flavor peptides from raw pork meat and dry-cured ham. In: Charalambous, G. (Ed.), Food Flavors: Generation, Analysis and Process Influence. Amsterdam: Elsevier Science, pp. 1323–1344.

Toldrá, F., Aristoy, M.C., 2010. Dry-cured ham. In: Toldrá, F. (Ed.), Handbook of Meat Processing. Ames, Iowa: Wiley-Blackwell, pp. 351–362.

Toldrá, F., Flores, M., 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. CRC Critical Reviews in Food Science and Nutrition 38, 331–352.

Relevant Websites

http://www1.clermont.inra.fr/tradisausage/
Agencia Estatal Consejo Superior de Investigaciones Científicas.

http://www.iata.csic.es/IATA/dcie/carn/
Instituto de Agroquímica y Tecnología de Alimentos, Research Group: Science of Meat and Meat Products.

Natural and Organic Cured Meat Products in the United States

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Glossary

Alternative curing The technology of using natural sources of nitrate and/or nitrite from plants, vegetables, etc. to cure.

Curing The addition of nitrite/nitrate with salt to a meat product to achieve improved preservation or the chemical entities of nitrite/nitrate.

Nitrite A pale yellow, nearly white, crystalline compound that is highly soluble in water and highly reactive functioning as an oxidizing, reducing or nitrosating agent, and is converted to a variety of related compounds when added to meat.

Nitrosamines It is also known as *N*-nitroso compounds. A class of chemical compounds classified as carcinogenic that can be formed in cured meat, primarily bacon, under special conditions where secondary amines are present, nitrite is available, and necessary pH and high temperatures exist.

Purified nitrite Nitrite that has been industrially produced by absorption of nitrogen oxides (NO_x), originating from the catalytic air oxidation of anhydrous ammonia, into aqueous sodium carbonate or sodium hydroxide.

Introduction

Natural and organic processed meats have been a very significant part of the explosive market growth that has occurred in natural and organic foods. Producers and processors have responded to consumer demand for foods perceived by many to be more healthy and wholesome than conventionally produced food products by offering more and more products labeled as 'natural' or 'organic.' To qualify as natural or organic in the US, foods must be produced and processed in accordance with United States Department of Agriculture (USDA) regulations that define these products. These products are also produced in other countries around the world, though specific regulations differ somewhat.

In most cases, natural and organic foods very closely resemble conventional products and do not differ in the typical characteristics expected by consumers. However, in the case of processed meat products such as hams, bacon, frankfurters, bologna, and others that are typically cured by addition of sodium nitrite, and sometimes sodium nitrate, the requirements for natural or organic marketing in the US do not permit addition of nitrite or nitrate and thus differences commonly exist. Therefore, a new category of 'uncured' processed meats often referred to as 'alternatively cured' products has been developed to provide consumers with the variety, convenience, and satisfaction of cured meat products while giving manufacturers the opportunity to meet consumer demand for natural and organic processed meat products.

Conventional Curing

Conventional Cured Meat Ingredients

Conventionally cured meat products are characterized and defined by the addition of nitrate and/or nitrite, which provide the distinctive properties that are common to all cured meat products. Saltpeter (potassium nitrate), first recognized as the original curing agent, was used in one form or another to cure meat for centuries before researchers, in the late 1800s, discovered that nitrate was actually being converted into nitrite by nitrate-reducing bacteria, and that nitrite was the true curing agent.

Nitrite is a highly reactive compound and it has become clear that the formation of nitric oxide (NO) from nitrite is a necessary prerequisite for many meat curing reactions. The addition of nitrite to cured meats fixes color, contributes to cured meat flavor, helps in the inhibition of the growth of microorganisms, specifically *Clostridium botulinum*, and effectively controls rancidity by inhibiting lipid oxidation. Sodium nitrite allows for the existence of meat and poultry products with unique colors, textures, and flavors that cannot be recreated by any other ingredient.

Regulations for the Conventional Curing Processes

Current regulations restrict the use of nitrite and nitrate in the US and vary depending on the method of curing used and the product that is being cured (see **Table 1**). Bacon is an exception to the general limits for using curing agents because of the potential for nitrosamine formation and as a result has more stringent curing regulations.

The curing accelerators permitted for use with nitrite are also restricted. Ascorbic and erythorbic acids, for example, cannot exceed 469 ppm (ppm) ingoing concentrations, while sodium ascorbate and erythorbate are limited to 547 ppm ingoing.

Alternative Curing – Systems and Labeling

Manufacturing Systems

Because the addition of purified nitrate and nitrite is prohibited for all 'natural,' 'organic,' or simply 'uncured' meat products to resemble traditionally cured meats, processors often utilize permitted natural ingredients and modified processing to achieve cured meat characteristics – a process that has become known as alternative curing.

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Table 1 Maximum allowable added levels for curing ingredients in meat and poultry in the United States[a]

Curing agent	*Curing method*			
	Immersion cured (ppm)	*Massaged or pumped (ppm)*	*Comminuted (ppm)*	*Dry cured (ppm)*
Sodium Nitrite	200	200	156	625
Potassium Nitrite	200	200	156	625
Sodium Nitrate	700	700	1718	2187
Potassium Nitrate	700	700	1718	2187

[a]Limits are based on total formulation/brine weight for immersion cured, massaged, or pumped and raw meat (green) weight for comminuted or dry cured products.

The term 'alternative curing' is not officially recognized by the USDA, but refers to the original, ancient process that is the true origin of all modern cured meats. Alternative curing refers to the microbial conversion of naturally occurring nitrate, present in the environment, to nitrite, which then cures the meat in the same manner as if purified nitrite had been added. Because nitrate is an essential part of the total nitrogen cycle, naturally occurring nitrate can be present in the soil, sea water, various 'sea salts,' and green plants (including vegetables). Furthermore, many types of microorganisms in the environment have the ability to convert this naturally occurring nitrate into nitrite; thus, combining these two natural ingredients (vegetables or sea salt+harmless food-grade microorganisms) can result in a natural preservation system for meat.

In general, natural curing processes now fall within one of the following three categories.

Culture system

This process involves using a natural nitrate source material and a suitable meat culture. Both ingredients are added separately to the meat formulation and the nitrate conversion occurs in the meat during processing. Starter cultures containing a nitrate-reductase enzyme facilitate nitrate-to-nitrite conversion. The culture system used for alternative curing is driven by the culture concentration, and thus higher nitrite levels result from a greater percentage of culture used in the product formulation.

The main advantage of the culture system is that some of the nitrite produced immediately reacts with the meat pigments to begin the curing process. The primary disadvantages are (1) the potential lengthening of the thermal process to allow adequate conversion and (2) the 'lag period' sometimes required to generate the nitrite can make some specific meat products and processes more vulnerable to the initial growth of undesirable microorganisms.

Prebrine system

This process also involves the use of both a natural nitrate source and a suitable meat starter culture; however, the nitrate-to-nitrite reduction is accomplished by the meat processor, either partially or completely, in brine (liquid) before addition to the meat. The nitrate source and all, or a portion, of the meat culture, is first added to the brine, preferably without other ingredients. The nitrite is generated in solution by the microbial reduction of the added nitrate and is then subsequently added to the meat mix directly or via injection, after remaining ingredients have been added to the brine.

The advantage of this system is that nitrite produced in the brine can be measured and is available immediately for reaction when added to the meat, thus eliminating any 'lag period.' The main disadvantages are controlling the nitrate reduction in the liquid system and stabilizing the resulting nitrite produced to avoid nitrite loss before addition to the meat takes place.

Preconverted system

This most recent and most commonly used system developed for alternative curing involves the intentional preconversion of nitrate to nitrite and stabilization of the nitrite produced, which is all done by the ingredient supplier. The resulting product in liquid or dry form originates from the same natural nitrate source, with the original nitrate mostly converted into nitrite. The preconverted product already contains the nitrite and is simply used by the meat processor as a curing agent, similar to conventional curing procedures.

The main advantages of this system are the simplicity of adding a known quantity of nitrite and avoiding the use of viable microorganisms. The main disadvantages are, in general, (1) lower potential nitrite concentrations ultimately available for curing as opposed to using nitrate plus starter culture and (2) the handling of the preconverted product that is reactive (i.e., as with traditional nitrite cures).

Labeling Terms for Natural, Organic, and Uncured Processed Meats

The labeling terms 'natural,' 'organic,' and 'uncured' refer to three distinct meat and poultry product categories governed by separate USDA labeling policies and federal regulations. By definition, none of the three types of products can have added nitrate and nitrite.

Natural

Processed meats that are labeled 'natural' must comply with the definition of the term provided by the *USDA Food Standards and Labeling Policy Book*. This definition requires that a natural product "does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and the product and its ingredients are not more than minimally processed." The definitions for flavorings, coloring, and chemical preservatives can be found, as noted above, in Title 21 CFR, Chapter 1, Part 101, Subpart B

(101.22)-subtitled Labeling of Spices, Flavorings, Colorings, or Chemical Preservatives.

Furthermore, the term artificial color or coloring means any 'color additive' as found by definition in Title 21 CFR, Chapter 1, Part 70-Color Additives, Subpart A(f). The second relevant part of 21 CFR 101.22 is item number 5, which addresses chemical preservatives as follows: "The term chemical preservative means any chemical that, when added to food, tends to prevent or retard deterioration thereof, does not include common salt, sugars, vinegars, spices, oil extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties…."

Organic

Products labeled 'organic' are much better defined and controlled than those labeled with 'natural' claims because organic products are governed by the Organic Foods Production Act (OFPA), first passed in 1990 as part of the 1990 Farm Bill. The OFPA authorized the USDA to create a National Organic Standards Board, which established a National List of Allowed and Prohibited Substances and developed National Organic Program (NOP) standards.

The NOP standards, implemented in 2002, specify methods, practices, and substances that are allowed for use for production, processing, and handling of organic foods. This means that products and ingredients used for organic foods must be certified as organic by a USDA-certified inspector. Meat, for example, must be raised using organic management and come from a certified farm. Ingredients used for processed products are clearly defined as permitted or prohibited in the OFPA National List.

Uncured

The Code of Federal Regulations (9 CFR 317.17) states that normal cured products ("to which nitrate or nitrite is permitted or required to be added…") can be made without nitrite or nitrate and labeled with such common or usual name or descriptive name when immediately preceded with the term 'uncured.' Additionally, specifically outlined, 'No nitrate or nitrite added' labeling is also required. All organic and natural products are uncured by definition, but not all uncured products are natural or organic.

General Labeling Regulations

The labeling for uncured, natural, and organic meat products is very confusing and has yet to be totally resolved, particularly with regard to 'alternative curing.' All three product categories do not permit the use of added purified nitrite or nitrate salts; however, natural sources of these chemicals are permitted. The main issue is the separate USDA regulation that requires the labeling of a traditionally cured meat product as 'uncured' if purified nitrate and nitrite are not added.

Specifically, "Any product, such as bacon and pepperoni, which is required to be labeled by a common or usual name or descriptive name in accordance with 9 CFR 317.2(c)(1) and to which nitrate or nitrite is permitted or required to be added may be prepared without nitrate or nitrite and labeled with such common or usual name or descriptive name when immediately preceded with the term 'Uncured' as part of the product name, provided that the product is found by the Administrator to be similar in size, flavor, consistency, and general appearance to such product as commonly prepared with nitrate or nitrite, or both. In addition, these products must bear the statements 'No Nitrate or Nitrite Added' and 'Not Preserved – Keep Refrigerated Below 40 °F at All Times' unless they have been thermally processed to 3 °F or more, fermented or pickled to pH of 4.6 or less, or dried to a water activity of 0.92 or less." Because these products are considered 'uncured' by the USDA, they must be processed accordingly.

With the growth of alternative curing, the USDA now requires 'uncured' products to have a qualifying statement, 'No Nitrates or Nitrites Added (except those occurring in sea salt and celery powder),' if any of the ingredients may contain naturally occurring nitrate or nitrite.

Only 'traditionally cured' products such as frankfurters, bacon, corned beef, pastrami, and pepperoni are required to follow the uncured labeling requirements for their natural, organic, and uncured alternatives. If the traditional product was not cured, such as oven roasted turkey breast, the natural alternative does not require uncured labeling.

Additionally, some alternatively cured product alternatives are exempt from the 'uncured' labeling requirements altogether if they meet the outlined USDA criteria such as (1) achieving a water activity of 0.92 or less or (2) having a brine concentration of ≥10%.

Alternative Curing – Manufacturing Ingredients

Alternative Curing Agents

Natural nitrate sources

Several natural nitrate sources are available for natural curing but the most common ingredients are based on celery juice or celery powder. This is a regularly available and consistent vegetable crop with relatively minimal negative sensory effects on meat product attributes such as flavor and color. Celery powder can also be labeled as 'natural flavor' according to USDA regulations and 'celery powder' is NOP approved for 'organic meat products' if 100% organic celery product with the same characteristics is not available. Celery juice is usually expressed as 'celery juice' on the label and is sold either in frozen or shelf-stable form depending on the concentration (brix) and pasteurization/packaging procedures. The main disadvantage of celery-based ingredients is that celery is considered an allergen or 'sensitizing agent,' and thus other vegetable ingredients suggested to be less allergenic than celery, such as Swiss chard, are being commercially developed. 'Sea salt' generally has not proved to be a consistent source of nitrate or nitrite and is no longer required for 'natural' labeling.

With any natural nitrate or nitrite source ingredient, it is important to understand and have specifications for 'nitrate' (NO_3^-) and/or 'nitrite' (NO_2^-) ions, which are the active components. Often expressed as 'sodium nitrate' ($NaNO_3$)

and/or 'sodium nitrite' ($NaNO_2$) as per the USDA regulations, the weight of the sodium salt is approximately 1.37x and 1.50x, respectively, of the nitrate or nitrite ions based on the atomic weight of each compound.

Natural preconverted nitrite sources

Most of the preconverted natural ingredients containing nitrite are manufactured by using similar natural nitrate sources (e.g., celery) and similar meat cultures for the nitrate conversion. In general, preconverted juices or powders contain concentrations varying from 10 to 25 000 ppm (1–2%) expressed as sodium nitrite. The final concentrations are limited by the initial nitrate concentrations present in the various vegetable materials. These products are available in both liquid and dry form and provide a designated minimum nitrite ion, either designated as the 'nitrite ion' concentration or expressed as 'sodium nitrite' salt concentration. As with any natural product, these 'preconverted' vegetable products exhibit some color and flavor attributes that make the products somewhat 'self-limiting' in usage. However, improvements in both nitrite concentration and flavor control have allowed for addition levels that can now attain the maximum regulatory limits outlined for purified nitrite.

Nitrate-Reducing Starter Cultures

Most meat cultures employed are harmless staphylococci strains that are also the most commonly used meat starter cultures worldwide. Originally, *Staphylococcus carnosus* was the most commonly used strain due to its nitrate reductase activity and previous successful use as a meat starter culture for color and flavor development. Because most existing *S. carnosus* strains were more active at relatively high temperatures (optimum at 90–100 F), the use of this starter culture in natural curing required elevated temperatures for the nitrate conversion, and thus the need for an 'incubation period.' Subsequently, other stains of staphylococci demonstrating higher nitrate reductase activity and functionality at lower temperatures were isolated and developed commercially. These 'second-generation' commercial meat cultures are a mixture of different *S. carnosus* strains (*S. carnosus* spp. *utilis*) and other staphylococci strains (i.e. *Staphylococcus vitulinus*). These culture blends are activated earlier in the process (at lower temperatures) and as a result are more efficient for nitrate conversion. The use of these blends results in overall higher nitrite generation and their use can minimize or even eliminate the need for an 'incubation period.'

Natural Curing Adjuncts

As with traditional curing, the addition of other natural compounds can enhance the natural curing process. Cherry powder and acerola juice and powder are products containing relatively high levels of natural occurring ascorbic acid, which is an oxygen scavenger (reductant) and metal ion sequestrant that serves as a curing accelerator. Citrus powders (e.g., lemon, lime) also contain naturally occurring ascorbic acid as well as citric acid, which are also effective as natural curing accelerators and antioxidant synergists.

Other Natural Ingredients

Antioxidants

A number of natural compounds with antioxidant activity exist. Rosemary and other herb extracts, as well as extracts of green tea and grape seed, serve as natural free radical scavengers inhibiting fat, protein, and meat pigment oxidation when incorporated into natural meat and poultry products.

Preservatives

Citrus juices and powders used as curing adjuncts are typically used at relatively low concentrations; however, when used at higher levels, they can serve a dual function as microbial inhibitors, particularly in combination with vinegar. Acetic acid, di-acetate, and acetates are proven antimicrobial ingredients and are available 'naturally' in the form of vinegar, specifically if the vinegar is considered 'minimally processed.' Technically, sodium lactates are not USDA approved for 'natural' labeling unless it can be shown that they are being used as 'flavoring agents' to extend shelf life and not as 'chemical preservatives' to extend shelf life. Generally, the acceptable level is less than 2%.

Binding and texturizing agents

Natural meat products commonly exhibit lower yields with looser texture and lower product pH because ingredients such as sodium phosphates are not allowed. For natural meat products some water-binding agents are approved if considered 'minimally processed.' Carageenan (seaweed) is the most commonly used, with some gums such as xanthan gum also being utilized. Raising the brine and/or product pH with added sodium bicarbonate or sodium carbonate can also achieve increased yields.

Flavorings

'Natural flavoring' has generally been defined by the USDA as the essential oil, oleoresin essence, or extractive, protein hydrolysate, distillate, or any product of roasting, heating, or enzymolysis that contains the flavoring constituents derived from natural sources such as spices, fruits, vegetables, plants, meats, and seafood. Celery, onion, and garlic powders are considered foods rather than spices and thus can be labeled as natural flavorings, while the respective juice derivatives must be labeled as 'juice,' for example, 'celery juice,' etc. (9 CFR, Part 318). Generally, if the flavoring is derived from natural sources and is considered minimally processed, it can be used in natural meat products. Often, the actual material from which the flavoring is derived must be either labeled or approved. In addition, 'spice oleoresins' are specifically mentioned as acceptable in natural meat products by the USDA.

Alternative Curing – Manufacturing Procedures

General Manufacturing Procedures

Aside from the replacement of normal curing ingredients, such as sodium nitrate and nitrite with natural curing ingredients, and the addition of an incubation step to allow for nitrate-to-nitrite reactions to occur (if a system with starter culture is

used), all other processing procedures can generally remain unchanged. Two steps in the process are, however, considered critical for the successful manufacture of alternatively cured products.

The first critical step is the level of ingoing nitrate/nitrite-containing source added. The highest amount possible should be added but care must be taken as levels that are too high can negatively affect finished product aroma or flavor. Ideally, the natural nitrate/nitrite-containing ingredient should be added at a level slightly lower than the amount that induces ingredient-related (e.g., vegetable-like) aromas and flavors in the finished product. This approach is preferred over following 'minimum recommended levels' suggested by manufacturers so that the greatest extent of curing can take place. The product itself, however, must also be taken into consideration because those products that are more heavily spiced (frankfurters, polish sausage, etc.) tend to allow for higher levels of nitrate/nitrite-containing ingredients before changes in aromas and flavors are detected.

The second critical step for successful alternative curing is incubation (if the culture system is used). The incubation step is where nitrate-to-nitrite conversion occurs and is commonly performed at the very beginning of thermal processing. However, in the case of multi-strain cultures, much of the necessary nitrate-to-nitrite conversion can potentially take place during product manufacture and before thermal processing. Thus, a thermal processing incubation step may be substantially shortened. Regardless of starter cultures used and their associated functional temperature properties, the amount of nitrite converted from nitrate during incubation is a function of both temperature and time. Incubation temperatures corresponding to the optimal activity of the specific starter culture are used in concert with an incubation time that provides enough time to allow for a high percentage of the nitrate to be converted to nitrite. As such, incubation is an extremely critical manufacturing step and proper control of this step can significantly impact the quality, shelf life quality, and safety of the finished product. A minimum of 2 h of incubation time, where product is held at optimal incubation temperature, is recommended.

Comminuted Sausage Manufacture

If ingredients that have been preconverted to nitrite are used for comminuted sausage, they may be directly added during the addition of other nonmeat ingredients. If a culture system is used, it is first advised to mix the culture in a portion of the formulation water to allow for better distribution when added to the meat mixture. In addition, because the starter cultures will go into suspension but will not go into solution when mixed with water, proper periodic agitation to maintain suspension is also important.

Whole Muscle Product Manufacture

Whole muscle product manufacture includes any product in which the incorporation of curing and/or other ingredients is accomplished by injection, tumbling, or immersion. If a preconverted system is used, ingredients may be directly added to the brine or solution with no additional processing changes needed. Thus, no changes in manufacturing practices are needed with the inclusion of preconverted ingredients. However, if a culture system is used, then special attention must be taken to ensure successful alternative curing. Because the starter culture is not water soluble, it must be injected or physically placed inside the meat so that it can come in contact and interact with the nitrate-containing ingredient. Tumbling or immersion methods would be ineffective, resulting in natural curing failure (e.g. uncured spots) because the starter culture would not be carried to the muscle interior by the tumbling or immersion curing process. As an example, a product in which tumbling is traditionally used as the means of incorporating a brine or solution must instead be injected. However, because vegetable juices/powders are water soluble, they could be carried to the muscle interior through tumbling or immersion practices.

'Alternatively Cured' Meat Product Challenges

Quality Implications

Lipid oxidation and cured meat color are the two quality-related challenges most often associated with alternative curing. Because lower levels of nitrite typically exist in alternatively cured products, the amount of unsaturated lipids in a product, the rate and extent of oxidation of those lipids at the time of processing, the length and type (frozen or refrigerated; aerobic or anaerobic packaging) of product storage, and the actual amount of nitrite generated during processing can have substantial effects on the oxidative stability of the lipids.

Cured color and the shelf life of that color are greatly impacted by good curing practices. Because little nitrite (<20 ppm) is necessary to induce a cured color and much more is needed to maintain that cured color throughout the stored shelf life ($\sim>50$–60 ppm), a false sense of product quality could result if inadequate alternative curing occurred because acceptable cured color could be present immediately after product manufacture but then rapidly fade within weeks, days, or even hours. To prevent this from occurring, maximizing the amount of nitrite generated will result in a more desirable amount of residual nitrite that can later be used for cured color regeneration during product shelf life.

The microbiological quality properties of alternative and organic meat products are also affected by alternative curing. As the level of nitrite and use of ingredients with antimicrobial properties decreases, nonpathogenic spoilage bacteria have greater ability to grow and can reduce product shelf life. Anaerobic packaging and temperature control can be used to help address microbiological quality changes and limit product quality deterioration.

Safety Implications

The safety of alternatively cured processed meats is a significant concern because nitrite is a very well-known and effective antimicrobial agent, particularly for preventing toxin production by *C. botulinum*. The issue for processed meats that use natural sources of nitrate in a culture system is that the

true amount of nitrite formed is unknown and impossible to measure because nitrite reacts quickly with meat components. Although the amount of detectable residual nitrite in these products is significant, it is typically less than that found in traditional, nitrite-cured products. Consequently, the microbial safety of processed meats manufactured with natural sources of nitrate is very difficult to assess without microbiological challenge studies. However, several studies have shown that the inclusion of ingredients possessing antimicrobial properties can significantly enhance the safety of the products. The preconverted system is not presented these same challenges, however care in ensuring adequate levels of nitrite are used to attain equivalent quality and safety results as with purified nitrite is important.

See also: Additives: Extenders; Functional. Curing: Brine Curing of Meat; Production Procedures. Microbiological Safety of Meat: *Clostridium botulinum* and Botulism; *Clostridium perfringens*; *Salmonella* spp.

Further Reading

Sebranek, J.G., Bacus, J.N., 2007. Cured meat products without direct addition of nitrate or nitrite: What are the issues? Meat Science 77, 136–147.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Love, J.A., Ahn, D.U., 2007a. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified, cooked sausages. Journal of Food Science 72, S324–S332.

Sindelar, J.J., Cordray, J.C., Sebranek, J.G., Love, J.A., Ahn, D.U., 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH and sensory attributes of ready-to-eat uncured ham. Journal of Food Science 72, S388–S395.

Terns, M.J., Milkowski, A.L., Claus, J.R., Sindelar, J.J., 2011. Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages. Meat Science 88, 454–461.

Terns, M.J., Milkowski, A.L., Rankin, S.A., Sindelar, J.J., 2011. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. Meat Science 88, 311–318.

USDA, 2005. Food Standards and Labeling Policy Book. United States Department of Agriculture-Food Safety Inspection Service. Available at: www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf (accessed 21.12.09).

Winter, C.K., Davis, S.F., 2006. Organic foods. Journal of Food Science 71, R117–R124.

Xi, Y., Sullivan, G.A., Jackson, A.L., Zhou, G.H., Sebranek, J.G., 2012. Effects of natural antimicrobials on inhibition of Listeria monocytogenes and on chemical, physical and sensory attributes of naturally-cured frankfurters. Meat Science 90, 130–138.

Relevant Websites

http://amif.org/ht/a/GetDocumentAction/i/62610
American Meat Institute Foundation.

http://www.meatscience.org/page.aspx?id=403
American Meat Science Association.

Physiology of Nitric Oxide

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Introduction

Salting as a means of preserving meat, poultry, fish, seafood, and vegetables predates written history and was essential in ancient times for providing nutrient-dense foods during scarcity or population migration and before refrigeration was an option. Meat curing is historically defined as the addition of salt to fresh meat cuts to remove moisture and reduce the water activity of the tissues to prevent spoilage. However, salt is poorly defined as there are many salts that can fulfill this activity although some are better than others. In ancient times, salt was obtained from crystalline deposits left by evaporating water from brine pools, seawater, or mining directly from the earth. As a consequence, it often contained natural contaminants such as sodium or potassium nitrate or nitrite that contributed directly to the curing reaction and the preservation process, although unrecognized at the time. These contaminants, nitrite and nitrate, as it was later learned, were the primary components in curing reactions. The reduction of nitrate (NO_3^-) salts to nitrite (NO_2^-) and then to gaseous NO and its subsequent reaction with myoglobin to form the nitrosyl–myoglobin complex forms the basis for cured meat flavor and color. It was also later realized that it is bacteria that first converts nitrate into nitrite, which is the mechanism underlying in the preservation of food. Nitrite in meat is responsible for inhibiting the growth of aerobic and anaerobic bacteria (especially the spores from *Clostridium botulinum*), retarding the development of rancidity during storage, developing and preserving the meat flavor and color, stabilizing the oxidative state of lipids in meat products.

At present most cured meats contain added sodium nitrite or cultured celery extract where the naturally contained nitrate is reduced to nitrite by a starter culture of bacteria. For many years, some epidemiological data have implicated that nitrite and nitrate in cured and processed meats are responsible for a number of human diseases including some cancers. Although modestly increased associations between consumption of foods containing nitrite and nitrate and certain cancers have been reported in some prospective epidemiological studies overall, findings across studies have been largely inconsistent and equivocal. Consequently, the overall burden of proof remains inconclusive. A biologically plausible mechanism for the carcinogenicity of ingested nitrate and nitrite involves endogenous *N*-nitrosation reactions. Although generally considered harmful due to the formation of *N*-nitrosamines, biomedical science over the past 20 years has recognized the nitrosation reaction as an essential fundamental process in mediated cell signaling. Despite this new emerging science on NO, nitrite, and nitrate, there are still very strict regulations of nitrate and nitrite levels in our food and drinking water.

In the early 1980s it was shown that, in addition to dietary exposure, nitrate and nitrite are also generated endogenously. Shortly thereafter, the entire L-arginine–nitric oxide synthase (NOS)-system was discovered and was found to be the major endogenous source of nitrate and nitrite, because NO is rapidly oxidized to these higher nitrogen oxides. Until recently, biologists considered nitrate and nitrite merely as inactive end-products of NO metabolism, but this view is rapidly changing. It is now clear that nitrite and nitrate can recycle *in vivo* and again form bioactive nitrogen oxides, including NO. Interestingly, commensal bacteria in the human oral cavity play a key role in the bioactivation of nitrate. A picture is emerging suggesting physiological, nutritional, as well as therapeutic roles for the nitrate–nitrite–NO pathway. Thus, instead of simply wasting the products of NO oxidation, mammals store and actively recycle it. Nitrite reduction to NO was first described in the stomach, where salivary nitrite forms NO non-enzymatically via acid-catalyzed reduction. Soon after this observation, researchers described NOS-independent nitrite reduction in the ischemic and acidic heart. In the past 10 years it has become evident that blood and tissue nitrite are reduced under physiological conditions to form NO and modulate blood flow. Subsequent studies show that a variety of enzymes and proteins can catalyze the one-electron reduction of nitrite to NO in blood and tissues. The authors will review the nitrate–nitrite–nitric oxide pathway in human physiology and highlight this fundamental pathway, which has been recently shown to afford enormous health benefits to humans. The picture that has emerged is that foods that contain nitrite and nitrate confer profound health benefits representing a complete change in paradigm requiring reconsideration of the risk-benefit analysis on nitrite and nitrate.

Nitric Oxide Biochemistry and Physiology

Before the nitrate–nitrite–NO pathway is reviewed, it is first necessary to describe the fundamental roles and production pathways for NO and its implications in health and disease to better appreciate the new-found role of nitrite and nitrate. The discovery of the mammalian biosynthesis of NO and its roles in the immune, cardiovascular, and nervous systems in the 1980s established a startling new paradigm in the history of cellular signaling mechanisms. Before that discovery, it was essentially inconceivable that cells would intentionally produce a toxic molecule as a messenger; NO was previously known as a common air pollutant, a constituent of cigarette smoke, and a toxic gas, which appears in the exhaust of automobiles and jet airplanes, causes acid rain, and destroys the ozone layer. Amazingly, despite this nasty reputation, it is now known that NO is one of a family of reactive signaling molecules, which includes both reactive nitrogen and reactive oxygen species that perform essential cellular functions in the body. This is, in fact, a hallmark example of the propensity of nature to seek out and exquisitely utilize the unique properties of unusual molecules. This same theme is emerging around

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nitrite. Once considered an unwanted, toxic food additive and now considered an essential nutrient, much of the chemistry that was described centuries ago is now reemerging in human physiology. NO is one of the most important signaling molecules in the body, and is involved in virtually every organ system where it is responsible for modulating an astonishing variety of effects. NO has been shown to be involved in and affect (just to list a few major examples) neurotransmission, memory, stroke, glaucoma, and neural degeneration such as in Alzheimer's disease, pulmonary hypertension, penile erection, angiogenesis, wound healing, atherogenesis, inflammation such as arthritis, nephritis, colitis, autoimmune diseases (diabetes, inflammatory bowel disease), invading pathogens, tumors, asthma, tissue transplantation, septic shock, platelet aggregation and blood coagulation, sickle cell disease, gastrointestinal motility, hormone secretion, gene regulation, hemoglobin delivery of oxygen, stem cell proliferation and differentiation, and bronchodilation. One can then imagine the host of diseases or conditions that may be caused or affected by the body's dysregulation of NO production/signaling. Maintaining NO homeostasis is critical for optimal health and disease prevention, and understanding foods and diets that promote NO activity will have a profound effect on public health.

The discovery of the NO pathway represented a critical advance in the understanding of cell signaling and subsequently into major new advancements in many clinical areas including, but not limited to, cardiovascular medicine. This seminal finding was viewed as so fundamentally important that the Nobel Prize in Physiology or Medicine was awarded to its discoverers, Drs. Louis J. Ignarro, Robert Furchgott, and Ferid Murad in 1998, a short 11 years after NO was identified. Dr. Valentin Fuster, then president of the American Heart Association, noted in a 1998 interview that "the discovery of NO and its function is one of the most important in the history of cardiovascular medicine." In fact, development of NO-based drugs and therapies is a major priority for big pharmaceutical companies. Drugs like Viagra and Cialis for erectile dysfunction are effective because they affect the NO pathway, which allows for blood vessel relaxation and blood flow into the corpus cavernosum for penile erection. Enhancing this effect through dietary means may provide a safer and more natural alternative.

What is clear is that continuous generation of NO is essential for the integrity of the cardiovascular system, and decreased production and/or bioavailability of NO is central to the development of many disorders. The production of NO from L-arginine is a complex and complicated biochemical process involving a 5-electron oxidation with many cofactors and prosthetic groups carried out by a group of enzymes called nitric oxide synthase (NOS). There are three isoforms of NOS, neuronal NOS (nNOS or NOS 1), inducible NOS (iNOS or NOS 2), and endothelial NOS (eNOS or NOS 3). It is the NO produced by iNOS that is responsible for killing of bacteria. There are many steps or factors that may be altered and may affect ultimate NO production. Once produced, NO can be quickly scavenged before it has a chance to perform its actions. It is, therefore, a war of attrition when it comes to producing bioactive NO, and is a remarkable feat that this short-lived gas is responsible for so many essential cellular activities. Understanding strategies to restore NO homeostasis will represent a major breakthrough in disease management and prevention.

Nitrite and Nitrate in Human Physiology

Although the L-arginine–NO pathway was the first to be discovered, it does not necessarily mean that it is the primary pathway for the endogenous production of NO. In fact, nitrogen cycling in bacteria and production of NO as an intermediate in denitrification may be one of the most primitive pathways known, dating back to the Archean era. The now recognized human nitrate–nitrite–nitric oxide pathway that still relies on bacteria may be a redundant system for overcoming the body's inability to make NO from L-arginine. It appears that we have at least two systems for affecting NO production/homeostasis. The first is through the classical L-arginine–NO pathway. This is a complex and complicated five-electron oxidation of L-arginine and if any of the cofactors become limiting, then NO production from NOS shuts down, and in many cases, NOS then produces superoxide instead. The enzymatic production of NO normally proceeds very efficiently. However, in disease characterized by oxidative stress where essential NOS cofactors become oxidized, NOS uncoupling, or conditions of hypoxia where oxygen is limiting, this process can no longer maintain NO production. Therefore, one can argue saliently that there has to be an alternate route for NO production. It is highly unlikely that nature devised such a sophisticated mechanism of NO production as a sole source of a critical molecule.

This alternate route involves the provision of nitrate and nitrite reductively recycled to NO. Inorganic nitrite and nitrate are still considered at present in the media and public predominantly as undesired residues in the food chain or as inert oxidative end-products of endogenous NO metabolism. However, from research performed over the past decade, it is now apparent that nitrate and nitrite are physiologically recycled in blood and tissues to form NO and other bioactive nitrogen oxides. Nitrite is an oxidative breakdown product of NO that has been shown to serve as an acute marker of NO flux/formation. Nitrite is in steady state equilibrium with S-nitrosothiols and has been shown to activate soluble guanylyl cyclase (sGC) and increase the second messenger cGMP levels in tissues, activities very similar to NO.

Sources and Estimates of Exposure to Nitrite and Nitrate

The health concerns for exposure to nitrite and nitrate have been focused on the levels of nitrite and nitrate in the diet and primarily their content in cured meats. As with any compound, dose dictates poison and at high concentrations, pure nitrite can be toxic. The reported LD50 on a material safety data sheet (MSDS) is 175–180 mg kg^{-1} in rodents. The lowest acute oral lethal dose of nitrite has been reported to vary from 33–250 mg kg^{-1} body weight, which might be applied to children or the elderly. Estimates of the lethal dose of potassium nitrate (KNO_3^-) have ranged from 4 to 30 g. A realistic estimate of a

lethal dose in adults is 20 g nitrate ion or 330 mg nitrate ion per kilogram body weight. The National Academy of Sciences in 1981 concluded that 39%, 34%, and 16% of the dietary intake of nitrite were derived from cured meat, baked goods/cereal, and vegetables, respectively. More recent reports have shown that less than 5% of the total ingested nitrite and nitrate are derived from cured meat sources with the remainder coming from vegetables and saliva. In an assessment of nitrate, nitrite, and *N*-nitroso compounds in the human diet, it was concluded that vegetables contribute more than 85% of the daily dietary intake of nitrate and that endogenous synthesis is an important contributor to humans' overall exposure to nitrate. The National Research Council has estimated, based on food consumption tables, that the average total nitrite and nitrate intake in the USA was 0.77 and 76 mg, respectively. However, more sensitive and accurate analytical methods and changes in agricultural practices over the past few decades may have changed our consumption values. International estimates of nitrate intakes from food are 31–185 mg per day in Europe and in the USA approximately 40–100 mg per day. A healthy 70 kg person produces 1.68 mmol NO per day (based on 1 μmol (kg h)$^{-1}$ NO production). Using the conservative estimates of an average daily intake of 0.77 mg of nitrite would equate to 11.1 μmol per day and 76 mg nitrate would equate to 894 μmol per day or approximately 1 mmol of nitrite and nitrate per day from diet. This almost matches what our body makes from NO if we assume most of the NO goes to stepwise oxidation to nitrite and nitrate. Therefore our steady state levels of NOx, which are routinely used as clinical biomarkers of NO activity come almost 50% from diet. In fact, it has been suggested that people consuming certain diets, such as the dietary approaches to stop hypertension (DASH) diet get more than 1000 mg of nitrate. Nitrate is also used on toothpaste formulations in the form of potassium nitrate, designed for sensitive teeth (approximately 5000 ppm) representing a major source of exposure.

Human Nitrogen Cycle: Reductive Pathways to Produce NO from Nitrite and Nitrate

The bioactivation of nitrate from dietary or endogenous sources requires its initial reduction to nitrite, and because mammals lack specific and effective nitrate reductase enzymes, this conversion is mainly carried out by commensal bacteria in the mouth and gastrointestinal tract and on body surfaces. Nitrate from the diet is rapidly absorbed in the upper gastrointestinal tract. In the blood, it mixes with the nitrate formed from the oxidation of endogenous NO produced from the NOS enzymes. After a meal rich in nitrate, the levels in plasma increase greatly and remain high for a prolonged period of time (plasma half-life of nitrate is 5–6 h). The nitrite levels in plasma also increase after nitrate ingestion. Although much of the nitrate is eventually excreted in the urine, up to 25% is actively taken up by the salivary glands and is concentrated up to 20-fold in saliva. Once in the mouth, commensal facultative anaerobic bacteria reduce nitrate to nitrite during respiration by the action of nitrate reductases. Human nitrate reduction requires the presence of these bacteria – suggesting a functional symbiosis relationship – as mammalian cells cannot effectively metabolize this anion. The salivary nitrate levels can approach 10 mM and nitrite levels 1–2 mM after a dietary nitrate load. When saliva enters the acidic stomach (1–1.5 L per day), much of the nitrite is rapidly protonated to form nitrous acid (HNO_2; pK_a ~3.3), which decomposes further to form NO and other nitrogen oxides. Nitrite excreted in saliva has significant antibacterial properties. Nitrite also contributes to the bactericidal effects of gastric fluids as demonstrated by some studies on the food-borne pathogens like *Escherichia coli*. Nitrite and nitric oxide are also shown to have antibacterial benefits against *Helicobacter pylori*, certain strains of bacteria responsible for dental caries and skin pathogens. This human nitrogen cycle is illustrated in Figure 1.

Once nitrite is absorbed and circulated, it is taken up by peripheral tissues and can be stored in cells. The one-electron nitrite reduction to NO can occur in a much simpler mechanism than the two-electron reduction of nitrate by bacteria. The one-electron reduction of nitrite can occur by ferrous heme proteins (or any redox active metal) through the following reaction:

$$NO_2^- + Fe(II) + H^+ \leftrightarrow NO + Fe(III) + OH^-$$

This is the same biologically active NO as that produced by NOS, with nitrite rather than L-arginine as the precursor, and is a relatively inefficient process. Much of the recent focus on nitrite physiology is due to its ability to be reduced to NO during ischemic or hypoxic events. Nitrite reductase activity in mammalian tissues has been linked to the mitochondrial electron transport system, protonation, deoxyhemoglobin, and xanthine oxidase. Nitrite can also transiently form nitrosothiols (RSNOs) under both normoxic and hypoxic conditions, and a recent research demonstrated that steady state concentrations of tissue nitrite and nitroso are affected by changes in dietary NOx (nitrite and nitrate) intake. Furthermore, enriching dietary intake of nitrite and nitrate translates into significantly less injury from heart attack. Previous studies also demonstrated that nitrite therapy given intravenously before reperfusion (restoration of blood flow) protects against hepatic and myocardial ischemia/reperfusion (I/R) injury. Additionally, experiments in primates revealed a beneficial effect of long-term application of nitrite on cerebral vasospasm. Moreover, inhalation of nitrite selectively dilates the pulmonary circulation under hypoxic conditions *in vivo* in sheep. Topical application of nitrite improves skin infections and ulcerations. Furthermore, in the stomach, nitrite-derived NO seems to play an important role in host defense and in regulation of gastric mucosal integrity. All these studies together along with the observation that nitrite can act as a marker of NOS activity (reflective of total body NO availability) opened a new avenue for the diagnostic and therapeutic application of nitrite, especially in cardiovascular diseases, using nitrite as a marker as well as an active agent.

Oral nitrite has also been shown to reverse NG-nitro-L-arginine methyl ester (L-NAME – a NOS inhibitor) induced hypertension and serve as an alternate source of NO *in vivo*. In fact, a recent research report demonstrated that plasma nitrite levels progressively decrease with increasing cardiovascular risk. Because a substantial portion of steady state nitrite concentrations in blood and tissue are derived from dietary

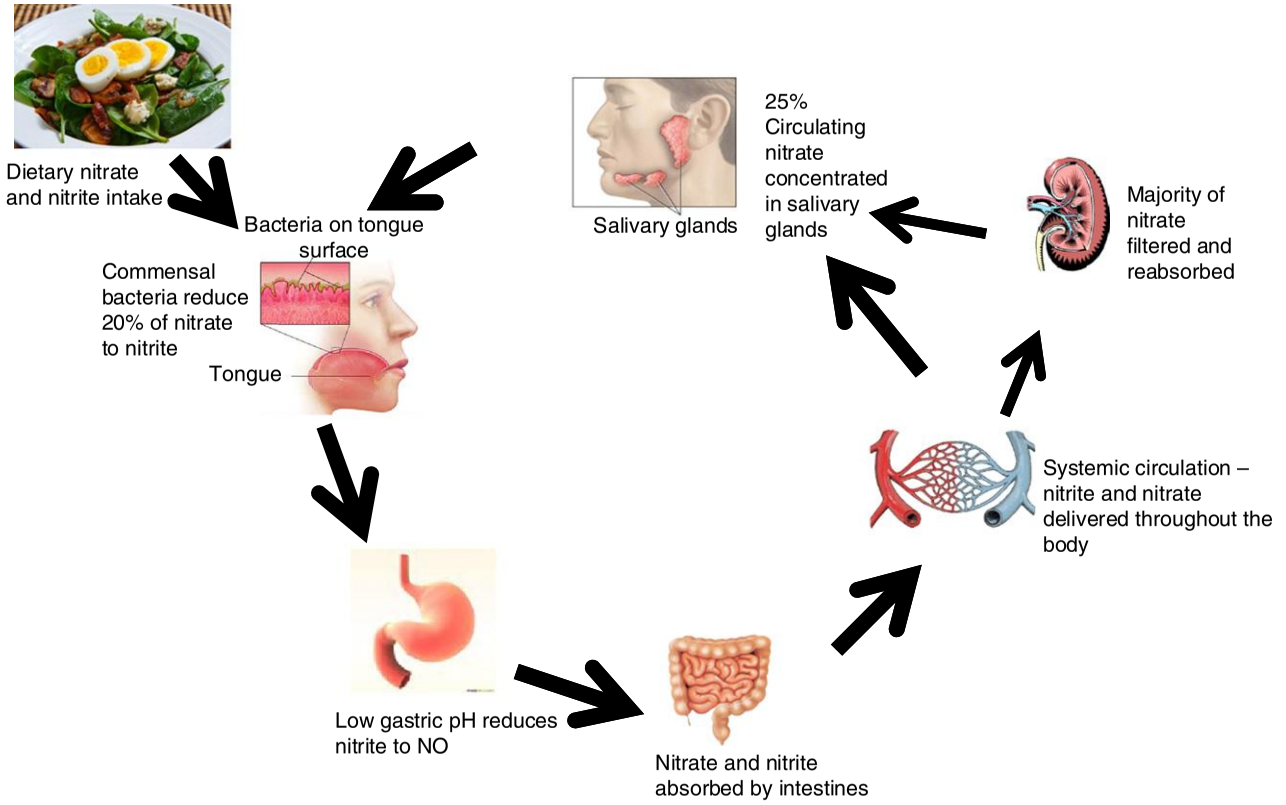

Figure 1 Human nitrogen cycle.

sources, modulation of nitrite and/or nitrate intake may provide a first line of defense for conditions associated with NO insufficiency. In fact it has been reported that dietary nitrate reduces blood pressure in healthy volunteers.

Conclusion

The emerging health benefits of nitrite and nitrate represent a profound change in paradigm from the past 50 years. Until now, scientists have operated under the paradigm of the L-arginine–NO pathway by NOS enzymes as the only pathway to produce NO. As shown in Figure 2, there are a number of recycling pathways to regenerate NO from dietary nitrite and nitrate. The emergence of a redundant pathway for maintenance of NO homeostasis by dietary nitrite and nitrate provides a new mode of intervention and a new paradigm for restoring NO homeostasis. The provision of nitrate and nitrite as sources of NO may then be viewed as a system of redundancy. Therefore, industry efforts to reduce nitrite and nitrate in the curing process may not be meaningful. In fact, nitrite and nitrate therapy or supplementation may restore NO homeostasis from endothelial dysfunction and provide benefit in a number of diseases characterized by NO insufficiency. If so, this will provide the basis for new preventive or therapeutic strategies and new dietary guidelines for optimal health. There are currently a number of clinical trials using sodium nitrite as a therapeutic agent (www.clinicaltrials.gov). From a public health perspective, better recommendations can be made on diet and dramatically affect the incidence and severity of cardiovascular disease and the subsequent clinical events. Replenishing nitrate and nitrite through dietary means may then act as a protective measure to compensate for insufficient NOS activity under conditions of hypoxia or in a number of conditions characterized by NO insufficiency. In fact, use of a rationally designed nitrite- and nitrate-enriched dietary supplement has been shown in a clinical trial to restore NO homeostasis and modify cardiovascular risk factors such as hyperlipidemia.

One cannot help but notice the emerging physiological data on nitrite are strikingly analogous to a vitamin. Vitamine or vital amine was the term coined by Casimir Funk (1884–1967) for the unidentified substances present in food, which could prevent the diseases scurvy, beriberi, and pellagra. A vitamin is by definition any of a group of organic substances essential in small quantities to normal metabolism, found in minute amounts in natural foods or sometimes produced synthetically: deficiencies of vitamins produce specific disorders. We may have identified a new vitamin, perhaps vitamin N. We know that nitrite is produced in relatively small quantities in normal metabolism of L-arginine and reduction of nitrate and is found in minute amounts in natural foods. Many animal studies reveal that nitrite insufficiency exacerbates I/R injury and increases mortality from I/R. There are a host of diseases that are associated with decreased NO availability as measured by nitrite. Becoming more evident is the

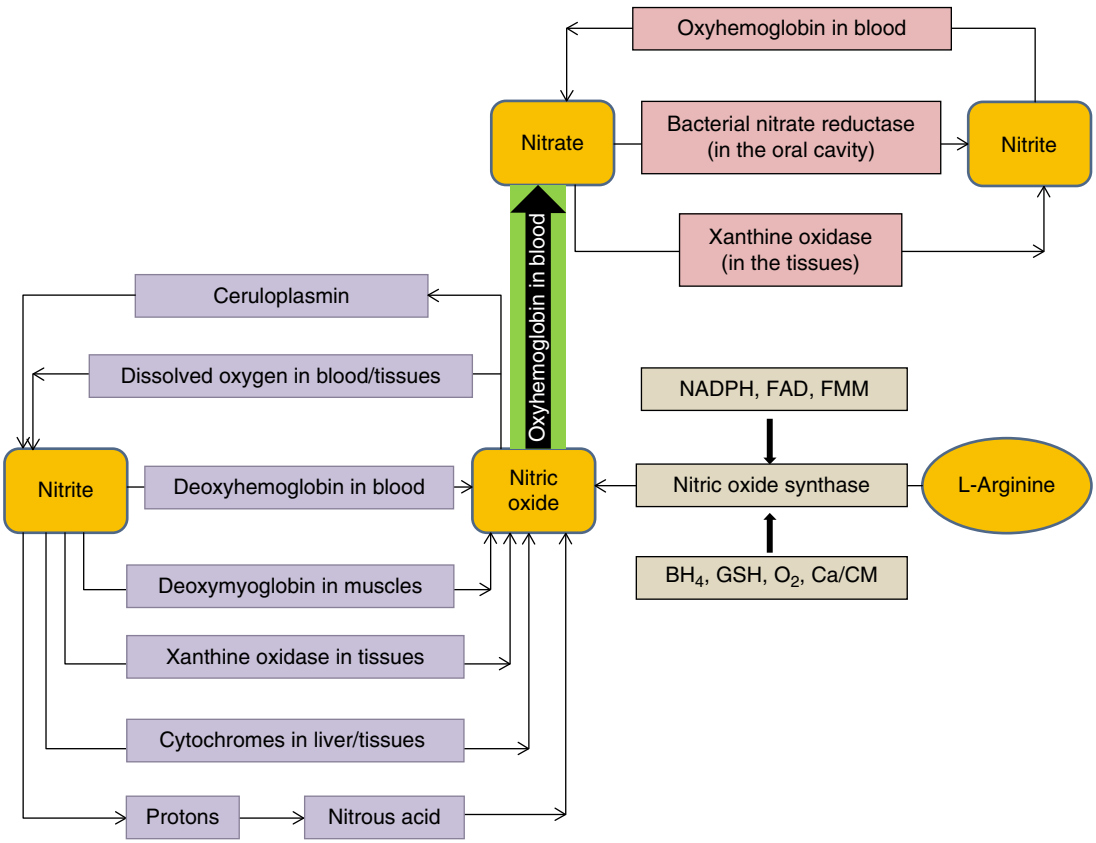

Figure 2 Different pathways of nitrite, nitrate, and NO in human body.

enormous benefit of exogenous dietary nitrite and nitrate in a number of disease models in animals and even in humans. Very little can affect our health more than what we choose to eat and our daily lifestyle habits. The realization of a nitrate–nitrite–nitric oxide pathway suggests that NO can be modulated by the diet independent of its enzymatic synthesis from L-arginine, for example, the consumption of nitrite- and nitrate-rich foods, such as leafy vegetables or meats to which nitrite is added. Diet and nutrition may be the key to NO-related therapies. After all it was Hippocrates who said, "Let food be thy medicine and medicine be thy food." The active agent of some medicinal foods may very well be nitrite.

See also: Additives: Functional. Chemical Analysis for Specific Components: Curing Agents. Chemical and Physical Characteristics of Meat: Color and Pigment. Curing: Brine Curing of Meat; Natural and Organic Cured Meat Products in the United States

Further Reading

Bryan, N.S., 2006. Nitrite in NO biology: cause or consequence? A systems-based review. Free Radical Biology & Medicine 41 (5), 691–701.

Bryan, N.S., 2009a. Cardioprotective actions of nitrite therapy and dietary considerations. Frontiers in Bioscience 14, 4793–4808.

Bryan, N.S. (Ed.), 2009b. Food, Nutrition and the Nitric Oxide Pathway. Pennsylvania: DesTech Publishing. ISBN: 978-1-932078-84-8.

Bryan, N.S., Calvert, J.W., Elrod, J.W., *et al*., 2007. Dietary nitrite supplementation protects against myocardial ischemia–reperfusion injury. Proceedings of the National Academy of Sciences of the USA 104 (48), 19144–19149.

Bryan, N.S., Fernandez, B.O., Garcia-Saura, M.F., *et al*., 2005. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nature Chemical Biology 1, 290–297.

Bryan, N.S., Loscalzo, J. (Eds.), 2011. Nitrite and Nitrate in Human Health and Disease. New York: Springer Humana Press. ISBN: 978-1-60761-615-3.

Bryan, N.S., Zand, J., Gottlieb, B., 2010. The Nitric Oxide (NO) Solution. Good for you Books Publishing. ISBN: 978-0-615–41713-4.

Butler, A.R., Feelisch, M., 2008. Therapeutic uses of inorganic nitrite and nitrate: from the past to the future. Circulation 117 (16), 2151–2159.

Elrod, J.W., Calvert, J.W., Gundewar, S., Bryan, N.S., Lefer, D.J., 2008. Nitric oxide promotes distant organ protection: evidence for an endocrine role of nitric oxide. Proceedings of the National Academy of Sciences of the United States of America 105 (32), 11430–11435.

Hord, N.G., Tang, Y., Bryan, N.S., 2009. Food sources of nitrates and nitrites: The physiological context for potential health benefits. American Journal of Clinical Nutrition 90 (1), 1–10.

Kevil, C.G., Kolluru, G.K., Pattillo, C.B., Giordano, T., 2011. Inorganic nitrite therapy: historical perspective and future directions. Free Radical Biology & Medicine 51 (3), 576–593.

Lundberg, J.O., Gladwin, M.T., Ahluwalia, A., *et al*., 2009. Nitrate and nitrite in biology, nutrition and therapeutics. Nature Chemical Biology 5 (12), 865–869.

Lundberg, J.O., Weitzberg, E., Gladwin, M.T., 2008. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. Nature Reviews Drug Discovery 7 (2), 156–167.

Milkowski, A., Garg, H.K., Coughlin, J.R., Bryan, N.S., 2010. Nutritional epidemiology in the context of nitric oxide biology: A risk-benefit evaluation for dietary nitrite and nitrate. Nitric Oxide 22 (2), 110–119.

Zand, J., Lanza, F., Garg, H.K., Bryan, N.S., 2011. All-natural nitrite and nitrate containing dietary supplement promotes nitric oxide production and reduces triglycerides in humans. Nutrition Research 31 (4), 262–269.

Relevant Website

http://www.uthouston.edu/imm/centers/texas-therapeutics-institute.htm
University of Texas.

Production Procedures

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Glossary

Brine A solution of salt (usually sodium chloride) in water. For meat curing, cure ingredients (sodium nitrite) are added to the salt solution to create a brine to affect a cure in meat.

Corned (beef) Granulated or grain salt was formerly called 'corn,' from old Old Norse, 'korn,' meaning grain.

Cure To preserve meat, as by salting with nitrates and/or nitrites, smoking, or aging.

Dry curing Salt containing nitrates and nitrites are rubbed by hand onto the surface of a meat cut or ham. The pork pieces are packed and left for a few weeks. The product is often overhauled during this period.

***N*-nitrosamines** Potential reaction products generated from nitrites and secondary amines of meat, most of which are carcinogenic. High temperatures, as in the frying of bacon, can enhance the formation of *N*-nitrosamines.

Prague powder 1 (Cure 1) A mixture of 1 oz of sodium nitrite (6.25%) to 1 lb of salt. Both Cure 1 and 2 contain a small amount of FDA-approved red coloring agent, which gives them a slight pink color thus eliminating any possible confusion with common salt; this is why they are sometimes called 'pink' curing salts.

Prague powder 2 (Cure 2) A mixture of 1 oz of sodium nitrite (6.25%) along with 0.64 oz of sodium nitrate (4%) to 1 lb of salt. Both Cure 1 and 2 contain a small amount of FDA-approved red coloring agent, which gives them a slight pink color thus eliminating any possible confusion with common salt; this is why they are sometimes called 'pink' curing salts.

Salometer A specially graduated hydrometer that measures the strength of brines. A 100° salometer reading is equivalent to a 100% saturated salt solution (26.5% salt).

Silent cutter Equipment for mixing and chopping meat and other products through the use of rotating knives.

Wet curing Sometimes called brine (salt and water), sweet pickle (sugar added), or immersion curing, this process has been traditionally used for larger cuts of meat like butts and hams. It is accomplished by placing the meat in a wet curing solution (water, salt, nitrites, and sometimes sugar). Sugar is added only when curing at refrigerator temperatures; otherwise, it may begin fermentation and start to spoil the meat.

Introduction

The origin of salting meats is lost in antiquity, but it is believed that the ancient Sumerian civilization, which flourished in the southern part of Mesopotamia during the fourth and third millenniums BC, was first to practice this process. From a historical perspective, meat curing can be defined as the addition of salt to meats for the sole purpose of preservation, that is, to inhibit or deter microbial spoilage. The preservation of meat resulted from necessity, so that products could be held for extended periods for later consumption. At some point, it was discovered that 'certain' salts (i.e., those containing saltpeter) could impart a unique color and flavor to meats. Granulated or grain salt was formerly called 'corn,' which comes from the Old Norse, 'korn,' meaning grain; thus, when beef was sprinkled with these salts, corned beef was the resultant product.

Scientific principles of meat curing were not applied until the early part of the twentieth century when the growing meat packing industry began to search for ways to improve quality and to extend the shelf life of products. It was discovered that nitrite, not nitrate as originally thought, plays a multifunctional role in the meat matrix: Nitrite is responsible for developing or 'fixing' the characteristic color associated with cured meats; for creating a special flavor so that one can distinguish the flavor of corned beef from that of roast beef; for imparting antioxidant activity to the cooked product, thereby extending its shelf life; and for suppressing the outgrowth and production of toxin from the anaerobic bacterium, *Clostridium botulinum*. Including nitrite at a minimum of 120 ppm also slows the growth of other pathogens such as *Clostridium perfringens* and *Listeria monocytogenes* as well as spoilage bacteria.

Meat color is an essential quality attribute of processed meat products and thus a key factor when consumers make their selections. The pigment responsible for the characteristic color of cooked cured meat products is nitrosyl myochrome/myochromogen (sometimes also referred to in the literature as nitrosyl hemochrome/hemochromogen). It is formed between the reaction of nitrite and the endogenous Fe–protoporphyrin (i.e., heme) meat pigments on thermal processing. In dry-cured ham such as Parma ham, salt is added but not nitrate/nitrite, and there is careful control of temperature and humidity during processing. During the long ripening period, a Zn–protoporphyrin with a stable bright red color is formed; this pigment has been isolated as the main component of red pigments found in these dry-cured hams.

The industry has evolved to the point that quite a diverse list of cured meat products offering great taste, convenience, and versatility is available to the consumer. On account of

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household refrigeration, the original need to cure meats no longer exists; nevertheless, consumers have become accustomed to certain products in their diet and the increase in variety of products that curing offers. For these reasons, as well as others, the public still demands the availability of cured meat products in the market.

Regulations

Before outlining some curing production procedures, it is important to note that each country has its own set of regulations as to what additives are permitted in a particular meat product, what additives are prohibited, to what maximum level an additive may be used based on the weight of the meat block or the entire formulation, how the finished product must be labeled, etc. It is not within the scope of this article to detail all of the guidelines and exceptions to meat regulations for each country. Therefore, any quantity of an additive specified in this treatise is used solely for the purpose of illustration. For example, sodium nitrite is employed at the level of 156 ppm (0.4 oz 100 lb^{-1} meat) in comminuted products in the US, but this level is based on the weight of the meat block and not that of the finished emulsion or pumped product. In most European countries, ingoing nitrite levels are 100 ppm based on the meat. Canadian regulations, in contrast, permit the use of nitrite in not only the meat but also the complete emulsion or pumped product at the level of 200 ppm. Bacon is an exception to the rule, as ingoing nitrite levels are reduced in most countries: The regulated level in Canada and the US is 120 ppm or 0.012% based on the weight of the pumped product. Knowledge of the regulations is critical for domestic production. For instance, the USDA considers anything with less than 120 ppm uncured and must be labeled as such. Furthermore, processors who want to export their wares to foreign markets must adhere to each country's meat regulations/standards of identity.

Basic Ingredients Needed for Curing

Meat

The meat used in formulations can vary markedly, so careful selection of species and muscle type is required to produce a consistent product. The concentration of myoglobin present in the meats selected will ultimately determine the color characteristics of the cooked cured product. The quantity of meat used in a formulation is called the meat block. Seasonings and other nonmeat ingredients are added based on the weight of the meat block. Typically, a meat processor will calculate the formulation of products based on 100 lb or kilogram batches, but, of course, the actual weight of the batches produced will depend on the equipment available to the processor.

Water or Ice

Water serves as the carrier for curing ingredients in most commercial ham and bacon processing operations. It replaces moisture lost during thermal processing and smoking. In some cases, additional water is pumped into hams, so that the weight of the finished product exceeds its fresh, uncured weight (i.e., the green weight). In the US, labeling of extended products that include added water and other ingredients is based on the minimum meat Protein Fat Free (PFF) percentage. For example, hams with a minimum PPF of 20.5% are labeled as ham. Hams with a minimum PPF of 18.5% are labeled as ham in natural juices. The quality of the water or ice added to meat products directly, or in the form of the brine, can have a profound effect on the product. Low-quality water, as a result of contaminates, pH fluctuations, different sources or seasonal variation, can cause a reduction in the shelf life and create aftertastes and off-colors in products.

Salt

Salt or sodium chloride is the most basic ingredient for curing and is used in every formulation. Although it still affords some preservative effects, salt is primarily added to flavor meat and to solubilize myofibrillar proteins for the product's yield and texture. The amount of salt used in dry cures and brines can vary considerably but to some extent is self-limiting. Too little salt can cause excessive product shrinkage (i.e., not sufficient water-holding capacity), poor binding or emulsion stability (i.e., not sufficient protein extraction), and a reduced shelf life, whereas too much salt can impart an undesirable taste. Only food-grade salt of high purity (i.e., free of nitrite, nitrate, and heavy metal ions) is used in meat processing, as impure salts can impart flavor and color defects to products. One type of salt that has been getting more and more attention is sea salt. Sea salts are as varied as the water from which they are made. This is a favorite additive in natural and organic products. These salts, however, can cause problems because of contaminates including nitrites, nitrates, and heavy metals. The level of salt ranges typically from 1.0% to 2.8% in finished cured meat products.

Cure

After salt, sodium nitrite is the most important ingredient in the cure. It provides the characteristic color and flavor and works with salt to offer bacteriostatic protection against anaerobic bacteria such as *C. botulinum* and *C. perfringens* in processed meat products. It is generally added to pickles or meat formulations in the form of a 'curing salt.' Because it is difficult to accurately weigh out the small quantities of nitrite needed for a formulation, nitrite is preblended with sodium chloride to give a commercial curing salt. Prague powder and Cure 1 are examples of blends for such a curing salt, which is routinely utilized in North America.

Prague powder 1 is a mixture of 1 part sodium nitrite and 15 parts sodium chloride (i.e., 1 oz $NaNO_2$ in 1 lb of salt, or 6.25% $NaNO_2$). The chemicals are combined and crystallized to assure even distribution. An anticaking agent such as glycerin or 1% (w/w) sodium carbonate is commonly incorporated, and in some preparations a red food-grade dye, FD&C Red Dye 3, is added so that one can distinguish this 'curing salt' from regular salt in the processing plant. Depending on the ingredient supplier, the curing salt goes by different names, including sure cure, insta-cure, speed cure, modern cure, and pink curing salt. Prague powder 2 is a mixture of 1 part sodium

nitrite, 0.64 parts sodium nitrate, and 14.36 parts sodium chloride (i.e., 1 oz $NaNO_2$ and 0.64 oz $NaNO_3$ in 1 lb of salt, or 6.25% $NaNO_2$ and 4% $NaNO_3$). It is 'primarily' utilized in dry curing of meat products that do not require cooking, smoking, and refrigeration; however, Prague powder 2 can also be used in slow cured products that are cooked. In Europe, it is often referred to as bacon-curing salt.

When purchasing cure units for specific products (e.g., pepperoni preparation), the curing salt is packaged in a separate bag from all other nonmeat ingredients. This is important because nitrite can react with amines in seasonings, leading to the formation of *N*-nitrosamines. For example, in a 500-lb meat block formulation (i.e., for a US product), 1.25 lb of curing salt at 6.25% nitrite would be placed in a separate bag and put inside or attached to the outside of the main seasoning/binder bag. The operator would only have to add a bag of each product to the formula without additional weighing of the seasonings.

Sweetener

Sweeteners in the form of table sugar (i.e., sucrose), brown sugar, dextrose, corn syrup solids, honey, sorbitol, or lactose are often added to meat products as a flavoring ingredient. Their addition level can vary from 1% to 3% and is product dependent. These sweeteners help to counteract some of the sharpness imparted by salt and have a moderating effect on flavor. Dextrose in particular assists in the formation of the characteristic brown color on the external surface of country hams and bacon during thermal processing via the Maillard reaction and caramelization. The term 'glucose' used by some in the meat industry is a bit of a misnomer; it refers to corn syrup solids or 'glucose solids,' which is a hydrolyzed starch product, and not dextrose itself. Unlike corn syrup, corn syrup solids have a low sweetening power; they have different dextrose equivalents and hence browning potentials. Corn syrup solids are added to meat products for bulk and their ability to hold some water. Lactose also has a weak sweetening capability and may contribute bitterness in certain meat products but is present in sausages when nonfat dried milk is included in the formulation.

Erythorbate

Sodium erythorbate and ascorbate are reducing agents/cure accelerators, which speed up the conversion of nitrite to nitric oxide and thereby shorten the time required to complete the curing process. Residual amounts of erythorbate will also help stabilize the finished product's color. Moreover, erythorbate and ascorbate help to prevent the formation of carcinogenic *N*-nitrosamines that may form from secondary amino residues and residual nitrite in bacon.

Polyphosphates

Phosphates are sometimes added to improve the retention of moisture and to reduce the shrinkage or purge (i.e., cookout) that occurs during the heat processing of hams and bacon. Alkaline phosphates raise the pH of the product, and in doing so, help to solubilize muscle proteins, to improve the bind, and to increase their water-binding capacity and thereby the yield of the finished product. When phosphates are added to brines, it is not uncommon to obtain finished yields for intact hams and shoulders (i.e., picnics) of more than 100%. Polyphosphates have also been reported to retard the development of warmed-over flavor (WOF) and lipid oxidation, supposedly by their action as a metal ion chelator. The maximum regulated level of phosphate in the product formulation in the US is 0.5% with typical usage for hams, bacon, and cured sausages at 0.3–0.5%. Metallic and soapy flavors in meat products have been detected if phosphate levels are greater than 0.45%.

Seasonings and Flavors

Whole or ground spices, spice oleoresins, and seasonings (i.e., compounds containing one or more spices, or spice extractives, which are added to a food during its manufacture or in its preparation before serving) may be used by processors to develop distinctive flavors and to create special cured products. Seasonings should not be overwhelming or diminish the product's natural flavor, but potentiate the product with a blended, well-rounded flavor with no perceptible, undesirable aftertaste. Most flavors are mixed with a carrier such as a sweetener or salt. In recent years, however, flavors have been mixed in or encapsulated by starches, milk ingredients, or soy protein carriers. Today, preformulated cure units with specific flavors are available to processors. For example, California ham spice is a cinnamon and clove flavor mixed into salt or sugar, which is incorporated into ham formulations at levels of $<0.3\%$. Maple seasoning is a flavor that is added to a sweetener to impart a maple flavor in ham and bacon products. However, honey flavor is usually added directly to the meat product from a pure source. When comparing full-fat formulations with reduced-fat formulations, it is important to note that there will be a difference in flavor between the two products even if the same amount of seasoning is added. Low-fat formulations typically use more water to help soften the texture of the finished product, and water will carry the flavor differently. Excess seasoning in low-fat products can at times impart a very unpleasant metallic or astringent taste which lingers on the palate. Thus, the choices and addition levels of seasonings to meat formulations are a bit of an art, as the final balance of flavors will be dictated by the specific product in question. Even if the quality of meat used during formulating is of the highest standard, it becomes of little importance when the product is not properly seasoned.

Many seasoning companies are known by the industry to be predominantly suppliers of meat seasonings. Historically, there are two reasons for this. First, most of the smaller seasoning houses were founded by large meat companies so as to provide seasonings to their processing operations. Second, some seasoning houses marketed meat seasonings because of the technical expertise available from their personnel. Not only did these companies sell seasonings, but they also provided other ingredients like smoke flavors and sausage casings as well as the technical support for the preparation of value-added meat products.

Formulating Meat Products

Formulating any food product is much more than simply recipe development. In fact, some may even call it an art. It involves detailing the required processing steps and in what particular order they must be carried out to produce a high-quality finished product. Decisions when formulating meat products can entail the selection of meats and their levels of addition, choice of nonmeat ingredients such as salt, sweeteners, binders and seasonings, method and length of curing, grind or chop size of meat and fat, oven/smokehouse schedules, type and diameter of casings, as well as type and method of packaging. Formulating meat products also requires preparing a product which meets recognized characteristics set out by the country's standards of identity for that product. For meat companies, innovation is one of the single most important factors in building and maintaining a successful product or brand. A brand can be well recognized, but if over time that brand does not continue to offer value to consumers, it will soon be eclipsed in the market.

When designing meat products, it is practical to formulate by ingredient weight, which is based on the amount of the meat block. When formulating the seasonings, the processor must work in the weight of seasoning per 100 lb or kilogram of meat. Quantities can then be easily converted to percentages and the seasoning formula determined. For meat blocks not in 100 lb or kilogram increments, the percent usage of spice, oleoresin, or seasoning is calculated per pound or kilogram and then multiplied by the weight desired to determine the quantity required. Many processors weigh out the dry ingredients in a controlled-access room where the temperature and humidity are carefully monitored. After weighing, the critical components are often packaged and then assembled for batch production. A checklist must be prepared and verified in order to ensure that all of the materials are accounted for. Meat processors keep very detailed records of all formulas, and most are proprietary. The formulas are designed so that costs can be easily calculated and updated as needed, especially in the case of least cost formulation products. Standard blending or formulation documentation includes a list of ingredients, the weight of the individual ingredients in one seasoning unit, the percent of each ingredient in a batch, as well as the laboratory and plant code numbers.

Brine Preparation

One of the most important steps in ham and bacon production is brine preparation. Brine is a water-soluble solution containing salt, cure (e.g., Prague powder), phosphate, sugar, sodium erythorbate, and seasonings. All of the flavoring materials should be water soluble. There are two schools of thought regarding the temperature of water used for brine preparation. Some processors use warm water to help the dissolution of added solids and then allow the brine to cool to 0–2 °C (32–35.6 °F) before its application to meats. Others have concerns that warm water may cause chemical reactions to occur prematurely (e.g., conversion of nitrite to nitric oxide and then release of nitrogen dioxide gas from the brine) and thereby reduce the brine's efficacy. Such proponents favor the preparation of brines using cold/ice water. Whichever approach is employed, a known weight of water is measured, and all quantities of brine additives are weighed out separately. If using phosphates in the brine preparation, they should be added first and mixed thoroughly with high-speed shearing to ensure their complete dissolution. Other ingredients are added to the brine based on their solubility. Sodium erythorbate and soy isolate or concentrate are both difficult to suspend. Soy, if used, should always be added following the manufacturer's instructions including how much and if shear is necessary to get the plant proteins into suspension. Any product that settles out during brine injection could potentially cause problems in the finished product. Salt is then added and dissolved, followed by the cure and any remaining ingredients such as sugars and water-soluble spice mixtures. Nitrite is almost always added last, or until just before the brine is used, to ensure that less is lost to the environment. Brines are usually covered and held in a cooler – to reach or maintain a temperature of 0–2 °C (32–35.6 °F) – before use. It is important to note that some additives like soy, which are not completely solubilized, require agitation when processing/injecting.

Application of Cures

There are a few means by which meats can be cured. The basic methods include the following:

1. Dry curing – in this technique the curing ingredients, usually salt, sugar, nitrite, and nitrate, are mixed together and rubbed over the surface of the meat cut. The product is then placed in a cool room and the ingredients are allowed to penetrate by diffusion through the muscle tissue. The main disadvantage of this approach is that it is slow and in thicker cuts of meat, spoilage organisms can begin growing before the preservatives reach all parts of the product. More details about dry curing of meats are provided in the preceding article.
2. Brine curing – a brine is prepared by combining the salt, cure, and seasonings in water, which serves as a carrier. The strength of a brine solution or 'pickle' is determined by the amount of salt present. A salometer is a specially graduated hydrometer that measures the strength of brines at a particular temperature (usually 40 °F/4.4 °C) and is calibrated to indicate the degree of salinity (this is essentially a measure of the brine's density). A 100° salometer reading is equivalent to a 100% saturated salt solution, which is typically 26.5% salt (depending on temperature). Larger processors prepare stock solutions of brine at a 100° salometer reading and then formulate working pickles with additional additives at lower strengths. The presence of sweeteners, phosphates, nitrite, and erythorbate in brine will affect salometer readings to an extent. Typical pickles have strengths of 60–70°, with 70° brine being the most common. For brine immersion or cover pickling, the product is simply immersed in the brine for a specified period. For example, hams and shoulders are normally cured for 2–2.5 days per pound in 70° brine. Even though the penetration of ingredients into the muscle tissue may be faster than in dry curing, this technique also suffers from

slowness and is not widely employed by industry. More details about brine curing of meats are provided in another article.

3. Multiple-needle (stitch) pumping – a brine is prepared and then injected mechanically under pressure through needles, which are perforated along the stem near the point, into primal cuts of meat. In this multiple-needle injection technique, a conveyor belt carries meat under a bank of offset needles through which brine is pumped until a desired target weight is achieved. The spacing of the needles, their size, the pumping pressure, spray pattern, and the dwell time between strokes are important variables to ensure good distribution and retention of the pickle. The brine injected into commercial mild-cured products is typically a 70° pickle. The main advantages of multiple-needle pumping include increased product yield, greatly reduced labor costs, and time required for production. After pumping, some products are cooked immediately, whereas others are further processed by immersing them in a brine cure for a period (e.g., Canadian bacon) or subjecting them to a mechanical operation such as tumbling.
4. Tumbling or massaging – although strictly not a basic curing technique, tumbling or massaging of pickle-injected meat cuts is employed to speed up the curing process, to facilitate extraction of salt-soluble proteins, and to improve the texture, bind, water-holding capacity, and yield of the finished product. Tumblers are large stainless steel units that rotate in a circular fashion for a period of time. Nowadays practically all units have vacuum capabilities. Inside, pieces of meat are continuously lifted up by baffles to the upper part of the machine. From here they fall, striking the meat mass below, and produce an intense mechanical action suitable for high-yield products. Muscle fibers are disrupted by this mechanical action, which makes cellular membranes more permeable and facilitates the distribution and absorption of brine. Some degree of massaging also occurs as the chunks slide over each other as the tumbler turns. Tumblers typically provide somewhat more of a destructive effect than massagers on account of the impact force generated from the mechanical action. Thus, not all cured meat products can be tumbled (i.e., bone-in hams). A fitting example for the benefits of tumbling comes from ham production: Hams processed in this fashion are more uniform, as brine uptake is more tightly controlled and pickle pockets are reduced. The tighter control of pickle uptake results from the ability to pump the hams at, or somewhat below, the target pump and then adjusting the product's uptake to the exact percent pump by adding pickle directly to the tumbler. Without the presence of tumblers and massagers in meat processing operations, the higher processing yields and lower production costs associated with a number of value-added meat products could not be achieved.
5. Chopping or blending – dry curing ingredients are distributed directly into ground meat products during the grinding, chopping, and emulsification steps involved in batter preparation. Particle size is important in this process. Larger particles will require more time for cure penetration than very small ones. The particle size can be controlled by the grinder plate size or time in the chopper.

Specific Cured Products

Bacon

In North America, bacon is manufactured from pork bellies. The preparation of the belly is dependent on the desired characteristics of the finished product. Bellies may be cured with or without skin attached. Skinless bellies produce a product that has smoke color and flavor on all sides, whereas skin-on-bellies are usually derinded just before slicing and are identifiable by their distinct white fat. In most preparations, the bellies are first skinned using a skinning machine and then trimmed of ragged edges by knives. Excessive lean at the butt end is trimmed and used for sausage production.

In former times, the bellies were placed in high-salt-containing brines with the lean side down for 4–5 days or were dry cured for 10–14 days. Modern processing operations, however, cannot afford these time or space constraints; green bellies (raw noninjected) are pumped with brine to a specific percentage using a multineedle pickle injector. The injection operation dramatically speeds up the curing processing and also results in weight gain and an even distribution of the cure in the product. In some operations, the bellies are tumbled or massaged after injection for a short period of time (i.e., less than 60 min) with additional brine. Brine pickups of 18–30% can be achieved with a uniform distribution of the cure. However, bacon in the US must return to green weight. The processed bellies are then placed on combs and hung on trees or trucks for thermal processing and smoking. The bacon is partially cooked in an oven/smokehouse according to either a one- or three-step cook schedule until a final internal temperature of ~57 °C (135 °F) is reached; even so, it is still considered as a raw product. Bacon is smoked during all or part of the cooking cycle, depending on the requirements of the desired final product. After processing, the bacon is chilled in a tempering cooler to an internal temperature of −4.4 or −3.3 °C (24–26 °F) before pressing, slicing, and vacuum packaging. Cooling the product allows the bacon to retain its shape during pressing and facilitates slicing. The pressing operation involves placing slabs of processed bacon in a large forming machine where they are compressed to a relatively uniform width and thickness. The pressed bacon is sliced to a set thickness using a high-speed slicer and then graded according to premium slices, secondary slices, and ends or pieces. The shingled bacon is then conveyed to a packaging machine where it is typically vacuum packaged.

Microwaveable bacon is a more recent product in the marketplace. It is bacon that has been thermally processed for a sufficient length to develop the characteristic flavor and texture of fried bacon. This fully cooked product is sliced and then packaged in special packs designed to enhance microwave heating. In this respect, the product can be prepared faster without significant fry out from fat. The convenience and little mess resulting during microwave processing afford an attractive product to food service operators and to the consumer.

Canadian-style bacon, which is sometimes referred to as back bacon, is manufactured using the center portion of boneless pork loins (i.e., longissimus dorsi). Thus, it is a very lean product. Boneless loins are pumped with brine and then held in a cover pickle for 2–5 days. Once removed from the

pickle, the loins are rinsed with cold water, stuffed into casings, and hung in an oven/smokehouse, where they are cooked to an internal temperature of ~70 °C (158 °F). Canadian bacon is sold either sliced or in chunk form.

Wiltshire bacon is made from selected hogs weighing between 68 and 90 kg with special cutting procedures for the carcasses; that being, the shoulder, loin, belly, and ham are left as one piece, whereas the foreleg is removed at the knee and the hind leg at the hock. Additionally, the tenderloin, ribs, neckbone, backbone, aitchbone, skirt, and loose fat are also cut out. The Wiltshire sides are cured by pumping and then placing them in a cover pickle for 7–10 days. After this period, the sides are removed from the pickle and product is held under refrigeration conditions for maturation, which lasts anywhere from 2 to 14 days. The sides are then usually smoked before being shipped to market.

Hams

Hams are very popular cured meat products that are prepared from the hind leg of pork. Meat sizes can vary from whole muscles to relatively small chunks, which are restructured. Some processors are matching muscles to make small 2–3 lb hams as opposed to large whole hams or chunked and formed hams. Various types of hams can be prepared and they include the following: traditional bone-in hams; semiboneless hams; boneless, premium hams; and boneless, sectioned/chopped and formed hams. Most hams are pickle cured with brine consisting of salt, cure, sugar, phosphate, and erythorbate. Once cured, hams can be stuffed into fibrous casings and processed in an oven/smokehouse or, as in the case of some sectioned/chopped and formed hams, be processed using cook-in-a-bag technology to produce a fully cooked ham.

Traditional bone-in and semiboneless hams

Bone-in hams are typically prepared from the whole pork leg with the foot removed. Hams are usually separated from the loin by cutting between the second and third sacral vertebrae parallel to the angle of the hock joint. The resulting whole intact hams contain only three bones – aitch, body (i.e., femur), and shank. When the aitch- and shankbones are removed, the ham is referred to as semiboneless. The aitchbone is sometimes removed alone when preparing spiral-cut country-cured hams. In older times, before processing, hams were trimmed of some collar fat by line workers. At present, processors who do not have abattoirs and bring in raw meat typically purchase Institutional Meat Purchase Specifications (IMPS) 401 or 402 series hams, which already have partially skinned collar fat removed or are fully skinned (402). A prepared brine is injected into the muscle tissue at a certain percentage pump. The product may be immersed in a cover pickle before netting and is then placed on a tree or truck for thermal processing. The ham is thermal processed (with or without smoke), chilled, packaged, and then boxed. Bone-in hams are comprised of the butt, center, and shank. The hams may be sold whole or cut into butt and shank sections.

For bone-in hams, special care is needed when using a multineedle pickle injector to avoid potential damage of the needles should one hit a bone during mechanical treatment. Specially designed bone-in injectors are recommended for use with bone-in hams. Consequently, in many small operations, hams are pumped by hand. Placing the hams in a tumbler or massager for a period to distribute the cure throughout the product aids in creating a uniform cure and prevents against bone souring; however, if tumbled too long, this can also create problems. Hams, and picnics to a lesser extent, may also be cured by artery pumping. This involves pumping brine directly into the tissue's vascular system. A needle is usually inserted in front of the branch in the femoral artery so that the pickle can be distributed throughout the entire ham. Care must be exercised, however, to ensure that blood vessels are not ruptured by excessive pumping pressures. The pumping schedule generally calls for adding 8–10% by weight of the pump pickle. There are some problems, however, with this technique. First, the arterial pathways in the muscle are not uniform, thereby resulting in uneven curing. Second, it is generally recommended to hold the ham under refrigeration conditions after injection to permit not only equilibration of the cure but also the fixation of the cured color. Most important, the success of arterial injection is dependent on attentive work during slaughter and cutting as well as subsequent handling procedures in order to guarantee that the arteries are left intact. Owing to the inherent difficulties with this technique, it is not as often employed as it once was.

Boneless, premium hams

The usual muscle classes for premium hams from the leg area of hogs include semimembranosus–adductor and biceps femoris–semitendinosus. The muscle tissue is generally trimmed, deseamed, and boned. The boneless meat is then pumped via a multineedle injector to a certain pump percentage and then subjected to massaging or tumbling to evenly distribute the cure. In instances where the targeted pumping gain in the hams has not been achieved, brine is added to the tumbler for pickup of the additional amount needed. After tumbling, the muscles are netted together or placed in casings; they are manufactured in either round or flat shapes. In some cases, ham molds are placed on the product before thermal processing to give them the required shape. Round boneless hams are held in cellulose casings, whereas flat hams are prepared by pressing boneless hams together. Before pressing, the hams are stuffed into stockinettes, cellulose, or collagen casings. The pumped meat is then thermal processed and smoked. Afterward, the ham is chilled and finally vacuum packaged. In some instances, the hams are cut in half or to specific pound quantities before packaging.

Boneless, sectioned/chopped and formed hams

These hams are made virtually in the same manner as premium hams except that the biceps femoris–semitendinosus muscles are chopped or sectioned into smaller pieces along with muscles from the shank and the knuckle. The meat from boned hams or ham pieces may be used fresh or after freezing and thawing. All excess surface fat and seam fat should be removed. Brine containing salt, cure, sugar, phosphate, and erythorbate is formulated and pumped into the pieces of ham (typically a 15–30% pump). Tumbling or massaging is used to extract myofibrillar proteins from the muscle tissue to cause the meat pieces to be glued or stuck together. In some cases, shank meat or lean ham trim is finely chopped in a bowl

chopper with added water, salt, phosphate, and cure. Approximately 5–10% of this mixture (based on the pumped weight of hams) is transferred to a massager containing pumped hams. The meats are massaged for 6–8 h and then tightly stuffed into fibrous cellulose or collagen casings of approximately 12.5 cm. The meat chunks should be tightly stuffed to force the pieces together so that they will bind during heating. The product is cooked in an oven/smokehouse. The amount of smoke applied will vary from none to heavy, depending on the desired characteristics for the finished product. During the thermal processing operation, all hams must reach a minimum of 60 °C (140 °F), as this temperature will kill any *Trichinella spiralis*. This nematode parasite is not really a problem anymore; present day microbiological concerns over *Salmonella* spp. and *Escherichia coli* dictate an end-point temperature of 70 °C (158 °F). Because thermal processing is employed to 'set' the premium and section and formed hams by means of heat coagulable proteins, these hams need to reach a temperature of 72 °C (~162 °F). Afterward the hams are cooled to approximately 2 °C (35.6 °F) before packaging. Chilling must be accomplished in a specific time frame to ensure food safety.

A chopped and formed ham is a variation of a sectioned and formed ham. In the former, the meats used are usually smaller pieces. A formulation for a Pullman ham, a typical chopped and formed ham, is given in Table 1.

Table 1 Formulation sheet for a chopped and formed ham[a]

Product: Pullman ham
Yield over green weight: 145%
Batch size: 116 kg

Ingredients	*Concentration in product (%)*	*Level (%)*	*Quantity needed (kg)*
Meat			
Ham meat			68
Shank meat			12
Brine			
Water/ice		77.573	27.30
Salt	2.3	7.411	2.67
Dextrose	1.4	4.511	1.63
Glucose (i.e., corn syrup solids)	1.4	4.511	1.63
Sodium tripolyphosphate	0.50	1.611	0.58
Sodium erythorbate	0.05	0.161	0.060
Prague powder	0.31	1.000	0.36
Flavor	1.0	3.222	1.16

[a]Courtesy of Mr. Daniel J. Prefontaine, President, Saskatchewan Food Industry Development Center, Saskatoon, SK.
Note: Instructions:

- Pass chilled ham meat through a kidney plate.
- Grind chilled shank meat through a 0.12 in. plate.
- Transfer ground shank meat with about half of the phosphate, salt, Prague powder, erythorbate and water/ice to a silent cutter.
- Cut until the product is creamy, then add all remaining dry ingredients and water/ice, followed by blending.
- If all meat in the silent cutter is lean then the temperature is not an issue, but if fats have been added, the temperature must be monitored.
- Once complete, transfer the mixture to a vacuum tumbler and add the coarse-ground ham meat to it.
- Tumble the meats under vacuum for 1 h at high speed.
- Stuff product into water-cook casings.
- Place in 4 × 4 molds.
- Cook product in a water bath/steam kettle to an internal temperature of 72 °C (~162 °F).
- Cool down product by immersing it in cold water.
- Transfer product to a cooler, chill, and when ready slice and vacuum package.

Dry-cured hams

Although dry-cured hams are not as common in North America (the possible exception being the US country-cured hams for southeastern markets in Virginia, West Virginia, Kentucky, Tennessee, North Carolina, South Carolina, and Georgia), they are extremely popular in the Mediterranean region and are revered for their unique flavor as well as other characteristic sensory attributes. In Spain, two important dry-cured products are Iberian and Serrano hams; their production in 1993 was approximately 181 500 ton. During processing there is a loss of water, and diffusion of salt throughout the ham leads to a gradual stabilization of the product, due to a drop in the water activity. Simultaneously, there is a slow degradation of proteins and lipids that results in an accumulation of free amino acids and fatty acids, respectively. Details on the processing of Iberian and Serrano dry-cured hams are described in Table 2, but briefly include the following steps:

1. reception and classification of hams, and then presalting where a mixture of curing ingredients (i.e., salt, nitrate, and/or nitrite) and adjuncts (i.e., ascorbic acid) are rubbed onto the lean muscle surface of the meat;
2. salting, where hams are then placed fat side down, entirely surrounded by salt and arranged in single layers without touching one another. As there is no water added, the curing agents diffuse slowly into the ham and are solubilized by the original moisture present in the muscle tissues. This period usually takes 8–10 days (i.e., 1–1.5 days per kg weight) at temperatures between 2 (35.6) and 4 °C (39°F);
3. during the post salting stage, a complete salt equalization within the hams occurs. The temperature is kept less than 4 °C (39 °F) for a period not less than 20 days but not exceeding 2 months;
4. the last and more complex stage is the ripening/drying stage. Hams are placed in natural or air-conditioned chambers and subjected to different time–temperature/relative humidity (RH) cycles. The temperature is usually maintained between 14 (57) and 20 °C (68 °F) with a RH decreasing from 90 to 70%. Aging of hams takes anywhere from 9 to 24 months. For example, the ripening period for Serrano hams is between 9 and 12 months and for Iberian hams, it can be extended up to 18 or 24 months.

The quality of these two hams depends on the raw materials and the ripening conditions employed. Iberian dry-cured ham is produced from an autochthonous pig that is found in the southwestern region of Spain. These swine feed on pastures or stubble fields during their growing period (until 12/16 months of age, 55/75 kg) and their nutritional requirements are completed with cereals such as corn and

Table 2 Scheme of the approximate conditions for the processing of Serrano and Iberian dry-cured hams[a]

	Serrano ham	*Iberian ham*
Salting	T 0–4 °C RH 75–95% $t > 0.65$ and < 2 days kg^{-1}	
Post salting	T 0–6 °C RH 70–95% $t > 40$ and < 60 days	
Dry curing		
First phase	T 6–16 °C RH 70–95% $t > 45$ days	T 6–16 °C RH 60–80% $t > 90$ days
Second phase	T 16–24 °C RH 70–95% $t > 35$ days	T 16–26 °C RH 55–85% $t > 90$ days
Third phase	T 24–34 °C RH 70–95% $t > 30$ days	T 12–22°C RH 60–90% $t > 115$ days
Fourth phase	T 12–20 °C RH 70–95% $t > 35$ days	
Total Time	$t > 190$ days	$t > 365$ days

[a]Abbreviations: RH, relative humidity; T, temperature; t, time in days.
Source: Reproduced from Toldrá, F., 2002. Dry-Cured Meat Products. Trumbull, CT: Food & Nutrition Press, Inc.

barley. During the fattening period, three types of feeding regimes, known as montanera, recebo, and cebo, are possible. For montanera, the basic food is the acorn (*Quercus ilex*, *Quercus rotundifolia*, and *Quercus suber*) and the feeding period lasts from October to December or until a final weight of approximately 160 kg is achieved. For recebo, the acorn is complemented with cereals and mixed feeds. For cebo, only cereals and mixed feeds are used. Meat from acorn-fed pigs commands the highest price and the dry-cured hams so prepared offer a high degree of marbling (resulting from the finishing lipid-rich acorn diet), firm texture, and exquisite characteristic flavor. The Serrano ham is produced from different crossbreeding of white pigs and has lower marbling, firm texture, and a typical flavor. The intensity of the flavor can be controlled by the length of time the ham is allowed to ripen/dry. Complex biochemical reactions, mainly enzymatic, proteolytic, and lipolytic in nature, occur during the dry curing process and contribute to the development of an adequate texture and characteristic flavor.

Corned Beef

Traditionally, corned beef is prepared from the brisket; however, the demand for leaner meat products has some processors preparing it from muscles of the round. The basic corning process used today is multiple-needle injection of pickle into the beef. The pickle used in the preparation of corned beef is similar to that employed in ham and smoked-meat manufacturing. If boneless briskets are used, they tend to be pumped to 120% of green weight, whereas rounds are generally pumped to only 110%. The injected beef is then placed in a cover pickle containing additional spices and herbs, such as bay leaves and allspice, for a few days. Prepackaged corned beef is sold either uncooked or as a ready-to-eat product. In the latter case, the corned beef is cooked in water or steamed to an internal temperature of 67 (~153) to 72 °C (~162 °F).

Table 3 Formulation sheet for bologna

Product: Bologna

Ingredients	*Level (%)*	*Quantity needed (kg)*
Lean pork trim, 80/20	39.24	39.24
Lean beef trim, 85/15	22.00	22.0
Pork trim, 50/50	22.00	22.0
Water (as ice)	10.0	10.0
Salt	1.80	1.80
Sodium tripolyphosphate	0.35	0.35
Garlic powder	0.05	0.05
Paprika	0.25	0.25
Modified food starch	3.5	0.75
White pepper	0.13	0.13
Prague powder	0.30	0.30
Sodium erythorbate	0.05	0.050
Onion powder	0.06	0.060
Nutmeg	0.17	0.17
Ginger	0.10	0.10

Note: Instructions:

- Weigh out all ingredients and keep them separate.
- Grind all chilled meats through a 0.12 in. plate, but keep meats separate from one another.
- Transfer ground lean meats, salt, Prague powder, erythorbate, and half of the ice into the silent cutter.
- Chop to 4 °C (39 °F) and then add ground pork trim (50/50) and the remaining dry ingredients along with the ice.
- Chop on high speed until a temperature of approximately 13 °C (~55 °F) is reached.
- Transfer the batter to a stuffer, and stuff into cellulose casings.
- Heat process and smoke in a smokehouse to 72 °C (~162 °F).
- Cold water shower to reduce internal temperature of product to 32 °C (~90 °F).
- Transfer product to cooler, chill and when ready peel and vacuum package.

Frankfurters, Cured Sausages, and Comminuted Meat Products

Frankfurters, cured sausages, and comminuted meat products such as bologna, salami, and pepperoni are prepared as a meat batter. In its simplest form, a meat batter contains water, protein, fat, and salt. The general steps in preparing sausage products include grinding of meat, chopping of meat, additive addition, stuffing, linking, cooking, peeling, slicing, and packaging. As a general rule of thumb, better meat quality will give a higher quality batter and final product. A typical formulation for bologna is provided in Table 3.

There is a particular order of steps which one must follow when preparing batters. Meats to be used are divided into groups according to how much fat is present. For example, there is the lean or 95/5 (i.e., 95% lean muscle tissue containing 5% intramuscular fat), followed by 80/20 and then the fatter 50/50 trim. In the case of products containing pork, the majority of fat and fatty trims should be hard fats from the

shoulder, ham, back fat, or jowls. Softer fats such as belly trimmings should be limited, as they can cause fat caps and soft texture in the finished product. Each meat type is ground separately to a final grind size, depending on the product of choice. Grinding is a particle size reduction step in which pieces of meat are continuously forced through the holes of a metal plate between the arms of a rotating multibladed knife. The holes bored into the plate have specific diameters (e.g., 3.2, 6.4, 9.5, 12.7 mm; or 0.12, 0.25, 0.38, 0.5 in., respectively). Meats must be comminuted in two or three steps with a sequential reduction in the grind size. It is important to remember, however, that the meats must be kept cold during any grinding operation to prevent smearing/liquidization of the fat.

Ground meats are transferred to a grinder mixer, a bowl chopper, or a silent cutter. Here the mechanical action of the vertically positioned rotating knives in combination with added salt and phosphate will extract myofibrillar proteins from the meat; extracted proteins will help to immobilize fat particles and form a three-dimensional network of filaments that contributes to the overall texture as well as the water- and fat-binding properties of the finished product. The sequence of batter preparation goes as follows: lean meat, phosphate, salt, cure, erythorbate, and half the ice/water are added to the chopper. The chopping process creates sufficient shear to comminute meat and fat into fine particulates as well as to extract protein. Chopping of the raw materials causes a temperature rise of the meat batter; therefore, the use of ice during formulation helps to ensure that the mixture is blended at 0–3 °C (32–37.4 °F). The remaining ice/water is added, followed by fatter meats (e.g., 80/20 or 50/50) and then binders, flours, and seasonings. The rationale for chopping lean meat with salt and water is that maximum solubilization of myofibrillar protein occurs at the higher ionic strength before 'dilution' by fatter meats and binders. This will help to prevent emulsion collapse or 'shorting out' resulting in fat caps, gelatine pockets, and a greasy surface on the cooked product. Reasons for the lack of functionality from the proteins may be due to poor quality meat (e.g., not enough lean meat protein, denatured protein from acidification, and too much collagen), addition of too small amounts of salt and phosphate, overchopping, and incorrect final batter temperatures.

The batter is chopped until a specific end-point temperature is reached, which is dependent on the fat properties of the animal species used. To create a stable emulsion, the added fat must exist as small pieces or droplets so that the protein matrix can coat the surface of fat particles, thereby reducing surface tension; this occurs when the temperature of the batter is raised. Not all products contain meat from only one species; therefore, the end-point temperature of a batter containing a majority of pork should be between 10 (50) and 13 °C (~55 °F); for poultry, 2 (35.6) and 7 °C (~45 °F); and for beef, 13 (~55) and 21 °C (70 °F). Bowl choppers can be used to manufacture both coarse and finely comminuted cured meat products. When a very fine emulsion is desired, some sausage manufacturers pass the chopped meat through a separate emulsifier before stuffing. In such cases, the batter is processed in a silent cutter only to a temperature ranging between 2 (35.6) and 4 °C (39 °F). As it passes through the emulsifier, the temperature goes up, and the emulsification step continues until the final end-point temperature of the product is achieved.

In some emulsion-type products, vacuum chopping will increase the protein extraction and density of the emulsion. Vacuum chopping/stuffing removes most of the air from the emulsion and gives greater product uniformity, reduction in voids, better protein extraction, better color retention, and an increased shelf life. For example, in bologna, which is a large-diameter product, vacuum chopping is desirable to eliminate air bubbles or pockets that could cause color fading and a soft texture to the final product.

Binders or fillers are commonly used in the manufacturing of cured sausages and are normally made up of a combination of soy, cereals, as well as native and modified starches. If a product is high in meat protein and moisture but low in fat, then a high moisture-absorbing binder like starch can be employed. However, in a product with greater fat content, such as a frankfurter, a high protein binder like soy or milk solids might be chosen. Functional protein binders are binders where the protein has not been denatured during their manufacturing and thereby bestow properties to the meat matrix. The benefits of adding functional binders to a formulation include the following: increased protein level, improved texture, reduction in shrinkage, and overall juiciness and cohesiveness to the product.

Once the batter is ready, it needs to be stuffed into a casing. There are special machines designed for this purpose. First, it is important that products be stuffed in casings to their proper diameter so that they resemble the product they are supposed to be. For example, one would not stuff the batter of a pepperoni stick into a salami casing and vice versa. In fact, frankfurters of a relatively large diameter casing, or in natural casings, are often referred to as wieners. Second, the product should not be under or overstuffed in the casing. Understuffing usually results in casings with wrinkles, whereas overstuffing can result in casing breakage during thermal processing. Most casing suppliers provide guidelines of recommended stuffing diameters for products and these take into account shrinkage after cooking. Finally, stuffed products are hung on trucks for thermal processing and smoking in an oven/smokehouse. Some meat plants are so sophisticated that they have continuous processing lines for frankfurter production. After thermal processing, the meat product is chilled, and when ready, it is peeled from its casing, packaged, and shipped to market.

Natural Nitrate- and Nitrite-Free Cured Meats

Owing to the negative perceptions of nitrite-cured meats held by some consumers, there has been an interest of late in the 'so-called' nitrate/nitrite-free natural meat products. Any traditionally cured product produced in the US that does not include addition of a chemically derived form of nitrite is labeled as uncured. As previously discussed, nitrite/nitrate addition to meat products develops a characteristic color and flavor associated with cured meat products to which there is no known substitute. Without its addition, natural processed meat products would appear brown and their flavor would

Table 4 Formulation sheet for a natural hot dog

Product: Natural hot dog		
Ingredients	*Level (%)*	*Quantity needed (kg)*
Lean pork trim, 72/28	52.6	52.6
Beef trim, 50/50	22.7	22.7
Water	20.3	20.3
Sea salt	1.28	1.28
Natural hot dog seasoning	3.10	3.10
Cane sugar, natural flavors, celery powder, onion powder, garlic powder, oleoresin paprika		
Starter culture	0.02	0.02

Note: Instructions:

- Weigh out all ingredients and keep them separate.
- Grind all chilled meats through a 0.19 in. plate, but keep meats separate from one another.
- Mix starter culture with water totaling up to 0.50% of the total batch.
- Mix/chop lean meats, adding in order, salt, half of the water, fatty meats, seasoning, and remaining water.
- Add diluted starter culture.
- Continue mixing/chopping until the meat blend temperature reaches 50–54 °F (10–12 °C).
- Emulsify to 62–64 °F (17–18 °C).
- Stuff and link.
- Place on smokehouse rack and process using the smokehouse schedule: (1) 110 °F (~43 °C), 60 min; (2) 140 °F (60 °C), 20 min; (3) 155 °F (~68 °C), 30 min; (4) 175 °F (~79 °C), 30 min; and (5) 185 °F (85 °C)/30% RH to 165 °F (~74 °C) internal temperature.
- Cold water shower to reduce internal temperature of product.
- Transfer product to cooler, chill and when ready peel and vacuum package.

Source: Reproduced from Sebranek, J. G., Bacus, J. N., 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues. White Paper Series Number 1. Savoy, IL: American Meat Science Association.

be appreciably less desirable to the consumer than those of conventionally cured counterparts. The USDA permits the manufacture of uncured versions of typical cured meats according to the Code of Federal Regulations Title 9, CFR 317.17 and 319.2. To circumvent nitrite regulation and labeling issues, natural curing is accomplished by employing sea salt and vegetable juice/concentrate/powder high in naturally occurring nitrates (e.g., celery has nitrate levels typically ranging from 1500 to 2800 ppm, whereas celery juice powder has been reported to contain ~27 500 ppm or 2.75% nitrate) in combination with a nitrate-reducing starter culture (e.g., *Kocuria varians, Staphylococcus xylosus,* and *Staphylococcus carnosus*) to 'indirectly' cure the product. Processors are using both preconverted nitrates (already converted to nitrite) from celery powder and unconverted celery powder with a starter culture that has a nitrate reductase enzyme to convert the nitrates into nitrite. This practice combined with labeling requirements for such products has resulted in a category of processed meats in the US that is confusing and perhaps even misleading to the consumer. Moreover, protection afforded by nitrite addition to meat products against spore germination of *Clostridium botulinum* is potentially compromised in these uncured products, because the conversion of nitrate present in celery into nitrite is not a well-controlled reaction. This raises the specter of the product's microbiological safety in vacuum-packed bags. For this reason, the USDA is also concerned about chilling rates on finished packaged products. If the processor is manufacturing a 'naturally' cured product, the residual nitrite level will more likely not be enough and a more restrictive chilling regime as outlined in Appendix B of the USDA meat regulations would be in order.

For the most part, the processing procedures of natural curing are similar to those operations using sodium nitrite. Nitrate is more stable than nitrite; hence, a sufficient time is required to allow the starter culture to reduce exogenous nitrate to nitrite. The time needed depends on a number of factors including temperature, pH, growth cycle of the starter culture (i.e., number of microorganisms), and level of nitrate in the added celery powder. A good distribution of the ingredients is essential to ensure a uniform cure in such products. If dry, the vegetable powder (i.e., natural nitrate source) is typically blended into the dry seasonings for comminuted products or added directly to curing brines. The starter culture is often first mixed with water before addition to comminuted products and then dispersed via agitation for optimal distribution in the meat product. The USDA allows a maximum 0.5% combined water and starter culture without labeling the added water. The finished product, however, is required to bear a label disclaimer such as 'no nitrates or nitrites added, except for that which occurs naturally in celery juice powder.' A typical natural cooked sausage product formulation and process is given in Table 4.

See also: Bacon Production: Bacon; Wiltshire Sides. Chemical Analysis for Specific Components: Curing Agents. Curing: Brine Curing of Meat; Dry; Natural and Organic Cured Meat Products in the United States; Physiology of Nitric Oxide. Ham Production: Cooked Ham; Dry-Cured Ham. Processing Equipment: Brine Injectors; Mixing and Cutting Equipment; Tumblers and Massagers. Residues in Meat and Meat Products: Residues Associated with Meat Production

Further Reading

Claus, J.R., Colby, J.-W., Flick, G.J., 1994. Processed meats/poultry/seafood. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods. Meat, Poultry and Seafood Technology. New York, NY: Chapman & Hall, pp. 106–162.

Ockerman, H.W., 1989. Sausage and Processed Meat Formulations. New York, NY: Van Nostrand Reinhold.

Pearson, A.M., Gillett, T.A., 1999. Processed Meats, third ed. Gaithersburg, MD: Aspen Publishers, Inc.

Pegg, R.B., Shahidi, F., 2000. Nitrite Curing of Meat. The *N*-nitrosamine Problem and Nitrite Alternatives. Trumbull, CT: Food & Nutrition Press, Inc.

Romans, J.R., Costello, W.J., Carlson, W.C., Greaser, M.L., Jones, K.W., 2000. The Meat We Eat, fourteenth ed UpperSaddle River, NJ: Prentice Hall.

Sebranek, J.G., Bacus, J.N., 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues. White Paper Series Number 1. Savoy, IL: American Meat Science Association.

Sindelar, J.J., Cordray, J.C., Olson, D.G., Sebranek, J.G., Love, J.A., 2007. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. Journal of Food Science 72, S551–S559.

Sindelar, J.J., Terns, M.J., Meyn, E., Boles, J.A., 2010. Development of a method to manufacture uncured, no-nitrate/nitrite-added whole muscle jerky. Meat Science 86, 298–303.

Toldrá, F., 2002. Dry-Cured Meat Products. Trumbull, CT: Food & Nutrition Press, Inc.

Toldrá, F., Flores, M., Navarro, J.-L., Aristoy, M.-C., Flores, J., 1997. New developments in dry-cured ham. In: Spanier, A.M., Tamura, M., Okai, H., Mills, O. (Eds.), Chemistry of Novel Foods. Carol Stream, IL: Allured Publishing Corporation, pp. 259–272.

Wakamatsu, J., Nishimura, T., Hattori, A., 2004. A Zn–porphyrin complex contributes to bright red color in Parma ham. Meat Science 67, 95–100.

CUTTING AND BONING

Contents

Hot Boning of Meat

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Glossary

Prerigor boning Process of removing meat from the bones of a meat carcass before the carcass has entered a rigor state.

Refrigeration May be defined as the process of removing heat from any substance to: (1) render colder – reduce temperature, (2) change its state – for example, water to ice, and (3) maintain its state – preserving foods, storing ice.

Introduction

Typically, meat is initially chilled after slaughter in the form of whole eviscerated and dressed carcasses or sides. The majority of carcasses/sides are chilled in conventional air chill rooms, nominally operating at one or sometimes two conditions during the chilling cycle. The rate of heat removal and the resulting rate of temperature reduction at the surface and within the carcass/side have a substantial influence on the weight loss, storage life, and eating quality of the meat produced. European Union regulations require that all red meat temperatures within the carcass/side must be reduced to not more than 7 °C before the meat is further processed, or moved from the chiller, whereas for poultry this temperature is 4 °C. Similar legislation is applied in many other parts of the world. Careful control is required to achieve conditions that will reduce the meat temperature in the designed time cycle. This has to be carried out in the most economic manner taking into account weight loss and energy consumption.

The concept of deboning a 'hot' carcass was first mooted in the early 1970s. Potentially, the hot boning of carcasss has distinct advantages over cold boning. The warm meat is soft and requires less effort to bone, thus occupational overuse injuries are less likely to occur, there is potential for improved yield, and expensive chilling of fat and bones is avoided. There are also benefits in terms of certain processing qualities when hot-boned meat is used to manufacture meat products. However, there are also potential disadvantages of hot boning; for instance, the potential for the meat to be tough, darker in color, and for some primals to be different in shape.

There is no strict definition of the temperature range over which boning can be considered as 'hot.' For example, in Australia, hot boning usually means boning carcasses that have a deep butt temperature of more than 20 °C.

It is useful to consider hot boning in two categories – (1) 'true' hot boning and (2) warm boning. In true hot boning, carcasses or sides are not cooled before they are boned. They are boned within 30–45 min of slaughter. Plants that perform true hot boning often use plate freezers to cool the hot meat. Even with plate freezers, it can be difficult to rapidly reduce the temperature of large beef primal cuts. Bulk-packed product, at average temperatures of 28–30 °C, can normally be cooled in sufficiently rapid time using plate freezers. In warm boning, carcasss or sides are boned after a period of prechilling. Typically, carcasses are prechilled for 30 min to 6 h. Short prechills are used for lamb/sheep and longer periods for beef. Warm boning allows for an increase in throughput on the slaughter floor without having to increase chiller space. After warm boning, primals and manufacturing meat can be cooled quickly enough in air blast or plate freezers to prevent microbial growth.

Microbiology of Hot-Boned Meat

A disadvantage of hot boning is that there is an increased risk that the meat can support the growth of pathogenic bacteria as it cools. In conventional boning, microbial growth on carcasss is controlled by a combination of drying and cooling of the carcass surface. When the meat is hot-boned and packed,

moist meat surfaces may be contaminated and provide an opportunity for microbial growth because they stay moist and warm. The surface temperature of boned meat prepared conventionally in accordance with regulatory requirements is usually less than 4 °C and the core of the meat below 7 °C. At these temperatures, the growth of pathogenic bacteria is stopped or is very slow. In the case of true hot-boned meat, the boneless meat surfaces could be 20–35 °C at the time of packing. At these temperatures, pathogenic bacteria can potentially adapt to their new environment within an hour or two and begin to grow quickly. Therefore, the meat must be cooled quickly to below 7 °C after it is hot boned in order to control the growth of pathogenic bacteria. Both New Zealand and Australia have performance criteria for the cooling of hot boned, boxed beef that are based on the growth of *Escherichia coli* calculated from temperature histories for the centers of boxes.

Quality of Hot Boned Meat

Immediately postmortem, there are two related processes that affect meat-eating quality: (1) a fall in muscle pH and temperature and (2) shortening of the muscle.

The combination of a very rapid fall in pH and slow cooling of the carcass can lead to heat or rigor shortening; whereas a slow fall in pH and rapid cooling can lead to cold shortening. Shortening is undesirable as it can cause moderate to severe toughness. A further important penalty of both cold and heat shortening is a reduction in the ability of the meat to tenderize during aging. Also, both may adversely affect functional properties of manufacturing meat.

Because of the lack of skeletal restraint, these phenomena are more important with true hot boning than with warm or cold boning. Ideally to avoid shortening problems, the aim is to achieve an optimal rate of pH fall during rigor mortis and cooling. However, this is not readily achievable with true hot boning because of the varying cooling rates within a carton of meat.

The application of an electrical current to the carcass on the slaughter floor accelerates pH decline. Electrical currents may be applied via electrical stunning, immobilization, hide-puller probes, or from conventional electrical stimulation (ES). The effects on the rate of pH fall are additive and lead to the early onset of rigor mortis. In some abattoirs where extra low voltage stimulation and downward hide pullers are used, beef sides have attained close to ultimate pH (5.5–6.0) within 60 min of stunning. 'Overstimulation' in this instance is undesirable as it can increase the risk of heat shortening, reduce the aging potential of the product, and in warm (and cold) boning can cause denaturation and paleness of slow cooling internal muscles.

Meat that is taken from a carcass prerigor and promptly subjected to further processing has manufacturing advantages, such as better fat emulsifying and water-holding properties. However, hot-boned meat is usually chilled, or frozen, before it is used for manufacturing. If hot-boned meat cannot be processed immediately after slaughter it is possible to preserve its superior manufacturing properties by very rapid (i.e., cryogenic) freezing. The meat must be frozen while it is still in the prerigor state and held at storage temperatures close to − 18 °C. In fresh beef, or mutton, freezing must be completed within 6 h of slaughter. If the meat is thawed before use, however, biochemical changes take place rapidly, and most of the advantages of prerigor meat are lost.

By adding at least 2% salt (sodium chloride) before freezing, or at the time of thawing (e.g., during chopping), the superior qualities of the prerigor meat can be retained after thawing. When minced thoroughly with the meat, 2% salt has been shown to preserve the water-binding power of fresh (unfrozen) prerigor meat for some days. Comminuted prerigor meat has a water-holding capacity similar to that of similarly treated postrigor meat to which polyphosphates have been added. In some countries, the use of phosphates and other nonmeat additives to improve water-holding capacity and fat emulsifying properties of manufactured meat products is either forbidden, or restricted. The addition of salt to prerigor boned meat before freezing is acceptable, however, as salt is included in the formulation of sausage meats. Therefore, there is an opportunity to better exploit the superiority of hot-boned meat for processed meat products.

Removing muscles from the carcass soon after slaughter changes their normal state. Some muscles are normally stretched on the carcass and become free to shorten when released from their attachments to bones. Others, which usually cool slowly because they are enclosed by other muscles, may be cooled much faster. These differences will affect tenderness development and can cause muscles that are tender when cold boned (e.g., tenderloin) to be toughened when hot boned.

With true hot boning, meat is still in the prerigor state at the time of boning even if electrical stunning, electrical immobilization, or ES has been used. This makes muscles susceptible to adverse temperature/pH combinations. Shortening is particularly likely. To overcome these problems systems such as the Pi-Vac Elasto-Pack system for the prerigor muscles have been developed. These stretch or constrain the muscle during chilling. The Pi-Vac Elasto-Pack system operates by stretching tubes of highly elastic films to the inside walls of a packaging chamber. After inserting the muscle into the chamber, the pressure is released, and the film returns to its original dimensions. The forces from the elastic film stop the diametrical muscle expansion, which would result from the longitudinal contraction of the muscle. It is claimed that it is possible to chill the meat rapidly without detrimental effects on tenderness and produce an attractive shape for cuts. Other systems having similar aims are Tendercut, Tenderstretch and SmartStretch™. Smartstretch™ uses external air pressure to stretch and reform hot-boned primals into a uniform size, using restraining packaging to ensure that the stretch is retained during rigor. The technique has been shown to improve the initial tenderness (0 days aged) of beef striploins from adult cattle, although no effect was found after a 2-week aging period. The treatment increased cooking losses, but had no influence on moisture losses in raw or frozen meat, or on meat color.

With warm boning, many muscles from electrically stimulated carcasses are at least partly in rigor at the time of boning. In this case, muscles are less likely to be affected by adverse postboning temperature/pH combinations. As with

cold boning, skeletal restraint minimizes the risk of the meat being affected by adverse temperature/pH combinations during carcass chilling.

The color and retail display life of hot-boned primal cuts is generally equivalent to that of conventionally boned product. However, in cold boning some of the deeper beef muscles are often paler than the more superficial muscles because of their slower temperature fall and faster pH fall. Hot boning can mean that these cuts are cooled more rapidly leading to less denaturation, leading to darker meat and less two toning than in their cold-boned counterparts. Some studies have also reported a greater color stability in hot-boned steaks. In addition, some staining of the fat of primal cuts with blood from superficial blood vessels has been experienced in hot boning.

Hot and Warm Boning Operations

Hot boning is generally considered to be easier than cold boning, but care needs to be taken when handling the cuts as they may be slippery in comparison. A major health and safety advantage with hot boning is that the problem of hard fat is not encountered. However, difficulty can be encountered in trimming fat from primal cuts. This can result in a fat thickness that is not up to specification and a less attractive cut. However, with the proper training and care, trimming can be done accurately.

In objective studies carried out in the United States, strain gauges were fitted on knife handles to measure the effort required to hot-bone beef. It was claimed that 49% less effort was required to hot-bone beef sides compared with cold boning. In addition, it has been reported that a yield improvement of 1.5–2.0% is achievable with true hot boning compared with conventional boning. This increased yield is a combination of reduced evaporation losses and more efficient removal of meat from the bones. Studies have shown that hot boning reduces the time taken to bone out a beef side from 18 to 14 min. However, it can take some time for boners to become fully competent with hot boning, thus yield advantages may not be immediately obvious.

Beef

The New Zealand meat industry has pioneered the application of hot boning with approximately 20% of beef production processed hot in 2006 with large plants processing up to 80 000 cattle per year. Historically, hot boning plants were used almost exclusively to process manufacturing beef from bulls and cows; however, it is now commonplace to process prime beef animals using hot boning procedures. Regular audits of product quality have demonstrated that eating quality and other quality attributes can match those of meat produced by more conventional cold boning procedures, as long as pH and temperature decline are effectively managed. In contrast it has been reported that Brazil, which is the leading beef export country in the world exporting 1635 million tons in 2011, makes no use of hot boning and only limited use of ES.

Immersion chilling has been investigated to improve chilling rates of hot-boned beef cuts. The commercial process that has developed involves vacuum packing hot-boned meat and transferring the packaged cut to a system similar to the auger system used for poultry processing.

ES accelerates the onset of rigor and can be used to minimize if not eliminate cold shortening induced toughness in hot-boned beef. However, studies on beef sides that were hot boned and chilled at -20 or 0 °C showed that the meat from beef chilled at -20 °C was tougher than cold-boned controls after 7 and 14 days of aging. Drip loss from meat chilled at -20 °C was also higher than that hot boned and chilled at 0 °C or cold boned.

Studies have been carried out to determine the effects of hot boning, low voltage ES, and chilling temperature on the tenderness of bovine M. longissimus dorsi (LD) and M. semimembranosus (SM) muscles. Hot-boned muscles, which were not electrically stimulated had higher Warner Bratzler shear force (WBSF) values and shorter sarcomeres than cold-boned muscles. Under fast and slow chilling regimes WBSF values were lower in ES hot-boned LD and SM muscles at days 2, 7, and 14 postmortem than those not electrically stimulated muscles. Hot-boned muscles subjected to slow chilling had longer sarcomeres than those subjected to fast chilling. In hot-boned SM muscles, ES resulted in longer sarcomere lengths. However, ES did not have a significant effect on the sarcomere length of LD muscles. As indicated by WBSF values, all muscles tenderized during aging, including muscles, were 'cold shortened.'

Lamb

As may be expected there appears to have been limited interest or work carried out on the hot boning of lamb. The small size of the lamb carcass makes it amenable to quick chilling and lamb cuts are often sold with the bone in. Few of the advantages of hot boning, therefore, apply to lamb. Nevertheless hot boning of lamb has been proposed as a means to reduce energy costs. Hot boning of lamb has been shown not only to improve flavor and juiciness and decrease cooking loss but it also can reduce tenderness.

In one study on hot boning of lamb after ES, carcasses were placed in a drying room for approximately 35 min with an average temperature of 8 °C to dry the surface of the carcass to limit bacterial growth before hot boning. Within 2 h of slaughter the hot-boned cuts were treated in a SmartStretch™ machine before chilling and aging. The results of the study were that, irrespective of treatments (aging, stretch, or stimulation), 100% of samples would have been acceptable after 0 days on display. However, after 24, 48, and 72 h on retail display, 72%, 86%, and 93% of the samples would be deemed unacceptable to consumers, respectively.

Studies on the eating quality of commercially processed hot-boned sheep meat showed that all samples were tougher than the recommended threshold for table meat. Only 13.5% of the samples met the 'good everyday' requirement following sensory assessment. The authors reported that the application of effective ES is not sufficient to ensure that hot-boned sheep meat will be suitable as a table meat.

Pork

Although cold shortening can produce toughening in pork it is potentially less of a problem than in beef or lamb. Consequently a whole range of rapid chilling and warm/hot boning technologies have been developed for pork. As early as the 1950s, several progressive sausage manufacturers in the United States, who were also engaged in pig slaughtering, deboned hot (less than 1 h postmortem) sow carcasses. The resulting prerigor muscles were treated with salt or sometimes polyphosphates and this procedure improved the water-holding capacity for the production of frankfurters. Today, the majority of sows and some boars, 15% of the total pork production, are hot boned and nearly all the musculature transformed immediately into sausages. This is the most extreme example of accelerated processing currently in commercial operation, from pig to sausage in less than 2 h. The adenosine triphosphate present in prerigor pork acts as a natural glue in the production of restructured products and with the trend toward additive-free food, such processing prerigor bears reexamination.

The rate of diffusion of salt through muscle becomes faster as muscle temperature rises. Also, the still intact arterial system of the pig immediately after slaughter provides a good distribution network for curing brine. Systems have been developed to hot cure bacon by arterially pumping cold brine into the carcass prerigor. This has the added advantage of partially cooling the meat, before immersion chilling in refrigerated brine.

In a hot boning system developed in the late 1980s, loins were removed 30 min postmortem, vacuum packed, held in a water bath at 11 °C for 5 h, and then brine chilled. Drip loss after storage for 21 days at 0 °C was less (0.55%) than the control and other rapidly chilled treatments. Other sensory parameters were similar. In warm processing work, carried out at the same time, loins were removed from carcasses 1, 3, or 5 h poststunning. Three rapid cooling treatments; immersion in brine at −23 °C; CO_2 chilling at −94 °C or packing in CO_2 at −68 °C, were used in the trials. These produced loin temperatures of −2 °C after 1.5–2 h of chilling with no significant difference between treatments. The crust frozen loins were then tempered and mechanically portioned. Pork chilled at 1 h poststunning resulted in high shear force values and short sarcomere length. For a delay time of 3 h or more there were no major differences in muscle color, pH, sarcomere lengths, drip, or taste panel determinations between treatments and a conventionally (0–2 °C chiller) chilled control.

Cold shortening in pork was first confirmed in trials where pork carcasses and sides were chilled immediately after slaughter using air below −30 °C and high air velocities. Further trials showed that ES before rapid chilling alleviated the toughening problems. Other trials have shown that the detrimental effects of accelerated boning on pork tenderness can be overcome with temperature conditioning at 14 °C and aging for 4 days postslaughter. Chilling of muscles at 0 °C following accelerated boning resulted in cold shortening as seen by the reduction in sarcomere length relative to muscles chilled at 14 or 21 °C. Furthermore, the reduced sarcomere length increased drip loss and produced pork of a darker color.

Warm boning as practiced in Denmark is another technology that allows same day processing and distribution. Immediately after dressing, chilling in air at −25 to −30 °C for approximately 80 min is commenced. This brings the surface temperature down to approximately −2 °C. It is therefore necessary to equilibrate the carcass for one or two hours before cutting and boning take place. The total chilling loss is approximately 0.6%. After boning the meat is either vacuum packed for storage and aging; wrapped, boxed and frozen, or cured and tumbled. Not all the heat is extracted during the short initial blast chilling operation and further cooling is required after cutting. Studies showed that there were no differences in the microbial and sensory qualities of the 'warm' processed pork compared with cold-boned controls. Overall yield was 0.8% higher than that from the controls.

It is not uncommon in Spain for pork carcasses to be boned out after a 90 min chilling period. With the exception of the hams the rest of the carcass is immediately butchered into primals, which are further chilled and distributed on the same day as slaughter.

Horse

Although little horse meat is eaten in comparison with other red meats, a considerable amount is produced. There is little data on either conventional or hot processing of horse; however, hot boning is likely to offer similar advantages and disadvantages as for beef. In one published study on the commercial warm boning of horse carcasses the cooling process for warm-boned meat met with standards for hot-boned beef cooling processes based on calculated growth of *E. coli* at box centers. In the plant studied, carcass sides were cooled overnight, or for between only 1 or 2 h. In the latter case, the warm carcasses are divided into quarters and prime cuts of meat removed from the hanging quarters placed directly into bags for vacuum-packaging followed by air blast chilling air at a temperature of −5 °C and a speed of approximately 4 m s^{-1}. For 80% of the temperature histories, the initial temperature was >20 °C and temperatures of 7 and 0 °C were attained within 13 and 26 h, respectively. Numbers of bacteria recovered from cooled carcasses or hot- or cold-boned cuts were generally similar. The microbiological condition of horse carcass quarters delivered to plants in Europe was claimed to be comparable with the microbiological conditions of hanging beef delivered from packing plants to distant customers within North America.

Poultry

Production of deboned poultry meat has rapidly increased since the 1990s. Hot boning of poultry results in similar advantages and disadvantages as for red meat. However, the onset of rigor is far faster in poultry than red meat. As with red meat, ES is often used to speed the onset of rigor, allowing for hot boning without toughening. Electrical stunning at 40 V and high frequency is claimed to significantly improve the texture of chicken and can produce hot-boned breasts with acceptable tenderness. Boning is commonly carried out at

1.5–2 h postmortem. However, breasts are usually boned out after 24 h postmortem.

There have been a number of studies on the eating quality of breast fillets boned at different intervals postmortem. Studies on the eating quality of cooked chicken fillets from either hot boned at 45 min postmortem or cold boned at 2 or 24 h postmortem showed little difference between hot and the 2 h boned fillets. However, the flavor profile of 24 h boned fillets was different from both hot and 2 h boned samples. The 24 h boned were rated less cardboardy and sweeter. In other studies on breasts boned from 0.25 h (hot boned) to 24 h all the meat produced before 6 h postmortem was judged to be tougher than that boned after 6 h or later.

Conclusion

Hot boning of beef, pork, lamb, horse, and poultry carcasses offers a variety of benefits to the processor such as increased boning yield and savings in refrigeration capacity and energy usage compared with conventional cold-boning operations. In addition manufacturers of further-processed products have realized the improved functionality of hot-boned muscles especially in the production of ground beef and pork items.

Common problems with early hot-boned meat systems usually included reduced tenderness, distortion of muscle shape, and darker lean color. However, the use of ES or muscle restraining and aging systems greatly reduces, or eliminates, many of these problems. Prerigor boning and chilling systems are applicable to the meat industry and provide a safe and high-quality product.

Although a number of countries, such as New Zealand and Australia, have embraced the benefits of hot boning, the lack of understanding of, and information about, hot processing, the operational changes required to make hot-boning systems work in current operations, and fear of reduced shelf-life has limited the uptake of hot-boning in other countries, especially in Europe and the Americas.

See also: Electrical Stimulation. Meat Marketing: Transport of Meat and Meat Products. Modeling in Meat Science: Refrigeration. Physical Measurements: Temperature Measurement. Refrigeration and Freezing Technology: Freezing and Product Quality; Thawing

Further Reading

International Institute of Refrigeration, 1986. Recent advances and developments in the refrigeration of meat chilling. Proceedings of the Conference of: IIR Commission C2, Bristol (UK). Paris, France: International Institute of Refrigeration.

International Institute of Refrigeration, 2001. Rapid cooling of food. Proceedings of the Conference of: IIR Commission C2, Bristol (UK). Paris, France: International Institute of Refrigeration.

James, C., Vincent, C., de Andrade Lima, T.I., James, S.J., 2006. The primary chilling of poultry carcasses – A review. International Journal of Refrigeration 29 (6), 847–862.

James, S.J., James, C., 2002. Meat Refrigeration. Cambridge, UK: Woodhead Publishing Limited. ISBN 1 85573 442 7.

Kerry, J.P., Kerry, J.F., 2011. Processed Meats: Improving Safety, Nutrition and Quality. Cambridge, UK: Woodhead Publishing Ltd.

Nollet, L.M.L., Toldrá, F., 2006. Advanced Technologies for Meat Processing. Boca Raton, FL: Taylor and Francis.

Relevant Websites

http://www.ecff.net/
Official Site for the European Chilled Food Federation.

http://www.fao.org/
Official Site for the Food and Agriculture Organization of the United Nations.

http://www.iifiir.org/
Official Site for the International Institute of Refrigeration.

http://www.chilledfood.org/
Official Site for the UK Chilled Food Association.

Traditional

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Glossary

Fabricate (as related to meat cutting) To reduce a carcass into its major and minor components for further use in the retail and foodservice channels.

Primal Large section of carcass usually associated with legs (e.g., round, leg, shoulder, chuck, rib, loin, etc.).

Subprimal Sub-divided portion of primal into smaller pieces more suitable for portioning into steaks, roasts, or chops.

Superior spinous process The most dorsal portion of the vertebrae.

Transverse process The lateral portions of the vertebrae most prominent in the lumbar area of the animal.

Introduction

One of the challenges of communicating cutting and boning information to a worldwide audience is that the nomenclature and terminologies used vary so widely. Many technical references now feature pictorial examples of cuts so that a visual description can then be matched with the word description of it. It is always best to use anatomical descriptions – muscle and bone names – where possible so that the best and most universal description of carcass cutting/boning can be made. One such source is the International Committee on Veterinary Gross Anatomical Nomenclature, which provides detailed muscle and bone names.

The scope of this article will focus on beef, pork, and lamb cutting and boning, but some of the methods and terminologies can be used for other livestock species of importance to some countries around the world. For the most part, prerigor cutting and boning is termed 'hot processing' or 'hot boning,' whereas postrigor cutting and boning usually is referred to as 'cold processing' or 'cold boning.' Because cold cutting/boning is the default method in the developed world, it may just simply be referred to as 'cutting/boning' because it is performed after chilling and limited cold storage. Cutting styles and nomenclature do not differ between hot or cold boning. The debate about the advantages and disadvantages of hot boning are numerous and will be discussed in the article on hot boning.

Early humans probably developed crude cutting/boning procedures as they obtained carcasses from dead animals, through hunting or later through domestication and harvest. People had to find a way to handle the large carcass mass and reduce it into smaller pieces for possible sharing or for consumption over time, especially if performed during the winter months where cold temperatures would have allowed for extended storage for consumption at other times.

Early cutting tools might have been made from stones before the advent of metal where various blade-type devices (e.g., axes and knives) would have been used to cut meat. The development of sawing tools would have assisted the carcass cutting process with band and reciprocating saws used commercially today. In fact, the thin blades of commercial band saws with the small amount of kerf they remove in the sawing process is of great economic importance for large-scale meat processors where even small savings in yields are magnified through the large volume of carcasses processed.

Terminology of Cutting and Boning

Describing the process of converting carcasses into smaller portions is often confusing. For example, in the US, beef carcasses are 'fabricated' into wholesale cuts, using the broad definition of 'fabricate' i.e., 'to make or build something.' This term is usually shortened to state that 'carcasses are being fabbed,' or to describe the room where this process is conducted is simply referred to as the 'fab room' or 'fab area.' These are nonstandard uses of this term, but this simply demonstrates how terminology has evolved to describe various processes in the meat industry in at least one country. For the most part, pork is simply 'cut' so the description of the process and where the location of the process occurs is referred to as 'pork cutting.'

Terms to Describe Wholesale Cuts of Meat

Nomenclature used to describe the wholesale portions from carcasses varies from country to country, and also the nomenclature used to describe the general terms for what these portions are varies widely. The term 'primal' probably has its origin to the fact that a section of the carcass was a 'prime' or 'primary' region and thus this term was developed. Several decades ago, with the advent of widespread vacuum packaged, boxed meat programs, the term 'subprimal' began to be used because it best described smaller portions of primals. For example, the primal loin in beef could be further reduced to the strip loin, tenderloin, top sirloin, and bottom sirloin cuts. The trend today is for even further separation of cuts with some

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now merchandized as an individual muscle (IM) with the term 'IM' being used in the names to signify such cuts.

Because specific nomenclature and exacting specifications are necessary in buyer–seller relationships, and because the US Federal Government purchases a tremendous volume of meat for various buying programs, the Agricultural Marketing Service of the US Department of Agriculture (USDA) developed the Institutional Meat Purchase Specifications (IMPS) program as a way to have clear descriptions for the ever-evolving number of cuts available in the marketplace. The IMPS system also is used in market-news reporting as a way to note the prices being paid for specific cuts of meat. There are many different IMPS programs, but the ones related to this topic can be obtained from USDA for the categories of beef, pork, and lamb.

In 1961, the National Association of Meat Purveyors (now known as the North American Meat Association) began publishing the *'The Meat Buyer's Guide®'* as a way to provide a pictorial depiction of these cuts. The scope of the guide has grown over the years to include beef, lamb, veal, pork, and poultry, and the guide has been published in a number of different languages to assist buyers and sellers in the world market for meat.

Beef Carcass Cutting

As mentioned earlier, methods of cutting/boning differ among countries and among companies even within a country. Beef carcass cutting is a method of cutting adapted from Savell and Smith, which reflects a US perspective of cutting/boning. Figure 1 depicts beef wholesale cut names used in this article.

Beef sides are separated into forequarters and hindquarters based on historic precedence and whether the ribeye/loin eye is to be evaluated for a grading/classification system. In the US, carcasses are ribbed between the 12th and 13th rib for grading purposes. The exposed ribeye muscle (M. longissimus thoracis) is evaluated for the voluntary USDA quality grade program using human visual or video-image analysis systems.

After grading and sorting by grade, weight, and other factors, sides are separated into forequarters and hindquarters by continuing the original ribbing cut through the plate/flank juncture perpendicular to the outside surface of the carcass. Before making this cut, the inside skirt (M. transverse abdominus) is loosened from its attachment in the flank area and dropped into the forequarter region so that it is not cut when separating the forequarter and hindquarter.

Forequarter Cutting

Here is a brief summary of forequarter cutting/boning that might involve table and on-the-rail methods, depending on facility layout and staffing.

- A knife cut is made between the 5th and 6th ribs through the lean and fat from the vertebrae to the sternum.
- The thoracic vertebra and sternum are sawn through between the 5th and 6th ribs to separate the chuck/brisket/foreshank from the rib/plate section.

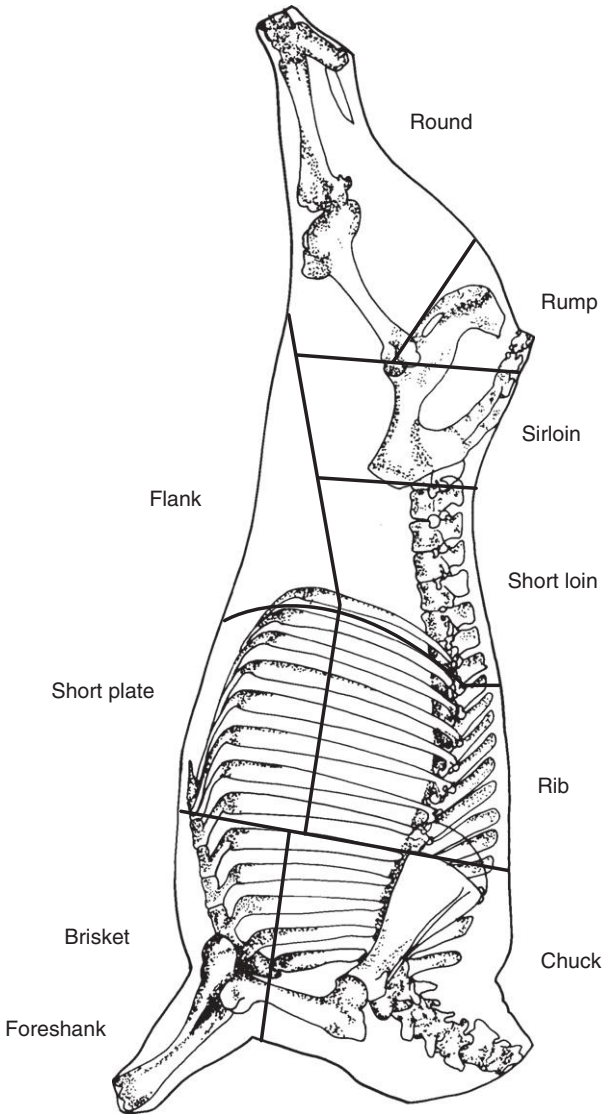

Figure 1 Beef wholesale cuts chart. Courtesy of the American Meat Science Association.

- The rib is separated from the plate by cutting through the ribs at a point measured from the lateral edge of the ribeye muscle (M. longissimus thoracis) on each end of the rib. This distance should be no more than 15 cm from the ribeye muscle on the loin end (posterior) and no more than 25 cm on the chuck end (anterior).
- The foreshank and brisket are removed from the chuck just above the lateral condyle of the humerus by making the cut parallel to the top of the chuck.
- The foreshank and brisket are separated at the natural seam between them.

Forequarter Boning

There are a number of major boneless subprimals that come from the forequarter. Here are a few and how they are prepared.

Beef Rib Ribeye, Lip-On (IMPS 112A)

This is the most common form of boneless ribeye marketed in the United States.

- The wholesale rib that is 15 × 25 cm (length of ribs from the lateral edge of the M. longissimus thoracis) is now cut so that the rib is 7.5 cm (measured from the lateral edge of the M. longissimus thoracis on the loin or posterior end) × 10 cm (measured from the lateral edge of the M. longissimus thoracis on the chuck or anterior end).
- The body of the vertebral column is removed on a band saw exposing a strip of lean between the feather bones (superior spinous processes of the vertebral column) and the rib bones. The feather bones are removed.
- The blade meat (portions of M. subscapularis and M. rhomboideus immediately below, and M. latissimus dorsi, M. infraspinatus, and M. trapezius immediately above the scapula) is removed starting at the ventral end of the rib near the seam in the exterior fat cover and continuing up to the dorsal end of the rib.
- Remove the ligamentum nuchae and the portion of the 'lip' (consists of the M. serratus dorsalis, M. longissimus costarum and associated fat tissues) that exceeds 5 cm from either end of the rib so that the ribeye is 5 cm × 5 cm. This ribeye is now a Beef Rib, Ribeye Roll, Lip-On, Bone In (Export Style), or IMPS 109E.
- Remove the back ribs beginning at the ventral end, being careful to follow the natural curvature of the ribs.

Beef Chuck, Shoulder (Clod) (IMPS 114)

In industrial settings, the shoulder clod (major muscle system that contains the M. triceps brachii, M. infraspinatus, and M. teres major) is removed from the arm chuck, which is a chuck that has the foreshank still attached, but the brisket has been removed.

- The arm chuck is suspended on-the-rail from the foreshank. A cut is made through the M. triceps brachii immediately below the elbow joint to the bone (humerus).
- The seam between the clod and the M. pectoralis profundus is opened.
- The M. triceps brachii is cut along the humerus to the knob portion of the bone (near the juncture of the humerus and scapula). At this point, the seam between the clod and the remaining portion of the chuck should be more evident.
- Continue cutting along the edge of the scapula paying particular attention to the complete removal of the M. infraspinatus from the scapula and the M. teres major from behind the scapula.
- The shoulder clod can be separated into these cuts: Beef Chuck, Shoulder (Clod), Top Blade (IMPS 114D), consisting of the M. infraspinatus; Beef Chuck, Shoulder Tender (IM) (IMPS 114F), consisting of the M. teres major; and Beef Chuck, Shoulder (Clod), Arm Roast (IMPS 114E), consisting primarily of the M. triceps brachii, caput longum (long head) and M. triceps brachii, caput laterale (lateral head).

Beef Chuck, Chuck Roll (IMPS 116A)

The scapula and M. supraspinatus are removed from the remaining portion of the arm chuck. The chuck portion is then separated from the foreshank before further processing.

- Using a band saw, the neck is removed from the chuck by cutting through the 5th-6th cervical vertebrae and parallel to the cut that was made between the 5th and 6th ribs. Make a cut that is immediately ventral to the body of the vertebrae and parallel to the line where the brisket was removed to separate the chuck short ribs and the pectoral muscle (M. pectorales superficiales) from the chuck roll.
- Remove all feather or superior spinous processes, vertebrae, rib bones, and ligamentum nuchae from the chuck roll.
- Remove the M. trapezius and exposed seam fat from the outside surface of the chuck roll along with the prescapular lymph gland.
- The remaining arm portion shall be excluded by a straight cut that is not more than 7.5 cm ventral from the M. longissimus thoracis at the rib end (posterior) and not more than 10 cm from the M. complexus at the neck end (anterior).

Hindquarter Cutting

- Separate the loin/flank and round on a line between the 4th and 5th sacral vertebrae and approximately 2.5 cm anterior to the aitch bone. Cuts through the bone are made with a saw, whereas cuts through the lean are made with a knife.
- Remove the flank from the loin by making a cut no more than 15 cm from the lateral edge of the loin eye muscle (M. longissimus lumborum) at the rib end (anterior) to no more than 2.5 cm from the lateral edge of the M. tensor facia latae in the round end (posterior). There is one saw cut (13th rib) and the remainder is made with a knife.
- Remove the kidney knob and pelvic fat leaving no more than 1.3 cm of fat at any point.
- Separate the short loin from the sirloin by cutting between the last two lumbar vertebrae parallel to the sirloin face (posterior).

Hindquarter Boning

There are a number of major boneless subprimals that come from the hindquarter. Here are a few and how they are prepared.

Beef Round, Sirloin Tip (Knuckle), Peeled (IMPS 167A)

- On the table, remove the caudal vertebrae from the round along with the lean and fat tissue around the top of the aitch bone. Remove the aitch bone paying particular attention to staying very close to the bone. Hang the round from the rail by the hock.
- Begin removing the knuckle by cutting just posterior to the knee cap. Follow the seams between the knuckle and the

M. biceps femoris and M. adductor and along the femur making sure to remove the periosteum as the knuckle is removed.
- Remove the M. tensor fasciae latae, fat, and skin tissue to make this a 'peeled' knuckle.

Beef Round, Top (inside) (IMPS 168)

- Make a knife cut beginning at the posterior section of the top round (M. semimembranosus and M. adductor are the major muscles) on the inside portion of the round.
- Continue removing the top round by following the natural seam and cutting along the femur.
- Finish removing the top round and trim external fat to purchaser's specifications.

Beef Round, Outside Round (Flat) (IMPS 171B) and Beef Round, Eye of Round (IM) (IMPS 171C)

These subprimals may be removed together as the Beef Round, Bottom (Gooseneck) (IMPS 170) before being separated into these two cuts.

- Make a knife cut immediately below the Achilles tendon being careful so that the hock will still be hanging on the hook.
- Continue the cut until the shank muscle seam is found.
- Remove the gooseneck round following the shank muscle seam and down along the femur.
- Remove the heel muscle from the gooseneck round. Separate the eye of round from the bottom round.
- Trim external fat from the eye of round to purchaser's specifications.
- Remove the opaque heavy connective tissue (often referred to as 'silver skin'), seam fat, lymph glands, and cartilage and ligaments. Trim external fat to purchaser's specifications.

Beef Loin, Tenderloin, Full, Side Muscle On, Defatted (IMPS 189A), Beef Loin, Strip Loin, Boneless (IMPS 180), and Beef Loin, Top Sirloin Butt, Boneless (IMPS 184)

These three subprimals are the major cuts obtained from the loin. To remove the full tenderloin, the loin has to remain intact and not separated into the sirloin/short loin sections as previously described.

Beef tenderloin

- From the full loin, remove the kidney and pelvic fat, being careful not to cut into the tenderloin.
- Remove the bottom sirloin flap and flank steak from the flank.
- Begin removing the tenderloin in the tail region by carefully cutting it from its attachment to the lumbar vertebrae.
- Continue cutting the tenderloin away from its attachment to the lumbar vertebrae by following along the body of the chine bones and the finger bones (transverse processes of the lumbar vertebrae).
- When the tenderloin is loosened near the sirloin/short loin juncture, begin removing it from the sirloin face (posterior).
- Carefully roll out the tenderloin by cutting the attachment of the wing (M. iliacus) to the pelvic bone.
- Trim the remaining fat from the tenderloin being careful not to separate the chain (M. psoas minor) from the body of the tenderloin (M. psoas major).

Beef strip loin

- Separate the shell loin from the sirloin by cutting between the last two lumbar vertebrate and parallel to the sirloin face (posterior).
- On the strip loin portion, using a band saw, cut the bodies of the vertebrae away so that there is clear separation between the superior spinous processes and the transverse processes.
- Using a knife, carefully remove the superior spinous processes, transverse processes, and the 13th rib from the strip loin.
- There are several purchaser-specified options that can be used for flank removal: a common one today is 2.5 cm × 0 cm, which refers to making a straight cut at a point 2.5 cm on the rib end (anterior) to a point 0 cm on the sirloin end (posterior) ventral to the loin eye muscle (M. longissimus lumborum).
- Trim the external fat to purchaser's specifications.

Beef top sirloin

- With the full sirloin, separate the bottom sirloin from the top sirloin using a knife and following the seam between the two. The bottom sirloin can be further separated into the Beef Loin, Bottom Sirloin Butt, Ball Tip, Boneless (IMPS 185B) and Beef Loin, Bottom Sirloin Butt, Trip-Tip, Boneless (IM) (IMPS 185C).
- For the top sirloin section, remove the hip bone and sacral vertebrae.
- Trim the external fat to purchaser's specifications.

Pork Carcass Cutting

Here are general procedures for cutting pork carcasses, with Figure 2 depicting wholesale cut name and location. The loin/ham break has some latitude and will vary based on the relative value of each of these cuts. When loin prices are higher than ham prices, the cut will be made closer to the ham; conversely, when ham prices are higher than loin prices, the cut will be made closer to the loin.

General Pork Carcass Cutting

- The leg or fresh ham is separated from the pork side by making a straight cut approximately perpendicular to a line parallel to the shank bones and that passes through a point that is not less than 3.7 cm and not more than 8.8 cm from the anterior edge of the aitch bone. There shall be no more than two sacral vertebrae, but no caudal vertebrae left on the loin.
- The shoulder is separated from the side by a straight cut that is approximately perpendicular to the length of the side. The

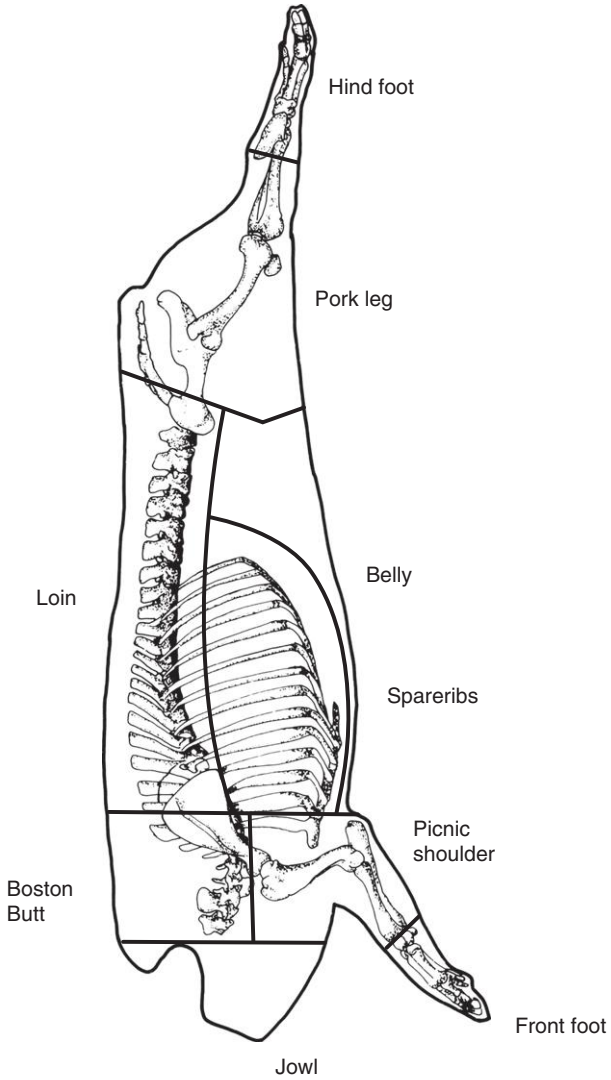

Figure 2 Pork wholesale cuts chart. Courtesy of the American Meat Science Association.

cut is made posterior to, so as not to expose, the elbow, but not more than 2.5 cm from the tip of the elbow. The outer tip of the M. subscapularis shall not extend past the dorsal edge of the base of the medial ridge of the blade bone.

- The belly is removed from the loin making a straight cut (a slight dorsal curvature is acceptable) that extends from a point that is ventral to, but not more than 7.5 cm from the M. longissimus thoracis on the shoulder end, to a point on the leg end ventral to, but not more than 1.3 cm from the tenderloin (M. psoas major).

Pork Shoulder Cutting

- The foot is removed at or slightly above the upper knee joint by making a straight cut approximately perpendicular to the shank bones.
- The jowl is removed by making a straight cut approximately parallel with the loin side that is anterior to, but not more than 2.5 cm from the innermost curvature of the ear dip, then the neck bones, ribs, breast bones, associated cartilage, and breast flap (through the major crease) are removed.
- The skin, dorsal to a straight line parallel to the dorsal side that starts at a point that does not exceed 25% of the distance from the elbow joint to the dorsal side, is removed.
- Fat exposed by the removal of the skin is trimmed to not exceed 1.3 cm or more from the edge of the skin collar, and traces of false lean (M. trapezius) are to be visible.
- The picnic shoulder is separated from the Boston butt by making a straight cut, dorsal to the shoulder point approximately 1.3 cm from the dorsal edge of the blade bone on the loin side, at an approximate right angle with the belly side.

Pork Leg (Fresh Ham) Cutting

- Remove the tail, vertebrae, flank muscle (M. rectus abdominis), M. cutaneous trunci, prefemoral lymph gland, and any other exposed lymph nodes.
- Remove the foot at or slightly above the hock joint.
- The skin and collar fat over the cushion (M. semimembranosus) is removed so that it is smooth and well rounded such that the innermost curvature of the skin is trimmed back at least half the distance from the stifle join to the posterior edge of the aitch bone.
- The skin overlying the medial side (inside) of the M. quadriceps femoris and fat close to the lean overlying the M. quadriceps femoris and pelvic area are removed.

Pork Loin Cutting

- The surface fat of the loin is trimmed to an average of 0.6 cm or less in depth except in the hip bone area (defined as the area contained within two parallel lines, 5 cm on either side of the anterior end of the hip bone and associated cartilage). Fat in the hip bone area is trimmed to the same contour as the rest of the trimmed surface of the loin. Lumbar and pelvic fat are trimmed to 1.3 cm or less in depth.
- At least 5 cm of the false lean (M. trapezius) shall be exposed lengthwise on the blade end of the loin, and the diaphragm and hanging tender are removed.

Lamb Carcass Cutting

Because of their size, lamb carcasses are not split in half during the slaughter-dressing process like beef and pork, so the style of fabrication differs somewhat from these species. Here are some general cutting procedures which Figure 3 depicts lamb wholesale cut name and location.

Carcass Primal Breaking

- The foresaddle is separated from the hindsaddle at the 12th and 13th rib by a cut that follows the natural

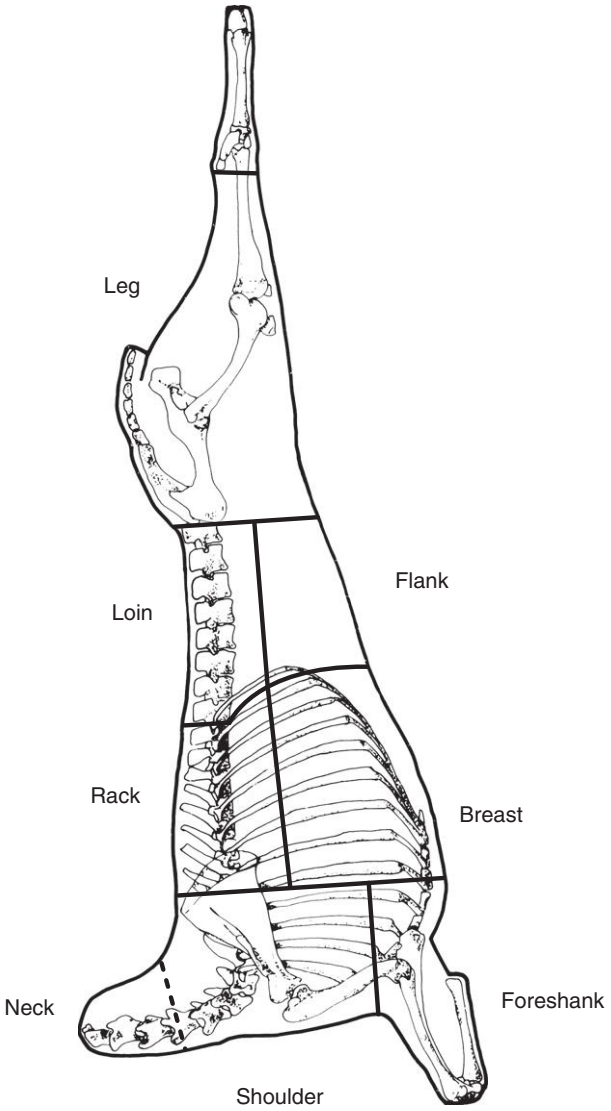

Figure 3 Lamb wholesale cuts chart. Courtesy of the American Meat Science Association.

curvature of the ribs. With this, there are 12 ribs that remain on the foresaddle and 1 rib on the hindsaddle.

- The foresaddle is separated into the shoulder/foreshank/brisket portion of the breast and bracelet (rack and breast) by making a straight cut between the 4th and 5th ribs perpendicular to the back. This leaves four ribs in the shoulder and eight ribs in the rack.
- The hindsaddle is separated into the loin (with the flank attached) and the leg by making a straight cut, approximately perpendicular to the length of the leg, passing anterior to the hip bone and hip bone cartilage.

Lamb Foresaddle Component Cutting

- The shoulder, square-cut is prepared by removing the foreshank and brisket portion of the breast by making a straight cut approximately perpendicular to the rack side (posterior) and through the cartilaginous juncture of the first rib. The neck is removed leaving no more than 2.5 cm of it on the shoulder. The thymus gland and heart fat is removed. If desired, the shoulder can be split into individual shoulders by making a saw cut through the vertebral column.
- The foreshank is separated from the brisket portion of the breast by cutting through the natural seam, which may contain a portion of the web muscle (M. pectoralis superficialis). The trotter or lower foreshank is removed at or above the knee joint.
- The rack is separated from the breast by making a straight cut no more than 10 cm from the lateral edge of the ribeye muscle (M. longissimus thoracis). The diaphragm and fat along the ventral side of the vertebrae of the rack are removed.
- If desired, the rack can be split into half racks by making a saw cut through the middle of the vertebral column.
- The breast can be made into 'Denver Style' ribs by removing the sternum and costal cartilages, fell membrane, M. cutaneous trunci, exterior fat cover, M. latissimus dorsi, and diaphragm.

Lamb Hindsaddle Component Cutting

- The flank is removed from the loin by making a straight cut no more than 10 cm from the lateral edge of the loin eye muscle (M. longissimus lumborum) on both ends of the loin. The diaphragm and hanging tender are removed from the loin.
- If desired, the loin can be made into half loins by making a saw cut through the vertebral column.
- The flank is separated into lean, fat, and bone (13th rib) portions.
- The legs are separated by making a saw cut through the vertebral column and pelvis. A short-cut leg can be made by removing the sirloin by making a straight cut approximately perpendicular to the length of the leg starting at the juncture of the last sacral and first caudal vertebra and passing just anterior to the protuberance of the femur (this exposes the ball of the femur). The short-cut leg can either have the tibia left in (French-style leg) or removed (American-style leg or shank-off leg).

Cutting and Boning Trends for the Future

The widespread adoption of many of the cutting and boning schemes used around the world would not have happened without the development of plastic packaging technologies in the last part of the twentieth century. Vacuum packaging of fresh meats led to extensive and centralized cutting and boning near the point of slaughter, which allowed for innovative styles to be developed. In addition, with the advent of packaging technology, the ability to export meats to great distances and to countries that demanded specific cuts that might have never been used domestically greatly increased the development of new styles of cutting and boning. It is likely that these trends will only increase in the future.

Further Reading

International Committee on Veterinary Gross Anatomical Nomenclature, 2005. *Nomina anatomica veterinaria*, fifth ed. Hannover, Germany: Editorial Committee; Columbia: Editorial Committee; Gent, Belgium: Editorial Committee; and Sapporo, Japan: Editorial Committee. Available at: http://www.wava-amav.org/Downloads/nav_2005.pdf (accessed 08.01.10).

North American Meat Processors Association, 2010. TheMeat Buyer's Guide™, sixth ed. Reston, VA: North American Meat Processors Association.

Savell, J.W., Smith, G.C., 2009. Meat Science Laboratory Manual, eighth ed. Boston, MA: American Press.

U.S. Department of Agriculture, 1996. Institutional Meat Purchase Specifications: For Fresh Lamb and Mutton – Series 200. Washington, DC: United States Department of Agriculture, Agricultural Marketing Service. Available at: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3003283 (accessed 30.12.13).

U.S. Department of Agriculture, 2010. Institutional Meat Purchase Specifications: Fresh Beef – Series 100. Washington, DC: United States Department of Agriculture, Agricultural Marketing Service. Available at: http://www.ams.usda.gov/AMSv1.0/getfiledDocName=STELDEV3003281 (accessed 30.12.13).

U.S. Department of Agriculture, 2010. Institutional Meat Purchase Specifications: Fresh Pork – Series 400. Washington, DC: United States Department of Agriculture, Agricultural Marketing Service. Available at: http://www.ams.usda.gov/AMSv1.0/getfiledDocName=STELDEV3003285 (accessed 30.12.13).

Relevant Websites

http://www.ams.usda.gov
Agricultural Marketing Service, United States Department of Agriculture.

http://www.americanlamb.com
American Lamb Board.

http://www.meatami.com
American Meat Institute.

http://meatscience.org
American Meat Science Association.

http://www.fmi.org
Food Marketing Institute.

http://www.beefretail.org
National Cattlemen's Beef Association, Retail Marketing.

http://www.pork.org
National Pork Board.

http://meatassociation.com
North American Meat Association.

D

DOUBLE-MUSCLED ANIMALS

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Glossary

Callipyge A genetic mutation that causes lambs to develop large and muscular rumps, from the Greek for 'beautiful buttocks.'
Double-muscled The exceptional muscular development as a result of a functional mutation in the myostatin gene.
Heterozygous animals Animals having two different alleles of a given gene.
Homozygous animals Animals having the same alleles of a given gene.
Muscular hypertrophy Exceptional muscular development.
Myogenesis The formation of muscle tissue during development of an embryo.
Myostatin A protein that acts as an inhibitor of the growth of muscle tissue.

Introduction

Double-muscled animals are of particular interest to meat producers because they are characterized by an increase in muscle mass and a decrease in fat deposition, resulting in a larger amount of lean meat than in normal animals. The term is mainly restricted to cattle and refers to an inherited condition that has been known for some time but of which the causal mutation in the myostatin gene was recently discovered. The condition is associated with altered physiological and histological characteristics that bring about differences not only in meat quantity but also in animal performance and in meat quality. Therefore, the use of double-muscled cattle in pure breeding or crossbreeding production systems has to be considered carefully, especially because of calving difficulties. Meat quality of double-muscled cattle differs from that of normal cattle in several respects and resembles as well as differs from meat of particular genotypes with enhanced muscularity in other species.

Genetic Background

Double muscling is an inherited condition that occurs in several cattle breeds. However, it is highly prevalent in only two breeds, i.e., the Belgian Blue and the Piedmontese. Different symbols have been used to differentiate between the double-muscled and normal phenotype, of which mh (muscular hypertrophy) and + are the most common. The locus was mapped to the centromeric end of bovine chromosome 2. Identification of double-muscled animals was long based on visual assessment of the degree of muscular hypertrophy, i.e., by looking at protruding muscles and intermuscular grooves under the skin. This is only accurate in classifying normal and homozygous double-muscled animals, but does not allow distinction of heterozygous animals. This has contributed to the controversy in the early studies on the mode of inheritance of the mh allele. Segregation analyses later revealed that the mh allele was partially recessive with the mh/+ animals being distinct but closer to the +/+ than the mh/mh animals. In 1997, several research groups uncovered the genetic cause of double muscling by mutations in the myostatin gene. Myostatin (MSTN, also called growth and differentiation factor 8, GDF8) is a member of the transforming growth factor β superfamily of growth and differentiation factors. First, it was shown that mice in which the myostatin gene had been knocked out had a two to three times increased muscle mass ('Mighty mice'). Because myostatin had been mapped before to the mh region of bovine chromosome 2, it was a very likely candidate gene for the mh locus. Sequencing of myostatin deoxyribonucleic acid from double-muscled Belgian Blue cattle revealed an 11-bp deletion in the third exon causing a frameshift in the active *C*-terminal domain of the gene. Double-muscled Piedmontese cattle have a G to A transition also in the third exon, that changes a cysteine to a tyrosine in the same highly conserved region of the gene.

Later, four additional myostatin loss-of-function mutations were discovered, disproving the initial hypothesis that double muscling would be genetically homogenous and, originating from the Shorthorn breed was then spread from the British Isles to the continent and the rest of the world. Several mutations are shared by more than one breed. Most breeds examined are genetically homogenous, but several show allelic heterogeneity. The molecular dissection of this trait now also allows correct genotyping of animals, and thus a better comparison of the three myostatin genotypes for important traits, a shortcoming that many earlier studies suffered from.

Naturally occurring functional mutations in the myostatin gene have further been described in humans, sheep, dogs, and horses, leading to similar hypermuscularity. In sheep, three different mutations have been reported so far. In the heavily muscled Texel breed, a single-nucleotide polymorphism in the 3′UTR of the gene was found that creates an illegitimate target site for two micro ribonucleic acids (miRNAs) that are highly expressed in skeletal muscle, causing a reduction in messenger RNA concentration and circulating myostatin protein levels, thereby contributing to the muscular hypertrophy. This hypomorphic allele segregates and causes an increase in muscle mass also in several other sheep breeds. No functional mutations in the myostatin gene have been discovered in other domestic farm animal species that have undergone intense selection for muscularity, such as pigs, poultry, and turkey. Only a putative mutation was found in the myostatin gene of Piétrain pigs, a breed that is also known for its heavily muscled phenotype. This fact could be fortuitous, but is likely indicating differences in myostatin physiology in different species.

The finding that an allelic series of myostatin loss-of-function mutations explain most of the cases of double muscling in cattle, demonstrates the important function of myostatin in skeletal muscle. Active myostatin seems to play a key role in regulating myogenesis and acts as an inhibitor of muscle development. The inactivation of the protein causes an increase in the number of late myoblasts because of increased myoblast proliferation or delayed differentiation into primary and secondary myofibres. Myostatin appears to inhibit myoblast proliferation by arresting cell cycle at the G_1-phase, and myoblast differentiation by downregulating the expression of differentiation related genes. Myostatin also maintains satellite cells in a quiescent state. The muscular hypertrophy of double-muscled cattle is mainly the result of muscle cell hyperplasia, i.e., individual muscle fibers are not larger but higher in number, though some muscle fibers are indeed larger. Recently, it was shown that postnatal inactivation of the myostatin gene in skeletal muscle is able to cause a generalized muscular hypertrophy of the same magnitude as that observed for constitutive myostatin knockout mice, demonstrating that myostatin regulates muscle mass not only during early embryogenesis but also throughout development. The growth inhibition of proliferating skeletal muscle myoblasts by myostatin appears to be widely conserved among not only mammalian vertebrates but also avians and fish.

The biological function of myostatin is, however, not restricted to suppressing skeletal muscle growth. Myostatin also appears to play a role in protein metabolism, and more specifically in the regulation of protein synthesis. It has also been shown to regulate glucose metabolism. The effects of myostatin on adipose tissue are less well established, but it is clear that inhibiting its activity results in a large decrease of the fat mass. Myostatin is expressed at low levels in adipose tissue, and increases fat accumulation and adipogenesis. Although this may simply be a consequence of metabolic changes in skeletal muscle, it is likely that myostatin has a direct role in adipose tissue also and in the cross-talk between skeletal muscle and adipose tissue. The bioactivity of myostatin is not solely mediated by increased synthesis or release from skeletal muscle, but requires proteolytic cleavages of the precursor protein. The propeptide, as well as several other ligands, for example, follistatin and GASP-1, prevent receptor binding and activity of myostatin. Follistatin is a potent myostatin antagonist, and transgenic Mighty mice overexpressing follistatin have even greater muscle mass than follistatin transgenics alone. Once activated, myostatin appears to signal by directly binding to its serine/threonine kinase receptor. Downstream intracellular signaling pathways for myostatin are multiple and involve Smad-mediated and non-Smad pathways. As mentioned above, a role for miRNAs in the regulation of myostatin expression has become evident. Conversely, myostatin may regulate the expression of miRNAs.

Evidently, muscle mass is under polygenic influence, and it should be stressed that as a result of ongoing selection, the muscular hypertrophy of the present-day Belgian Blue cattle is much more pronounced than the one of the mh/mh animals three decades ago. Similarly, there are large differences in muscularity between double-muscled animals of identical myostatin genotype from different breeds. However, it is unlikely that there are many other genes with comparable large effects on muscularity as the myostatin gene. The reason why myostatin null alleles, despite intense selection for improved carcass quality, still segregate at low or intermediate frequencies in most breeds tolerating double muscling, is that adverse side effects (see further) do not outweigh the tremendous benefits observed in lean meat yield from cattle possessing this mutation.

Another gene with a major effect on muscularity is the callipyge gene in sheep located on chromosome 18, subject to a special mode of inheritance, i.e., polar overdominance. Similar to double-muscled cattle, callipyge lambs have improved feed efficiency and carcass quality, but because the callipyge condition manifests postnatally, dystocia is not a problem. Unlike double-muscled animals, callipyge sheep show muscle cell hypertrophy, and meat of callipyge lambs consistently has lower tenderness scores, resulting from a reduced degree of calpain-mediated postmortem proteolysis (see Section Carcass and Meat Quality). Pigs having the mutation in the ryanodine receptor gene on pig chromosome 6 leading to the stress susceptibility syndrome as a result of a disturbance in the Ca^{2+} transport of skeletal membranes have also improved carcass quality compared to normal pigs. The biological implications of this mutation are now well established, including large effects on meat quality, but the mechanism for the increased muscle mass, characterized by muscle cell hypertrophy, in stress-susceptible pigs is not elucidated yet.

With the molecular genetics tools now being exploited, other genes affecting muscle development are likely to emerge, for example, the recently described regulatory mutation in the insulin-like growth factor II (IGF-II) locus in pigs with a major

effect on carcass quality and without apparent effects on meat quality. The increased muscularity associated with these genes is associated with differences in animal physiology, in muscle fiber histology and biochemistry and consequently also in meat quality. Similarities but also distinct differences in meat quality compared to the effects of the myostatin gene are thereby observed.

Physiology and Metabolism

Differences in circulating levels of various hormones and several other physiological parameters have been found in double-muscled compared to normal cattle, indicating an altered metabolism in favor of protein synthesis and lipolysis at the expense of lipid synthesis. The production of growth hormone is generally higher, whereas blood concentrations of insulin and IGF-I are normally lower. Concentrations of thyroid hormones are either not different or slightly lower. Small and mostly nonsignificant differences have been found for blood levels of cortisol, testosterone, glucose, α-amino nitrogen, and urea. Blood concentrations of triacylglycerols and nonesterified fatty acids have tendency to be lower and higher respectively. The blood level and urinary excretion of creatinine are clearly higher, in line with the higher muscle mass, whereas the opposite is the case for creatine. Other differences have been found too and it should be mentioned that the evolution of these parameters during development and growth, and the effect of management and nutrition conditions therein (e.g., compensatory growth) may differ at some points for double-muscled compared to normal animals.

Though considerable variation is observed across muscles, double-muscled cattle possess nearly 25% more muscle mass than those cattle lacking the mutation. Conversely, there are sizable reductions (approximately 5–40%) in bone and fat masses as well as significant reductions in the digestive tract size (Table 1). The skin is thinner and external genitalia are less developed. Newborn calves frequently have an enlarged tongue (macroglossia). Normally this disappears at young age, creating only temporary problems when suckling. When persisting, grazing is disturbed in adults. Macroglossia is typical for double-muscled calves. Other congenital and/or inherited disorders are not restricted to double-muscled animals only, but are nevertheless seen more frequently in this kind of animals. Brachygnathia superior and brachygnathia inferior, i.e., an abnormal shortness of the maxilla (upper jaw) and mandible (lower jaw) respectively, and locomotory problems such as extreme flexing as well as extreme stretching of the fore and hind legs, spastic paresis, and other joint problems are common. Some of these disorders apparently do not harm the animal and may even disappear with age. However, locomotory problems may become problematic when gaining weight. Double-muscled animals up to 1 year are more vulnerable to respiratory diseases, increasing calf mortality. The likely reason therefore is the underdevelopment of the cardiorespiratory system. The lower respiratory capacity in combination with the increased muscle mass is also at the origin of the greater susceptibility to exercise fatigue and heat stress. Reduced oxygen transport, aerobic metabolic activity of the muscle, larger heat production, and lower capacity for heat dissipation all contribute to greater stress susceptibility. It has also been suggested that double-muscled animals have a more excitable temperament, but research data to support this are scarce and this statement can be questioned in view of the generally very docile temperament and the lower level of spontaneous activity of these animals experienced in commercial practice.

Table 1 Effects of double muscling (myostatin loss-of-function) in cattle

	Double-muscled phenotype (mh/mh genotype)
Meat production	
Fertility	−
Calving difficulties	+ + +
Birth weight	+ +
Daily weight gain	− 0
Feed intake capacity	−
Feed conversion efficiency	+
Rusticity	− −
Carcass quality	
Dressing proportion	+ +
Organ weights	−
Carcass lean proportion	+ +
Carcass fat proportion	− − −
Carcass bone proportion	−
Cutability	+ + +
Meat quality	
Muscle fiber hyperplasia	+ + +
Muscle fiber hypertrophy	0
Color lightness	+
Myoglobin content, oxidative metabolism	−
Water-holding capacity	− 0
Myofibrillar weakening, proteolysis	−
Connective tissue content	− − −
Tenderness (high connective tissue content muscles)	+ +
Tenderness (low connective tissue content muscles)	0 +
Juiciness and flavor intensity	−
Intramuscular fat content	− − −

Note: The number of '+' or '−' indicates the degree of increase or decrease in double-muscle animals compared to normal animals, and '0' indicates no major effect.

Double-muscled cattle have reduced appetites and feed intakes compared to normal cattle as a result of the reduction of the size of the digestive tract (Table 1). Hence, they require adapted feeding systems for optimal or maximal production. When fed diets that meet their requirements, a more positive nitrogen balance and a reduced feed conversion ratio will be obtained. Urinary nitrogen excretion is significantly reduced, resulting in more efficient nitrogen retention. At the muscle protein level, the fractional rate of net synthesis appears similar, but the fractional rates of degradation and total synthesis are lower compared to normal animals. The rate of protein turnover, defined as the ratio of the fractional rates of net to total synthesis, is thus slower, explaining the higher efficiency of protein retention.

Reproduction, Growth, and Management

In general, fertility is reduced in double-muscled compared to normal cattle (Table 1). Sexual behavior is less distinct, making estrus detection more difficult for artificial insemination. Delays in puberty in heifers and males have been reported, but estrus characteristics and ovarian activity appear similar to normal animals. In males, testicular weight and semen volume and quality are reduced, but this poses little problem for reproduction. Reduced fertility in females is primarily manifested by a somewhat later age at first calving, longer intervals between calving and first estrus and between calving and first service, and a larger number of services per pregnancy. However, gestation length; rate of abortion; and incidence of placental retention, endometritis, and ovarian cysts are apparently not influenced. The most prominent difference with regard to reproduction is, however, the well documented higher frequency of calving difficulties (dystocia) due to a foeto-maternal morphological imbalance at calving. The higher birth weight and width of the calf especially at the trochanters in combination with reduced pelvic dimensions of the dam make delivery more difficult. Assistance on calving and ultimately cesarean section then become necessary in many cases. Part of the reduction in female fertility and rebreeding problems are associated with these calving difficulties. As expected, milk yield is substantially reduced in double-muscled cows.

Birth weight of double-muscled calves is significantly higher compared to normal calves. Postnatal growth rate is not much different or is slightly lower. The combination of comparable average daily weight gain and lower feed intake results in significantly improved feed conversion efficiencies. The improved feed conversion is primarily due to the altered composition of body weight gain toward relatively more protein and less fat deposition, and likely not the result of changes in feed digestibility or maintenance requirements. Feed digestibility is only slightly affected or is decreased on high roughage diets. It is unclear at present if maintenance requirements are really lower in double-muscled animals. Data on total energy requirements during growth and development are also inconclusive, but feed protein requirements are obviously higher in view of the altered composition of weight gain. For finishing, double-muscled animals need high energy (concentrate) diets due to the lower feed intake, and their performance is reduced on high-fiber diets. More generally, they require higher skilled management and are less adapted to harsh environmental conditions.

Animal performances are subject to the influence of many factors including breed, nutrition, and management. Hence, the effects of the double-muscled gene mentioned above may differ according to the breed, the ration, and the management conditions that are considered. However, it is clear that there are distinct differences for many traits that are either beneficial (efficiency of lean growth) or disadvantageous (reduced fertility and dystocia) to the overall production efficiency. The use of pure-breed double-muscled cattle in production systems around the world is limited, mainly because of the calving difficulties, and restricted to Europe where marketing systems favor high carcass yield. However, there is increasing interest for the use of double-muscled crossbreds in many beef producing countries. There is a large variability in the performances of progeny from crossing double-muscled and normal cattle. The likely strategy is to cross double-muscled males with normal females, hereby reducing reproductive problems and maintaining a considerable part of the superior carcass characteristics. Although many of the problems associated with the double-muscling condition are not experienced in crossbreds, these systems still require pure-breed herds to be maintained. The need for routine cesarean sections in pure-breeding systems is, however, sometimes also criticized on animal welfare grounds. More generally, the social acceptance of the use of extremely selected animals is expected to decrease in many societies. Hence, it is unclear at present if or to what extent double-muscled cattle will be utilized in the long term.

Carcass and Meat Quality

The largest merit of double-muscled animals lies in their superior carcass characteristics (Table 1). Because of the slower rate of fat deposition, slaughter maturity is delayed. Inversely, animals of this genotype can be finished to higher slaughter weights. Dressing proportion is significantly increased (approximately 5%) compared to normal animals because of the reduced digestive tract and the lower weight of skin and organs. At similar age or weight, carcasses of double-muscled animals have higher proportions of lean meat and lower proportions of fat and bone. Although prominence is generally given to the muscle hypertrophy in describing the double-muscled condition, the reduced development of the fat tissues is relatively much more distinct. The size but not the number of the fat cells is decreased. The reduction in bone proportion is more moderate. The muscle hypertrophy and the fat and bone hypotrophy are general but not uniform throughout the body. Especially superficial muscles and the hindlimbs compared to the forelimbs are most affected, but differences between studies as to the relative muscle hypertrophy are noticed. Bones of the limbs are shorter and thinner according to the same gradients observed for the muscles. The muscle to bone ratio is maximal at the level of the shoulders and the thigh where the hypertrophy is also most visible. At a comparable level of subcutaneous fat cover, a lower overall carcass fat content is found for double-muscled compared to normal animals. The nonuniform muscle hypertrophy and greater conformation in general results in a different size and shape of most meat cuts and in a higher proportion of more expensive cuts. In commercial practice, this effect of conformation and carcass cutability may add substantially to the difference in carcass value of double-muscled animals, irrespective of the difference in lean meat content. The combination of increases in dressing proportion, carcass lean content, and upgrading of some cuts may yield a difference in the proportion of high value cuts on a live weight basis that amounts to more than a quarter for pure-bred double-muscled compared to normal cattle.

As mentioned above on the genetic determination, the myostatin-deficient condition leads to an increase in muscle fiber number (Table 1). The contractile differentiation during the first two-thirds of gestation and the metabolic differentiation of aerobic oxidative metabolism during the last third of

fetal growth are delayed in double-muscled fetuses. A higher proportion of glycolytic muscle fibers at the expense of oxidative and oxido-glycolytic fibers are thus found at birth and throughout life in double-muscled cattle. Most reports indicate no major changes in the muscle fiber dimensions, and slightly lower as well as higher fiber sizes have been reported. Hence, the relative area of type IIB fibers is increased and the overall muscle metabolism is more glycolytic.

The more glycolytic muscle fiber type results in a faster muscle pH fall postmortem in double-muscled animals, whereas ultimate pH values are generally not different. Concomitantly, the meat is paler, illustrated by higher CIE L* values (Table 1). A lower ratio of CIE a*/b* values corresponds to a less red color tint in line with reduced levels of myoglobin. The higher rate of glycolysis early postmortem, in combination with the increased muscle mass, also leads to slightly higher muscle temperatures postmortem, and consequently an increased degree of protein denaturation. This is expected to affect water-holding capacity unfavorably. However, data on several measures of water-holding capacity have been variable. Slightly higher drip and purge losses are generally found, but lower, unchanged as well as higher cooking losses have been reported. Differences in color and water-holding capacity in comparison with changes in other traits are relatively moderate.

With respect to meat tenderness and palatability in general, literature concerning double-muscled cattle are coherent on most but not all points (Table 1). Meat tenderness and tenderisation are complex phenomena determined by a number of factors. The content and nature of connective tissue content together with the postmortem weakening of the myofibrillar and cytoskeletal network are considered the most important factors, provided that no extreme muscle shortening occurs during rigor development. No difference in sarcomere length in meat of double-muscled animals is observed under normal slaughtering conditions. A large reduction (approximately 25%) in muscle collagen content in double-muscled animals is reported in almost all studies. The perimysial connective tissue network is thinner, but the nature of the perimysial collagen in terms of solubility and crosslink concentrations on a collagen molar basis is not affected. The much lower content of connective tissue explains the upgrading of lower quality cuts to more expensive cuts, allowing for a larger and more homogenous distribution of high quality meat throughout the carcass. In muscles with a low content of connective tissue, like the Longissimus, the positive effect of double muscling on tenderness may be mitigated by reduced myofibrillar and cytoskeletal protein degradation that normally occurs during the tenderisation process. Double-muscled cattle have consistently lower μ-calpain, calpastatin, and cathepsin levels in the Longissimus, associated with changes in protein breakdown and in line with the reduced *in vivo* protein turnover. Total proteolysis and tenderization during full ageing seem to be lower in double-muscled animals. However, observations in the Longissimus indicate that proteolysis occurs at a faster rate early postmortem in double-muscled beef animals, consistent with the more glycolytic muscle fiber type and the earlier rigor development. Data for other muscles on enzyme activities and postmortem proteolysis are very scarce. The overall effect on shear force values is variable, depending on the muscle studied and on the time/temperature treatment of the meat. Across studies and muscles, shear force values of raw meat have always been lower due to the lower collagen content. The literature shows that cooking meat for 1 h at 75 °C, the recommended standard preparation method for shear force determinations, yielded higher values for double-muscled animals, but not in all studies. Because of extensive solubilization of collagen, this procedure of shear force determination can be regarded as a measure of myofibrillar toughness, but is not necessarily a good indication of overall tenderness. The higher myofibrillar toughness of double-muscled animals as a result of reduced proteolysis is apparently only reflected in higher shear force values in heated low-collagen muscles in some studies. Indeed, taste panel tenderness evaluations on cooked meat do always show higher tenderness ratings, although the benefit may be lower for muscles low in connective tissue. Hence, in general meat from double-muscled animals is more tender. Meat of double-muscled animals is particularly suited for raw consumption or after short time heating only, culinary preparation methods prevalent mainly in Western and Southern Europe. Regarding other taste panel parameters, lower juiciness, and beef flavor ratings have been reported, in line with the lower intramuscular fat content.

The meat composition in double-muscled animals is changed according to the altered carcass composition. The meat protein content is higher and, because of the lower collagen content, protein quality in terms of essential amino acids content is improved. The intramuscular fat content is approximately 25% lower when compared to normal counterparts. Differences in fatty acid composition in different fat depots have also been reported. In intramuscular fat, the triacylglycerol content is greatly reduced as a result of the lower fat deposition, whereas the phospholipid content is only slightly lower in line with the lesser amount of cell membranes of the more glycolytic muscles. Accordingly, the contents of saturated and monounsaturated fatty acids are significantly reduced, whereas the contents of polyunsaturated fatty acids are similar or slightly reduced. Consequently, the molar proportions of polyunsaturated fatty acids are significantly higher and those of saturated and especially monounsaturated fatty acids are lower. The ratio of intramuscular polyunsaturated to saturated fatty acids is thus higher in meat from double-muscled animals. Similar but less marked changes are to be expected in other fat depots. There are also indications for alterations in the n-6 and n-3 polyunsaturated fatty acid metabolism, based on differences in the proportions of the long chain fatty acids resulting from elongation and desaturation of linoleic and linolenic acid. The content of conjugated linoleic acids is similar relative to the sum of fatty acids, but is lower on muscle weight basis. Meat oxidative stability of double-muscled and normal animals has not been properly compared at present, but there are no indications for large differences.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Chemical and Physical Characteristics of Meat: Chemical Composition; Palatability; Water-Holding Capacity. Classification of Carcasses: Beef Carcass Classification and

Grading. **Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat:** Aging; Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. **Growth of Meat Animals:** Adipose Tissue Development; Endocrinology; Muscle; Physiology. **Muscle Fiber Types and Meat Quality. Sensory and Meat Quality, Optimization of. Species of Meat Animals:** Cattle. **Tenderizing Mechanisms:** Chemical; Enzymatic; Mechanical. **Tenderness Measurement**

Further Reading

Arthur, P.F., 1995. Double-muscling in cattle: A review. Australian Journal of Agricultural Research 46, 1493–1515.

Clinquart, A., Hornick, J.L., Van Eenaeme, C., Istasse, L., 1998. Influence du caractère culard sur la production et la qualité de la viande des bovins Blanc Bleu Belge. INRA Productions Animales 11, 285–297.

De Smet, S., Claeys, E., Demeyer, D., 2002. Muscle enzymes in relation to meat quality and muscularity. In: Toldrá, F. (Ed.), Research Advances in the Quality of Meat and Meat Products. Kerala, India: Research Signpost, pp. 123–142.

Fiems, L.O., Cottyn, B.G., Boucqué, Ch.V., *et al.*, 1997. Effect of beef type, body weight and dietary protein content on voluntary feed intake, digestibility, blood and urine metabolites and nitrogen retention. Journal of Animal Physiology and Animal Nutrition 77, 1–9.

Fiems, L.O., Van Hoof, J., Uytterhaegen, L., Boucqué, Ch.V., Demeyer, D., 1995. Comparative quality of meat from double-muscled and normal beef cattle. In: Ouali, A., Demeyer, D.I., Smulders, F.J.M. (Eds.), Expression of Tissue Proteinases and Regulation of Protein Degradation as Related to Meat Quality. Nijmegen: ECCEAMST/Audet Tijdschriften, pp. 381–393.

Gariépy, C., Seoane, J.R., Cloteau, C., Martin, J.F., Roy, G.L., 1999. The use of double-muscled cattle breeds in terminal crosses: Meat quality. Canadian Journal of Animal Sciences 79, 301–308.

Georges, M., 2010. When less means more: Impact of myostatin in animal breeding. Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry 10, 240–248.

Grobet, L., Poncelet, D., Royo, L.J., *et al.*, 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Mammalian Genome 9, 210–213.

Hanset, R., Michaux, C., 1985. On the genetic determinism of muscular hypertrophy in the Belgian White and Blue cattle breed I. Experimental data. Genetics, Selection and Evolution 17, 359–368.

King, J.W.B., Ménissier, F. (Eds.), 1982. Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production. The Hague: Martinus Nijhoff Publishers.

Raes, K., De Smet, S., Demeyer, D., 2001. Effect of double-muscling in Belgian Blue bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Animal Science 73, 253–260.

Rodgers, B.D., Garikipati, D.K., 2008. Clinical, agricultural, and evolutionary biology of myostatin: A comparative review. Endocrine Reviews 29, 513–534.

Wheeler, T.L., Shackelford, S.D., Casas, E., Cundiff, L.V., Koohmaraie, M., 2001. The effects of Piedmontese inheritance and myostatin genotype on the palatability of longissimus thoracis, gluteus medius, semimembranosus, and biceps femoris. Journal of Animal Science 79, 3069–3074.

Wray-Cahen, C.D., Kerr, D.E., Evock-Clover, C.M., Steele, N.C., 1998. Redefining body composition: Nutrients, hormones, and genes in meat production. Annual Reviews in Nutrition 18, 63–92.

Relevant Website

www.ncbi.nlm.nih.gov/omia/1280
Online Mendelian Inheritance in Animals Database.

DRYING

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Glossary

Effective water diffusion coefficient The amount of water (kg) that passes through a unit area (m^2) in a unit of time (s) under the influence of a concentration gradient of one unit. Diffusion coefficient of water during drying of meat products depends on several factors such as temperature, water, and salt content. The SI unit of diffusion coefficient is $(kg\ (m^{-2}\ s)^{-1})$. The value of this coefficient for diffusion of water in meat typically ranges from 10^{-10} to 10^{-11}.

Heat capacity The amount of heat required to raise the temperature of the material by 1 °C. The SI unit of heat capacity is $J\ kgK^{-1}$. For nonprocessed meat values range approximately from 15 000 to 30 000.

Quick-dry slice process (QDS process®) QDS process is an accelerated drying process for sliced meat products that is applied to the sliced product directly after the fermentation step before the long drying phase. This process results in a dramatically reduced total processing time.

Thermal conductivity A measure of the ability of a substance to transfer heat. The SI unit of thermal conductivity is $(W\ mK^{-1})$. For nonprocessed meat values range from 0.1 for fat to 0.5 for lean.

Vapour pressure of pure water (p_o) The pressure at which distilled water vapour is saturated at a given temperature. The SI unit of p_o is Pa.

Vapour pressure of water in food (p) The pressure exerted by the water vapor in the foodstuff which is in equilibrium with the surrounding air. The SI unit of p is Pa.

Water activity (a_w) Water activity is defined (for practical purposes) as the ratio of the vapor pressure of water in a material (p) to the vapor pressure of pure water (p_o) at the same temperature. When vapor and temperature equilibrium are obtained, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percent $a_w = p/p_o = \text{ERH}\ (\%)/100$.

Introduction

Drying is a process in which water is removed from a material by evaporation, sublimation, or an osmotic process. Evaporated water is carried away by a stream of air, or it diffuses to an absorber or to a cold surface on which it is deposited as ice (frost) or water drops. Drying with the use of air is called convective drying, whereas diffusion of water molecules predominates in vacuum drying and freeze drying. Evaporation of water from the surface of the material being dried takes place at any temperature when there is a water activity gradient, but the higher the temperature the higher is the rate of drying, especially at the beginning of the process.

The aim of drying is to increase the shelf life of the product and to create new, sometimes unusual, properties in the final product. Dry-cured ham, semidry, and dry sausages are good examples of controlled drying that imparts and develops special texture and flavor in the product. It is worth mentioning that sometimes drying is undesirable because of weight loss and an unpleasant appearance of the dry surface; freezer burn is an example.

Drying is probably one of the oldest methods of food preservation. It takes advantage of the fact that only part of the water in food has the properties of bulk water, i.e., it is a good solvent and an environment in which biological reactions take place. Removing that part of water from food ensures its microbial stability and limits or inhibits chemical and enzymatic reactions.

The state of water in food results from the structure of the water molecule and its interactions with the remaining food constituents. The phenomenon of interaction between water and solute molecules is termed hydration. The nature and extent of hydration shells surrounding the solute molecules depend on the kind of hydrated food constituent. Few water molecules surround ions and small molecules, whereas macromolecules such as proteins or polysaccharides can be hydrated by hundreds of water molecules.

The properties of water in hydration shells differ from those of bulk water. Numerous experiments have shown that

[†]Deceased.

part of water in food is so strongly associated with food constituents that it is not able to form crystals during freezing. The state of water in food is expressed by its activity coefficient, a measure of the thermodynamic chemical potential of water in the system. Under isobaric conditions and at temperatures typical for food processing (−20–120 °C), the properties of water vapor do not differ much from those of an ideal gas. Hence, the activity coefficient can be expressed as the ratio of the vapor pressure of water in food (p) to the vapor pressure of pure water (p_o) at the same temperature and total pressure. The activity coefficient is called the water activity (a_w) and is expressed by the equation:

$$a_w = \left[\frac{p}{p_o}\right]_{P,T}$$

The water activity of pure water is 1 and for absolutely dry material it equals 0. Fresh meat, in the lean portion, has $a_w = 0.98–0.99$. The water activity of meat products is dependent on the additives used and the processing applied. Sausages such as Bologna type, liver sausage, or blood sausage all have water activities in the range 0.93–0.98. Dry-cured ham has a water activity in the range 0.88–0.96, and the value depends strongly on the position within the ham and on the process used. Country-cured hams, ripened or dried for months, have much lower water activities than do raw hams subjected to a short ripening process. Fermented sausages usually have a similar range of water activity but, in some products, can reach a value as low as 0.72. Dried products usually have water activity below 0.7.

Foods that have water activity from 0.60 to 0.90 and moisture between 10 and 40% are called intermediate-moisture foods (IMF). Such meat products as dry-cured ham, semidry and dry sausages, and cabanossi and salami all belong to the IMF classification.

Drying is one method to reduce water activity in food. Evaporation of water removes mostly that portion of the water that has the properties of bulk water, i.e., water activity close to 1. Increasing the proportion of water associated in hydration shells reduces the water activity of the food. The lower the water content, the lower the water activity of the food. However, the water activity and water content are not directly proportional (linear). The actual relationship at a given temperature is called a sorption isotherm (Figure 1). This enables one to predict the water activity of a given food at a prescribed water content. The sorption isotherm is characteristic for a given food, and its shape and course depends on the temperature, chemical composition, and structure of the material.

Most foods have sigmoid isotherms, which means that at the low a_w and high a_w ends of the curve, large changes in water content are needed to change the water activity by a small fraction. In the middle, usually at water activities between 0.2 and 0.6, comparatively small changes in water content cause large changes in water activity. The sigmoid type of isotherm is typical of foods containing proteins and polysaccharides. Foods containing mostly small molecules, for example, sugar or acids, show isotherms that are concave downward.

Drying as a method of reducing water activity in food can be assisted by the addition of substances that lower the water activity of the material. One of the effective depressors of water activity is sodium chloride (common salt, NaCl). At a concentration of 10% w/w, the water activity of the solution at 20 °C is 0.935; at 20% the water activity is 0.839. A saturated NaCl solution has a water activity of 0.755. Hence, by combining salting and drying, products with a sufficiently low water activity can be obtained without excessive dehydration. Good examples of products manufactured this way are charqui, an important meat product in rural Brazil; biltong, produced in South Africa; cecina, characteristic of the province of Leon in Spain; basturma (also known as pastirma), produced in many counties throughout the Caucasus, Balkans, and Middle East; and the traditional Cuban tasajo. All these products belong to the IMF classification.

Drying of Solid Foods

The design and organization of food dehydration depends, in the first place, on the state and kind of material subjected to

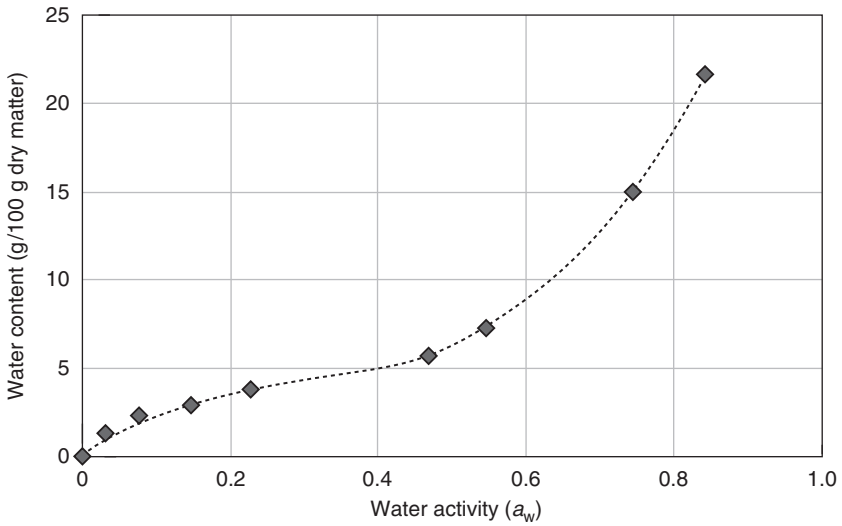

Figure 1 Water sorption isotherm of dried beef.

drying. The state of the food, liquid or solid, determines the equipment, process design, and arrangement.

Drying of solid food is a process in which water present within the microstructure of the solid is evaporated to the surrounding environment. During drying, two processes occur simultaneously:

- Transfer of energy.
- Transfer of water from the interior to the surface of the solid and its subsequent evaporation to the surrounding environment.

Transfer of energy as heat occurs, in most cases, as a result of convection and conduction. In some cases, radiation is also used as a means of energy transfer. Using dielectric, radio-frequency, or microwave energy, heat is generated internally within the solid.

In convective drying of solids, heat is transferred from the hot air to the surface of the solid. This process is dependent on air velocity and its temperature and humidity. The direction of air flow, the size and shape of the solid, and its degree of agitation also affect the rate of heat transfer. Heat transferred from the hot air to the surface of the solid is subsequently conveyed into the interior of the solid. In solid food, conduction is the prevailing mechanism of heat transfer within the solid. It depends on the difference between the temperature of the surface of the solid and the temperature of the coldest point within the solid, on the structure of the solid (its porosity), and on its thermophysical properties (heat capacity and thermal conductivity). In fibrous foods, the direction of heat flow in relation to the orientation of the fibers (parallel or perpendicular) is also important.

Heat transfer in the drying of solids involves two processes: external and internal movement of heat (Figure 2). Hence, two sources of resistances to heat transfer operate: external and internal; these are also referred to as resistance to convective and to conductive heat transfer, respectively. Resistance to conductive heat transfer is usually larger than that for convective heat transfer and is strongly dependent on the water content in the solid. The lower the water content, the larger is the internal resistance to conductive heat movement. As a result, heat transfer within the solid during the final stages of drying is more difficult than at the beginning of the drying process.

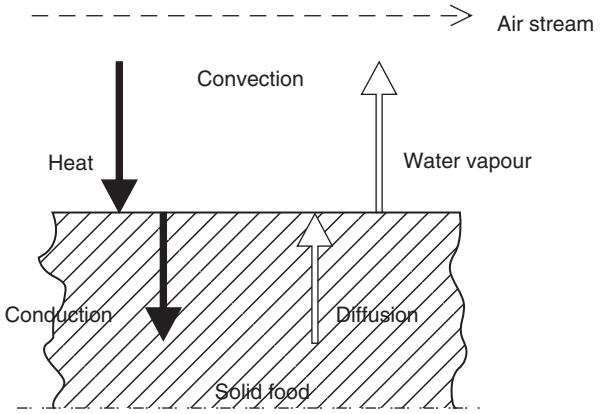

Figure 2 Heat and mass transfer during solid food drying.

The relationship between external and internal heat transfer resistances is important for the quality of the dried product and course of the drying process. A large external heat flux (due to a large difference between the hot air temperature and the temperature of the surface of the solid) sometimes cannot be conveyed within the solid at a sufficiently high rate. In this case, the surface of the solid absorbs most of the energy, water is evaporated rapidly, and a dry layer that is less permeable to water is formed on the surface of the solid. Further evaporation of water is slowed down; drying is reduced and overheating and scorching of the surface can occur.

In some drying methods, heat is supplied to the solid by conduction, which requires very good contact between the surface of the solid and the surface of the heating plate. Hence, this means of heat transfer can be used for drying paste-like materials, which can be spread on to the heating surface, or materials formed (cut) as parallelepipeds. Being in contact with the heating plate, the surface is excluded from the water evaporation process.

Transport of water in the solid being dried proceeds again in two steps (Figure 2). First, water is transported from the interior to the surface of the solid. Different mechanisms for water movement within the solid are applicable, but diffusion and capillary flow prevail in foods; capillary flow occurs only in porous bodies. Second, water is evaporated at the surface of the solid and is transferred as vapor to the surrounding air. This process is a convective mass transfer. Hence, two resistances to transport of water operate during the drying of solids: internal and external mass transfer resistances. The internal mass transfer resistance depends on the effective water diffusion coefficient (which accounts for all possible mechanisms of water transport in the solid) and the temperature of the solid. In moist foods, the effective water diffusion coefficient is weakly dependent on water content and is assumed constant. However, at low water contents (below 20–30% w/w) the dependence of the effective water diffusion coefficient on the water content is very strong. A decrease in water content by a few percentage points can lower the effective diffusion coefficient by two or three orders of magnitude. The external mass transfer resistance depends on the same variables that are responsible for convective heat transfer.

The relationship between external and internal mass transfer resistances affects the course of drying. If the external resistance is larger than or equal to the internal resistance, the flux of water reaching the surface of the solid is constant and drying proceeds at a constant rate. Drying at a constant rate can occur with very moist foods (e.g., some vegetables and fruits) but usually does not occur when the initial water content in a material undergoing drying is below 70–80%. If the internal resistance is larger than the external, less and less water is transported to the surface of the solid. The flux of evaporated water decreases with time and the drying proceeds at a falling rate. The course of drying can be presented as the rate of drying curve (Figure 3), and the transition from the constant to the falling rate of drying occurs at the critical moisture content.

The mechanism of solids drying presented above shows that drying is a simultaneous process of heat and mass transfer in which four resistances occur. In general, internal mass transfer resistance is the most important factor and determines the rate of drying and time the process takes. There are ways to

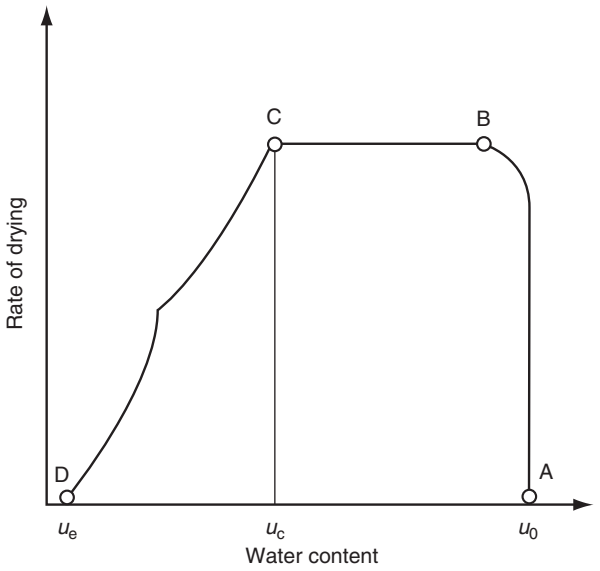

Figure 3 Rate of drying curve for moist food. AB, initiation of drying; BC, period of constant rate of drying; CD, period of falling rate of drying; u_0, initial water content; u_c, critical water content; u_e, equilibrium water content. Reproduced with permission from Lewicki, P.P. (Ed.), 1999. Inzynieria Procesowa I Aparatura Przemyslu Spozywczego (Food Processing Engineering and Food Processing Equipment). Warsaw, Poland: Wydawnictwa Naukowo-Techniczne, © 1999 Wydawnictwa Naukowo-Techniczne.

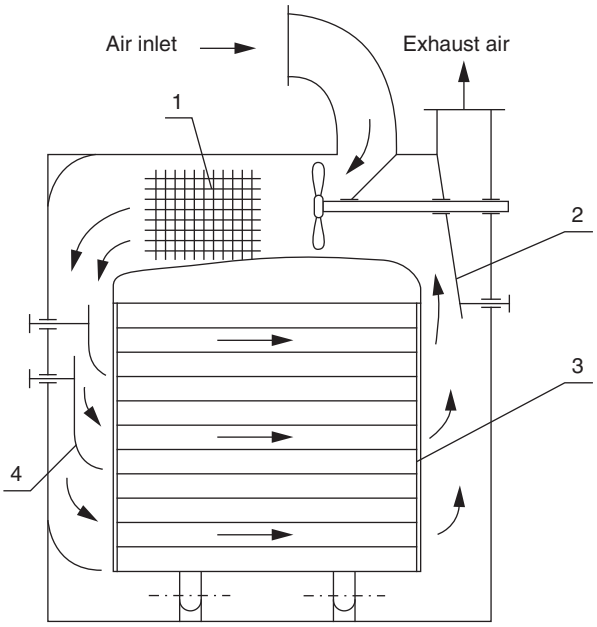

Figure 4 Cabinet dryer: 1, heater; 2, recirculation damper; 3, trolley with shelves; 4, baffles. Reproduced with permission from Lewicki, P.P. (Ed.), 1999. Inzynieria Procesowa I Aparatura Przemyslu Spozywczego (Food Processing Engineering and Food Processing Equipment). Warsaw, Poland: Wydawnictwa Naukowo-Techniczne, © 1999 Wydawnictwa Naukowo-Techniczne.

affect the internal resistance to mass transfer and to take advantage of an increased value in designing the drying process.

The internal resistance to mass transfer can be modified by the predrying treatment of raw material, by its size and shape, and by the variables of the drying process. Predrying treatments include mincing, heating, cooking, freezing, and thawing. Mincing destroys internal microstructure, breaks cell walls and cell membranes, and releases the liquid phase. Minced material forms a capillary porous body in which cavities are filled with liquid, which can easily be transported to the surface for evaporation. Heating and cooking of food causes denaturation of proteins. In effect, cell membranes lose their semipermeable properties, and water movement in the tissue becomes easier. Moreover, denatured proteins are hydrated to a lesser extent than native ones and hence some water is released from the hydration shells. Freezing, especially slow freezing, leads to the formation of large ice crystals, which injure the tissue. Subsequent thawing yields material with destroyed or degraded microstructure, and the drying properties of the material become similar to those of minced material.

The size and shape of the solid are also important. Transport of water from the wet center to the surface is dependent on the concentration gradient (the difference in water concentrations divided by distance). The larger the concentration gradient, the larger is the water flux. Because the concentration gradient is inversely related to the distance over which the water is transported, the water flux or the rate of drying is smaller for larger pieces of food. The shape of the solid also affects the drying rate. For the same distance of water transport, a long cylinder dries twice as fast, and a sphere three times as fast as a plate (slab). The numbers are theoretically derived, but in practice a sphere always dries more rapidly than a cylinder and a cylinder loses water more rapidly than a plate.

Temperature, air velocity, and humidity are important variables of the drying process. Transport of water is faster at higher solid temperatures. Hence, increasing the air temperature causes faster drying. The velocity of the air affects external heat and mass transfer resistances; increased velocity reduces the resistances. However, high air temperature and velocity can lead to rapid evaporation of water from the surface and formation of a crust. The humidity of the air affects the external mass transfer of water; high humidity causes slow drying and prevents crust formation on the surface of the solid. In some processes, the drying air is specially humidified in order to slow down the rate of water evaporation.

Solids can be dried in a variety of types of dryers, but drying of meat and meat products can only be done in certain dehydrators: cabinet dryers, belt dryers, and vacuum tray or rotary dryers.

Cabinet Dryers

A cabinet dryer (Figure 4) is built as an insulated chamber with trays stacked one above another on which material is loaded. The trays may be arranged onto trolleys or may be stacked individually into the slots of the cabinet. Air, forced by a fan, passes through the heater and is then baffled parallel to or across the trays loaded with the product. The flow of air through the layers of the product creates better conditions for

heat and mass transport. Cabinet dryers can be equipped with a recirculation damper, which makes it possible to take part of the exhaust air, mix it with fresh air, and use the mixture as a drying medium. This not only saves energy but also primarily increases the humidity of the air and prevents case hardening and crust formation on the surface of the solid.

Cabinet dryers are batch dryers that are relatively easy to set and control the optimum conditions of the process. They are thus good for drying heat-sensitive materials.

Belt Dryers

A belt dryer is suitable for drying small pieces of cut food. It comprises a long, rectangular drying chamber through which a finely woven wire mesh moves on rollers (Figure 5). Air is supplied from the bottom of the belt and can be recirculated. The speed of the belt is low and can be regulated over a broad range. At a speed of 0.1 m min^{-1} with a drying section 45 m in length, the residence time of the material undergoing drying is 7.5 h. This drying time can be too short for large pieces of food. In this case the belt dryer is used to remove most of the water from the material and final drying is done in another type of dryer, the bin dryer. The bin dryer is a container with a wire mesh bottom in which partly dried material is placed in a thick layer. Air is forced through the layer, and water evaporates until the required moisture content of the material is reached.

Belt dryers have large yields and can be used in large-scale production. Some products can stick to the belt and can be difficult to remove at the discharge end of the dryer. A long drying section can be divided into sections in which the temperature of the air can be adjusted according to the heat sensitivity of the material being dried.

Vacuum Dryers

Rapid drying under atmospheric pressure requires temperatures as high as 70 °C and large volumes of air in contact with the product. This affects quality disadvantageously. To lower the drying temperature and eliminate contact with the air, drying under vacuum can be applied. In vacuum dryers, heat is supplied by conduction and external mass transfer resistance is much larger than that in convection dryers. Hence, drying is a long process, but the quality of the final product is superior to that obtained in convection dryers.

Vacuum cabinet dryers are designed as hermetically insulated chambers in which solid trays are heated by circulating a suitable heating medium. Contact between the food and tray surface is very important as well as the amount of food loaded on the tray. Product sticking to the tray surface can create a problem with emptying and cleaning the dryer.

The vacuum rotary dryer is a stationary, jacketed, cylindrical shell, mounted horizontally with a revolving center tube on which a set of paddle arms with blades is mounted (Figure 6). The heating medium is delivered to the jacket, hollow center tube, and paddle arms. Solid pieces of food are loaded into the cylinder, the vacuum is pulled down, and stirring or agitation is initiated by rotating the center tube. Mixing the material prevents sticking to the heating surface, exposes the material's surface to evaporation, and facilitates heat transfer by conduction. Vacuum rotary dryers are batch dryers; they provide good control of the process parameters and the rate of drying is faster than that in vacuum cabinet dryers.

Freeze Dryers

Freeze drying is another method of drying under vacuum. However, in this case the food is frozen before drying and drying parameters are below the triple point of water (Figure 7). Pure water can exist in all three phases – as gas (vapor), liquid, and solid (ice). The triple point is that combination of pressure and temperature at which all three phases are in equilibrium and can coexist. When the parameters are above the triple point, the change from solid to vapor must go through the liquid phase: hence the melting of solid occurs. When temperature and pressure are kept below the triple point values, sublimation occurs and a direct change of ice to vapor

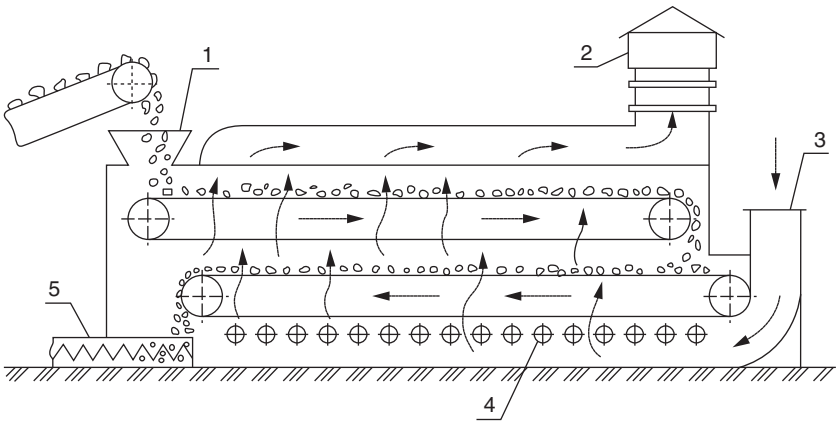

Figure 5 Belt dryer: 1, feed; 2, exhaust air chimney; 3, fresh air inlet; 4, heater; 5, dry product outlet. Reproduced with permission from Lewicki, P.P. (Ed.), 1999. Inzynieria Procesowa I Aparatura Przemyslu Spozywczego (Food Processing Engineering and Food Processing Equipment). Warsaw, Poland: Wydawnictwa Naukowo-Techniczne, © 1999 Wydawnictwa Naukowo-Techniczne.

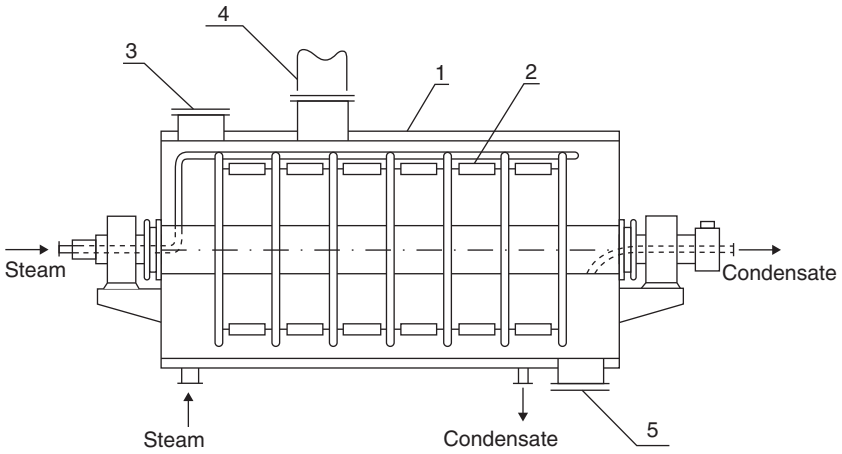

Figure 6 Vacuum rotary dryer: 1, jacketed cylinder; 2, paddles; 3, feed; 4, connection to vacuum pump and condenser; 5, dry product outlet.

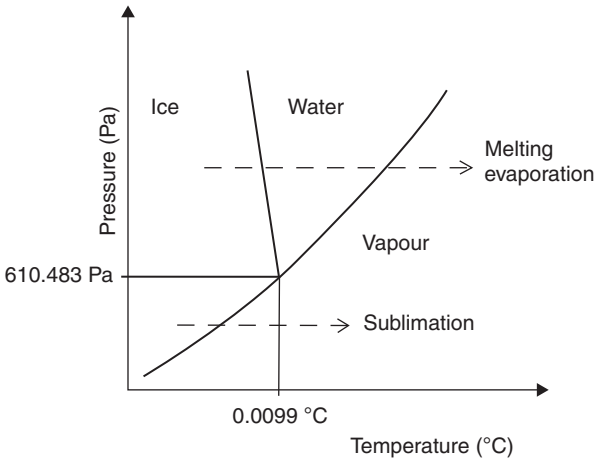

Figure 7 Pressure–temperature phase diagram for pure water.

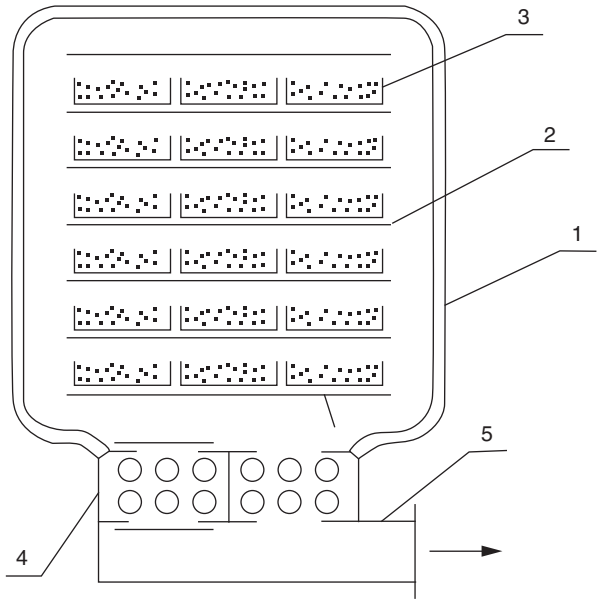

Figure 8 Freeze dryer: 1, drying chamber; 2, heated shelves; 3, product; 4, condenser (resubliming surface); 5, connection to vacuum pump.

takes place. Because the water vapor pressure over food is lower than over ice, the pressure in a freeze dryer must be kept well below 610 Pa; it is usually 200 Pa or below. The temperature of the material is also kept well below the freezing point of the food; usually it is below −10 °C.

Before the freeze drying process, the food is frozen in auxiliary equipment and then loaded to the freeze dryer. Freezing is a very important process and strongly affects both the sublimation process and the quality of the freeze-dried product. The larger the ice crystals and, in consequence, the more injured the tissue, the easier will be the transport of water vapor through the dry porous tissue, leading to a relatively short freeze drying period. However, the adverse influences of slow freezing on food quality are well known and, in designing the freeze drying process, a compromise must be made between process time and the quality of the product.

A freeze dryer (Figure 8) is usually built as a cabinet dryer equipped with shelves heated electrically (for small-scale production) or with circulating hot mineral oil. Frozen product is loaded onto shelves, vacuum is pulled down to the required value, and controlled heating is started. The heating is controlled in such a way that the product never reaches a temperature at which melting would occur. Water vapor sublimed from the product is resublimed (i.e., it forms ice) on the condenser, whose temperature is well below the sublimation temperature (−40 to −50 °C).

Heat and mass transfers in freeze drying are hampered by large internal and external resistances. Heat is conducted through the frozen layer to the zone of ice sublimation, whereas water vapor must pass the already dry layer and reach the condenser by diffusion. Large resistances are responsible for long freeze drying times, which are several hours and may exceed 24 h.

Freeze-dried products are superior in quality to those dried under atmospheric pressure. However, freeze drying is the most expensive method of drying because of very low pressure, the freezing of the food, and long process time.

Figure 9 Drying room for fermented sausages.

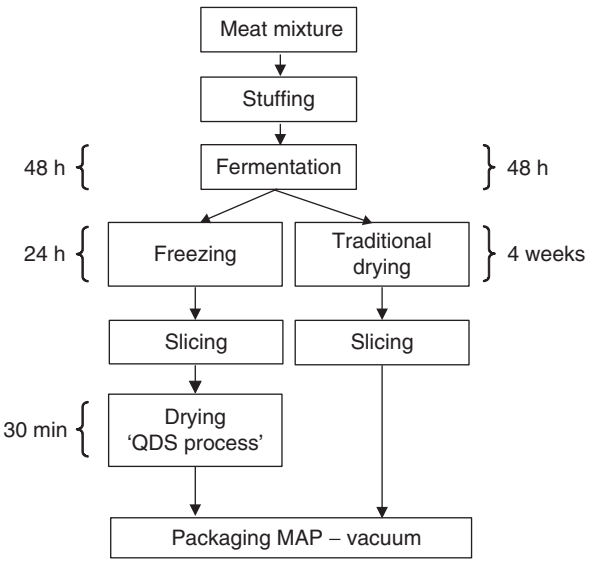

Figure 10 Comparison between traditional and Quick-dry slice process for sliced fermented sausages.

Traditional Meat-Drying Facilities

Some traditional meat products such as dry-cured ham, semidry, and dry sausages are obtained by removing a certain amount of water from the material by evaporation. This is a drying process, but it is done under specific conditions in drying rooms (Figure 9).

The design of a drying room makes it possible to control temperature, humidity, and air flow. Usually the air circulates in the room, and this can be achieved by natural or forced convection. Drying of dry-cured ham is done at increasing

Figure 11 Quick-dry slice process facility for sliced fermented sausages.

temperatures ranging from 3 °C to room temperature. During that time 30–40% of initial weight is lost and the water activity of the product (especially on the surface) decreases sufficiently to prevent microbial spoilage. Circulating air is changed 15–50 times per hour.

Drying of sausages depends on their variety and the diameter of the product. Stuffed sausages are first fermented by lactic acid bacteria and then hung in a drying room with air relative humidity of approximately 80%. The sausage loses some water but case hardening is avoided. In some cases, the air temperature is lowered to approximately 10 °C and the relative humidity to 70%. The air is circulated by a fan dimensioned to move from 15 to 75 times the chamber volume per hour. The process continues for 15–60 days, and the sausages lose 25–50% of their original weight. The growth of inoculated molds on the surface of sausages can be promoted by controlling the air's humidity and temperature to produce special types of sausages. Addition of glucono-δ-lactone to the batter of raw fermented sausage (salami type) strongly accelerates the acidification of sausage chubs and thereby substantially shortens the period of ripening.

Recently, a new drying-maturing process, quick-dry slice (QDS), has been proposed for sliced products (Figures 10 and 11). Sausages are fermented to the desired pH and are then frozen, sliced, and dried in a continuous system that combines

both convective and vacuum drying. With the QDS system, the traditional drying process could be reduced to 30 min, without any negative effect on safety. The QDS process reduces the acid taste and increases the color intensity of fermented sausages. Moreover, some sensitive colorants (e.g., Ponceau 4R) do not fade during the process.

Drying of Solutions and Suspensions

The most versatile method of drying liquids is spray drying. A solution or suspension with appropriate viscosity or consistency is sprayed into a cylindrical chamber in which it comes into contact with hot air. Spray drying consists of three steps: atomization of the fluid, the drying of droplets, and the separation of dry powder from the air stream (Figure 12).

Atomization is achieved using a rotating wheel or a nozzle. This is the most important operation in the spray drying process. The method of fluid atomization and its variables determine the size and the size distribution of the drops, and their trajectory and speed in the drying chamber. The size of the drop determines the heat and mass transfer surface and influences heat and mass transfer resistances. Generally, the smaller the droplet diameter, the faster is the drying. Another important variable characterizing the spray is the size distribution. Because the rate of drying is inversely proportional to the droplet diameter, it is evident that the variability of the drying time will increase with the variability of the drop size. However, the stream of hot air conveys the drops, and their residence time in the drying chamber is not very dependent on their size. In consequence, small particles leave the drying chamber with a low water content and can be overdried, whereas large particles can be moist and underdried. The difference in moisture contents of small and large particles can be detrimental to the quality of the product during storage.

Drops formed by a rotating wheel atomizer are 20–250 μm in diameter. The area for evaporation is large and drying takes a few seconds. Rapid evaporation maintains a low droplet temperature. Hence, high drying air temperatures can be applied, in the range of 150–200 °C. Particles leaving the drying chamber have temperatures between 70 and 90 °C.

The drying chamber is a cylinder with a conical bottom in which the spray makes contact with the hot air. The most common design is a concurrent system in which the droplets fall down the chamber with the air flowing in the same direction. Circulation of air in the drying chamber and trajectories of the particles undergoing drying should prevent the deposition of wet particles on the chamber wall.

The dry powder is collected at the bottom of the drying chamber. It can be removed together with the exhaust air and product in the powder/air separation system. Commonly, the exhaust air is removed at some height above the bottom of the drying chamber. The product then accumulates at the bottom of the chamber and is discharged by means of a valve. Exhaust air carries only the fine particles, which are separated from the gas stream in the separation system.

Separation systems consist of cyclones though, in some cases, bag filters are used. In cyclones, particles with a diameter larger than 15 μm are removed with high efficiency. Bag filters remove particles with diameter larger than 2 μm.

Because of low product temperature and short drying time, spray drying is suitable for the drying of heat-sensitive products. However, economic spray drying can be done only with large-scale production. Meat extracts and purées can be dried by a spray drying technique.

One product commonly manufactured by spray drying is dried blood plasma. This is concentrated by evaporation and is then fed to a spray dryer. The inlet temperature of the hot air is between 120 and 190 °C, but the outlet temperature should not exceed 70 °C. The drying time is very short and ranges from 2 to 4 s. The moisture content of dried blood plasma is between 5 and 8%.

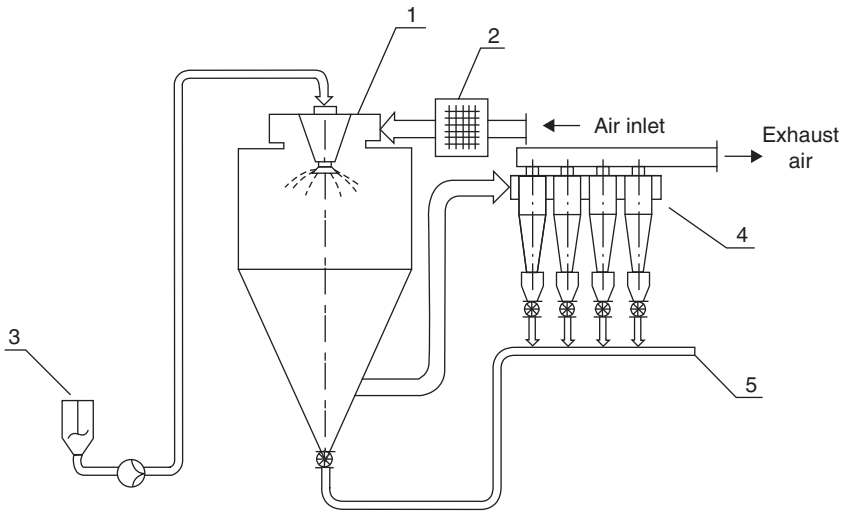

Figure 12 Spray dryer: 1, drying chamber; 2, heater; 3, feed; 4, cyclones; 5, dry powder outlet. Reproduced with permission from Lewicki, P.P. (Ed.), 1999. Inzynieria Procesowa I Aparatura Przemyslu Spozywczego (Food Processing Engineering and Food Processing Equipment). Warsaw, Poland: Wydawnictwa Naukowo-Techniczne, © 1999 Wydawnictwa Naukowo-Techniczne.

Quality of Dried Products

Drying causes numerous physical and chemical changes in food. Some of these changes can be used to enhance the quality and nutritional value of the food or to create new properties that are appreciated by consumers.

Physical changes caused by drying include shrinkage, shape alteration, surface modification, and modification of mechanical properties. Most of these texture modifications are due to moisture and temperature gradients occurring in the solid being dried. The larger the gradients, the larger are the stresses developed in the material. Under the stress, the material shrinks, its shape is deformed, and the surface develops wrinkles and creases. Shrinkage, shape distortion, and surface modification depend on the method of drying and the extent of dehydration. Convective drying at a low drying rate creates small moisture gradients. Hence, the stresses developed in the material are small; the body shrinks uniformly; there is no shape change; and the surface, owing to the material's elasticity, evenly follows the change of size of the solid body. Under these conditions, shrinkage is equal to the volume of the evaporated water. The traditional method of dry-cured ham production is a good example of such a process.

Convective drying done at high drying rates can produce large shrinkage stresses. The dry surface loses elasticity and cannot follow the change of volume. Consequently, the surface wrinkles and creases. If the drying is too fast, internal cracks and fissures are formed. These are the typical events occurring during spray drying, though they cannot be seen by the naked eye. Meat dried by convection or under vacuum is also exposed to these physical changes, some of which take place during production of semidry and dry sausages.

In freeze drying there is no liquid phase, hence these are no moisture concentration gradients. Temperature gradients are too small to create large stresses and freeze-dried material retains the shape and size of the frozen material. Freezing is the step in the freeze drying process that causes injury to the internal structure of the material.

Evaporation of water and increased concentration of solids change the mechanical properties of the solid. Resistance to deformation, chewiness, juiciness, ease of biting, cutting, slicing, etc., are altered and the extent of the change depends on the kind of dried material, the degree of dehydration, drying mode, and predrying treatment applied.

Chemical changes caused by drying are mainly due to elevated temperature and increased concentration of solubles. In some cases, contact with air can have deleterious effects on the quality of the final product. Increased concentration of solubles can affect the quality of the product at water contents at which water can act as a solvent. Hence, changes of the degree of ionization, redox potential, solubility, and the catalytic activity of food constituents can be expected in material undergoing drying at high and moderate water contents. Proteins can be denatured or their spatial conformation can be changed, and high concentrations of reactants can promote chemical reactions. Nonenzymatic browning reactions can occur, and precursors can be formed that facilitate color changes during storage of dried product. Contact with oxygen causes oxidation of lipids and oxidation of pigments that occur naturally in food.

Physical and chemical changes caused by drying usually affect the quality of the product unfavorably. However, with an appropriate choice of drying variables and mode of drying, suitable equipment, and predrying treatment, physical and chemical changes can be controlled and high-quality or novel sensory attributes can be obtained. Near infra-red equipment has been proposed as an on-line system to measure the water content and water activity at the product surface. This information can be used to set up the drying conditions in the drier (relative humidity, temperature, and air flow) as a function of the product characteristics.

See also: Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Germany; Mediterranean; Poland. Ham Production: Dry-Cured Ham. Packaging: Modified and Controlled Atmosphere. Refrigeration and Freezing Technology: Applications; Thawing. Sausages, Types of: Dry and Semidry

Further Reading

Bender, A., 1992. Meat and meat products in human nutrition in developing countries. Food & Nutrition Paper 53. Rome: FAO.

Chang, S.F., Huang, T.C., Pearson, A.M., 1996. Control of the dehydration process in production of intermediate-moisture meat products: A review. Advances in Food and Nutrition Research 39, 71–161.

Collell, C., Gou, P., Picouet, P., *et al*., 2012. NIR technology for on-line determination of superficial a_w and moisture content during the drying process of fermented sausages. Food Chemistry 135, 1750–1755.

Doe, P.E. (Ed.), 1998. Fish Drying and Smoking: Production and Quality. Lancaster, PA: Technomic Publishing.

Greensmith, M., 1998. Practical Dehydration. Cambridge, UK: Woodhead Publishing.

Hui, Y.H., Wai Kit, Nip, Rogers, R.W., Young, O.A. (Eds.), 2001. Meat Science and Applications. New York: Marcel Dekker.

Kudra, T., Mujumdar, A.S. (Eds.), 2001. Advanced Drying Technologies. New York: Marcel Dekker.

Lewicki, P.P., 1998. Effect of pre-drying treatment, drying and rehydration on plant tissue properties: A review. International Journal of Food Properties 1, 1–22.

Mujumdar, A.S. (Ed.), 1995. Handbook of Industrial Drying, Vols. 1 and 2. New York: Marcel Dekker.

Oetjen, G.W., 1999. Freeze-drying. Weinheim: Wiley–VCH.

Rahman, S. (Ed.), 1999. Handbook of Food Preservation. New York: Marcel Dekker.

Stawczyk, J., Muñoz, I., Collell, C., Comaposada, J., 2009. Control system for sausage drying based on on-line NIR a_w determination. Drying Technology 27, 1338–1343.

Turner, I., Mujumdar, A.S. (Eds.), 1996. Mathematical Modeling and Numerical Techniques in Drying Technology. New York: Marcel Dekker.

van't Land, C.M., 1991. Industrial Drying Equipment. Selection and Application. New York: Marcel Dekker.

Varnam, A.H., Sutherland, J.P., 1995. Meat and Meat Products. London: Chapman and Hall.

Relevant Websites

http://www.frigomeccanica.it/
Frigomeccanica S.p.A.

http://www.kide.com
Kide S.Coop.

http://www.metalquimia.com
Metalquimia S.A.

http://www.refrica.com
Refrigeración Casassas S.A (Refrica).

http://www.travaglini.it/
Travaglini S.p.A.

http://en.wikipedia.org/wiki/Drying
Wikipedia.

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ECONOMICS

Meat Business and Public Policy

NC Speer, Western Kentucky University, Bowling Green, KY, USA

Glossary

Antibiotics These are the drugs utilized to treat and/or prevent various bacterial infections.
Concentrated Animal Feeding Operation (CAFO) It is defined by the US Environmental Protection Agency describing animal production units that potentially fit specific pollution and/or waste profiles.
Mandatory country-of-origin labeling (COOL) This is the US law dictating country of origin for various meat products.
North American Free Trade Agreement (NAFTA) It is the agreement between the US, Canada, and Mexico detailing trilateral trade law among the three countries.
Rights These are the moral and/or legal rights of animals recognized by society.
Traceability It details various production and/or attributes maintained throughout the supply chain.
Welfare It is the general sense of animal well-being.
World Trade Organization (WTO) It deals with legal rules of trade among nations ensuring that trade flows smoothly and freely.

Background

The past half century ushered in a period of sustained economic growth across the globe. Flourishing economies generally establish a higher standard of living that includes greater consumer demand for conveniences and measures of health and safety. More specific to the meat industry, economic growth also leads to changes in tastes and preferences and commonly includes pursuit of improving one's eating standards often characterized by a desire to include more animal protein within the diet. The expansionary period that began in the 1960s occurred largely within the developing regions (vs. developed countries) and strongly underpinned new opportunities for consumer spending on meat and meat products within those countries.

Convergence of shifting consumer preferences coupled with an ever-expanding population strongly boosted meat demand. This subsequently led to the need for increased meat production throughout the world. Global meat production (beef, veal, pork, broiler, and turkey combined) grew more than 500% between 1960 and 2010 (45.447 vs. 243.396 mmtons, respectively).

Despite steady growth and new market opportunities across the globe, the meat production business has proven especially challenging in recent years. Gains in overall production equate to a larger, more defined, and more easily targeted industry. That reality, along with advancements in technology and communication, has culminated to bring about increased attention and scrutiny for all participants associated with animal agriculture and meat production.

Stakeholders at all levels are dealing with new and developing concerns surrounding the business. Aside from normal, routine consumer market issues, a number of nonroutine developments have arisen. Primarily, there is increasing desire for public involvement in various issues associated with meat production. Moreover, transition of economic growth away from developed countries to developing regions highlights the meat industry's connectedness to a variety of interacting forces throughout the world.

As noted above, increasing economic prosperity often leads to increased meat consumption. In most businesses, that would typically prove favorable. However, for meat production, that reality also serves to be a double-edged sword. Once economic prosperity becomes sufficiently high, consumer

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priorities tend to shift across a variety of attributes. For example, attributes associated with the production process (such as animal welfare) become more heavily weighted in purchasing decisions. In other words, fundamental attributes such as food safety, nutrition, taste, convenience, etc. become relatively less important items (albeit still essential), whereas other attributes become relatively more important. Therein enters many of the intricacies of decision making and policy navigation for the meat industry going forward.

Many policy issues and challenges are relatively uncharted and potentially redefine how the industry carries out business from a strategic perspective. Key areas of policy arise in several key categories oriented around: (1) meat products; (2) production processes and the supply chain, and (3) overarching legal and trade issues. Several key issues are highlighted below. The list is neither comprehensive nor clear cut, given the variety of issues and potential for crossover among the categories. However, such imprecision further underscores the increasing complexity surrounding meat production.

Product-oriented Policy Issues	Production-oriented Policy Issues
Food safety (pathogen-induced and residue derived)	Animal welfare and well-being
Health and wellness attributes	Environmental stewardship and regulation
	Animal health/Antibiotics
Trade/Legal Issues	Industry Business regulation (including biofuels)
Labor/immigration regulation	Source verification and traceability
International trade regulations	Biotechnology
	Interaction with the biofuel industry

Animal Welfare

Consumers generally expect respective food purchases to be nutritious, flavorful, and safe. However, rising affluence also leads to alternative attributes being increasingly important across the food system. Increasing interest and awareness among consumers of farm-to-fork connectedness have especially driven production-oriented issues to the forefront in recent years. The outcome being that all participants in the supply chain are investing more time and resources to dealing with such issues. Foremost among those issues includes animal welfare.

The industry has traditionally countered broad concerns about animal welfare by demonstrating that animals grown in mainstream production systems are increasingly well cared for. In fact, overall management knowledge has never been more advanced. The upshot of those developments leads to better care and subsequent animal comfort. That reality is ultimately reflected by enhanced productivity across all of animal agriculture. There exists any number of favorable trends when considering items such as improved animal health, increased growth rates, and advances in reproductive efficiency.

However, from a policy perspective, for many individuals, improved animal health, increased growth rates, and advances in reproductive efficiency are inadequate measures of animal care and/or welfare. Such opponents of the meat industry contend that well-being of an animal also include more intangible factors such as freedom from fear and/or freedom of social interaction. In particular, policy discussions often revolve around animal housing for animals involved in production – especially those housed in confinement operations. The issue typically includes topics such as gestation crates for sows, battery cages for laying hens, veal crates, and feedlot pens. Opponents of such housing argue that animals should be allowed opportunities for social interaction while also providing opportunity to turn around, lie down, stand up, and/or fully extend their limbs at all times. There have been a number of efforts, especially notable in the US, to pass state regulations to limit confinement-type operations to provide such attributes.

Meanwhile, animal activists are also increasingly calling other management practices into question including concerns around beak trimming, castration, dehorning, clipping needle teeth, tail docking, etc. The primary argument is that meat production during the past few decades has dramatically changed. Activists advocate that modern meat production has increasingly moved to 'industrial' facilities emphasizing a concern only for productivity and completely disregards humane treatment of animals.

Meanwhile, a recent rise of undercover investigations appearing on the internet from groups such as Humane Society of US, People for Ethical Treatment of Animals, and Mercy for Animals have provided ammunition for those calling for sweeping changes within animal agriculture, including potential elimination of raising livestock for consumption purposes. Such movements have led to laws in several states in the U.S. banning specific production practices (e.g., sow gestation crates, veal crates, and battery cages). The threat of ongoing lawsuits and/or ballot initiatives has led to several states (e.g., Ohio and Kentucky) to be proactive in developing animal care oversight boards. Simultaneously, throughout the European Union many such initiatives are underway.

Public and social media outlets have also led to increasing awareness among consumers about animal welfare/animal care issues. Many large companies are responding accordingly. For example, McDonald's recently announced (February 2012) that it would work with suppliers to implement the phasing out of gestation crates associated with normal production practices. Similarly, the Humane Society of the US and the United Egg Producers announced a cooperative agreement to replace conventional battery cages with new housing systems providing hens more room and to prohibit sale of eggs and egg products that do not fall within the newly established guidelines (July 2011). Finally, the Compass Group – the largest global food and support service company – has also announced similar requirements for its pork and egg supply chain (March 2012).

It should be noted that animal welfare is a very different concern from animal rights. Animal rights groups typically promote vegetarianism and believe that animals should not be utilized for any purpose, that is, some groups advocate a liberation agenda contending that animals have rights (similar to humans) and hence should not be utilized for any purpose (food, recreation, or research).

Environmental Stewardship and Regulation

Concerns around animal welfare and well-being often intersect with environmental stewardship and regulation. Growing adoption of production models emphasizing economies of scale draw the attention of those concerned about environmental degradation and subsequent societal costs. The forefront of environmental policy issues typically surrounds several key topics: waste or nutrient management, water quality and utilization, and air pollution or odor.

Waste management becomes an especially important issue to manage when considering public perception and management of concentrated animal feeding operations (CAFOs), that is, such production units typically house larger numbers of animals in greater density (compared with more traditional production methods) and thereby larger amounts of waste are collected and/or stored in relatively small areas (e.g., manure lagoons). Regulation surrounding CAFOs has become increasingly stringent and rigorous over the years – all CAFO operators must maintain updated operating permits and undergo constant monitoring by regulatory agencies.

However, opponents argue that if/when such resources are mismanaged and/or neglected, these units can potentially produce environmental problems – either acute or chronic in nature. Concerns about waste management inherently also involve policy discussions revolving around water quality: The concern being that CAFOs may represent increased potential for water contamination, be it point source (directly identifiable) or nonpoint source (diffuse) pollution. Therefore, animal agriculture finds itself dealing with regulatory pressures revolving around specific nutrients within various watersheds. And finally, increased regulatory pressure also comes on the air pollution/odor front, especially in areas where the urban/rural interface is mounting.

From a more overarching, international perspective, the most notable environmental policy concern revolves around the very existence of meat animal production itself. Much of that attention in recent years result from the release of the United Nation's Foreign Agriculture Organization's publication entitled Livestock's Long Shadow (2006). The report asserting, "…climate change is the most serious challenge facing the human race. The report states that the livestock sector is a major player, responsible for 18 percent of greenhouse gas emissions measured in CO_2 equivalent. This is a higher share than transportation." The report has since been demonstrated to include several major miscalculations but nonetheless served to provide significant traction toward policy discussion and development.

Meanwhile, environmental policy pressure also arises relative to land and/or resource utilization coupled with the integration of ecological and social equity. Those broader concerns invoke the concept of 'sustainability' and will more likely be an important driver of policy evolution within meat production systems in future years. A recent paper by Pretty *et al*. (see further reading) summarizes, "Vital work needs to be done to establish more precisely what 'sustainable food' represents, and to identify best practice standards across a wide range of activities throughout the [food supply chain]."

Nonetheless, the concept has led to several key companies, along with external partners, to proactively address the issue. For example, 2012 marked a new venture entitled the Global Roundtable for Sustainable Beef – a coalition of stakeholder companies and organizations. The founding members include: AllFlex, Allianca de Terra, Cargill, Elanco, Grupo de Trabalho da Pecuaria Sustentavel, JBS, McDonald's, Merck Animal Health, National Wildlife Federation, Rainforest Alliance, Roundtable for Sustainable Beef Australia, Solidaridad, The Nature Conservancy, Walmart, and World Wildlife Fund. The endeavor's mission is to assess and promote more efficient, environmentally sustainable beef production practices.

Antibiotics

There generally exists a large degree of confusion and ambiguity about antibiotic use in livestock production. That is largely because such usage is a relatively nuanced issue often being commingled with the broader connotations surrounding consolidation and/or industrialization of animal agriculture. Therefore, there is a high degree of potential for misleading claims. Regardless, the use of antibiotics in food animal production derives broader concerns about antimicrobial resistance in humans (AMR).

A variety of public interest groups are highly interested in the subject of AMR. Such concern is especially amplified when considering nontherapeutic use within livestock production. Opponents often claim that general and rampant overuse of antibiotics in animal agriculture leads to drug-resistant bacteria, an increasing threat to human beings.

Such arguments are predicated on the following logic: Antibiotics fed to livestock at subtherapeutic levels facilitate establishment of resistant strains of bacteria and absolute containment at the local farm environment proves elusive. That scenario inevitably put citizens at risk because such strains prove unresponsive to treatment if they are able to cause illness. Therefore, the argument is that such use must be curtailed and future approval of new antibiotics in livestock should be preempted (new development should be saved for human usage).

AMR concern often gets leveraged to advance ideologies and/or policy proposals to limit antibiotic use in farm animals. The most commonly cited example supporting an antibiotic ban comes from Denmark, where nontherapeutic antibiotic use has been banned in animal agriculture. However, since the ban, therapeutic use in animal production has increased. Moreover, no scientific documentation exists that reflect antibiotic resistance in the human population has declined since the ban.

Policy implementation suggesting simple fixes or solutions leads to false sense of security. That is because numerous complexities surround development of antibiotic resistance. Several other risk factors also contribute to development of AMR, including large hospitals, socialized care of elderly persons, and increased social interaction via international travel. Therefore, policy must key on proper assessment and subsequent management of risk – comprehensive, science-based strategies in both livestock production and human health. Moreover, AMR results from intricate interactions among (1) specific antibiotic class, (2) specific pathogen, and (3) host population. Therefore, predicting and/or preventing

broad or wide-scale resistance, based on simple policy solutions, is highly challenging because of the large number of specific antibiotic/pathogen/host combinations. As a result, there is no scientifically documented link establishing antibiotic use in livestock and increased resistance in humans. In fact, quantified research assessment of potential farm-to-patient resistance represents a 'very low risk of human treatment failure'.

Finally, policy pressure revolving around antibiotic resistance often invokes the previous two issues discussed: animal welfare and environmental regulation. That is because antibiotic usage in animal production commonly gets tied to the overall 'factory' or 'industrial' farming model – the argument being that antibiotic utilization would be unnecessary if animals were not housed in confinement. Therefore, the three issues often become inherently linked with policy discussion.

Dietary Guidelines

Obesity progressively receives attention as a global epidemic as obesity rates continue to climb. For example, the Center for Disease Control estimates that two-thirds of the US population is now overweight; more than half of that segment, or fully one-third of the total population, falls into the obese categorization. To that extent, human weight management is not simply an individual matter; rather, it is now a collective, public health concern stemming from lost productivity and rising health care costs associated with obesity. Rising prevalence of obesity and subsequent health problems (claims revolving around type-2 diabetes, heart disease, various cancer types, etc.) have served as a platform to advance a vegan and/or vegetarian agenda. From a public health point of view, many argue that the parallels among increased meat consumption and obesity rates represent a meaningful relationship. Therefore, reduced meat consumption is often promoted as conducive with healthier lifestyles and hence should be eliminated from the diet. These opposition groups often utilize rhetoric revolving around a general theme of meat being unnecessary in the diet and detrimental to individual health. The dietary issue has been especially leveraged of late around other matters within animal agriculture, that is, food choice and/or availability converges with other matters of policy (such as environmental degradation, resource exploitation, animal welfare, etc.). Stated another way, policy discussions addressing healthcare costs (due to heart disease, obesity, type-2 diabetes, and the like) are sometimes aimed directly at animal agriculture, citing meat production and increasing corporate pressures as contributing to broader health concerns.

International Trade

Structural shifts in developing countries have moved economic activity to new areas of the world. Meanwhile, technology gains in transportation and communication have driven international trade of all types of material goods as costs of logistics and supply chain coordination have declined. However, as global interconnectedness increases, managing global governance and trade policy becomes both increasingly difficult and important. That reality is evidenced by several key, long-standing trade embargoes around meat and/or meat products. When such embargoes are principally unfounded, it often escalates to retaliatory trade measures that include other products including grain and/or other manufactured material goods.

Animal health is an especially important consideration with regard to meat production and international trade policy. Therefore, moving animals and/or meat products across international borders requires more stringent sanitary and phytosanitary policies. International trade requirements increasingly mandate some form of identity preservation and source verification. For such verification to be meaningful, traceability must exist throughout the entire supply chain. Therefore, many countries now require individual animal identification and meat traceability as precursors for import status.

Traceability is often a mandate to ensure that animal disease requirements dictated by the World Organization for Animal Health (OIE) are strictly followed. The OIE promotes international cooperation to control the spread of transboundary animal diseases while also providing expertise to establish public health standards and improve legal framework for trade. Countries with such systems generally possess increased competitive advantage when negotiating market access with those not possessing such systems. However, implementation of such systems can prove cumbersome, complicated, and often controversial. That has been especially evident in the US as the country progresses through various attempts at mandatory animal identification. The attempts are made to ensure rapid tracking capabilities in the event of serious animal disease events and/or outbreaks (e.g., bovine spongiform encephalopathy or foot and mouth disease, respectively).

Animal disease traceability is very different from market-driven traceability – the latter more focused on value-added credence attributes such as age verification, natural programs, animal welfare, etc. A secondary level of such discussion revolves around country-of-origin labeling (COOL). Implementation of a mandatory system within the US (effective 2008) has played an especially important role within the North American Free Trade Agreement (NAFTA) framework. Legality of the labeling law is now in question due to a 2011 World Trade Organization (WTO) ruling: COOL is not WTO compliant, as per NAFTA requirements, and effectively represents a nontariff trade barrier. Regardless of the dispute's outcome, the law will more likely lead to important ramifications around a whole host of products in future trade negotiations.

Finally, many countries often must manage trade policy from a more internal approach. Those discussions can be especially contentious with food and/or food production. Many antitrade proponents, regardless of the country in question, promote a sense of national security – food production should not be included in discussions and/or negotiations revolving around trade. That perspective argues food should remain immune from the broader forces of global economic change. That influence makes trade development policy especially difficult because consumers in all countries desire goods that come from free trade – which is often in the form of meat or

meat products. That ultimately creates an inconsistency between protectionism on one side and free market capitalism on the other.

Interaction with the Biofuel Industry

Introduction of biofuels policy in the US initiated some important influences on the meat production business. It represents a whole new dimension for agriculture that has arisen during the past five years or so. The dynamics have dramatically shifted the business environment for all of agriculture (not to mention its indirect influence on consumers across a number of venues). From a policy perspective, it has initiated a more wide-reaching debate about food versus fuel.

More directly, the influence of ethanol policy on the meat production business has been derived primarily in the form of higher feed and other input costs. However, it has also created an inexorable linkage among commodities of all types. That connectedness among markets along with increasing speed of commerce and globalized trade ultimately means that biofuel policy in any country has ramifications to the meat industry throughout the world. Specifically, it has given rise in recent years to renewed concerns about food costs and the role of speculators on all commodity prices across the globe – speculators often being accused of unduly profiting from their respective positions while the rest of the world (especially the impoverished) are unduly punished by rising costs.

Internal Business Regulation

Much of the discussion above focuses primarily on public–industry interaction. However, the meat industry also wrestles with several ongoing and contentious internal policy struggles because many of the traditional trade and production strategies have blurred over time. Most notably, there is divided ideology about adaptation strategies and future competitiveness – much of it centered around production sector competitiveness issues. Consolidation remains a very important and controversial issue within agriculture with distinct and well-entrenched opinions about its long-term impact on the industry.

To date, much of agriculture's transition stems from consolidation in the food processing, manufacturing, wholesaling, retailing, and service sectors. The industry has evolved to become more efficient to meet rising consumer demand across the globe. Coordination of the supply chain facilitates improved cost controls, more efficient scheduling of inputs, and processing assets. The meat industry has transitioned accordingly – from relatively uncoordinated commodity-sorting systems to growing adoption of more specialized production. Restaurant and retail companies desire to offer high quality, competitive products while also facilitating consistent and predictable inventory turnover; in turn, processors seek development of specified capabilities from producers to deliver those attributes.

These endeavors increasingly enhance the bottom line for companies, especially in the face of rising costs; information-based supply chain coordination efforts improve efficiency while emphasis on differentiated, value-enhanced production boosts revenue. That has not occurred at the exclusion of the production sector (albeit comparatively more pronounced in the pork and poultry sectors compared with the beef complex). The trend to larger operations has occurred to leverage some economies of scale and management, but it is done not only to implement cost efficiencies but also to facilitate better input quality control and to ensure that specifications are met at all levels of production.

However, the meat business, at least at the production level, remains relatively large, diverse, and fragmented. Consequently, there exists wide opinion about how business should be conducted: The struggle often vacillates between pressures to evolve to ever-increasingly synchronized systems approach versus maintaining a more traditional independent, segmented structure (adhering to other more important values surrounding production systems). Those arguing for the latter have seemingly been bolstered with renewed consumer interest in the food system – that is reflected by success of several popular books during the past decade along with various documentaries. Such coverage tries to engage the audience in seeing the connections between retail and restaurant food business and ensuing production of the products which they merchandise.

Conclusions

Meat production and animal agriculture are increasingly an important, dynamic, and demanding business from a number of perspectives. Animal agriculture must continue to pursue increased output and efficiencies in order to satisfy consumer demand and meet the needs of a growing global population. Simultaneously, those same consumers increasingly view themselves as stakeholders in the production and marketing system. Food activism has steadily increased with each passing decade since the 1970s, gaining significant traction in recent years. Regulatory reform must consider both cost competitiveness and social implications.

See also: Animal Health Risk Analysis. Human Nutrition: Meat and Human Diet: Facts and Myths. Manure/Waste Management: Manure Management; Waste Management in Europe. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms; Potential Environmental and Wildlife Sources of Microorganisms in Meat. Preslaughter Handling: Preslaughter Handling. Professional Organizations. Residues in Meat and Meat Products: Feed and Drug Residues. Slaughter, Ethics, and the Law

Further Reading

Armbruster, W., Stenholm, C., Armstrong, J., *et al.*, 2006. The Future of Animal Agriculture in North America. Oak Brook, IL: Farm Foundation.

Field, T.G., Taylor, R.E., 2008. Scientific Farm Animal Production. ninth ed. New York: Prentice-Hall.

Hurd, H.S., Doores, S., Hayes, D., *et al.*, 2004. Public health consequences of macrolide use in food animals: A deterministic risk assessment. Journal of Food Protection 67 (5), 980–992.

NIAA, 2011. Consumers Stake in Today's Food Production: Meeting Growing Production Demands With Integrity. Colorado Springs, CO: National Institute for Animal Agriculture.
Pretty, J., Sutherland, W.J., Ashby, J., *et al.*, 2010. The top 100 questions of importance of the future of global agriculture. International Journal of Agricultural 8 (4), 219–236.
Steinfeld, H., Gerber, P., Wassenaar, T., *et al.*, 2006. Livestock's Long Shadow: Environmental Issues and Options. Rome: Food and Agriculture Organization of the United Nations. ISBN: 978-92-5-105571-7.

Relevant Websites

http://www.soundagscience.org/
Animal Agriculture Alliance.

http://www.fao.org/
Food and Agriculture Organization of United Nations.

http://www.animalagriculture.org/
National Institute for Animal Agriculture.

http://www.animalagriculture.org/Solutions/Proceedings/Annual%20Conference/2011/White%20Paper.pdf
National Institute for Animal Agriculture.

http://www.usmef.org/
US Meat Export Federation.

http://www.oie.int/
World Organization for Animal Health.

http://www.wto.org/
World Trade Organization.

ELECTRICAL STIMULATION

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Glossary

Aging The process of meat tenderization that is an enzymatic process occurring over time.
Calpains Components of the enzyme system acting on cytoskeletal proteins during meat tenderization.
Current Electrical term defining flow of electric charge.
Cytoskeletal proteins A set of structural proteins (includes titin, nebulin, and desmin) that are denatured by calpains.
dpH/d*t* The rate of fall in pH that takes place following the electrical stimulation.
Drip or purge Water that increases over time arising from the protein denaturation as meat tenderises – it is additional to that from prerigor myosin denaturation that can also occur.
Electrical stimulation (ES) The application of an electric current through a carcass postmortem that accelerates the rigor process.
Hot boning A process when the meat is removed from the carcasses before rigor mortis in contrast to cold boning when meat is removed after rigor mortis.
Myofibrillar proteins The muscle contractile proteins, actin and myosin.
ΔpH The actual fall in pH that occurs immediately after electrical stimulation.
Postmortem The period after harvest when the pH falls until rigor mortis and subsequent aging.
Preslaughter The period before slaughter where factors such as stress can affect meat quality.
Protein denaturation A process whereby proteins lose their tertiary and secondary structure such as by application of acids or heat – water that is part of the tertiary structure can be released.
Pulse waveforms Characteristics of specific electrical stimulation parameters.
Rigor A term for individual muscle fibers that have been depleted of adenosine triphosphate, whereas rigor mortis is a term where muscles stiffen after all muscle fibers enter rigor.
Shear force The force (N) applied to a standardized piece of meat to shear it.
Shortening A process that occurs when prerigor muscle is cooled below 10 °C – additionally it also occurs as muscles enter rigor (rigor shortening).
Ultimate pH The pH that is reached when muscles reach rigor mortis.
Voltage Electrical term defining the electrical potential difference.

Introduction

'Electrical stimulation' (ES) or 'stimulation' are general terms used for describing a passage of any electrical current through a muscle or carcass and the abbreviation ES is used in this article generally to apply to the process in which an electric current is passed through carcasses with the aim of ensuring that the meat is tender. Tenderness is a major consumer requirement (toughness is its inverse) and it is quantified using either consumer panels, trained sensory panels, or objective measurements such as shear force (force required to shear through a cooked piece of meat). ES has been used to improve the tenderness of meat from deer, goats, sheep, cattle, and various poultry species, and in certain circumstances for pigs, and is perhaps one of the recent most significant factors in improving meat quality. In some situations its application has introduced small problems of its own such as human safety considerations, but overall the improvement in tenderness far outweighs these minor problems. Although it is not possible to increase meat tenderness beyond that limited by connective tissue, it is possible to ensure the meat routinely reaches the highest degree of tenderness it is capable of achieving.

ES of muscle from harvested animals hastens the process of rigor mortis (defined when adenosine triphosphate (ATP) production ceases). It does this by causing muscles to undergo work via anaerobic glycolysis, resulting in an initial pH fall (ΔpH) followed by a change in the rate of pH fall (dpH/d*t*). The combined effect is that the muscles enter rigor mortis before the muscle temperature falls to values producing cold shortening and toughening.

Early post slaughter, stimulation can be applied using relatively low voltages that effectively operate via the nervous system. With increasing delays before stimulation, higher voltages are then required to directly stimulate the muscles.

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The electrical parameters generally used must consider the appropriate waveform and pulse frequency, duration, prestimulation delay, chilling rate, and type of species involved.

Although ES ensures that cold shortening is avoided, aging also starts at a higher temperature and is consequently more rapid. However, evidence suggests that there are other mechanisms involved in tenderization (defined as the reduction in toughness postrigor), such as modification of the enzyme systems and possibly fiber disruption and protection of the enzymes responsible for tenderization.

ES must be considered as part of a total process from slaughter through chilling to final sale, and has particular advantages for hot boning, where the shortening and toughening conditions that would occur for nonstimulated muscles during chilling are avoided. With appropriate ES and chilling rates, hot-boned meat is as tender as normal cold-boned meat, especially if a wrapping procedure is also used to avoid shortening.

History

The association between muscle/meat and electricity started with Galvani, although the earliest reported use for meat improvement was by Benjamin Franklin, in 1749, who electrocuted turkeys, with the result that they were 'uncommonly tender.' During the 1950 s it was shown that ES could improve meat tenderness of beef, but no commercial application of the process occurred. Stimulation of horse muscle was used experimentally to examine the microbiology of pre and postrigor meat from the same animal at the same temperature.

The incorporation of a practical system into the slaughtering process was first used in New Zealand and then Australia to avoid toughness resulting from cold shortening. It is now widely used in many other countries with a variety of parameters (Box 1). In New Zealand, ES was originally used to accelerate rigor mortis before sheep meat and beef was frozen. For cattle, shortening is less of an issue as temperatures fall more slowly than sheep, but ES in many circumstances enhances tenderization with a general improvement in quality and reduction in the differences among cattle breeds.

In initial commercial operations, loins and legs from nonstimulated lamb carcasses put into a blast freezer (−5 °C within 2 h of slaughter) and later cooked from the frozen state were exceedingly tough, with only approximately 1% of the loins assessed as acceptable on the basis of shear force. In contrast, nonaged loins from similarly processed, but high voltage-stimulated lamb carcasses gave approximately 75% of shear force values in the acceptable range. For small, easy-to-chill lambs, the improvement is therefore dramatic, ensuring that the majority of rapidly frozen meat is acceptable. For cattle, shortening is less of an issue, but ES still enhances the rate of tenderization. The combination of ES and a specified further aging period to achieve a desired tenderness level underpins the accelerated conditioning and aging (AC&A) standard that currently applies in New Zealand abattoirs for sheep and lambs.

Description

Events During Electrical Stimulation

ES involves passing an electric current through the bodies or carcasses of freshly harvested animals. This electric current causes the muscles to contract, increasing the rate of glycolysis and results in an immediate reduction in muscle pH (ΔpH) that ranges from 0.6 pH units at 35 °C to 0.018 units at 15 °C, suggesting that ES of warm carcasses should take place soon after slaughter to maximize efficacy. Following the pH fall, there is a temperature-dependent acceleration of the rate of glycolysis (dpH/dt) and subsequent early rigor mortis development (Figure 1).

The increased rate of pH fall after ES seems to occur with a wide range of electrical parameters and even occurs as a consequence of high frequency electrical stunning and kicking movements post slaughter. Although the ΔpH is generally lower with low-voltage ES, the same rate of poststimulation pH fall is achieved as with high-voltage systems.

The rapid development of rigor mortis ensures that postmortem aging starts earlier, improving meat color, and provides increased muscle stiffness that facilitates early boning.

Electrical Stimulation Systems

There are numerous physical methods by which ES can be applied and many different possible electrical specifications (see Box 1 for representative electrical parameters). A new generation of systems developed in Australia for both sheep/lambs and beef were designed to impart the response observed with high-voltage ES, but without the danger associated with such systems. The pulse width is reduced and the peak voltage

Box 1 Typical ES parameters in common commercial use. It should be noted that these are representative and many variations are used

- 200 V, 60 Hz applied in bursts of 1 s on, 1 s off for 60 s duration for beef in the US
- 1130 V peak, 14.3 alternating pulses per s applied for 90 s within 30 min of slaughter for sheep are lambs in New Zealand
- 80 V peak, 15 unipolar pulses per s, square or half sine wave, applied for 15–30 s within 5 min of slaughter for beef in New Zealand
- 800 V rms bidirectional half-sinusoidal, 14.3 pulses per s applied within 40 min postslaughter for beef and lamb
- 80 V peak, 15 unipolar pulses per s applied for 30 s within 20 min of slaughter for beef in Sweden
- 40 V peak, 15 unipolar pulses per s applied using an anal probe with durations for 40–50 s within 5 min of slaughter for beef in Australia
- Voltage controlled to deliver up to 2 A peak for 30 s at 15 pulses per s up to 2.5 ms duration applied within 30–40 min for sheep and lambs in Australia. A variation to this includes modulated frequency, for example, for a 6 electrode system 10, 15, 25, 10, 15, and 25 Hz
- 400 V peak 2 ms pulses at 15 pulses per s in Australia for beef

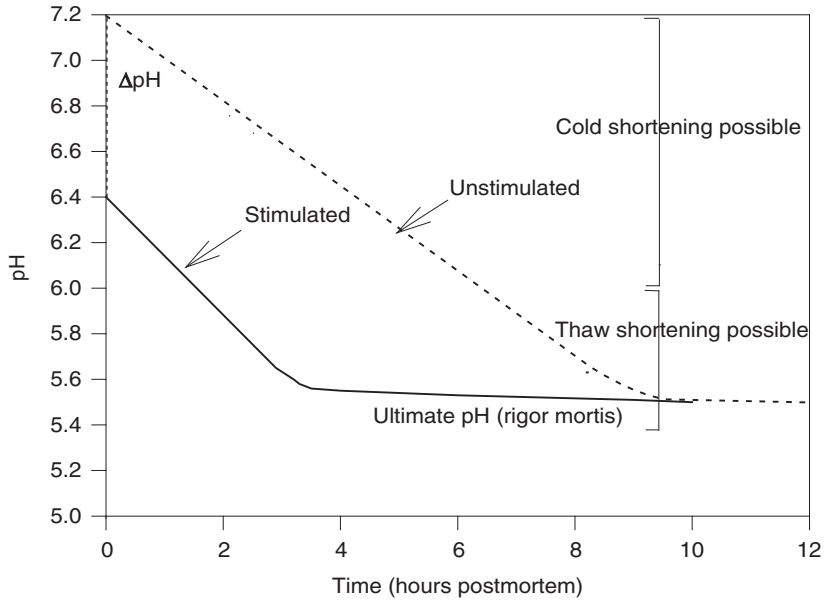


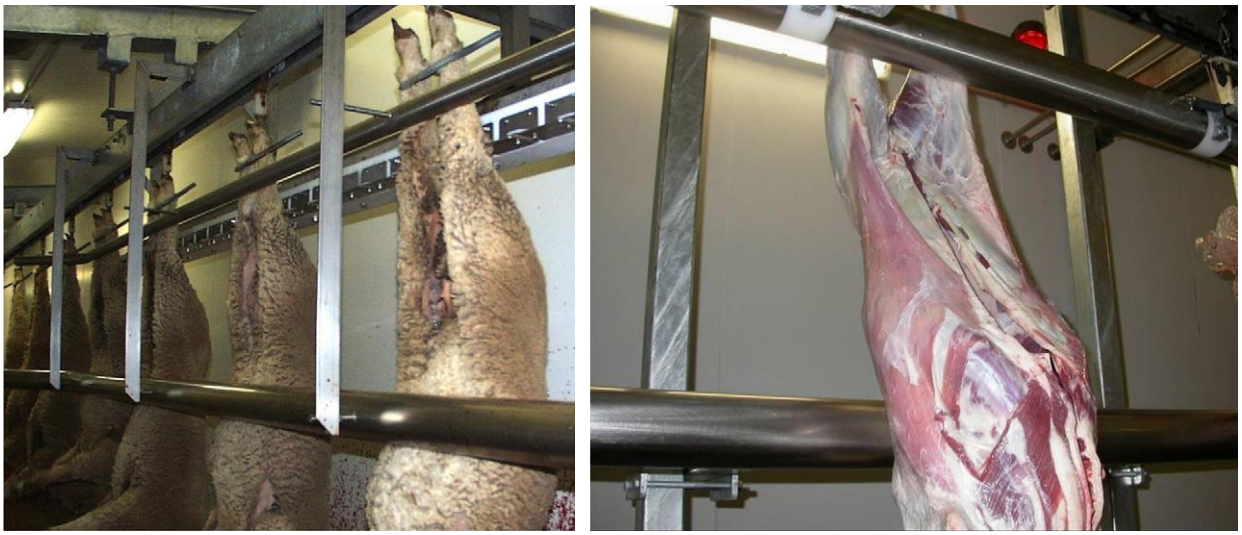
Figure 1 Postmortem pH fall in muscles after ES and held at a constant 35 °C. During stimulation, the muscle pH falls (ΔpH). Nonstimulated beef muscle has a rate of pH fall (dpH/d*t*) of 0.18 pH units per hour. After stimulation, the dpH/d*t* is increased to 0.3 pH units per hour. The time that muscle is at cold shortening temperatures is clearly reduced.

is decreased compared with high voltage ES, but the lower rms voltage developed (rms is the 'average' voltage and is explained later) ensures that the systems are much safer for workers (see Box 1). Regardless of species, ES can be applied immediately after slaughter or at any point in time thereafter until the muscles become unresponsive. The time until muscles fail to respond is related to the natural rate and extent of glycolysis and the voltage and or current being applied, the duration of ES, and the type of response expected. Procedures range from stimulating stunned but not bled animals, to whole bodies, skinned bodies, carcasses, or sides or even primal cuts.

Most commercial ES systems employ the carcass rail as ground and a live electrode contacts some other point of the body, carcass, or side as shown in Figures 2, 3a,b, and 4. In the most basic systems, the live electrode contact is a clip manually applied to the head or neck of the body (such a clip would replace the lower rubbing bar in Figure 2) as it is suspended by one or both hind legs, resulting in a current flow to the grounded rail support or top electrode. A range of other systems have also been developed for beef ES, covering both batch and continuous operations. The batch systems might involve manually inserted electrodes or electrode bars that move out to make contact with the body or carcass. In these systems, the carcass or carcasses are enclosed within a shielded cabinet during ES. Continuous systems consist of stationary rubbing electrodes (Figures 2 and 3) or, where the ES is applied to carcasses before inspection, the electrode system consists of a moving series of electrodes. For poultry, heads can be placed in a trough filled with water with the rail being the other electrode.

In New Zealand, electrical stunning of cattle is widely used; and the stunning current is applied to a restrained head in a stunning box and followed by application of additional currents to cause cardiac arrest (if nonhalal) with reduced poststun carcass movements. Following stunning, current is often immediately applied to the body for ES before the animals are ejected from the stunning box in some New Zealand plants. Some carcass electrical immobilization may cause a pH fall (although some recent immobilization parameters do not cause a pH fall and are exempt) as will the application of electrodes to the back muscles to stiffen them during downward hide pulling. New immobilization systems have been developed in Australia for sheep/lambs and beef.

Figure 2 Low-voltage ES of cattle in a meat processing plant. The current (120–400 mA, 15 pulses per s) passes between the top rubbing bar electrode and a lower rubbing bar electrode (often earthed). An alternative arrangement can use a clip attached to the nose. The duration of ES ranges from 30 to 60 s. Bleeding out rate is generally increased. Photograph courtesy of Meat and Livestock Australia.



(a) (b)

Figure 3 (a) A medium voltage ES system for electrically stimulating lamb bodies prior to dressing at the start of the chain, typical of Australian new generation systems for sheep predressing. The application of ES is through the rail to the hind leg hocks- also commercially referred to as transverse leg stimulation. Photograph courtesy New South Wales Primary Industries, Australia. (b) A medium voltage ES system for electrically stimulating lamb bodies postdressing at the end of the chain, typical of Australian new generation systems for sheep postdressing. The application of ES is from the electrode at top rail to the hind leg with only one carcass on any electrode at any one time. Photograph courtesy New South Wales Primary Industries, Australia.

Figure 4 A typical high-throughput system using a high voltage ES facility for sheep postdressing, in which the current passes through the carcass between two rubbing bars. Photograph courtesy New South Wales Primary Industries, Australia.

More sophistication and protection for humans is required as voltages increase, such as in high-voltage systems for sheep and lambs (Figure 4). However, safety is enhanced with the development of systems with shorter pulse widths and increased voltages utilizing reduced rms voltages. In one New Zealand abattoir, an effective single in-line high-voltage ES system coped with a peak kill of 20 000 carcasses per day. Typically, for high voltage situations, the ES is applied less than 30 min after slaughter where dressed carcasses are suspended by metal or plastic skids and gambrels from a grounded rail and moved through a stimulation tunnel to make contact, at shoulder level, with an electrode supplied with high voltage pulses (see Figure 4).

Electrical Stimulation Parameters

Any electric current above a certain threshold will stimulate muscles, and for this reason stunning or immobilization currents can have a beneficial effect on tenderness by also accelerating glycolysis. The current flow is dictated by the applied voltage and carcass characteristics such as pelt cover, animal size (determining resistance) and fatness (potential insulation), and contact area (in particular reduced contact with shin). However, in commercial situations where high voltage ES is used, large peak current flows occur (e.g., in excess of 2 A peak per carcass). In situations where many carcasses are stimulated simultaneously on the same electrode system, very sophisticated power supplies, delivering up to 60 A total, are needed for the pulsed currents and currents are not necessarily shared equally between carcasses. Development of new systems for sheep/lambs and beef in Australia using short pulse widths and moderate voltages use segmented electrodes to ensure that each electrode only contacts one carcass at a time. This allows computer-controlled electronics to give a precise, but adjustable current to each carcass to match the requirements of a particular carcass type (Figures 2 and 3a,b). The current pulses in these systems use very rapid rise times that appear to provide a greater stimulation effect with lower peak current to give very effective results (Box 2).

Voltages used vary from 32 to 3600 V (historically). The value specified might be that of the peak or the rms (root mean square) voltage, or in some cases the average over the total time. The rms voltage is the effective value or heating

Box 2 Meat characteristics following ES. Color (redness (a^*), color stability at 630/580 nm, shear force (*M*), pH, predicted temperature at pH 6.0 (°C), for the m. longissimus of electrically stimulated (800 mA, pulse width 0.5 ms, peak voltage of 300 V, 15 Hz, for 60 s) and nonstimulated lamb carcasses (40 per treatment). Chilled at 4.2 °C. All values are predicted means (s.e.d.)

Trait	*Stimulated*[a]	*Nonstimulated*	*s.e.d.*
Initial loin pH	6.34a	6.79b	0.04
Predicted temperature at pH 6.0	24.8b	13.9a	1.50
Shear force (*M*) at 1-day aging	36.0a	44.0b	2.40
Redness (a^*)	7.70a	7.00a	0.32
Color stability (630/580 nm)	3.20a	3.00a	0.14

[a]Stimulation treatment was at a current of 800 mA with a pulse width of 0.5 ms for duration required. Means followed by the same letter in a row are not significantly different ($P = .05$).

Source: Adapted from Toohey, E.S., Hopkins, D.L., Stanley, D.F., Nielsen, S.G., 2008. The impact of new generation pre-dressing medium-voltage electrical stimulation on tenderness and colour stability in lamb meat. Meat Science 79, 683–691.

capacity of a waveform. For a sine wave, the rms value is the peak voltage divided by $\sqrt{2}$. For 1130 V peak, 50 Hz, the rms voltage is 800 V. However, for many derived (nonsinusoidal) waveforms the rms may be quite different and ineffective. For one version, termed the Meat Industry Research Institute of New Zealand (MIRINZ) waveform, every seventh half-sine wave of a 50 Hz sine wave is used and the rms voltage is the peak voltage divided by $\sqrt{14}$. Figure 5 illustrates the meaning of the different terms used to describe voltages and waveforms. Defining a waveform with a frequency (expressed in Hz) is likely to lead to confusion unless the waveform is also defined in terms of shape, duration, and pulse spacing. Square waves also can be used and may be unipolar or bipolar and applied as discrete pulses or even as pulse trains.

Extensive research in Australia particularly in sheep and lambs has demonstrated that ES systems must be validated and optimized to ensure effective operation – in other words mere application of electricity does not guarantee a satisfactory result. In instances where this does not happen or system monitoring is not employed ES can be relatively ineffective. In some situations with multielectrode systems, lights are used to indicate when each electrode is operating, to limit ineffective ES. Although any stimulation increases dpH/d*t*, it is only optimum parameters (duration, peak voltage, and pulse characteristics) that increase the fall ΔpH significantly. It is likely where ES is not regarded as effective or useful the resultant ΔpH has not been sufficient.

Safety

Occupational safety has been of utmost importance during experimentation and implementation of ES, especially when peak voltages as high as 1130 V are used. In some instances, safety concerns have effectively prevented commercial adoption of the process but, as indicated, modifications to pulse width resulting in much lower rms voltages have meant that the safety concerns are negligible. When required, isolating switches, warning lights, and proximity switches are used. For frequencies of 50 or 60 Hz, cardiac arrest is highly likely with any form of contact by personnel, but cardiac arrest is less likely with the pulsed waveforms now used. Systems have been developed for ES of beef carcasses ranging from continuous operations to manual insertion of electrodes. In past systems, the carcasses were enclosed within a shielded cabinet during ES. The new narrow-pulse systems have eliminated the need for this shielding. There are also food safety issues to consider. For example, where the stimulation is applied to carcasses before inspection, but after the hide is removed, the electrode system needs to be sterilized between carcasses.

Until recently, plants have applied ES to beef carcasses with an electrode on the nose or stick wound and grounding via the rail from which the carcass is suspended. Under these conditions the resistance of the narrow portion of the hind leg with high bone and tendon but low muscle content can be very high. The relatively high resistance encountered is due not to the bone component per se but to the narrow points of contact at the rigid interface, thus reducing contact area and severely limiting current flow. An anal electrode has been used in Australia for beef to provide good contact in the hindquarter, and using this system effective low-voltage ES has been achieved. The new narrow-pulse systems allow automatic application (Figures 2 and 3a,b) using rub bars as higher peak voltages can be used safely to overcome the increased electrical resistance of the bars.

Other Meat Quality Responses to Stimulation

In most instances ES is applied with the aim of ultimately improving tenderness, but there have been reported some minor adverse effects such as ES-mediated meat color changes. In most cases the stimulation has been suboptimal/ineffective and the chilling conditions inappropriate. It seems unlikely that electricity on its own affects meat color. The potential interaction of low pH and high temperature on prerigor meat are only present for a short time due to early rigor mortis (see below). Because of early rigor following ES, color changes are not identical at 24 h (typical results in Box 2). This is expected as electrically stimulated meat is in fact biochemically further along the postmortem pathway, including being more tender – this makes a slight difference at 24 h but not is not significant for aged meat.

Similarly, glycolysis is rapid following ES so the consequent early rigor mortis, even with a slow temperature decline of very large/fat beef carcasses, is so brief and does not produce significant myosin denaturation (it does not therefore mimic pale, soft, exudative (PSE) pork). Any drip that does occur following ES is expected as a consequence of early rapid tenderization, but the extent is similar to that without ES for the same tenderness (see below).

Denaturation of muscle protein (myosin) during the prerigor period, can occur to a small extent even for nonstimulated carcasses if the chilling rate is slow and even occurs to some degree under normal chilling conditions for all species – it is a characteristic of prerigor muscle, so it is not a direct stimulation effect. The temperature and pH conditions that

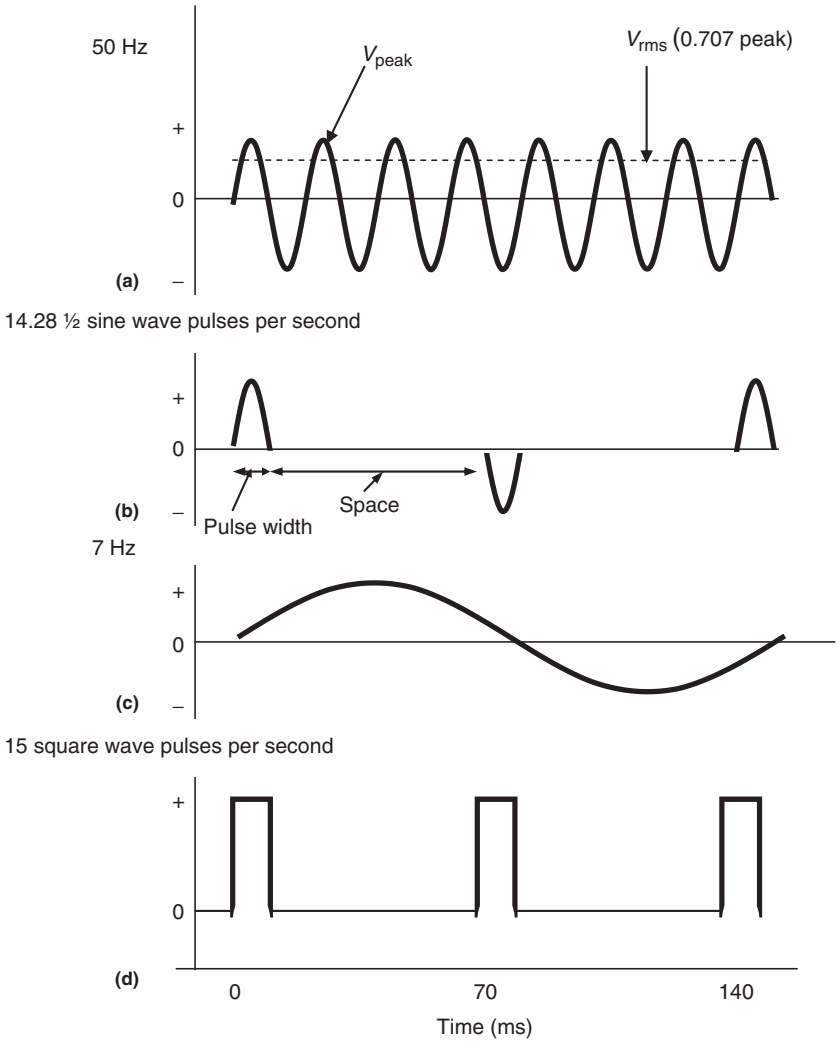


Figure 5 Terms used to describe pulses and waveforms illustrated by sinusoidal (a and c), half sinusoidal (b) and square wave pulses (d). In (a) there are 50 sinusoidal cycles per second (100 half sine wave pulses); peak and rms voltages are indicated. The pulses in (b) are obtained by cutting out half-sinusoidal pulses, which in this case gives 10 ms duration pulses, 14.28 pulses per second, with the same peak voltage. The pulse width (mark) and space between pulses give the mark-to-space ratio used to specify a single repetitive cycle, with the polarity of pulses and the number of cycles per second required to complete the description. The waveforms (b) and (c) both have the same period (inverse of frequency) and peak amplitude, but have different shape characteristics. Square wave pulses (d) can have variable widths depending on the characteristics desirable, and only a particular stimulation waveform is shown here.

result in myosin denaturation and drip have been modeled by Gerald Offer for pork and beef during glycolysis and cooling and he showed that once rigor occurs there is no further myosin denaturation. In particular, Offer stated, "If the rate of glycolysis is very high, although the muscle experiences particularly severe denaturing conditions so that the rate constant of denaturation reaches high levels, these are experienced for a sufficiently short time that the total amount of myosin denatured is lower rather than higher." This means that the brief prerigor exposure to elevated temperatures, following ES, reduces myosin related drip compared with longer prerigor exposure without ES at lower temperatures. In fact increasing stimulation effectiveness increases ΔpH and this in turn reduces the time to rigor and thus exposure to denaturation conditions.

Without ES, muscle held at constant elevated prerigor temperatures appears to have a reduced tenderization by inhibiting calpain activity. In contrast, with ES not only the prerigor exposure duration is reduced but also some fibers enter rigor almost immediately and the rest of the muscle fibers rapidly follow – the degree depending on initial prerigor glycogen – so full tenderization can then take place.

Postrigor cytoskeletal denaturation and tenderization is associated with production of free water. This means that the early appearance of drip as muscles tenderize rapidly at high temperatures, is a consequence of rapid and extensive tenderization, but there is no more drip for equivalent tenderization. The appearance of drip is unfortunate, but its appearance means meat has tenderized. Conditions that reduce drip generally also reduce tenderization.

Factors That Influence Effectiveness of Stimulation

Fall in pH upon Stimulation

The magnitude of ΔpH is governed by muscle fiber type, initial glycogen stores within the muscle, the electrical characteristics (current, frequency, pulse shape, and stimulation duration), temperature of muscle, and the time after death at which ES is applied. Postmortem delay, through a combination of lower muscle temperature and lower initial pH reduces ΔpH, but not the extent of pH reached – ES effectiveness and tenderness is not reduced.

Effects of Muscle Type on Stimulation Response

There is a difference in response to stimulation between various muscles that depends on muscle fiber type and the ratio of fiber types. Fast-twitch beef m. cutaneous trunci, largely composed of white muscle fibers, gives higher values for ΔpH (and dpH/d*t*), whereas in the slow-twitch m. masseter, composed of red fibers, there is neither a distinct ΔpH nor an acceleration of dpH/d*t*, which is naturally rapid (0.4 pH units per h).

Frequency, Voltage/Current, Pulse Shape, and Polarity Effects

Most beef and sheep muscles have a greater physical response, prolonged contraction, and a concomitantly greater ΔpH in the range from 9 to 16 pulses per second than at any other frequency, and most ES systems encompass these frequencies (Figure 6). In general, the higher the current (at a constant resistance, current increases with increased voltage) the greater will be the effect. This response will be asymptotic to some maximal value, however, so continually increasing the current will not lead to a continuing increase in effect once the ES parameters are supramaximal. The advantage of pulsed currents is that the energy of the electrical input is lower, with a smaller chance of melting structures in the current pathway such as the Achilles tendon.

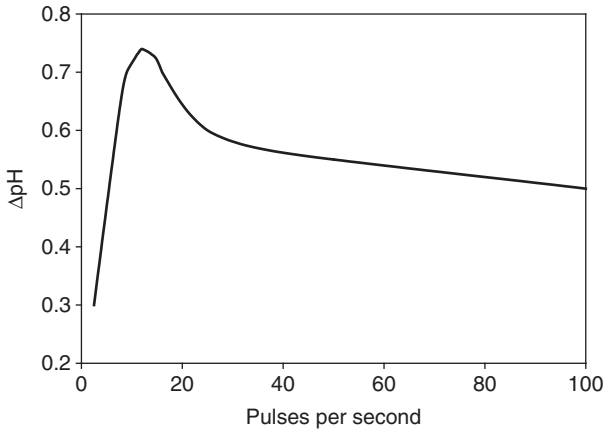


Figure 6 The effect of pulse frequency on the ΔpH value. The greatest effect lies between 7 and 15 pulses per second and these frequencies are chosen in most ES systems, although the effect is significant at all frequencies. With pulsed waveforms, the electrical input to the carcass is low and this is a major advantage, with lower heating.

There is recent evidence that the pulse shape (at a constant peak voltage/current) has an influence on the ES effect. Rapid-rise time rectangular pulses appear to have a greater stimulation effect compared with sinusoidal pulses of the same peak voltage/current. The width of the pulses also influences the stimulation effect.

Recent studies in Australia have explored this in some detail and have found that as the pulse width is reduced the effectiveness of ES diminishes slightly, but this reduction can be more than compensated for by an increase in the current. The consequence is that the rms voltage (a function of pulse width, frequency, and peak voltage) increases only slightly, but the effective ES effect is dramatically increased. With low-voltage ES, the greatest ΔpH values were obtained when the positive electrode was attached to the cranial end of the animal.

Changes in the Rate of pH Fall (dpH/d*t*) upon Stimulation

Almost any stimulation can affect dpH/d*t*, including unexpected stimulation arising from electrical stunning, immobilization of carcasses, electronic bleeding and even effects from current application during downward hide pulling. Rates of pH fall are slowest in nonstimulated muscles from curarized animals and there are even increases in dpH/d*t* with any vigorous muscle movement during slaughter. There can be a twofold increase of dpH/d*t* with stimulation bursts as short as 5 s. Unfortunately, coupled with the other adverse situations in susceptible species such as pork (e.g., stress and nondesirable genetics), a poor outcomes results. Some studies have shown when stimulation is sufficient (in other words a high ΔpH) this does not occur, but this has not been explored fully.

These increases in glycolytic rate can be explained by stimulation causing a reduction in the energy of activation (the amount of energy needed to start the reaction in excess of that already possessed by the molecules). If the activation energy is high, the rate is low and vice versa. The energy of activation (E_a) for the rate of pH fall of nonstimulated muscle has been calculated in a range of situations, with values from 40 to 110 kJ mol^{-1}. In one study for stimulated muscle, the E_a was 70 kJ mol^{-1} and for nonstimulated muscle from the same animal the E_a was 50 kJ mol^{-1} suggesting that fundamental changes, perhaps in enzyme activity, are induced by ES. In addition, dpH/d*t* is strongly affected by temperature, being faster at higher temperatures, so that an increase in temperature (increase in the kinetic energy of the molecules and hence in the rate) has a greater effect on dpH/d*t* of stimulated muscle than that of nonstimulated muscle. These changes are possibly a consequence of irreversible changes to ATPase activity that dictate the rate of ATP hydrolysis and therefore pH decline. For example, Ca^{2+}-activated actomyosin ATPase activity increases following ES, possibly owing to increased Ca^{2+} sensitivity, which in turn could account for the lower E_a.

Electrical Stimulation and Hot Boning

Hot boning (i.e., the process of boning the carcasses before attainment of rigor mortis in most muscles) has many

economic advantages (savings in energy, space, labor, and time). The major constraints to the use of hot boning have been the slippage of one muscle relative to another within a primal cut, which is considered visually less appealing, and the extra shortening of excised muscles subjected to rapid chilling. Aging is effective when muscle shortens by less than 20% (but reduces if shortening is greater), so hot boning and aging is feasible if temperatures are controlled.

The role of ES in hot-boning applications is clearly to hasten the onset of rigor mortis, so that cold shortening and rigor shortening are minimized. Rapid cooling subsequent to rigor mortis, with its greater control of microbial proliferation, especially in vacuum packaging, can then be used without irrevocably toughening the product. More effective ES is required when very rapid chilling systems are employed. ES by itself cannot prevent cold shortening encountered within rapidly chilled hot-boned meat.

In general, hot boning without ES (and also without any prevention of shortening) has resulted in a disproportionately greater increase in the toughness of beef muscles compared to those that are normally stretched when carcasses are supported by the Achilles tendon (e.g., beef m. psoas major). The sarcomere length of cold-boned psoas has been recorded at 3.3 μm (stretched), whereas when hot-boned product it has been fallen to 1.95 μm – close to that of the rest length of most muscles in the body, but further shortening occurs in other muscles. The observation that rigor at approximately 15 °C produces the most tender meat, whether hot-boned or restrained, suggests that further temperature control will result in significant tenderness improvements, but ES still significantly further improves tenderness.

Scientific Basis for Tenderization Involving Electrical Stimulation

Rigor Mortis, Cold Shortening, Rigor Contracture Calcium Levels, and Optimum Tenderization

If there is sufficient ATP in some fibers, cold shortening can still occur when muscle temperatures fall below 8 °C. A rule of thumb, for the prevention of cold shortening is to maintain temperature above 10 °C until muscle pH falls below 6.0.

Start of Tenderization

Proteolysis arising from the action of calpains takes place takes place to a minor extent prerigor, but significant tenderization through cytoskeletal proteolysis commences at rigor mortis, and can continue even in shortened muscle (without the meat becoming tender). If the longissimus muscle has been stretched (e.g., 'Tenderstretch' or SmartStretch™), then the beneficial changes due to tenderization occur earlier, but not necessarily to a greater extent. Under the same chilling regimen, stimulated muscles enter rigor mortis and commence to age at a higher temperature than nonstimulated muscles and hence initially experience faster tenderization. Early tenderness measurements will be substantially different for stimulated muscles compared with nonstimulated muscles.

Structural Effects

Histological images of stimulated muscle have shown on occasion the appearance of contractile bands containing predominantly stretched, ill-defined, and disrupted sarcomeres. The linkage between improved meat tenderness and physical disruption is plausible, as ES treatment has improved tenderness under circumstances where no cold shortening was evident. However, it is unclear whether it is the physical disruption per se that has caused the effect or whether the physical disruption facilitates aging in other ways, such as enhancing proteolysis. Contracture bands are not a direct consequence of electric current passing through the muscle, but are rather due to the supercontracture caused through localized excessive release of calcium ions from the sarcoplasmic reticulum (and also failure by the sarcoplasmic reticulum to pump calcium out). It could be this extra calcium that assists tenderization, since the key proteases are calcium dependent.

It is possible that physical stretching/tearing leads to an acceleration of proteolysis as a result of greater exposure of proteolytic substrates within muscle fibers, in addition to the direct effect of physical tearing. Stretched longissimus muscle (Tenderstretch) is initially more tender and the proteolysis starts from this greater tenderness, but the final tenderness is likely to be similar, being limited by connective tissue cross-linking with the final tenderness therefore not being affected. It has been shown that red muscles such as the masseter do not exhibit an increase in rate of pH fall upon ES, but do show evidence of supercontracture. However, white muscles such as the cutaneous are not so susceptible to cold shortening and show almost no supercontracture, yet the rate of pH fall is increased by ES. This raises the question whether structural alteration itself significantly affects meat tenderness of stimulated muscles, whereas there is no direct solid evidence available at the present time to discount the importance of physical alteration.

Impact of Physical Disruption on Ions

If the physical disruption is great enough to cause early release of calcium ions from the sarcoplasmic reticulum and mitochondria into the sarcoplasm, this will have a direct effect on activation of the calpain enzyme system and muscle shortening. It has been estimated that free calcium concentration could be raised to more than 100 μmol l^{-1} by an influx of extracellular calcium ions into the myofibrillar space. However, the evidence indicates that low-voltage ES per se does not lead to an increased release of 'free' calcium ions into the sarcoplasm. Calcium concentration in the intracellular space increases during ES, but the released calcium ions are taken back to the resting state into the sarcoplasmic reticulum if energy reserves are not completely depleted during ES treatment. This suggests that at the same temperature, stimulated muscle will be momentarily exposed to higher levels of 'free Ca^{2+}' and thus increased proteolysis. Under normal circumstances this extra Ca^{2+} is sequestered back into the sarcoplasmic reticulum. However, this reuptake process may be retarded if the sarcoplasmic reticulum pumps are affected. In addition, the stimulation accelerates pH decline, which is mirrored by an increase in 'free' Ca^{2+} and consequently an early activation of the tenderization process. Recent studies

have suggested that stimulation irreversibly damages the calcium pump so that it is less efficient at sequestering Ca^{2+} from the cytosol. This could also give rise to increased proteolysis via calpain system.

Effect of Stimulation on Calpain Enzyme Activity

There are several possible explanations why ES might increase the activity of specific enzymes such as the calpains (in addition to being fully available after rigor mortis). It may be due to some intrinsic effect associated with the rapid pH decline, with a low pH at elevated temperatures, that affects the processes governing the activation and inactivation of the calcium dependent proteases, or it could be due to a flow-on effect associated with a significant increase in 'free' calcium, which leads to activation of the calpains, particularly μ-calpain.

Because ES alters the postmortem pH–temperature relationship, it is reasonable to expect some effect on endogenous proteolytic enzyme systems (especially the calpain system) and the rate and extent of postmortem proteolysis. *In vitro* estimations show that the endogenous calpains within skeletal muscle can degrade the myofibril component within an hour, suggesting that ES might confer an advantage to the enzymes responsible for aging at a higher rate of proteolysis in those

Box 3 Clarification of issues relating to ES with questions and answers that explain positive unexpected outcomes from early rigor and early tenderization

Issues raised	*Answers*
Are there any disadvantages of stimulation?	No, other than safety requirements that have to be met. No clear evidence that there are adverse effects in heavy fat carcases
Can there be too much stimulation?	No, provided the correct pulse frequencies are used, otherwise there could be resistive heating. Stimulation produces its effect through the muscles doing work and this ceases when the pH falls sufficiently. Thus over stimulation is not possible – it is self-limiting
What are the optimum stimulation parameters?	There are a range of frequencies around 12–20 pulses per second. The pulse width can range from 2–10 ms and the stimulation duration must be sufficient to result in a significant pH fall (40+s). The required duration therefore depends on the applied voltages from 80 to 1100 and the time of application after slaughter. See **Box 1** for examples
How important is contact?	Good contact is important for the maximum current to flow. It is this current rather than applied voltage that needs to be standardized and some units are current controlled to ensure reproducible results. More reproducible results ensure if the pelt is removed before stimulation, as contact is better
What is the effect of stimulation on drip?	As meat tenderises drip is produced. Drip appears early following stimulation but there is the same drip for the same tenderization
Can stimulation produce a PSE-like condition?	No, PSE in pork arises from severe myosin denaturation prerigor and in worst cases a lot of drip is produced because myosin is a major muscle protein. Tenderization occurs via cytoskeletal denaturation postrigor mortis of smaller amounts ($<10\%$) cytoskeletal proteins so no extremes of drip thus not PSE
Following stimulation the temperatures are high and pH low and myosin denaturation could occur	Partially true but not significant. Myosin denaturation will occur to some extent even at low temperatures even without stimulation. Following stimulation rigor occurs rapidly for most muscle fibers and when these are in rigor they are protected from serious adverse effects. The remaining fibers are minimally affected by a brief exposure
What is the difference between high- and low-voltage stimulation?	High-voltage stimulation stimulates the muscle directly. Low-voltage stimulation acts via the nervous system. There are a wide range of useful parameters. Thus the pH falls are not identical. As the voltage rises the duration of stimulation needed to produce optimum effects decreases
Does stimulation work mainly through reducing cold shortening?	Only to a small extent. Cold shortening is an issue in small carcasses like sheep when rapidly chilled, but even when cold shortening does not occur meat is still more tender
What is the main advantage of stimulation?	Meat tenderises faster and to a greater extent and therefore customer-ready earlier. The reduction of refrigerated storage capacity required for long aging should lower costs
Are there disadvantages with long storage following stimulation?	No, stimulation ensures meat is ready for the customer earlier, but this takes the order of days. Chilled storage in transit takes weeks and a day or two earlier does not matter. However, meat tenderises fully following stimulation
Are there texture differences through stimulation?	Has been raised as a theoretical issue, but has not been discernable commercially (other than being more tender)
Can a heat ring occur?	Yes, this may occur with slow cooling but then disappears early during aging
Can stimulation upgrade cuts?	No, but it can ensure a given cut reaches its potential tenderness and it might appear to be upgraded compared with unsatisfactory processing
Does stimulation affect some breeds more than others?	Without stimulation there can be significant tenderness differences between breeds, but with stimulation this difference largely disappears and both are more tender than without stimulation

muscles. ES however, does not appear to have a role in activation of lysosomal enzymes under normal chilling conditions.

Unclear Interpretations of Stimulation Effects

ES is often regarded as a variation to normal meat processing – merely enhancing entry into rigor mortis and avoiding cold shortening, but this is only partially true. Without ES, problems with prolonged high rigor temperature cause both myosin protein denaturation and reduced aging – this does not appear to occur to the same extent with electrically stimulated muscles where similar exposure to high rigor temperatures are brief. Extrapolations from unstimulated to stimulated systems are therefore problematic as the two situations are not comparable as mentioned above. Even so, in some experimental studies without chilling it appears that even with ES, prolonged prerigor temperatures above 38 °C may inhibit tenderization to a minor extent.

Adverse effects of ES appear to be rare and many controversial issues arise possibly arising from ineffective ES with poor chilling (hence the emphasis on optimum ES parameters and optimum chilling above) or not measuring comparable indices at equivalent times. The implication that ES increases total drip appears misplaced, for example, and experimental evidence is either unclear, minor, or is not supported. The pre- and postrigor sources of drip therefore need to be explored in more detail to characterize this situation fully.

As overall muscle responsiveness ceases when the pH falls to approximately 6.3 during ES, it is physiologically not possible to over stimulate as muscles cannot respond. Although ES is necessary where cold shortening is an issue, several studies show that ES also ensures the greatest possible tenderness.

Elevated rigor temperatures in the absence of ES inhibit tenderization at the end of aging. Although early tenderness levels may be superior, final tenderness may not reach the levels for meat entering rigor at lower temperatures – thus without ES it is necessary to cool carcasses to temperatures where maximum tenderization occurs. The Meat Standards Australia (MSA) temperature window for optimum chilling was designed with this in mind. For other classes of carcasses (lambs/sheep and lighter beef) ES is needed to achieve the relevant MSA temperature/pH windows.

Is ES effective for pork? With optimum ES the answer is sometimes unexpectedly yes for some breeds. Other very stress-susceptible breeds such as Pietrain do not give good results. Surprisingly when successful, problems of excess drip do not appear to arise (other than as the expected consequence of tenderization). Insufficient ES, however, appears to set in place conditions that result in PSE as mentioned above.

In the case of unstimulated poultry, fast rigor entry and subsequent aging is normally rapid. An even faster rigor entry after ES allows wing removal, breast to be trimmed and portioning early without rigor toughening (adverse rigor effects seem to be more severe in poultry than other meats) in approximately 1 h without quality deterioration.

Conclusion

It is clear that ES improves meat quality and there are no clear disadvantages with proper chilling (**Box 3**), and optimization of parameters produces significantly better results. This improvement is maximized when meat is rapidly chilled to reach temperatures close to 15 °C and some advantages are summarized in **Box 2**, for tenderness, color and color stability. However, ES does not significantly improve inherently tender meat beyond baseline tenderness and cannot improve on the toughness associated with intemediate pH meat (i.e., ultimate pH 5.8–6.2) or improve upon the tenderness achieved from procedures such as Tenderstretch. Drip that appears is a consequence of degradation of cytoskeletal proteins during aging appears earlier for the same level of tenderization with or without ES.

Although ES of carcasses hastens the onset of rigor mortis and reduces cold-induced shortening and toughness, there can be other effects such as early activation of the calpain system, possibly involving elevation of calcium at critical times, which can hasten cytoskeletal protein degradation and generally positively contribute to tenderness, with concomitant fiber disruption potentially contributing. Because ES alters the postmortem pH–temperature relationship it is reasonable to expect some effect on endogenous proteolytic enzyme systems (primarily the calpain system) and subsequently the rate and extent of postmortem proteolysis and enhancement of the activity of the enzymes responsible for aging by the elevated temperatures that exist at that time.

See also: Carcass Chilling and Boning. Chemical and Physical Characteristics of Meat: Color and Pigment; Palatability; pH Measurement; Water-Holding Capacity. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Cutting and Boning: Hot Boning of Meat. Exsanguination. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Modeling in Meat Science: Meat Quality; Refrigeration. Muscle Fiber Types and Meat Quality. Refrigeration and Freezing Technology: Applications; Equipment; Freezing and Product Quality; Principles. Sensory and Meat Quality, Optimization of. Slaughter-Line Operation: Cattle; Sheep and Goats. Stunning: Electrical Stunning; Slaughter: Immobilization. Tenderizing Mechanisms: Chemical; Enzymatic; Mechanical. Tenderness Measurement

Further Reading

Chrystall, B.B., Devine, C.E., 1978. Electrical stimulation, muscle tension and glycolysis in bovine sternomandibularis. Meat Science 2, 49–58.

Devine, C.E., Ellery, S., Averill, S., 1984. Responses of different types of ox muscle to electrical stimulation. Meat Science 10, 35–51.

Devine, C.E., Wahlgren, N.M., Tornberg, E., 1999. Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef m. longissimus thoracis et lumborum. Meat Science 51, 61–72.

Ferguson, D.M., Jiang, S.-T., Hearnshaw, H.R., Rymill, S.R., Thompson, J.M., 2000. Effect of electrical stimulation on protease activity and tenderness of M. longissimus from cattle with different proportions of *Bos indicus* content. Meat Science 55, 265–272.

Gursansky, B., O'Halloran, J.M., Egan, A., Devine, C.E., 2010. Tenderness enhancement of *Bos indicus* and *Bos taurus* cattle through electrical stimulation. Meat Science 86, 635–641.

Hopkins, D.L., 2011. Processing technology changes in the Australian sheep meat industry: An overview. Animal Production Science 51, 399–405.
Hwang, I.H., Devine, C.E., Hopkins, D.L., 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness – A review. Meat Science 65, 677–691.
Jeacocke, R.E., 1977. The temperature dependence of anaerobic glycolysis in beef muscle held in a linear temperature gradient. Journal of the Science of Food Agriculture 28, 551–556.
Jeacocke, R.E., 1984. The kinetics of rigor onset in beef muscle fibres. Meat Science 11, 237–251.
Offer, G., 1991. Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. Meat Science 30, 157–184.
Pearce, K.L., van de Ven, R., Mudford, C., *et al*., 2010. Case studies demonstrating the benefits of optimising medium voltage electrical stimulation of lamb carcases. Animal Production Science 50, 1107–1114.
Rosenvold, K., North, M., Devine, C.E., *et al*., 2007. The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. Meat Science 79, 299–306.
Strydom, P.E., Frylinck, L., Smith, M.F., 2005. Should electrical stimulation be applied when cold shortening is not a risk? Meat Science 70, 733–742.
Taylor, A.A., Tantikov, M.Z., 1992. Effect of different electrical stimulation and chilling treatments on pork quality. Meat Science 31, 381–395.
Toohey, E.S., Hopkins, D.L., Stanley, D.F., Nielsen, S.G., 2008. The impact of new generation pre-dressing medium-voltage electrical stimulation on tenderness and colour stability in lamb meat. Meat Science 79, 683–691.

ENVIRONMENTAL CONTAMINANTS

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Introduction

Environmental contaminants arise in meat as a result of chemicals present in the areas where the food is produced and from the use of contaminated animal feeds in the production process. Most organic contaminants arise initially as a result of industrial processes combined with the properties of the compounds themselves in that they persist in the environment as a result of their chemical stability and other physicochemical properties. These compounds are termed persistent organic pollutants (POPs). Elements and some radionuclides may also be present as a result of the geology and geography of the regions where the animals, birds, or fish are farmed or produced, combined with the location of production and type of feed ingredients used. Stable environmental contaminants may also be resistant to metabolism in plants or animals, and this can lead to bioaccumulation as higher trophic levels of the food web are reached. Because fish are at or close to the top of the aquatic food chain, and farm animals are similarly placed in the terrestrial food chain, levels of these persistent ubiquitous contaminants can accumulate in the tissues of fish and meat-producing animals. Where meat and fish by-products are used for animal feed, there is further scope for the elevation in levels of these compounds, unless they can be removed during processing.

Pesticides

Residues of pesticides are more commonly associated with foods of plant origin than with meat and other food products of animal origin. Nevertheless, there is the potential of residues arising, for example, from the use on animals of insecticides, such as organophosphates, pyrethroids, and carbamates; however, these are rapidly metabolized and therefore unlikely to be found in high concentrations or long after application. Some pesticides are classed as POPs and hence residues of them can be found in the environment and can also be present in animal feed ingredients, for example, cocoa bean husks. The occurrence of organochlorine residues in cows' milk produced in countries where the organochlorine pesticides have not been used for some years may be attributed to the use of contaminated animal feed or animal feed ingredients imported mainly from less developed countries where the pesticides are still in current use. Such pesticides include dichlorodiphenyltrichloroethane (DDT), lindane (hexachlorocyclohexane, γ-HCH) and other HCHs, hexachlorobenzene (HCB), aldrin and dieldrin, chlordane, etc. together with their degradation products and metabolites. In more developed countries, the application and use of pesticides is legally controlled in such a way that residue levels occurring in food are minimized. Where they are used according to good agricultural practice, residues of these pesticides should not exceed maximum residue levels (MRLs), which are set on the basis of what is achievable by best practice, i.e., correct application rates and minimum harvest intervals.

Most of the more developed countries have in place monitoring programs to examine both home-produced and imported food; although the emphasis of these programs is directed toward foods of plant origin, there is a significant level of monitoring of animal and fish products. The number of MRL exceedences in fruits and vegetables is typically a few per cent (usually between 3% and 5%), whereas the number of violations for meat and animal products is generally well below 1%.

Persistent pesticides may also be found in aquatic systems. They may arise from direct use in wetlands where they may be used to control vector insects (e.g., DDT has been used to control the spread of malaria by mosquitoes) and may also be used in fish farming (e.g., some organophosphates are used to control sea lice infections of farmed salmon). Pesticides, especially herbicides, can also enter river systems as a result of rainwater and irrigation wash-off from agricultural land into rivers. The potential for these compounds to biomagnify and to accumulate in fish and other aquatic fauna is strong. The residues will reenter the land-based food chain if fish are eaten by wildlife or are caught for human consumption.

The organochlorine pesticides are highly lipophilic and can quickly accumulate in oily fish. There have been particular problems with eels caught in river estuaries, partly because of their oily nature and longevity and also because of the environments they inhabit.

Toxaphene

Toxaphene, or camphechlor, is a complex mixture of polychlorated bornanes (CHBs) and other camphenes. It was one of the most heavily used chlorinated pesticides in the world, with the total quantities used estimated in megatonnes, which is comparable to the usage of polychlorinated biphenyls (PCBs) (see Section Dioxins and Polychlorinated Biphenyls). Toxaphene has been shown to undergo long-range transport and is recognized as a ubiquitous environmental contaminant. Like other organochlorine pesticides, it has also been shown to bioaccumulate in aquatic organisms. Human exposure comes largely from human milk – for breast-fed infants (especially if the mother has been eating a diet rich in oily fish) – fish, and

seafood. Although toxicological data are scant, toxaphene is a probable carcinogen and is a known endocrine disruptor. Owing to weathering and biotransformation, the residue composition in food of marine or animal origins will not necessarily reflect the original pesticide mixtures used.

Dioxins and Polychlorinated Biphenyls

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) (collectively referred to as 'dioxins') arise as a result of combustion processes, as by-products in the manufacture of organochlorine compounds, or as a result of activity of the chlorine industry. They are chemically stable and are ubiquitously present in human tissues, even when there is no history of occupational or accidental exposure. Although exposure could occur through inhalation of air, dermal absorption, consumption of drinking water, and consumption of food, the last is the predominant route for the general population and accounts for more than 90% of human exposure.

Although there are a total of 210 dioxins, only the 17 laterally substituted congeners, i.e., those that contain chlorine at the 2, 3, 7, and 8 positions persist and accumulate in animal tissues. These congeners form only a small proportion of the total output from many sources and environmental pollution. These 2-, 3-, 7-, and 8-substituted congeners are regarded as significantly toxic and have thus been the main focus of most exposure studies. They are highly lipophilic and are thus found primarily in fatty tissues, such as human and animal fats and fish oils.

PCBs are a group of compounds that were manufactured until the 1980s for use in various ways, including electrical products (e.g., as a dielectric in transformer oil). They are ubiquitous environmental pollutants, and it has become widely accepted that some PCBs elicit dioxin-like biochemical and toxic responses. These are the coplanar PCBs, i.e., those with no or only one *ortho* substituent. Assessment of the health risks of exposure to dioxin-like chemicals must, therefore, consider these PCBs in addition to the dioxins. The amount of information pertaining to dioxin-like PCBs in foods is somewhat less than for PCDDs/PCDFs themselves but is growing rapidly. However, PCBs have a variety of other biological effects, and although consideration of 'dioxins' is incomplete without the inclusion of dioxin-like PCBs, the different types of toxic effects of these and other PCBs should be taken into account.

Because of their toxicity, both dioxins and dioxin-like PCBs need to be measured at extremely low concentrations in food, and the sum of dioxins and dioxin-like PCBs present is usually expressed in picograms ($1\ \text{pg} = 10^{-12}\ \text{g}$) of dioxins (as toxic equivalents to the most toxic 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD)) per gram of food. Analysis at these concentrations is technically extremely challenging and expensive and is probably the most complex chemical analysis that is carried out as part of regular monitoring programs. The majority of PCB analyses are carried out by gas chromatography using more routine methods, but these often do not measure the dioxin-like PCBs, which are present at much lower concentrations in the environment than other congeners.

In addition to the general environmental contamination from dioxins and PCBs, there have been specific isolated events that have resulted in the release of these compounds into the environment and hence to their incorporation into food within a localized area. Such incidents have included the accident in Seveso in 1976, when a manufacturing plant producing a chlorinated herbicide exploded, scattering several kilograms of 2,3,7,8-TCDD (the most toxic of the dioxins) around the immediate locality of the factory; the spraying of contaminated 'Agent Orange' herbicide (2,4,5-trichlorophenoxyacetic acid) in Vietnam in the 1960s conflict in order to defoliate the jungle; the Yusho- and Yu-cheng-contaminated rice oil incidents in Japan and Taiwan, respectively; and the contamination of animal feed with transformer oil containing PCBs and (to a lesser extent) dioxins in Belgium in 1999. In 2008, animal feed derived from waste food contaminated by dioxins in heating oil used to dry the feed was supplied to 7 pork producers and 38 cattle farms in Ireland, resulting in a recall of all Irish domestic pork and pork products that had been exported to 23 countries.

Tolerable Intake

In 1990, the WHO established a tolerable daily intake (TDI) of 10 pg per kg body weight (bw) for 2,3,7,8-TCDD, but in 1998, an expert consultation concluded that the TDI should include other dioxins and PCBs that exhibit a similar toxic effect. The concentrations of the toxic congeners were weighted according to their toxicity using Toxic Equivalency Factors (TEFs) to give units expressed as toxicity equivalents (WHO-TEQs) and were established as a range of 1–4 pg WHO-TEQ per kg bw per day. The concept of TEFs was developed to facilitate risk assessment. These TEFs have been established to express concentrations of mixtures of 2,3,7,8-substituted PCDDs and PCDFs and some nonortho and monoortho chlorine-substituted PCBs that possess dioxin-like activity in toxic equivalents (TEQs) of 2,3,7,8-TCDD. Concentrations of the individual substances in a given sample are multiplied by their respective TEF and subsequently summed to give the total concentration of dioxin-like compounds expressed as a TEQ.

$$\text{TEQ} = \sum[\text{PCDD}_i \times \text{TEF}_i] + \sum[\text{PCDF}_i \times \text{TEF}_i] + \sum[\text{PCB}_i \times \text{TEF}_i]$$

More recently, at the end of May 2001, the Scientific Committee on Food (SCF), an expert committee that advises the European Commission, decided that the tolerable intake should be expressed on a weekly rather than a daily basis and set a tolerable weekly intake (TWI) of 14 pg WHO-TEQ per kg bw per week. The WHO/FAO Joint Expert Committee on Food Additives (JECFA) established, in June 2001, a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ per kg bw per month. Current estimates of consumer exposure show that intake of dioxins and dioxin-like PCBs is between 1.2 and 3 pg WHO-TEQ per kg bw per day, which is a range that overlaps with the range of the recommended limits. It is, therefore, important that steps are taken to reduce the amount of these substances found in food by the implementation and enforcement of pollution control measures.

Legislation

Although regulatory limits for dioxins in food have been set on an ad hoc basis by various authorities in the past, the European Union (EU) became the first body to set extensive and comprehensive limits for these compounds. These EU regulations came into force in July 2002, and include limits for PCDDs/PCDFs in food and animal feed. There was a stated intention to revise these limits with a view to making them stricter before the end of 2004. In 2005, the WHO reevaluated the weightings given to individual dioxin and PCB congeners and produced a revised set of toxic equivalence factors (TEFs) to use when calculating the TEQ. New EU limits were eventually proposed in 2011 and came into force on 1 January 2012. They use the new TEF scheme and have been lowered for some food types. An additional set of limits for the nondioxin like PCBs was also introduced, based on the sum of six marker PCB congeners. Mandatory targeted testing for animal feed has been proposed.

Concentrations in Meat and Fish

Because PCDDs/PCDFs and PCBs are lipid soluble, for most food types containing more than approximately 2% fat, the concentrations found are reported on a fat-weight basis rather than on a whole-product basis. This gives more consistency for comparisons of samples containing variable concentrations of fat, such as dairy products, which show more variability with respect to dioxins on a whole-weight basis than on a lipid basis. For some samples, however, reporting on a fat-weight basis may lead to confusion. Fish can show large seasonal variations in fat content, which can result in an illusion of variation, even if the body burden with respect to dioxins remains constant.

As pollution control measures are introduced and come into effect, levels of dioxins and PCBs in meat have started to show a downward trend. The same is true, but to a lesser extent, for fish.

Most available data suggest that mean dioxin levels on a fat basis in pork in most cases are lower than for beef, poultry, or mutton; concentrations on a fat basis in animal livers are higher than in other tissues for the same species.

There is a widespread data reported for fish, probably because of the large number of species, and also the geographical differences in the levels of contamination in the various fishing grounds from which the fish originate. Typically, many species of white fish contain lower levels than oily fish species and some shellfish species. This is especially true when sourced from a relatively highly polluted area. Certain fish species originating from the Baltic region are recognized as containing high concentrations of PCDDs/PCDFs and PCBs. A significant proportion of fatty fish from this region, such as Baltic herring and Baltic salmon, are unlikely to comply with the EU limit for dioxins, and these fish would, therefore, be excluded from the Swedish and Finnish diets. There are indications that such an exclusion would have a negative health impact in Sweden and Finland due to the nutritional importance of omega-3 fatty acids, vitamin D, and other ingredients in the fish, some of which are especially beneficial in low-light countries. Consequently, there is a local exemption to compliance with this legislation. Sweden and Finland have in place a system that informs consumers of the dietary recommendations about the consumption of fish from the Baltic region, in order to avoid possible health risks.

Another source of dioxins in meat can be from pentachlorophenol (PCP)-treated wood (for preservative purposes) used to house farm animals and poultry. PCP can contain traces of dioxins in a characteristic congener profile, and there have been incidents when this was thought to account for the elevated contaminant levels found in meat.

How do these become residues in meat? It says above that: "They are POPs and behave in the environment in a similar way to the dioxins and PCBs."

Trace Elements

The main sources of metals and other elements in food come from the environment. Some of these, such as arsenic, can be endogenous in some circumstances (e.g., in Bangladesh, where there is groundwater contamination), whereas others, such as lead, normally arise as a result of pollution from industry and other human activities. Elements can also arise in food as a result of certain agricultural practices; for example, cadmium from impure or contaminated phosphate fertilizers can pollute farm land. Manufacturing processes are also potential sources of contamination; for example, tin can be introduced to the food supply from the canning process. It is also possible to introduce trace metals during food preparation when metals, glazed ceramics, or enameled utensils are used.

Trace elements are of interest because of their possible health effects. Unlike the other categories in this section, some of the elements such as iron and calcium have health benefits. Others have no known beneficial biological functions and long-term high-level exposures may be harmful to health. Elements in this class include mercury in organic mercury compounds, which are known neurotoxins; lead, which can impair neuropsychological development; inorganic arsenic, which is an acute poison and a human carcinogen; and cadmium, which can cause kidney failure. High levels of tin can cause acute abdominal problems. Many metals such as copper, chromium, selenium, and zinc are essential to health at appropriate levels but become toxic at higher levels of exposure.

Toxicity and bioavailability of trace elements can depend on the form in which they are present in food. For example, organic forms of mercury, such as methyl mercury, are much more toxic than inorganic elemental mercury. In contrast, it is the inorganic form of arsenic that is more toxic than the organic compound forms, such as arsenobetaine.

Trace elements are assessed for safety by comparing dietary intake estimates with recommended safe levels. The figures usually used are the provisional tolerable weekly intake (PTWI) values and provisional maximum tolerable daily intake (PMTDI) values as recommended by the FAO/WHO Joint Expert Committee on Food Additives. These are estimates of the amount of a substance that can be ingested on a weekly or daily basis over a lifetime without any known risk to health.

Some of the routes of the largest exposure to toxic trace elements, especially mercury and arsenic, are from fish and

shellfish that can bioaccumulate these contaminants from polluted waters.

Fish can take up mercury from marine sediments, and fish and farm animals can be exposed as a result of pollution from industrial emissions. The high exposure of human populations that consume large quantities of fish to mercury has led to interest in the neurological development of children from such areas, for example, Faeroe Isles and Republic of Seychelles.

Similarly, arsenic and aluminum may be found at elevated concentrations in fish. Other elements, such as copper, lead, and cadmium, may be found at higher levels in offal, although this is generally eaten in relatively small amounts and exposure to toxic elements from this source is, therefore, still small for most segments of the population. Concentrations of lead in meat and other foods are following downward trends because of measures to reduce lead in the environment in recent years following the introduction of a number of important pollution control measures, such as the move to unleaded fuel for motor vehicles and unleaded paints.

Radionuclides

Natural and artificial radioactive elements can be found in food. Both types normally enter the food chain as a result of general environmental contamination. Like their nonradioactive counterparts, deposition from the atmosphere or from contaminated water results in direct exposure for farm animals or can result in uptake from contaminated soil or surface deposition onto plants that are, in turn, consumed by farm animals and result in contamination of meat.

Natural Radioisotopes

Three different types of natural radioactive elements can occur in food. The first are those that have always been in the lithosphere and have half-lives measured in millions of years, such as uranium 238, thorium 232, and potassium 40. The second group is composed of daughter elements resulting from the disintegration of the longer life parent elements. Radium 226, produced from uranium 238, is an example of this type. Radium, in turn, can decompose to produce radon, which is also unstable and can generate lead 210 and polonium 210. The third group consists of isotopes formed by the action of cosmic rays in the atmosphere. An important product generated in this way is carbon 14, made by the transmutation of nitrogen.

Natural radioisotopes account for approximately a quarter of the total background dose of radiation to which one is exposed.

Artificial Radioisotopes

Artificial radioisotopes that can be found in food increased greatly following the explosion of the Hiroshima bomb in 1946. Increases were also particularly rapid during the 1960s, when the nuclear powers carried out many atmospheric tests. In addition, smaller amounts of artificial radioactivity are released into the environment by nuclear power stations and their associated plants that process the waste materials. A still smaller contribution arises from medical and research uses.

When an atomic bomb explodes or a nuclear reactor operates, a mixture of radioactive elements is produced from heavy isotopes such as uranium 235 and plutonium 239. Lighter radioactive isotopes may be produced as a result of fission, such as strontium 90 (half-life 28 years), cobalt 60 (half-life 5.3 years), ruthenium 106 (half-life 1 year), cesium 137 (half-life 30 years), and iodine 131 (half-life 8 days). There are also various activation products, including zinc 65 (half-life 245 days) and carbon 14 (half-life 5760 days). Activation products are not produced directly by the fission process but are produced alongside as a result of the action of atomic radiation, especially neutrons, on elements naturally present where the reaction occurs. Elements used in medicine, industry, and research can also be produced by a process of neutron bombardment of selected target atoms in specially designed reactors.

In addition to environmental levels, the relative uptake of radioactive isotopes is an important consideration when considering food and meat contamination. Plutonium can be extremely hazardous when directly ingested, but plants take up plutonium compounds only with difficulty. Its salts are less soluble than those of cesium or strontium, so it is unlikely to enter the food chain in significant quantities by this route. Surface contamination of plants and direct ingestion are of greater concern for this type of element.

Nuclear reactors can result in the contamination of seas and rivers by the discharge of low-level waste or by accidental release into either the atmosphere or the aquatic ecosystem. Some forms of marine life can concentrate cesium 137 and other radioactive isotopes in their tissues, as they do with some nonradioactive isotopes. Flatfish concentrate this isotope by a factor of 20 compared with surrounding seawater concentrations. Zinc 65 has been found to bioaccumulate in the flesh of oysters near the discharge pipes of nuclear power stations. Monitoring programmes for radioactivity in food place a large emphasis on fish and marine products because of this possibility, although so far no serious concern to health with regard to biomagnification of these isotopes has been identified.

Incidents Resulting in the Contamination of Food with Radionuclides

Chernobyl

The world's worst nuclear power accident occurred at Chernobyl in the former USSR (now Ukraine) in April 1986. A reactor at the Chernobyl nuclear power plant, located 80 miles north of Kiev, went out of control, creating a chain reaction that resulted in explosions and a fireball that blew off the reactor's heavy steel and concrete lid, releasing a large radioactive cloud into the atmosphere.

The Chernobyl accident killed more than 30 people immediately, and as a result of the high radiation levels in the surrounding 20-mile radius, 135 000 people had to be evacuated.

Levels of radioactivity resulting from the explosion are still unexpectedly high and are expected to remain so for another 50 years. Levels of radioactivity found in fish caught from lakes

in the UK and Norway, and in lambs grazing on upland hills, were found to be unexpectedly high after the incident owing to contamination, particularly with cesium 137. During the first 5 years following the accident, concentrations of this radioactive element in most foodstuffs and water decreased by a factor of 10, but since then, the rate of decrease appears to have slowed down. At the end of 2011, the UK Government proposed to remove all remaining controls on the movement of sheep from the restricted areas, based on the assessment that the risk to consumers from radioactivity in sheep meat resulting from the Chernobyl nuclear accident is now very low. Berries, mushrooms, and fish from some areas of the former Soviet Union are more likely to continue to be restricted from sale for some years, and fish from some lakes, such as Lake Kozhanovskoe, are thought to remain under restriction for approximately another 50 years.

Fukushima

On 11 March 2011, an earthquake of magnitude 9.0 hit off the northeast coast of Japan. The subsequent tsunami struck the Fukushima nuclear power plants and radionuclides were released into the environment. As part of the risk management process, provisional regulation measures for radionuclides in foodstuffs were taken. For radiocesium, uranium, plutonium, and transuranic α emitters, these limits were set to keep the committed effective dose (an estimate of the radiation dose to a person resulting from inhalation or ingestion of a given amount of radioactive substance) less than 5 mSv year^{-1}. For radioiodines, they were set to keep the committed equivalent dose to the thyroid less than 50 mSv year^{-1}. Tap water, raw milk, and some vegetables were the first foodstuffs found to be contaminated, but fish with radionuclides above levels of concern were detected soon afterward.

Meats, including beef, did not generally exceed the provisional regulation value for radiocesium, but measures were taken to insure that farm animals did not graze in the affected area and were not given contaminated feed.

As a result of the incident, it was possible to detect low levels of radionuclides in foods produced around the globe, but the major impact was on food produced around the plant. Many countries placed restrictions on the import of food from Japan. A massive program of monitoring was brought into effect by the Japanese authorities and, at the time of writing, restrictions are still in place.

Note

Environmental contaminants might be associated with specific incidents but many classes are ubiquitous within the environment and are present in all of the food we eat. The concentrations of many of these contaminants in food are very low, and we should be reminded of the work of Paracelcus who lived from 1493 to 1541 and taught us that 'the dose makes the poison.' Having said that, although the concentrations of these contaminants might be very small (e.g., a few parts per trillion or even quadrillion for dioxins and similar organic contaminants), these concentrations in foods give rise to exposure close to the levels shown to give rise to adverse health effects in experimental animals. But any risk assessment should form part of a risk-benefit analysis and many of the foods that contribute significantly to the source of these contaminants, such as oily fish, also have many health benefits associated with their consumption. Pollution control measures together with efforts to regulate food contaminants in some parts of the world have resulted in a reduction in the amounts of some of these contaminants in food over recent years.

Disclaimer

The views expressed in this article are those of the author and do not necessarily represent the opinions or policies of the UK Department for Environment, Food and Rural Affairs.

See also: Canning. Chemical Analysis: Analysis of Final Product Composition for Labeling; Raw Material Composition Analysis; Sampling and Statistical Requirements. Chemical Analysis for Specific Components: Curing Agents; Major Meat Components; Micronutrients and Other Minor Meat Components. Hazard Analysis Critical Control Point and Self-Regulation. Human Nutrition: Cancer Health Concerns; Macronutrients in Meat; Micronutrients in Meat; Vegetarianism. Microbiological Analysis: Standard Methods. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Allsop, M., Erry, B., Stringer, R., Johnston, P., Santillo, D., 2000. A Review of Persistent Organic Pollutants in Food UK: Greenpeace Research Laboratories, University of Exeter. ISBN: 90–73361–63–X.

Annual Report of the Working Party on Pesticide Residues, 1992. Supplement to the Pesticides Register 1993. London: HMSO. Available at: http://www.pesticides.gov.uk/committees/PRC/ (accessed 24.03.03).

Ebdon, L., Pitts, L., Cornelis, R., *et al.*, 2001. Trace Element Speciation for Environment, Food and Health. London: Royal Society of Chemistry.

European Commission Task 3.2.5. Brussels, 2000. Assessment of dietary intake of dioxins and related PCBs by the population of EU Member States. Reports on tasks for scientific co-operation. Available at: http://europa.eu.int/comm/dgs/health_consumer/library/pub/pub08_en.pdf (accessed 24.03.03).

Hamada, N., Ogino, H., 2012. Food safety regulations: What we learned from the Fukushima nuclear accident. Journal of Environmental Radioactivity. doi:10.1016/j.jenvrad.2011.08.008.

Radioactivity in Food and the Environment, RIFE-7 (and previous and later editions). Compiled by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) on behalf of the Food Standards Agency and the Scottish Environment Protection Agency (SEPA). Available at: http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CC4QFjAA&url=http%3A%2F%2Fwww.sepa.org.uk%2Fradioactive_substances%2Fpublications%2Fidoc.ashx%3Fdocid%3D59116ab2-635e-4ee6-b96f-90b1287cb5c2%26version%3D-1&ei=PGlhUqTKA8Oh0QXVh4CADg&usg=AFQjCNEwga8dWV59XG7yaoZAGH3nrAoVZA&sig2=io87—1lxkoJmc2yN7mJg&bvm=bv.54176721,d.d2k&cad=rja (accessed 18.10.13).

Schecter, A., Gasiewicz, T.A., 2003. Dioxins and Health, second ed. New York: Wiley.

Sheppard, C., (Ed.), 2000. Seas at the Millenium: An Environmental Evaluation. Oxford: Elsevier Science.

ENVIRONMENTAL IMPACT OF MEAT PRODUCTION

Primary Production/Meat and the Environment

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Glossary

CH_4 Methane.
CO_2 Carbondioxide.
GHG Greenhouse gas.
GWP Global warming potentials.
Glyphosate (Common commercial trade-name roundup) Is the most used herbicide in the world. It is a broad-spectrum and nonselective herbicide.
Mton Million ton.
N_2O Nitrous oxide.
N Nitrogen.
P Phosphorus
ppb Part per billion.

Introduction

In 2007, global meat production was close to 280 million ton (Mton), corresponding to approximately 40 kg meat per person as a global average, of which pork dominated with approximately 40% of total supply followed by poultry representing approximately 30% (Figure 1). For the past 20 years, production from mono-gastric animals has expanded much faster than from ruminants, and also milk production has had a smaller growth rate than pig and poultry. According to reports from the Food and Agriculture Organization (FAO), the demand for animal products is predicted to double by 2050 relative to a year 2000 baseline. The FAO report 'Livestock's Long Shadow' highlighted the environmental impacts of the fast-growing global livestock sector and this issue has gained a lot of attention in society over the past years. Recently, also dietary shifts, reducing overall intake of animal products in high-income countries, are increasingly discussed as a necessary mitigation option.

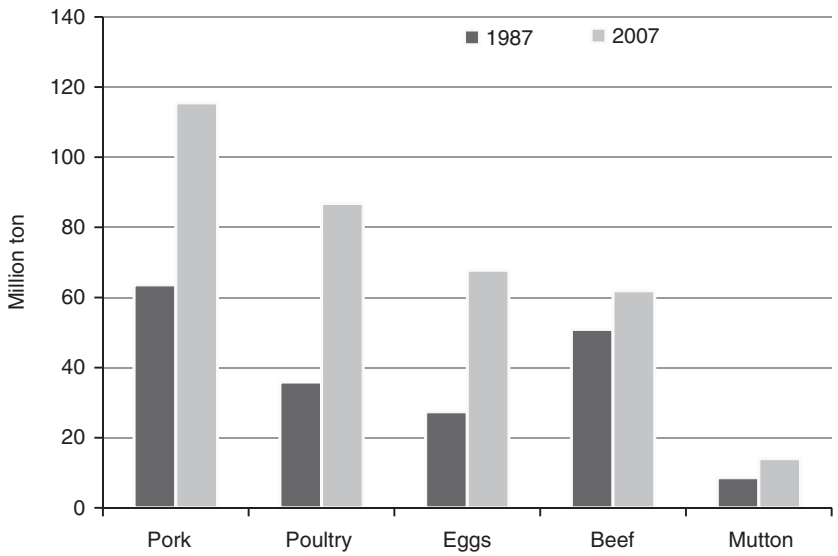

Figure 1 Global production of meat and eggs, 1987 and 2007. Reproduced from FAO, 2009. The state of food and agriculture – Livestock in the balance. Available at: www.fao.org/docrep/012/i0680e/i0680e.pdf (accessed 20.01.14).

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This article gives a short overview of the most important environmental aspects of livestock production: effects of land occupation and land conversion, interference in nitrogen and phosphorus cycle, emissions of greenhouse gases, and use of chemicals, and it is suggested that solutions must be found in both production and consumption phase of animal products.

Impacts Related to Land Use

The livestock sector today occupies approximately 30% of the global land surface and is thus the largest anthropogenical use of land. Approximately one-third of total arable land is dedicated for feed crop cultivation and in all, livestock production accounts for 70% of all agricultural land on the planet according to 'Livestock's Long Shadow.' The expansion of agriculture over the past 200 years has been dramatic, in 1750, total agricultural land was less than one billion hectare and this has increased almost fivefold in only 200 years. Agricultural expansion has had an enormous impact on habitats, biodiversity, carbon storage, and soil conditions such as erosion and organic matter. Globally agriculture has already converted 70% of grasslands, 50% of savanna, 45% of temperate deciduous forests, and 27% of tropical forests. Expansion of livestock production is a key factor in deforestation, especially in South America where approximately 70% of deforested land is occupied by pastures and feed crops (mainly soybeans) cover a large part of the remainder.

Biodiversity

One of the most direct drivers of biodiversity loss is land use change. Examples of this is conversion of temperate grasslands into arable land, or tropical forests into pastureland resulting in local extinction of most plant species and the associated animals whose habitat is largely determined by the composition of plant species. According to the Millennium Assessment, over the past few hundred years, humans have increased species extinction rates by as much as 1000 times over background rates that was typical in Earth's history. Because future scenarios predict that further 10–20% of grasslands and forestlands are projected to be converted mainly into agriculture by 2050, the impact of livestock's land requirement for feed and fodder production on biodiversity losses must be better understood and dealt with.

Rapidly growing demand for meat is the most important driving force for expansion of soybeans in South America. In Brazil, much of the soybean expansion has taken place in the cerrado biome where more than half of approximately 200 million hectare has been transformed into pasture with monoculture grass species and cropland in the past 35 years. The cerrado biome has the richest flora among the world's savannas and high levels of endemism (species not found elsewhere in the world). Deforestation rates have been higher in the cerrado than in the Amazon rainforest, and conservation efforts have been modest: only 2.2% of its area is under legal protection. Numerous animal and plant species are threatened with extinction.

Effects of livestock on biodiversity in Europe are described in a report from the Joint Research Centre of the EU Commission, investigating greenhouse gas (GHG) emissions and other impacts from European animal agriculture. Agricultural intensification has resulted in homogenization of large areas of European rural landscape. Of special importance to livestock production is farming system specialization (livestock vs. arable) with the loss of mixed farming system, larger farming units leading to removal of noncropped areas and field boundaries. But there are also positive impacts of cattle, the biodiversity in European seminatural grassland is very high and their management is dependent on grazing livestock. Very large proportions of Europe's most threatened bird species, vascular plants, and insects live in these grasslands and other 'high nature value farmland.'

Impacts Related to Nutrient Use

Mismanagement of nutrients in primary livestock production is the major reason for the large negative human interference with the global nitrogen (N) and phosphorus (P) cycles and this is informatively described in research papers of Erisman *et al.* (2008) and Cordell *et al.* (2009). In 2005, more than 100 Mton synthetic fertilizer-N and N in leguminous plants were an input in global agriculture whereas only 17 Mton N was consumed by humans in food. And as for P, it is estimated that less than 20% of the mined phosphate rock aimed for fertilizers ends up as P in humans' food. Important for the low overall use of nutrients in world agriculture is the fact that so much of the biomass produced is used for feeding the livestock. Utilization of N in an animal's feed is normally in the range of 10–35% (with cattle in the lower and poultry in the higher range) meaning that the majority of feed N ends up in manure. This is also the case for P, with utilization rates between 15% and 40%, varying for livestock categories and feeding systems.

Nitrogen

The yearly anthropogenical nitrogen input in the biosphere is estimated at approximately 140 Mton N of which ~85 Mton is industrial fertilizer production, ~33 Mton biologically fixed in leguminous crops, and ~21 Mton produced in combustion processes and emitted as nitrogen oxides. This nitrogen input is in the form of reactive N as opposed to the major constituent of the atmosphere, inert N_2. Consequently, each year, approximately 120 Mton reactive N enters agricultural production. The recently published book 'The European Nitrogen Assessment' provides the most comprehensive analysis of the nitrogen problem in agriculture and society.

Ammonia (NH_3) from livestock manure is a major pathway of losses of reactive N from agriculture. Globally, total nitrogen excreted in manure is estimated at approximately 112 Mton N (range 93–132 Mton) per year, thus amounting to the same magnitude as the input of new reactive N in agriculture. Ammonia losses provide approximately 20% of excreta-N in average, and there are great potentials for better use of manure in global agriculture, thus reducing emissions

and lowering input of synthetic N-fertilizer, which are based on fossil fuels in production.

Yearly losses of N in leaching and erosion from agricultural land are estimated at approximately 40 Mton N globally. The indicator 'Nitrogen loading indicator' is used for assessing watershed nutrient loads and shows that in Europe, water pollution from nitrogen is mainly the result of livestock production and fertilizer use. In India and southern parts of South America, livestock production is the dominant contributor whereas in North America and China, synthetic fertilizers dominate the total N load. However, when applying a life cycle-perspective on these findings, a considerable share of N-fertilizers is used in the cultivation of feed crops (e.g., 60% of European grains are used for feed) and leaching from the fertilizer use in these crops production is a result of demands in the livestock sector.

Emissions of the GHG nitrous oxide (N_2O) also represent a loss of reactive N from agriculture, but in absolute numbers, this is much lower than those of ammonia and nitrate. This is further discussed under in Section GHG Emissions and Global Warming.

Phosphorus

Mined phosphate deposits are mainly used in food production; as fertilizers (80%) and mineral feed (5%), whereas the remainder goes to industrial uses, mostly detergents. With current use it is estimated that today's economically exploitable resource will be depleted within 125 years and total reserves (the 'reserve base') within 340 years.

In the early 2000, global fertilizer use was roughly 15 Mton P per year in agriculture and approximately the same amount is produced in livestock manure annually of which approximately half is returned to agriculture and the rest is lost via land-fills, nonarable soils, and waters according to Cordell *et al.* (2009). Phosphorus in human excreta makes up approximately 3 Mton P per year and of this, only approximately 10% is returned to agriculture. It is obvious that livestock manure and human excreta must be better recycled to be used in agriculture to reduce the continuously growing use of fertilizer phosphorus.

When cereals are used as feed, there are large P flows from the fertilized croplands to animal manure at livestock production units. Besides this P-flow, there are also substantial P fluxes in byproducts from cereals and pulses used in concentrates, via food and feed industry and finally ending up in manure. For example, after milling wheat, the majority of P in the grain ends up in the byproduct wheat bran whereas the flour for human consumption has a low P content. After extraction of soybeans and rapeseed, most of the crops' uptake of P is destined for the feed coproduct meal/cake and the vegetable oil produced only holds a small amount of P. Production of especially pork and poultry animals leads to large P flows from mined fertilizer phosphates to crop production (grain and soybeans), via feed and food industry to feed concentrates.

Eutrophication and Acidification

Aquatic eutrophication means nutrient enrichment mainly from N and P of the aquatic environment. Excess input of nutrients increases the primary production of fast-growing algae and plants adapted to low-nutrient conditions decreases. Also in the fish community there are shifts due to changes in the habitats. Under severe conditions, nutrient enrichment of coastal stratified waters (having sharp temperature gradients preventing mixing surface and bottom waters) can cause anaerobic or nonoxygen conditions and result in significant bottom fauna mortality and fish mortality.

Terrestrial eutrophication is mostly an effect of excess input of N, as vegetation in natural ecosystems is mainly controlled by the limited availability of this nutrient. Atmospheric N deposition from human activities (most important NH_3 from manure and NO_x from traffic) leads to increased loads of N and, from this, follows changes in structures and functions in N-limited ecosystems. As explained earlier in the Section Nitrogen, NH_3 volatilization from manure represents a significant N loss from agriculture and of total N excreted by the livestock, as much as 20–40% can be lost as NH_3, depending on farming system, feeding routines, application methods, etc. Ammonia can also be an acidifying pollutant because it has a strong acidifying effect as a result of soil nitrification involving the conversion of ammonium into nitrate by microorganisms. Depending on the state of the ecosystem where the ammonia is deposited, the acidifying impact varies. Up to a certain level, a natural ecosystem can absorb deposited N, but above that level, excess nitrogen is leached and thus, the soil is 'N-saturated.' In forests saturated with N, nitrification and leaching of base cations and nitrate are usually the most important mechanisms behind soil acidification. There is a close interaction between terrestrial eutrophication and acidification.

GHG Emissions and Global Warming

Unlike industrial and transport systems, carbon dioxide (CO_2) from fossil fuel use is the least important GHG emitted from the animal sector. Instead, it is emissions of methane (CH_4) and nitrous oxide (N_2O) that contribute mostly to livestock products' GHG emissions. Also CO_2 emissions due to land use and land use change (LULUC), most important from deforestation, are important sources of greenhouse gases, see Figure 2. The global warming potential (GWP) for different greenhouse gases is normally calculated for a 100-year time horizon in kilogram carbon dioxide equivalents (kg CO_2e): CO_2 1, CH_4 25, and N_2O 298. FAO has estimated that global livestock production make up approximately 15% of total GHG emissions when land use-related CO_2 emissions also are included.

Methane

As seen in Figure 2, enteric fermentation is the most important source of CH_4 emissions from livestock production. CH_4 is also emitted from slurry storages and in a warm climate, this source can be substantial. Methane is approximately 25 times more effective in trapping heat in the Earth's atmosphere than CO_2 and its atmospheric concentration has increased from approximately 715 ppb preindustrial to 1774 ppb in 2005, i.e., by close to 150%. According to 'Livestock's Long Shadow,'

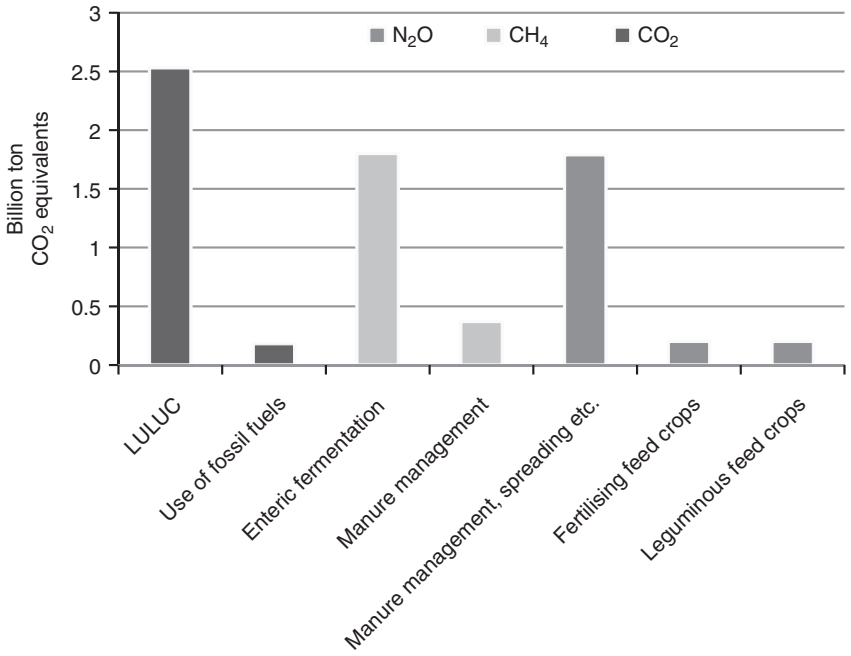

Figure 2 Emissions of greenhouse gases (CO_2, CH_4, and N_2O) from the global livestock sector. Reproduced from FAO, 2006a. Livestock's long shadow – Environmental issues and options. Available at: www.fao.org/docrep/010/a0701e/a0701e00.HTM (accessed 20.01.14).

global emissions from enteric fermentation and manure management in 2004 were 85.63 and 17.52 Mton CH_4, respectively. This corresponds to approximately 2.58 billion ton CO_2 representing approximately 5.3% of total global GHG emissions in 2004.

Nitrous Oxide

Emissions of the GHG nitrous oxide (N_2O) also represent a loss of reactive N in agriculture, but in absolute numbers, much lower than the losses of reactive N as ammonia and nitrate. Globally, it is estimated that approximately 2.8 Mton N yr^{-1} (1.7–4.8) is lost as N_2O–N from agriculture, mainly due to denitrification and nitrification processes in the soil and also nitrogen transformations in manure.

Manure storage, manure spreading in fields, and ammonia emissions from manure give rise to substantial N_2O emissions from the global livestock sector (Figure 2). Nitrous oxide is a strong GHG that is present in very low concentrations in the atmosphere. It is approximately 300 times more effective than CO_2 in trapping heat and has a very long atmospheric life-time (>100 years). Preindustrial concentration was approximately 270 ppb N_2O, which has grown to 319 ppb in 2005, i.e., an 18% increase. N_2O emissions have become more important in the second half of the twentieth century as a result of the strong increase in synthetic N fertilizer use in agriculture.

Carbon Dioxide

As seen in Figure 2, emission of fossil CO_2 is of minor importance for the global livestock sector's GHG emissions. But this is not the case for a highly industrialized region, such as the EU27, where the Joint Research Center of the EU Commission estimates that approximately 20% of the European livestock sector's GHG emissions come from the use of fossil fuels in animal production.

CO_2 emissions from land-use change processes are closely connected to expanding agricultural production and land clearance. Carbon emissions from forest clearing constituted approximately one-third of total anthropogenical CO_2 emissions in the period 1850–2005. In the past 50 years, there has been a stabilizing (or even decrease in agricultural land) in many regions but in the tropics, deforestation is still occurring rapidly. During the 1990s, it is estimated that tropical deforestation gave rise to CO_2 emissions in the order of 3.7–8 Gton CO_2 per year, comprising 14–25% of total anthropogenical emissions. Today, land use change, mostly deforestation, accounts for approximately 10% of global CO_2 emissions, and the emission trend has been falling over the past 10 years compared to levels during the 1990s. There are large uncertainties in estimates of GHG emissions from deforestation.

Indicators of a Changing Climate

The Intergovernmental Panel on Climate Change (IPCC) reports about a changing climate: The average global temperature has increased by 0.74 °C during the past 100 years (1906–2005) and during that period, average Arctic temperature has increased almost twice the global average. This global warming has led to a number of observed changes, for example, mountain glaciers and snow cover have declined on average in both hemispheres, global average sea level has risen at an average rate of 1.8 mm per year from 1961 to 2003, long-term trends from 1900 to 2005 have been observed in precipitation amount over many large regions, more intense and

longer droughts have been observed over wider areas since the 1970s, particularly in the tropics and subtropics, and widespread changes in extreme temperatures have been observed over the past 50 years. Cold days, cold nights, and frost have become less frequent, whereas hot days, hot nights, and heat waves have become more frequent.

Chemicals

Pesticides are chemical substances used to kill or control any pests, mainly weeds, insects, and fungus-attacking crops. There are large economic incentives to use pesticides to reduce the occurrence of pests and hence increase the quality and quantity of crop yield. In addition, use of pesticides is less labor-intense than traditional agricultural management practices. The disadvantages are pesticides potentially have toxic effects on humans and ecosystems and there are increasing risks of resistant pests. The impact of pesticides on different organisms varies greatly depending on their toxicity (direct and indirect), persistence, and fate. The use of pesticides and associated changes in management practices may also lead to biodiversity decline.

Extensive use of and reliance on one or only a few pesticides may also result in increased resistance to the pesticide(s) due to natural selection in the targeted weed-, fungi-, insect populations. An immediate example that is related to livestock production is a growing number of weed species that have evolved resistance to the important herbicide glyphosate, mainly occurring in areas where farmers grow feed crops (most importantly soybeans, but also maize) that have been genetically engineered to tolerate glyphosate. Owing to adaptation and natural selection in weed populations, the increased and often exclusive reliance on glyphosate to manage weeds in genetically engineered crop systems have led to that resistance to glyphosate having evolved in some weed species.

Veterinary medicines are another important chemical group used in livestock production and antibiotics are, by far, the most commonly used veterinary medicines and of great significance to modern livestock production, not only to treat diseases but also to promote growth and improve feed efficiency. Not all antibiotics are absorbed by the animal; sometimes 30–90% are excreted in manure. After excretion, the metabolites may still be bioactive and transformed back to the parent substance. The effect of pharmaceuticals on the environment has emerged as a scientific field over the past years.

Also for medicines, resistance is a growing problem. The main concern regarding the widespread use of antibiotics in veterinary medicine is the development of resistant bacterial strains, a health risk to both humans and livestock animals. Of special concern is antibiotic therapy in food-producing animals. Direct contact with animals, and thereby the risk of bacteria spreading via the food chain, enables the selection of bacterial strains resistant to antibiotics used in human therapy.

Conclusion

Livestock production is a key driver of environmental change. Already at present, the global livestock sector is one of the top two or three most significant contributors to some of the most important environmental problems, at every level, from local to global. The FAO prognosis of doubling global animal production to 2050 means the task of cutting the environmental impact per unit of livestock production by 50% to maintain the level of damage at the present level. This presents an enormous challenge to stakeholders in the livestock sector to reduce the sector's environmental impact. It involves improving efficiency in crop and animal production, reducing enteric CH_4 emission, improving manure management and handling, solutions discussed by de Boer *et al.* (2011). In recent years, there have been increasing discussions about the total intake of animal products in high-income countries and the possible need in future for reducing intake of animal products so that food-related emissions will meet future climate targets, this issue is further discussed in papers by Wirsenius *et al.* (2011), Garnett (2011), and Cederberg *et al.* (2012).

See also: Curing: Natural and Organic Cured Meat Products in the United States. Meat, Animal, Poultry and Fish Production and Management: Meat Production in Organic Farming; Red Meat Animals. Modeling in Meat Science: Meat Quality

Further Reading

Beusen, A.H.W., *et al.*, 2008. Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. Atmospheric Environment 42 (24), 6067–6077.

Bouwman, A.F., Beusen, A.H.W., Billen, G., 2009. Human alteration of the global nitrogen and phosphorus soil balances for period 1970–2050. Global Biogeochemical Cycles 23, GB0A04, doi:10.1029/2009GB003576.

Cederberg, C., 2010. Improving nutrient management in agriculture to reduce eutrophication, acidification and climate change. In: Sonesson, U., Berlin, J., Ziegler, F. (Eds.), Environmental Assessment and Management in Food Industry: Life Cycle Assessment and Related Approaches. Oxford, UK: Woodhead Publishing Limited, pp. 3–15. Woodhead Food Series No. 194.

Cederberg, C., Hedenus, F., Wirsenius, S., Sonesson, U., 2012. Trends in greenhouse gas emissions from consumption and production of animal products in Sweden – Implications for a long-term climate target. Animal, doi:10.1017/S1751731112001498.

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus: Global food security and food for thought. Global Environmental Change 19 (2), 292–305.

de Boer, I.J.M., Cederberg, C., Eady, S., *et al.*, 2011. Greenhouse gas mitigation in animal production: Towards an integrated life cycle sustainability assessment. Current Opinion in Environmental Sustainability 3, 423–431.

Denman, K.L., *et al.*, 2007. Couplings between changes in the climate system and biogeochemistry. In: Solomon, S.D., Qin, M., Manning, Z. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK and New York, NY, USA: Cambridge University Press, pp. 539–546.

Erisman, J.W., Sutton, M.A., Galloway, J., Klimont, Z., Winiwarter, W., 2008. How a century of ammonia synthesis changed the world. Nature Geoscience 1 (10), 636–639.

European Commission, Joint Research Center (JRC), 2010. Evaluation of the livestock sector's contribution to the EU greenhouse gas emissions (GGELS) – Final report. Available at: http://ec.europa.eu/agriculture/analysis/external/livestock-gas/full_text_en.pdf (accessed 20.01.14).

FAO, 2006a. Livestock's long shadow – Environmental issues and options. Available at: www.fao.org/docrep/010/a0701e/a0701e00.HTM (accessed 20.01.14).

FAO, 2006b. World agriculture: Towards 2030/2050, Interim Report. Available at: www.fao.org/docrep/009/a0607e/a0607e00.htm (accessed 20.01.14).

FAO, 2009. The state of food and agriculture – Livestock in the balance. Available at: www.fao.org/docrep/012/i0680e/i0680e.pdf (accessed 20.01.14).
Foley, J., *et al*., 2011. Solutions for a cultivated planet. Nature 478, 337–342.
Garnett, T., 2011. What are the best opportunities for reducing greenhouse gas emissions in the global food system? Food Policy 36, S23–S32.
Klink, C.A., Machado, R.B., 2005. Conservation of the Brazilian Cerrado Conservación del Cerrado Brasileño. Conservation Biology 19 (3), 707–713.
Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65 (5), 725–759.
Smit, A.L., Binraban, P.S., Schröder, J.J., Conijn, J.G., van der Meer, H.G., 2009. Phosphorus in Agriculture: Global Resources, Trends and Developments. Wageningen, The Netherlands: Plant Research International B.V.
Waltz, E., 2010. Glyphosate resistance threatens Roundup hegemony. Nature Biotechnology 28 (10), 1129.
Wirsenius, S., Hedenus, F., Mohlin, K., 2011. Greenhouse gas taxes on animal food products: Rationale, tax scheme and climate mitigation effects. Climatic Change 108 (1–2), 159–184.

Relevant Websites

www.nine-esf.org/ENA-Book
The European Nitrogen Assessment.
www.globalcarbonproject.org
The Global Carbon Project (GCP).
www.ipcc.ch/publications_and_data/ar4/wg1/en/ch2.html
The Intergovernmental Panel on Climate Change (IPCC).
www.millenniumassessment.org/documents/document.354.aspx.pdf
The Millennium Assessment.

EQUIPMENT CLEANING

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Glossary

Chemical energy Energy derived from cleaning agents used to disrupt the chemical interactions in a soil–surface interface.
Cleaning agent A chemical used to disrupt and solubilize soils.
Contact time Time allotted for cleaning agent to interact with a soil.
Food contact surface Equipment surface that physically contacts food during production.
Mechanical energy Physical (i.e., kinetic) methods (e.g., scrubbing, pressure sprays, turbulent flow) used to create energy for the disruption of soil–surface interactions.
Planktonic cells Bacterial cells that are suspended in a medium (i.e., not attached to a surface).
Sanitizer Chemical agent used to inactivate microorganisms.
Soil Any substance present in a food production environment that should not be present.
Surface energy The energy associated with a soil–surface interaction that must be overcome for effective soil removal.
Thermal energy Heat energy used to facilitate disruption of soil–surface interactions.

Introduction

The maintenance of a hygienic plant environment is a fundamental requirement for the consistent production of food that is safe, wholesome, and stable during the specified shelf-life. An inability to reliably and effectively clean and subsequently sanitize food production equipment may lead to increased risks of product spoilage and/or contamination by allergens and/or pathogenic microorganisms. Accordingly, appropriate efforts should be made to minimize the presence of soils before, during, and after production of food.

Soil removal is essential before the application of sanitizing chemicals. It is well established that cleaning and sanitizing contribute equally to reductions in numbers of microorganisms on equipment surfaces. For this reason, appropriate cleaning agents and strategies can be selected only with knowledge of the types of soil expected from individual raw materials and from processed food products. Inadequate cleaning may lead to soil accumulation. Biofilms may then form on equipment surfaces, and cause corrosion, reductions in processing efficiency, and problems with finished product quality. When deciding on procedures for control of soils, the benefits from cleaning and sanitation must be weighed against the related costs. Although increases in the number or duration of breaks in production for cleaning and sanitation will enhance soil control (Figure 1), they also result in higher costs for cleaning/sanitation chemicals, water, sewage, energy, and labor (Table 1). Accordingly, to balance cleaning-related costs against production efficiency and control of microbiological contamination, environmental trend analysis of microbiological data may be used to fine-tune production–sanitation cycles.

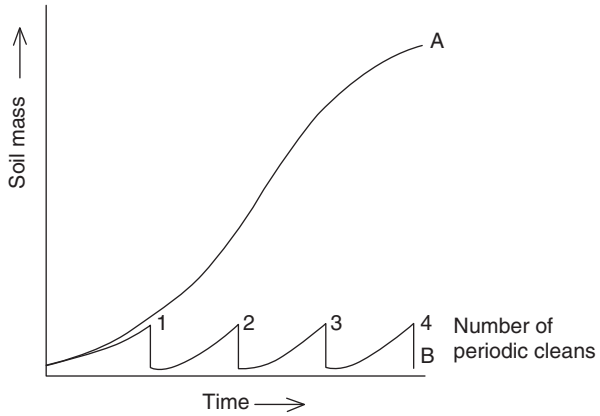

Figure 1 Accumulation of soils over time as impacted by two cleaning approaches: (A) no periodic cleaning and (B) with periodic cleaning. Adapted with permission from Holah, J.T., 2003. Cleaning and disinfection. In: Lelieveld, H.L.M., Mostert, M.A., Holah, J., White, B. (Eds.), Hygiene in Food Processing. Cambridge: Woodhouse Publishing Limited (Chapter 13).

Table 1 Costs associated with cleaning and sanitation

Input	*% Contribution to total cost*
Labor	46.5
Water and sewage	19.0
Energy and utilities	8.0
Cleaning agents and sanitizers	6.0
Corrosion damage	1.5
Other	19.0

Source: Adapted with permission from Marriot, N.G., 1997. Essentials of Food Sanitation. New York: International Thompson Publishing and Chapman and Hall (Chapter 7).

 doi:10.1016/B978-0-12-384731-7.00212-9

Soils, Surfaces, and Cleaning

The original definition of a soil was any material or substance that is, but should not be, present on a solid substrate (i.e., surface) of interest. Although this definition recognizes that a soil may be any sort of material (e.g., dirt, dust, grease, food derivatives, etc.), it does not take account of the complex natures of many soils. Subsequent definitions categorize soils on the basis of their solubility in acid, alkali, or water. These definitions are highly relevant to the selection of cleaners that will appropriately interact with and thus solubilize a soil. More recent classifications of soils recognize their essential chemical properties. Thus, soils are characterized as mineral (lime, milk stone, etc.), organic, or microbiological, or composite when a soil is composed of two or more of the simpler soils; but not all soils conform to this classification. Nonconforming soils include dust and debris. Organic soils are further categorized as carbohydrate (CHO), lipid, or protein based, and these subcategories may be further characterized. For example, CHOs may be identified as being water soluble or producing Maillard reaction products following heating. Such information is important as water-insoluble soils and Maillard reaction products are more difficult to remove than water-soluble CHOs. For proteins, it is essential to know the denaturation temperature and the appropriate pH to use for cleaning. Processing or cleaning waters at temperatures above the denaturation temperature or cleaning solutions of inappropriate pH may cause partial or total denaturation of soluble proteins, which consequently become insoluble and difficult to remove from surfaces. In addition, the fluidity and solubility of soil lipids should be considered, that is, whether the lipids are water soluble or insoluble, and whether they are liquid or solid at room temperature. Knowledge of these matters allows realistic considerations of the complex nature of soils, and facilitates appropriate cleaner selection.

Another key factor that affects soil deposition on equipment surfaces is the nature of the surface. It is well established that surfaces that are corroded, have obvious defects, or are porous are more difficult to clean than smooth surfaces, and may have microbial harborage sites in which biofilms can develop. The difficulty with removing soils from such surfaces is a result of the much greater area available for contact with soils than is available on smooth surfaces. Further, while suspension of a soil in water requires the input of energy, soil adherence to surfaces is an energetically favorable process. The more interaction there is between a soil and a surface, the more energy is required to disrupt the interactive forces in order to displace and remove the soil. Cracking and pitting of smooth, impermeable surfaces increases areas for adherence of soil, and therefore equipment with such defects should be avoided.

Interactions between soil and surface are determined by the chemical composition of the surface as well as its roughness. Thus, to maximize cleanability, all surfaces in food production environments should be smooth and made of hygienically acceptable materials (Table 2). Therefore, equipment should be constructed from hygienic grade plastics, rubbers, stainless steel, etc., whereas porous materials, such as wood, should be strictly avoided. The chemical reactivity of the surface has a critical effect on the deposition of soils on both food contact surfaces (FCS) and nonfood contact surfaces (nFCS). Various grades of stainless steel are available in which the contents of chromium, nickel, and other metals alloyed with iron are adjusted to improve corrosion resistance under different conditions of use. These formulations balance cleanability, durability, and resilience to oxidizing compounds routinely used in sanitation programs. Importantly, sanitation crews should report any corrosion, pitting, or damage to equipment to allow the matter to be corrected before product safety or quality is compromised.

Surface Energy

To effectively remove a soil, the surface energy (SE), i.e., the interaction between the soil and the surface, must be overcome. Energy used to overcome the SE may be chemical,

Table 2 Characteristics of hygienically acceptable and unacceptable surfaces observed in food production environments and equipment

Material	*Characteristics*	*Precautions/suggestions*
Wood	Draws moisture and lipids into porous structure; alkalis degrade it	Its use should be avoided due to hygienic and structural concerns
Black metals	Acids and chlorinated compounds will corrode them	Tinning or galvanizing minimizes corrosion. Neutral detergents should be used for cleaning
Tin	May be corroded by strong acid or alkali cleaners	Tin surfaces should be used only for surfaces that do not contact food
Concrete	Etched by acidic foods and cleaning compounds	Acid-resistant, dense concrete should be used in production environments; alternative, use acid brick
Glass	Should be smooth and impervious, but may be etched by strong alkaline cleaners	Mild cleaners should be used for cleaning
Paint	May be etched by strong alkaline cleaners	Nontoxic paints may be used in production environments if issues with peeling are unavoidable
Rubber	Firm, nonporous rubber should be used. Organic solvents and strong acids may degrade it, though it is resistant to alkaline detergents	Rubber cutting boards warp and dull knives
Stainless steel	Smooth, impervious, resistant to corrosion, may oxidize at elevated temperature, nonmagnetic, easy to clean	Expensive. Halogen compounds may corrode stainless steel

Source: Adapted with permission from Marriot, N.G., 1997. Essentials of Food Sanitation. New York: International Thompson Publishing and Chapman and Hall (Chapter 7).

mechanical (i.e., kinetic), or thermal, or a combination of these. Cleaning chemicals provide chemical energy to break apart and disperse soils, and keep removed soils suspended in solution so that rinsing is effective for carrying away the dislodged soil. Mechanical energy may be generated by scrubbing, pressure sprays, or turbulent flow in pipes, vessels, etc. that are cleaned using a clean-in-place (CIP) system. One of the most effective yet commonly overlooked means of overcoming SE is scrubbing. Thermal energy enhances most chemical and physical methods of reducing soil–surface interactions. For example, heated cleaning water increases the efficacy of chemical cleaners approximately twofold for every increase of 10 °C. This is particularly useful for lipid-based meat soils, as application of heated cleaning solutions that exceed the melting temperature of the fat greatly enhances its removal. However, if soils include proteinacious components, care must be taken not to exceed 55 °C because above this temperature protein denaturation may occur with the formation of insoluble residues that are difficult to remove. Moreover, at temperatures >50 °C, sublimation of iodine-based sanitizers may occur, reducing the antimicrobial activity of the sanitizer.

In addition, time is a key factor for the effectiveness of a cleaning program. In general, the longer cleaning-related chemicals remain in contact with soils, the more successful the soil removal process will be. Conversely, if the contact time between soil and cleaning compounds is not sufficient, stubborn soils may persist on equipment surfaces. Costs linked to chemical, mechanical, and thermal energy inputs may be reduced by prolonging the contact time between cleaning agent and the soiled surface; and combining chemical, mechanical, and/or thermal energy inputs in a cleaning regime will reduce the respective energy input required from each energy source. That is, less detergent will be required if the cleaning water is heated and the surface is scrubbed. However, if cleaning efficacy is enhanced by increased scrubbing or the use of higher concentrations of detergent, the cost of labor, or cleaning agent will increase. Suggested combinations of energy input are detailed in Figure 2. Overall, smaller items such as utensils, pails, and molds require a high input of mechanical energy, with soil removal being improved with soaking in a solution of chemical cleaner before scrubbing. Disassembled equipment that can be transported to soaking tanks should be treated in a similar manner. In contrast, for larger equipment, manual and thermal energy inputs are often necessarily limited, resulting in increased reliance on chemical and thermal energy. However, reduced concentrations of chemical cleaners and lower water temperature may then have to be used to minimize risks to workers engaged in cleaning. In such circumstances, increased cleaning agent contact time may be used to aid in soil removal.

The quality of water used for cleaning is also important. Hard water with high concentrations of calcium and magnesium salts, sulfates, and bicarbonates reduces the activities of some cleaning and sanitizing agents. Therefore, it is advisable to soften cleaning water with chelating agents or sequesterants. In addition, softening of cleaning water will minimize the deposition of stubborn mineral deposits on equipment surfaces. Obviously, potable water must be used for cleaning all food production equipment and facilities.

Composition of Soils

The composition of soils deposited during the production of food will reflect the raw materials used, the end product, and the processing technology or technologies employed. In addition, microorganisms present in or on raw materials or in the production environment may contaminate surfaces of equipment and, ultimately, the food being produced. In the production of raw and processed meat products, predominant soils consist of lipid and proteinacious components. These soils often accumulate to high levels during long production runs, thereby limiting the effectiveness of CIP cleaning and

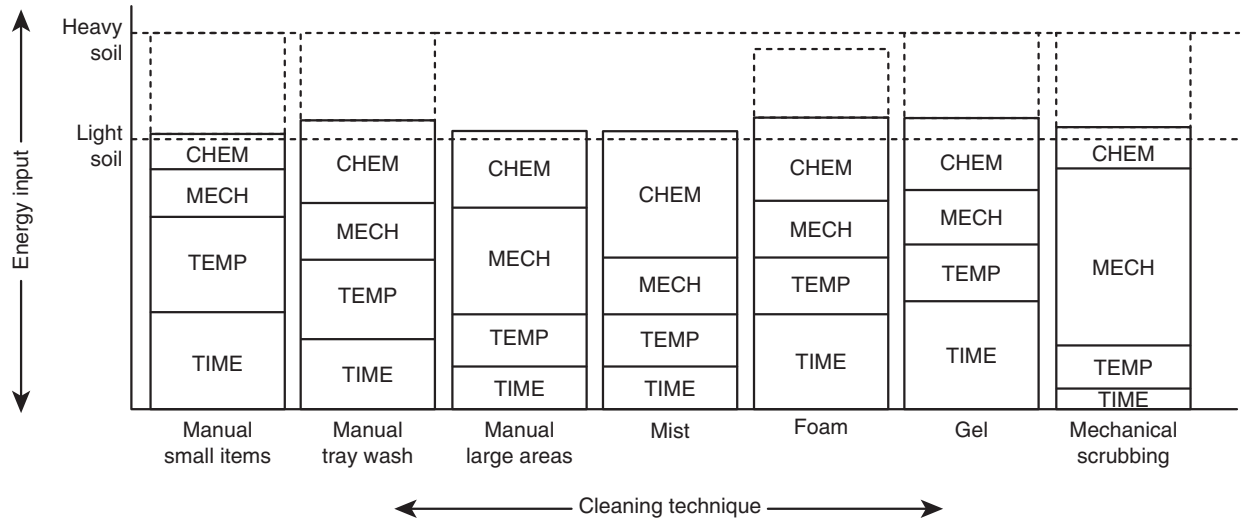

Figure 2 Suggested combinations of energy input for the removal of light and heavy soils. Adapted with permission from Holah, J.T., 2003. Cleaning and disinfection. In: Lelieveld, H.L.M., Mostert, M.A., Holah, J., White, B. (Eds.), Hygiene in Food Processing. Cambridge: Woodhouse Publishing Limited (Chapter 13).

Table 3 Soil solubility characteristics and suggested cleaning agent properties

Nature of soil	*Solubility characteristics*	*Recommend cleaning agent*
Carbohydrates, organic acids, salts	Water soluble	Mild alkaline detergent
Proteinacious (e.g., fish, meat, poultry)	Alkali soluble, slightly acid soluble, water soluble	Chlorinated alkaline detergent
Starch-rich (e.g., fruits, tomatoes)	Alkali soluble, partially water soluble	Mild alkaline detergent
Lipid-rich (e.g., adipose, butter, oils, etc.)	Alkali soluble, water insoluble	Mild-to-strongly alkaline detergents
Inorganic soils (e.g., hard water deposits, milk stone)	Acid soluble, alkaline insoluble, water insoluble	Acid cleaners

Source: Adapted with permisson from Elliot (1980).

sanitation strategies. Solubility characteristics of soils and the type of cleaning agents recommended for use with each type of soil are shown in Table 3.

Soil Removal

Strategies for effective cleaning are specific to each food production facility. However, a general step-wise strategy for effective soil removal in most production and processing facilities is as follows: (1) gross soil should be physically removed before moving any equipment, and then utensils and movable equipment should be disassembled and moved to a location suitable for cleaning activities; (2) gross soils along the production line and throughout the production environment should be removed, with larger soils being picked up to prevent the unnecessary spreading of soils; (3) because lipid- and protein-rich soils can be expected in meat production facilities, equipment should be subjected to an initial rinsing with hot water at temperatures $<55\,^{\circ}C$; (4) remaining soil should be disrupted using chemical, mechanical, and thermal energy, while adhering to the concentrations and contact times recommended for each cleaning agent; and (5) equipment should be rinsed with preferably pressurized hot water at $<55\,^{\circ}C$ to facilitate dispersion of soils from equipment surfaces being cleaned.

Following soil removal and sanitation, potable water should be used to remove cleaning and sanitizing agent residues from equipment surfaces. Although levels of microorganisms should be greatly reduced by cleaning, microorganisms may still be present on surfaces and/or in hard-to-clean areas. As such, after rinsing it is important to remove excess water from FCS and nFCS throughout the production environment. Moisture facilitates the survival and proliferation of microorganisms. Therefore, after cleaning, equipment and the processing facility should be dried as much as is practicable.

It should be noted that the cleaning process itself may serve to disperse soils in undesirable ways. If cleaning agents are not effective in keeping soils suspended, re-deposition of soils on equipment may occur, at their original sites or elsewhere. In addition, the use of pressure sprayers to remove large accumulations of soil will lead to increased soil deposition throughout the production environment. Both high-pressure/low-volume and low-pressure/high-volume sprayers generate aerosols, which have been shown to redistribute bacteria and soils within a production area. Accordingly, it may be necessary to limit the use of pressure sprayers in areas where risks of microbiological contamination of product are high.

Hygienic Design of Equipment and Facility

The persistence of bacterial pathogens in food processing environments poses major risks of processing and/or post-processing contamination of product. Equipment that is not hygienically designed or is improperly installed or maintained will compromise the efficacy of a cleaning and sanitation program by introducing harborage sites in which pathogens will be protected from sanitizing treatments. For example, if meat slicers are not designed to permit easy and quick disassembly, routine cleaning may not be carried out effectively. Accordingly, slicers have been identified as the sources of product contamination in several listeriosis outbreaks linked to ready-to-eat meats. Similarly, equipment used in facilities for breaking beef carcasses have been shown to present major sanitation challenges. Therefore, hygienic design of the food processing equipment and premises is a critical prerequisite for effective cleaning and sanitation.

Standards for sanitary fabrication, construction, and design of food processing equipment have been developed by a variety of standards organizations, such as 3-A Sanitary Standards, Inc., the National Sanitation Foundation, and the European Hygienic Energy & Design Group. Although there are some differences among these standards, the objective of all is the application of sound sanitary principles in the design and manufacture of food processing equipment. The general requirements for hygienic meat processing equipment are:

1. Equipment should be made of compatible materials. Materials used for construction of food processing equipment shall be completely compatible with the product, the processing environment, and cleaning and sanitizing procedures. To ensure durability, materials should be corrosion and abrasion resistant and, where applicable, capable of being shaped.
2. Self-draining. All pipelines and equipment should be self-draining. Residual liquids in or on equipment harbor and promote microbial growth or, in the case of cleaning fluids, result in chemical contamination of product or corrosion of equipment surfaces (Figure 3).
3. No dead spaces and niches. Hollow areas should be hermetically sealed and introduction of dead spaces during installation should be avoided. Equipment parts should be free of pits, cracks, corrosion, and other visible defects (Figure 4).
4. Accessible for inspection, maintenance, cleaning, and sanitation. Equipment should be designed so that all product contact surfaces can attain the required sanitized or sterilized condition. Equipment with obvious defects should be replaced.

5. No microbial ingress. Where appropriate (e.g., aseptic processes), equipment should be designed to prevent microorganisms migrating from the external environment onto product contact surfaces, either directly or via soils.
6. Hygienic design of maintenance enclosures. Maintenance enclosures and human–machine interfaces, such as push buttons, valve handles, and panels, should be designed so that they prevent the entry and/or accumulation of soils. Enclosure surfaces should be sloped to an outside edge to avoid them being used for storage (Figure 5).
7. Validated cleaning and sanitizing protocols. Procedures for cleaning and sanitation should be clearly described and proven effective. Care should be taken to ensure that chemicals recommended for cleaning and sanitation are compatible with the equipment and manufacturing environment.

In general, if care is not taken to ensure the hygienic design of the processing facility and equipment, effective cleaning will be severely compromised because of microbial harborage sites and biofilm development.

Cleaning Compounds

The primary function required of a cleaning compound is to disrupt soil–surface physicochemical interaction. Major considerations in cleaning compound selection are: (1) the physicochemical characteristics of the soil to be cleaned; (2) the chemical characteristics of the cleaning agent(s); (3) the application method; and (4) the surface area and

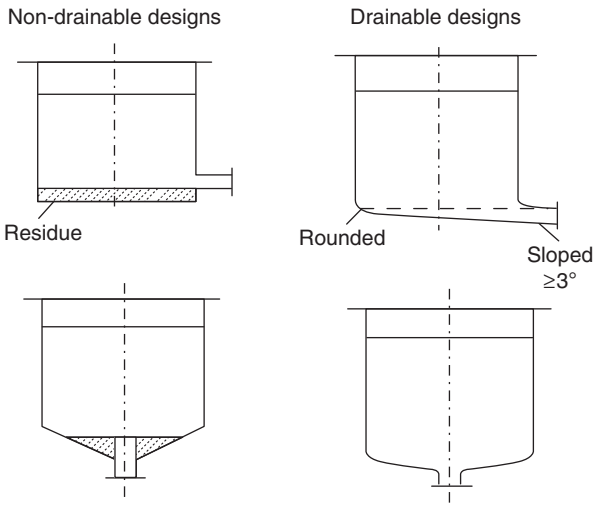

Figure 3 Examples of acceptable vs. unacceptable self-drainage design for tanks and vessels. Adapted from EHEDG, 1995. Hygienic design of equipment for open processing. Trends in Food Science and Technology 6 (9), 305–310.

Figure 5 Hygienic design is a necessity for easy-to-clean production systems in hygiene-critical processes in the food industry. Reproduced with permission from Schmitt, H., Koch, H.R., 2011. Hygienic Design of Enclosure Boxes in Relation to High Pressure Cleaning?

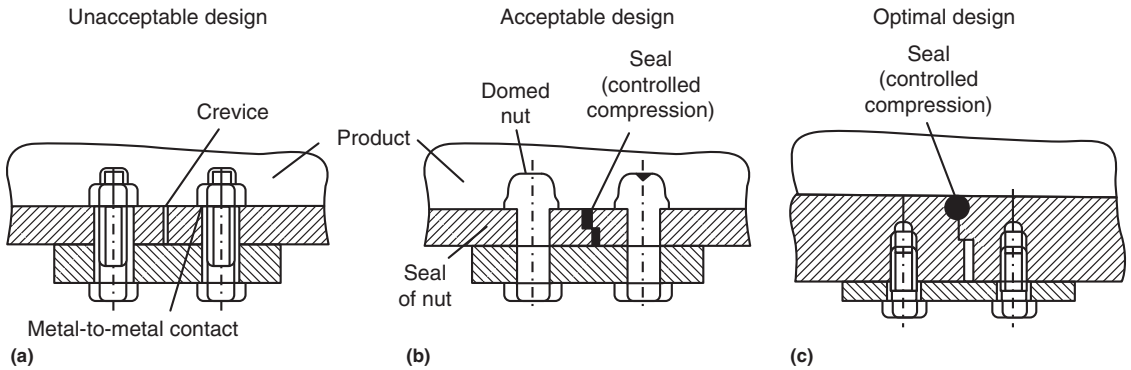

Figure 4 Design of dismountable joints: (a) crevices between sheet rims may lead to hygenic risks; (b) if exposure of screws to product is unavoidable, domed nuts, metal-backed seals and sealed rims on the overlapped sheets should be used; (c) optimal design uses sealed sheet rims and screw nuts on the reverse side to avoid direct contact with the product. Adapted from EHEDG, 1995. Hygienic design of equipment for open processing. Trends in Food Science and Technology 6 (9), 305–310.

design of the equipment to be cleaned. Generally, meat soils comprise lipids and proteins. To effectively remove these, alkaline cleaners with a pH of 11 or higher are commonly used. Specific types of cleaning compounds used for meat soil removal are:

1. Strong alkaline cleaners. These are used to saponify fats and remove heavy meat soils on metal surfaces, such as those found in smokehouses, boiling tanks, soak tanks, rail trolleys, and hooks. These agents pose serious risks of chemical injury to workers and may corrode surfaces and darken aluminum equipment. Components of strong alkaline cleaners include caustic soda and silicates having high N_2O:SiO_2 ratios. The addition of silicates to caustic soda, however, reduces corrosiveness and improves soil penetration and rinsing properties.
2. Heavy-duty alkaline cleaners. The active ingredients of these cleaners may be sodium metasilicate, sodium hexametaphosphate, sodium pyrophosphate, or trisodium phosphate. Although these generally do not corrode aluminum, the addition of sulfites may be necessary to reduce corrosion of tin alloys. These agents are frequently used for CIP, and for cleaning high-pressure rinsing equipment and mechanized equipment found in meat and poultry plants. They can, however, corrode metal parts and may cause eye damage and irritation of the skin and respiratory tract.
3. Mild alkaline cleaners. Mild alkaline cleaners commonly contain sodium bicarbonate. Liquid mild cleaners are frequently used for hand cleaning lightly soiled areas in meat processing plants, and are compatible with most surfaces. In the meat industry, they are used as general-purpose cleaners for hide washing equipment, floors, walls, and equipments made of rubber or plastic.

Although increased chemical energy may be applied by increasing cleaning agent concentrations, concentrations exceeding manufacturer recommendations may result in denaturation of proteins, thereby reducing cleaning effectiveness.

Biofilms

Biofilms form on surfaces. They are complex communities of microorganisms that are embedded in a matrix of polysaccharide materials secreted by the microbes themselves. Biofilms can form on any surface that is exposed to nonsterile water or other liquids, including but not limited to walls, floors, drains, process lines, pipes, and surfaces on/in refrigeration and air handling units. When bacteria colonize a surface, the cells secrete extracellular polysaccharide, proliferate, and eventually form a mature biofilm. Biofilms may not be effectively removed by cleaning agents because the bacteria within them are more resistant to heat and sanitizers than their planktonic forms. Biofilms may also enhance the corrosion of stainless steel, and bacteria from biofilms are continuously sloughed off into the foods and/or food processing environment. Biofilm formation is of particular concern with equipment that is difficult to clean, such as slicers and equipment requiring disassembly for cleaning. With such equipment, cleaning agents may not be effectively delivered to all parts of the equipment, leading to ineffective soil removal. Persistent soils will provide substrates for microbial growth and biofilm development, potentially leading to quality and/or safety issues. For example, two US listeriosis outbreaks 12 years apart were linked to a single turkey meat producer. The causative strains in both outbreaks were shown to be identical by molecular typing, and had been recovered from the food production facility in between the outbreaks. Biofilm formation throughout a food production facility should be closely controlled by preventing the establishment of harborage sites.

Biofilms may also impact operating efficiency. Accumulation of biofilms on processing equipment may reduce processing-related heat transfer, promote corrosion, or foul filters.

At this time, there are no specific procedures for the removal and disinfection of biofilms from equipment surfaces. As bacteria within biofilms may be up to 1000 times more resistant to biocides than planktonic organisms, biofilm removal cannot be achieved by increasing sanitizer concentration alone. Rather, combinations of chemical and mechanical energy must be used to improve the delivery and efficacy of cleaning and sanitizing agents. The use of cleaners and sanitizers in combination may enhance removal of biofilms. A suitable combination of cleaner and sanitizer can dissolve the biofilm and the organic material to which it adheres, allowing the sanitizer to inactivate the released cells. Current information also suggests that the application of heat with chemical sanitizers can be more effective against biofilms than chemical sanitizers alone. However, the application of heat may lengthen sanitation time, incur higher energy costs, negatively impact parts of equipment such as seals and gaskets, and increase risks to workers.

Difficulties associated with biofilms may be increased by the increasing duration of production runs. To optimize production efficiency, production runs in the food industry generally are becoming longer, with minimal down-time for sanitation. Although the use of hygienic equipment, inspection/maintenance, and the appropriate use of cleaning and sanitation chemicals may efficiently control microbiological contamination when run times are not extended, longer production runs inevitably lead to increased soil deposition and longer periods before any biofilms are disturbed. This may contribute to increased opportunity for biofilm formation and issues with maintenance of a hygienic production environment. Novel measures for control of biofilms are being sought. These include the use of enzyme-based detergents that may degrade biofilms, bacteriophages that can infect biofilm cells, and bacterial metabolites that affect the activities of cells in biofilms. These methods would be highly specific, nontoxic, and usable in-process, and thus could be feasible approaches for controlling microorganisms commonly found in food plant biofilms. However, they have as yet not been successfully applied in the food industry.

Assessing the Effectiveness of a Cleaning Program

Evaluation of the efficacy of cleaning should be carried out before sanitation. The methods available for rapid assessment of cleaning efficacy include: visual inspection of the equipment and environment; adenosine triphosphate (ATP) bioluminescence monitoring to detect the presence of ATP from

eukaryotic (i.e., meat tissues) and prokaryotic cells; and protein indicator strips to detect protein-based soils remaining on equipment. Failure to meet the standard specified for a test of cleaning should result in repetition of the equipment cleaning protocol.

Summary

Effective cleaning of equipment used in the processing and production of meat and meat-based products is an ongoing challenge. Raw materials and processed meat products contaminate equipment surfaces with soils rich in fats and proteins, which are difficult to remove. A thorough understanding of expected soil chemical properties is required to select appropriate cleaning agents, most of which are alkaline based. Combinations of chemical, thermal, and mechanical energy inputs should be used to maximize the removal of stubborn soils while minimizing costs of energy, labor, and cleaning agents. If food production equipment is not hygienically designed and properly maintained, or is made of inappropriate materials, the cleanability of equipment surfaces is diminished. Under such circumstances, microbial harborage sites develop in which organic substrate and resident microorganisms accumulate, resulting in biofilm development. The goal of a cleaning program should be to minimize opportunities for biofilms to form. If equipment cleaning is effectively executed, issues with premature spoilage, allergens, and/or pathogens in end products will be minimized.

See also: Biofilm Formation. Chemical Analysis for Specific Components: Major Meat Components. Cooking of Meat: Physics and Chemistry. Potential Chemical Hazards Associated with Meat. Processing Equipment: Battering and Breading Equipment; Brine Injectors; Mixing and Cutting Equipment; Smoking and Cooking Equipment; Tumblers and Massagers. Residues in Meat and Meat Products: Residues Associated with Meat Production

Further Reading

Carpentier, B., Cerf, O., 2011. Review – Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology 145, 1–8.

Detry, J.G., Sindic, M., Deroanne, C., 2010. Hygiene and cleanability: A focus on surfaces. Critical Reviews in Food Science and Nutrition 50, 583–604.

Gram, L., Bagge-Ravn, D., Ng, Y.Y., Gymoese, P., Vogel, B.F., 2007. Influence of food soiling matrix on cleaning and disinfection efficiency on surface attached *Listeria monocytogenes*. Food Control 18, 1165–1171.

Jackson, L.S., Al-Taher, F.M., Moorman, M., *et al*., 2008. Cleaning and other control and validation strategies to prevent allergen cross-contact in food-processing operations. Journal of Food Protection 71, 445–458.

Lelieveld, H.L.M., Holah, J., White, B., Mostert, M.A. (Eds.), 2003. Hygiene in Food Processing: Principles and Practice. Boca Raton, FL: CRC Press.

Marriott, N.G., Gravani, R.B., 2006. Principles of Food Sanitation. New York, NY: Springer.

NSF International, 2009. Hygiene Requirements for the Design of Meat and Poultry Processing Equipment (Tracking Number 14159-1i8r1). Available at: http://standards.nsf.org/apps/group_public/download.php/6462 (accessed 12.09.13).

Parker, S.G., Flint, S.H., Brooks, J.D., 2003. Physiology of biofilms of thermophilic bacilli – Potential consequences for cleaning. Journal of Industrial Microbiology and Biotechnology 30, 553–560.

Relevant Websites

www.meatami.com/
American Meat Institute.

http://chemstation-chesapeake.com/index.php?option=com_content&view=article&id=46&Itemid=69
Chemstation Chesapeake – Meat Processing Chemicals.

www.ehedg.org/
EHEDG, European Hygienic Engineering & Design Group.

http://www.fao.org/docrep/003/x6557e/X6557E04.htm
Food and Agriculture Organization – Slaughterhouse Cleaning and Sanitation.

http://www.nsf.org/
NSF International.

http://www.3-a.org/
3-A Sanitary Standards, Inc.

ETHNIC MEAT PRODUCTS

Contents

Biltong: A Major South African Ethnic Meat Product

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Glossary

Enterotoxin (not to be confused with endotoxin) A protein toxin released by a microorganism that targets the intestines.

Gamma radiation It is also known as gamma rays, denoted by the Greek letter γ that refers to electromagnetic radiation of high frequency and therefore high energy per photon.

Humectant A substance that absorbs or helps another substance to retain the moisture.

Osmophiles Microorganisms adapted to environments with high osmotic pressures, such as high sugar concentrations.

Water activity It is a measure of the availability of free water, and its value is governed by the degree to which ionic or other more weakly charged substances bind water.

Introduction

Biltong is a traditional South African meat snack consisting of strips of salted, flavored, and dried lean meat. The word 'biltong' is derived from the Dutch words 'bil', which means round or buttock, and 'tong' (or tongue), which describes the long strips of meat. For centuries, mankind has endeavored to preserve meat. Salting and drying of raw meat was a practice born of necessity by many of the forbearers who did not have any other facilities or methods with which to preserve meat. Through innovative upmarketing and subjection to modern processing and packaging technology, biltong has evolved from a food security type of commodity food to a market-driven premium snack.

The Traditional Method of Making Biltong

Biltong has probably been made since the 1650s when Jan van Riebeeck hosted a halfway house at the southern point of Africa. Later, biltong became a staple protein source of the pioneers on their expeditions into Southern Africa (1700–1800). During the Second Anglo-Boer War (1899–1903), Boer soldiers relied on biltong as a protein source when fresh meat from game and cattle became scarce. In earlier days, the whole carcass was used to cut biltong, but in modern times mostly muscles low in connective tissue from the round (buttock) and sometimes from the loin and tenderloin are used. In the mother tongue, biltong cut from the eye of the round (m. semitendinosus) is called 'predikantsbiltong' (Dutch for church minister's biltong), that from the fillet (m. psoas), 'ouma se biltong' (Dutch for grandma's biltong), and any cuts with high levels of connective tissue, are called 'vrinnebiltong' (Dutch for friend's biltong).

Meat from relatively young and lean carcasses is preferred for making biltong, as fat does not preserve well and becomes rancid, whereas meat from old animals will produce tough, sinewy biltong. The meat is processed by first removing all excessive connective tissue and trimming excessive fat if necessary. Depending on the muscle type and drying conditions,

the meat is usually processed into long strips varying between 25 and 100 mm in width. Coarse salt, in the proportion of 2–3%, is added by sprinkling the thin pieces and hand rubbing the thicker ones. Other spices, such as pepper and freshly roasted ground coriander, and ingredients such as brown sugar are often included as part of the traditional recipe. Sugar added at low levels prevents the hardening effect of salt without sweetening the final product (humectant function). Vinegar is sometimes sprinkled over the meat strips before they are packed in a container and left to pickle for 12–24 h. Brown or balsamic vinegar or wine or apple cider vinegar may be used. Excess salt is then removed by washing the pickled meat strips with a weak solution of warm vinegar water. This also helps to preserve the meat during the initial drying by delaying microbial growth. The salted meat strips are hung on small wire hooks on a piece of wire or a rod in the shade in a cool draughty place away from flies and dust. In humid environments, thinner strips are cut to prevent mold formation and spoilage. In addition, bicarbonate of soda is sometimes used to prevent spoilage, whereas saltpeter (a nitrate) is often added to bring out a red color in the final product through curing.

Biltong can be consumed after it has lost 50% of its weight, but in the absence of chilling or freezing facilities, further drying is advised for prolonged preservation. At 50% weight loss, beef biltong is at its best with a dry brown layer on the outside and soft, moist red tissue underneath. Meat from different game species is also very popular for making biltong, but owing to its peculiar taste, it is preferred when thoroughly dried ($>60\%$ weight loss).

Preservation, Storage, and Shelf Life of Biltong as an Intermediate Moisture Food

According to definition, biltong can be described as an intermediate moisture food (IMF). An IMF is characterized by a moisture content of approximately 15–50% and a water activity (a_w) between 0.6 and 0.85, which is less than what is normally present in natural fruits, vegetables, or meats but more than what is left in conventionally dehydrated products. In addition, IMF contains sufficient dissolved solutes to decrease water activity below that required to support microbial growth. As a consequence, IMF does not require refrigeration to prevent microbial deterioration.

There are two factors of major importance to produce microbiologically stable and safe biltong, namely, the salt and moisture contents. These factors can actually be related to one another through the concept of water activity, or a_w. The a_w of microbiologically stable biltong should be less than 0.70, with pH 5.5. However, a wide variation in a_w is encountered, because specific standards for processing of biltong do not exist. The a_w of commercial biltong varies from 0.30 to 0.92 (average 0.74). Bacterial growth usually ceases at an a_w of <0.75, where some yeasts and fungi continue to grow at levels as low as 0.62. A survey of the mycoflora of biltong indicated that moulds of the *Aspergillus glaucus* group were most frequently implicated in biltong spoilage. Although these osmophiles, which are capable of growth at low water activities, are not noted mycotoxin producers, their growth on biltong is nevertheless undesirable and results in economic losses. Studies indicated that yeasts are the predominant component of the mycoflora of biltong. In addition, the risk of pathogenic contamination, *Staphylococcus aureus* and *Listeria monocytogenes* in particular, is also high because many consumers prefer biltong with moisture content higher than 40% ($a_w > 0.85$). These conditions could favor the growth of these pathogens and the production of enterotoxins because both are tolerant of salt and reduced a_w. Reasons for contamination are (1) Raw meat from abattoirs that may be significantly contaminated, (2) lack of proper hygiene practices by manufacturers, and (3) the frequent selling of an unpacked product together with raw meat in butcheries. In commercial biltong production, proper hygiene practices are, therefore, essential. Proper drying of products, if accepted by the consumer, will decrease initially high counts of microorganisms to levels acceptable for most food safety regulations. Alternative interventions include the use of gamma irradiation, and low levels of irradiation (≤ 4 kGy) even showed improved flavor development of moist biltong, which could normally have a bland taste.

Commercialization of Biltong

Over time, biltong developed from a basic, staple, preserved food of pioneers and provisions of soldiers to a traditional food kept by farmers slaughtering their own cattle in the time before urbanization increased. Even during the earlier days of urbanization, biltong was a commodity mostly preserved by the local butcher, but today, it is increasingly becoming a branded delicacy that is grabbing more space on snack shelves. Biltong is still produced on the small scale for commercial purposes as well as for private use because of the fairly low entry costs in terms of facilities. However, it is believed that large-scale operations are more likely to survive price fluctuations of the required raw material (fresh, high-quality meat). More importantly, consumers are becoming ever more concerned about the quality and consistency of what is now becoming a luxury snack. Small-scale operations cannot offer the required quality standards or the required marketing and distribution backup. Furthermore, export opportunities are growing but are impossible to exploit without an EU and hazard analysis and critical control points (HACCP) certified factory.

Although small-scale and domestic operators are using more modern procedures such as artificial drying methods in cabinets and tunnels, larger operations operate in factories with wet and dry restricted areas (adoption of HACCP and ISO 9001), sophisticated portioning and cutting machinery, drying rooms (four day turnover), and packaging and labeling machines. Today, interesting variations on the basic product offer the consumer a range of choices from the traditional biltong to spicy 'snapstix' (very dry and thin meat strips) to paper-thin tearing biltong ('wafers' or 'leaves'). In addition, the basic flavor ingredients are complemented by hints of cloves, ginger, mace, garlic, chili powder, allspice, aniseed, and/or herbs such as thyme. Pineapple juice could be included in the manufacturing process to improve tenderness. Modern packaging methods, such as nitrogen flushing or vacuum packaging, ensure an extended shelf life, and the snack image is further promoted by offering different pack sizes from as small as

50 g. Long, thin perforated plastic packets allow moisture to evaporate before and after it is sold, but molds can still develop on the surface if the biltong is too moist.

Apart from product development strategies, innovative marketing by larger producers also adds to biltong's commercial success. One such strategy has been to use television to capitalize on biltong's strong links with sport, especially rugby, in South Africa. Brand names such as 'Halftime' are used in this regard.

From a traditional meat product born out of the need for food preservation, biltong has progressed through a production-driven commodity food to a market-driven and branded premium snack.

See also: Curing: Dry. Ethnic Meat Products: North America

Further Reading

Atwell, E., 2003. Biltong wakes up. South African Food Review 30 (2), 15–19.
Basson, D.S., 1970. The manufacture of ostrich biltong. Food Industries of South Africa 22 (12), 23.
Burnham, G.M., Hanson, D.J., Koshick, C.M., Ingham, S.C., 2008. Death of *Salmonella* serovars, *Escherichia Coli* 0157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* during the drying of meat: A case study using biltong and droëwors. Journal of Food Science 28, 198–209.
Dzimba, Flávia J.M., Faria José de Assis, F., Walter, E.H.M., 2007. Testing the sensory acceptability of biltong formulated with different spices. African Journal of Agricultural Research 2 (11), 574–577.
Nortjé, K., Buys, E.M., Minnaar, A., 2005. Effect of χ-irradiation on the sensory quality of moist beef biltong. Meat Science 71, 603–611.
Osterhoff, D.R., Leistner, L., 1984. Suid-afrikaanse bees biltong – weer eens onder die soeklig. Journal of the South African Veterinary Association 55, 201–202.
Taylor, M.B., 1976. Changes in microbial flora during biltong production. South African Food Review 3 (2), 120–123.
Van den Heever, L.W., 1972. The control of yeast on biltong with sorbic acid. Food Industries of South Africa 25 (5), 11.
Van der Riet, W.B., 1976a. Studies on the mycoflora of biltong. South African Food Review 3 (1), 105–111.
Van der Riet, W.B., 1976b. Water sorption isotherms of beef biltong and their use in predicting critical moisture contents for biltong storage. South African Food Review 3 (6), 93–96.
Van der Riet, W.B., 1981. The preservation of biltong with sorbic acid. South African Food Review 8 (2), 63–69.
Van der Riet, W.B., 1982. Biltong – a South African dried meat product. Fleischwirtschaft 62 (8), 1000–1001.

Brazil and South America

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Glossary

Dried meat A method of meat preservation which can be achieved through dehydration of fresh meat. It is one of the most ancient methods of extension of shelf life used in meat. The process can be carried out with a very low level of investment (using only solar energy), or may be performed in a more mechanised environment, with various processing steps during the manufacturing process.

Lamini group A group of mammals, which include the four species (alpaca, vicuña, guanaco, and llama) of South American camelids.

Salting A preservation process that considers the use of salt, followed by a long period of drying.

Sausage Product usually made from minced and/or ground meat from different food producing animals. In the traditional sausage production system, after processing, meat is stuffed in tripe, hence the typical cylindrical form of sausages. However, nowadays sausage casing can be done in natural or synthetic products (edible or not). Sausages may be preserved, amongst other methods by curing, drying, smoking, etc.; but they can also be fresh, hence needing cooking before consumption.

Water activity (a_w) Availability of water, defined as the ratio of the equilibrium of vapor pressure of water over the system and the vapor pressure of pure water at the same temperature.

Introduction

Though the South American countries have many cultural similarities, they also have many differences between them. These differences are reflected in the variability of their meat products.

Before the arrival of the Spanish, in 1492, the population in the Americas relied on their autochthonous fauna as a meat source, among these the most relevant were alpaca (*Lama pacos*), capybara (*Hydrochoerus hydrochaeris*), guanaco (*Lama guanicoe*), llama (*Lama glama*), nutria (*Myocastor coypus*), collared peccary (*Tayassu tajacu*), greater rhea (*Rhea americana*), lesser rhea (*Rhea pennata*), yacare (*Caiman crocodilus yacare*), tegu lizard (*Tupinambis merianae*), and green iguana (*Iguana iguana*).

After the arrival of the conquistador European, domestic livestock were gradually introduced, these breeds are the ones that currently dominate the local market, and make South America the largest meat producer in the world.

As well as the introduction of new domestic animals for human consumption, several meat products were brought from Spain, Italy, and Portugal. The production of these meat products, in some cases, was modified from the European original version to adapt the product to the local taste, climate, and availability of raw ingredients.

Regional Products

Dried Meats

There has long been a requirement to preserve meat products in a way that allows their consumption at a later time. The most common, and oldest methods, to extend the shelf life of meat products are salting and drying, which by reducing the water activity (a_w) of the product can help to extend shelf life and control the development of pathogenic microorganisms.

To obtain a more homogenous effect, the treated meat is normally cut into small pieces. Though traditionally the meat used in this process has been sun-dried in the open, nowadays meat dryers are widely used, allowing a faster, hygienic, and more controlled process.

In South America, as well as all around the word, dried meat products are still used in traditional cooking. Among these products the authors highlight *carne de sol*, *cecina*, and *charqui*.

Carne de Sol

This is a dried meat product consumed in Brazil. It is produced from beef and sometimes from goat meat.

The meat used to produce carne de sol is cut in thin strips, which are salted and then dried by exposing the meat to air-drying in a covered place from 2 to 4 days. Though the direct translation of its name means 'sun meat,' during its processing the meat is never exposed directly to sunlight. Instead, the meat is dried for a short time resulting not only in a hard and salty surface, which works as a protection barrier against microorganisms, but also keeping a juicy and tender inside.

After this process the product can be stored without the need of refrigeration for a long period of time.

Cecina

This product is originally from Spain and it has become popular in Paraguay, Peru, and few other South American countries. It appears that the name is derived from the latin word 'siccus,' which means dry.

It can be produced from beef and also from horse meat. As with other dry meats included in this section, traditionally cecina is salted and dried under the sun. However, this product is not necessarily dried in thin strips as entire meat cuts are used for the process.

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It is a ready-to-eat food product that does not need further processing.

Charqui

The name of this product comes from the quechua 'ch'arki.' It has been produced in South America from before the arrival of the conquistador and can still be found in all South American countries. Originally, it was elaborated with meat from the native species of the Lamini group (llamas, alpacas, and guanacos) but nowadays, due to the introduction of new species during the colonization period, this product is produced mostly from beef and horse meat (Figure 1).

The meat used for charqui is normally lean and is cut into thin pieces, followed by salting and drying under the sun. It can be consumed directly or as an ingredient in Latin American cuisine, mainly in dishes like soups and stews.

Sausages

Most of the most popular sausages in South America have European roots, not only Spanish and Portuguese but also with a large Italian and German influence. Chorizo, mortadela, salchichón, and salame are only few examples of how European immigrants influenced the development of the charcuterie in South America.

Like many other sausages, the most popular products in South America, linguiça and longaniza, are generally served as part of a heavy meal, typically accompanied by rice, beans or potatoes. Feijoada, for example, is a traditional dish, very common in Brazil, which incorporates linguiça with beans and other foods.

There are many types of meat products in South America with a wide variety of colors, flavors, and textures. These products constitute an important part of the local economy and tradition. Indeed, owing to the economic growth the elaboration has become more mechanized, but the processing is still based on the traditional manufacturing processes.

This section will highlight four food products that stand out for either their penetration in the region and/or for the modifications that have experimented to adapt to local ingredients and taste.

Butifarra

Butifarra is a traditional Spanish product that has become highly popular in the northern part of South America, especially in Colombia.

This cooked fresh sausage is elaborated using beef, but butifarras made of chicken or pork can also be found. In some areas a mix of these three kinds of meats are used in its production.

Lean meat, fat, and spices are mixed and then cased in edible tripe. Whereas the Spanish version of this product has a cylindrical shape, the Colombian one is more spherical. After casing, the butifarras are cooked in boiling water and can be consumed immediately because they do not require a ripening period.

Linguiça

Linguiça is a sausage that has its origin in Portugal and is very popular in Brazilian cuisine.

There are many varieties of this product, so it can be produced from pork or from more than one kind of meat, can be smoked or not, can be cured or not, can be added with fat or produced more lean, and it can be cased in natural or artificial edible casing (Figures 2–4).

Most commonly it is prepared with pork and up to 20% of beef plus the added seasoning.

Linguiças must be stored for a period of time to develop the desired organoleptic characteristic of the product. In case the product is intended to be smoked, the storage time can be in the smoking chamber. After the ripening period, the product must be stored under refrigeration until commercialization.

Longaniza

This meat product is originally from Spain; however, it is highly popular not only in South America, but also in Mexico, the Caribbean, and the southern regions of the USA.

Figure 1 Beef charqui.

Figure 2 Brazilian sausage Linguiça Calabresa. Courtesy of Eduardo A. Norkus (DVM).

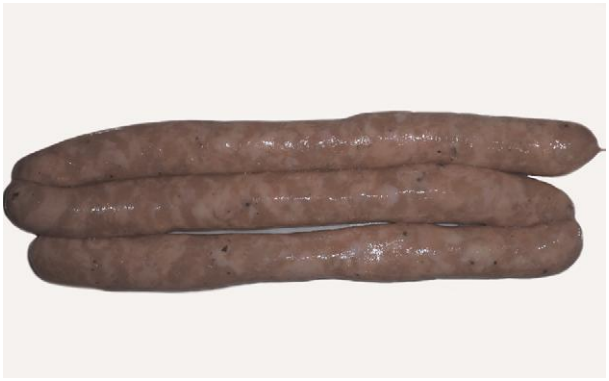

Figure 3 Brazilian sausage Linguiça carne frango (with chicken meat). Courtesy of Eduardo A. Norkus (DVM).

Figure 4 Brazilian sausage Linguiça carne mista (pork and beef). Courtesy of Eduardo A. Norkus (DVM).

Longaniza (Figure 5) is a sausage filled with minced pork mixed with fat (usually belly pork) and spices. Normally natural intestine (from pig) is used for casing, but also synthetic collagen is used at times. Synthetic collagen casing is preferred in large companies, because it helps to standardize the product and reduces the risk of biological contamination in the food product.

This sausage is normally long and relatively thin in size. It can be consumed raw if the longaniza has been cured and dried (a process that takes several months), but most commonly this sausage is commercialized as a fresh sausage, hence must be consumed cooked (traditionally fried or in barbecues).

Salchicha de Huacho

This product, also known as salchicha huachana, is typical from Peru. Its elaboration is similar as in longaniza; hence, pork, pork belly, and spices are normally used in the elaboration of this product. After mixing the ingredients, either natural or synthetic edible collagen can be used for casing.

Figure 5 Chillán's longaniza, the most famous Chilean sausage.

Figure 6 Prieta or morcilla, sausage made from pork blood.

A peculiar characteristic of this sausage is its bright yellow color due to the use of annatto in its production. Annatto is a natural colorant used in food production, which is extracted from the South American plant *Bixa orellana*.

Salchicha de Huacho is a fresh sausage and it must be cooked (usually fried) before consumption.

Miscellaneous

Chunchul

Chunchul is not technically a meat product, it is instead elaborated from the small intestine of cattle and its name and variations (Argentina, chunchuli; Chile, chunchules; Colombia, chunchurria; Peru, chinchulin) came from the quechua ch' unchul (intestine). This product varies in its name and preparation across South America, but basically the small intestine of cattle is emptied and washed. The product can be presented as twisted and braided intestines, or similarly as a sausage. It must be cooked before consumption and it is very popular in barbecues.

Prieta or Morcilla

Prieta (Chile) (Figure 6) also known as morcilla (Argentina, Peru) or rellena (Colombia) is a sausage made mostly of the blood from pigs. In some countries blood from cattle and/or goat is also used. It is a food product that was introduced to the region by Spanish settlers.

In addition to the basic ingredient (blood) it can also contain fat, rice, nuts, and spices. The ingredients used will vary according to the country. Once the mix is ready, an edible casing (natural or synthetic) can be used.

It can be consumed hot immediately after cooking, or cold; though the latest is normally blood sausages that have been dried and ripened before consumption.

See also: Drying. Ethnic Meat Products: Mediterranean. Sausage Casings. Sausages, Types of: Dry and Semidry

Further Reading

Brasil, Ministério da Agricultura e do Abastecimiento, 2000. Instrução Normativa N° 22 de 31 de julho de 2000. Anexo XIV Regulamento tecnico de identidade e qualidade da linguiça colonial.

Frentz, J.-C., 1982. L´Encyclopédie de la Charcuterie. Vesoul, France: Soussana.

Garcia, F.A., Mizubuti, I.Y., Kanashiro, M.Y., Shimokomaki, M., 2001. Intermediate moisture meat product: biological evaluation of charqui meat protein quality. Food Chemistry 75 (4), 405–409.

González, F., Paulsen, P., Smulders, F.J.M., *et al*., 2003. Exotisches für die Fleischtheke. Fleischwirtschaft 10, 32–37.

Judge, M.D., Aberle, E.D., Forrest, J.C., 1989. Principles of Meat Science, third ed. Dubuque, IA: Kendall/Hunt Publishing Co. 351 p.

Manhoso, F.F.R., Rudge, A.C., 1999. Aspectos microbiológicos, físico-químicos e histológicos das lingüiças tipo frescal comercializadas no município de Marília/SP. Higiene Alimentar, São Paulo 13 (61), 44.

Saadoun, A., Cabrera, M.C., 2008. A review of the nutritional content and technological parameters of indigenous sources of meat in South America. Meat Science 80, 570–581.

Salvá, B.K., Fernández-Diez, A., Ramos, D.D., Caro, I., Mateo, J., 2012. Chemical composition of alpaca (*Vicugna pacos*) charqui. Food Chemistry 130 (2), 329–334.

Siguelnitzcky, W., 2003. Elaboración de charqui de guanaco (Lama guanicoe) en la Isla Tierra del Fuego, XII Región. Tesis Medicina Veterinaria. Universidad de Concepción, Chile. Facultad de Medicina Veterinaria.

Soto, N., 1988. Alternativas de elaboración de charqui de Guanaco (Lama guanicoe). Tesis Medicina Veterinaria. Universidad de Concepción, Chile. Facultad de Ciencias Agropecuarias y Forestales, Departamento de Medicina Veterinaria.

Toldrá, F., 2002. Dry-Cured Meat Products. Trumbull, CT: Food & Nutrition Press, pp. 1–238.

Torres, E., Pearson, A., Gray, J., Ku, P.K., 1989. Lipid oxidation in charqui (salted and dried beef). Food Chemistry 32, 257–268.

Relevant Websites

http://www.fao.org/docrep/010/ai407e/ai407e00.HTM
Food and Agriculture Organization of the United Nations (FAO).

http://www.fao.org/docrep/010/ai407e/ai407e17.htm
Food and Agriculture Organization of the United Nations (FAO).

http://www.ccmv.ufpr.br/2011/EMYLI.pdf
Universidade Federal do Paraná.

China and Southeast Asia

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Glossary

Angkak Red fermented rice powder.
Soy sauce A condiment made from a fermented paste of boiled soybeans, roasted grain, brine, and *Aspergillus oryzae* or *Aspergillus sojae* molds. It originated in China in the second century BCE and spread throughout Asia and today is also used in Western cuisine and prepared foods.

Introduction

Chinese cuisine has become ubiquitous in most countries of the world. This article does not directly cover the various cuisines originating from China or nearby Southeast Asian countries as these are extensive and are more appropriately covered by the websites. Through trade many of the Asian cuisines, such as Indonesian, Malaysian, and Thai, have blended. This article covers the way various meat products are made and used.

There are more than 100 different types of traditional Chinese meat products (TCMPs) in mainland China. Many of these products can also be found in Southeast Asian countries and in metropolitan cities where there is a sizeable ethnic Chinese population. These products can readily be produced in home kitchens as well as in restaurant kitchens. A few of the TCMPs are now manufactured in meat processing plants using the latest meat processing technology and machinery, while maintaining the characteristics of Chinese dietary customs and product formulations; examples include Chinese-style pork sausages (Figures 1 and 2) and Jinhua ham. Many TCMPs are named after cities or geographical regions. For example, Jinhua is a city in Zhejiang province, which is in southeastern China and situated south of the Yangtze River Delta.

As a result of the relatively low water activity (a_w) of these meat products (e.g., the a_w of the different grades of Chinese-style pork sausages ranges from 0.59 to 0.71), a high degree of dehydration (e.g., Jinhua ham has a moisture content of 52% at 40 days postproduction, and 34% when 1-year-old) (Figure 3) and high cooking temperature, TCMPs are generally marketed unrefrigerated. A majority of Chinese dried meat products are in fact intermediate-moisture foods that are stable at ambient temperatures and can be consumed directly from the package.

Product Characteristics

The bulk of TCMPs are made from pork, the most common meat of choice for the majority of the Chinese people. There

Figure 1 Unpackaged Chinese sausage.

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are also a few TCMPs that are made from beef, mutton, chicken, duck, rabbit, and game meat.

The basic product formulation is a combination of different amounts of salt, sugar (cane sugar and malt sugar are commonly used), soy sauce (either light or dark), Chinese wine (e.g., rice wine and the rose wine *Mei Kuei Lu Chiew*), vinegar, and monosodium glutamate. Also included in the formulation is a variety of spices and seasonings including star anise, cinnamon, clove, fennel, nutmeg, ginger, orange peel, garlic, and green onion (scallions).

A natural food coloring that is used in some TCMPs is red fermented rice powder (*angkak*). This is a product of rice fermented with the fungus *Monascus purpureus*. The addition of a small quantity of this powder, approximately 1.5–3 g per kilogram of sausage meat, results in maximum red color, which is stable and retains the color in the final product. It is an excellent natural substitute for nitrite in meat curing solutions.

Many of the dried or semidried TCMPs have a relatively high salt content of more than 4%. In the case of cured meat, for example, the Nan An pressed-cured ducks (*Ban Ya*), the salt content is as high as 9–10%.

Another characteristic of Chinese dried meat is the sweet taste. For example, dried beef has a sugar content of 15%, and the sugar content of the different grades of Chinese-style pork sausages ranges from 10% to 25%.

Figure 2 Short Cantonese dried sausages.

Figure 4 Siu mei platter including roast pork (bottom), roast goose (top), smoked ham (left), and unroasted white cut chicken and jellyfish (center).

Figure 3 Jinhua ham.

To a very large extent, TCMPs are characterized by regional flavors:

- The northern (Beijing-style) flavor has a long history from the Ming dynasty (AD 1368–1644). The products are characterized by a strong flavor and liberal use of many types of spices. Representative products are roasted mutton, and cooked and seasoned beef and pork.
- The southern (Suzhou-style) flavor is characterized by a rich and heavy flavor tinged with sweetness from a liberal use of wine and sugar. Representative products are roasted meats and dried meats.
- The Cantonese flavor is characterized by a sweet pleasant taste from the liberal use of sugar (Figure 4). The main processing method is drying by hot air or smoke generated from burning charcoal made of special fragrant wood. The products are brightly red colored. The best-known products are roasted suckling pigs and Cantonese-style Chinese sausages (*lap cheong*).
- Sichuan flavor (also Szechuan) cuisine is characterized by spiciness, the result of liberal use of hot chilli, black pepper, sesame oil, ginger, and *huajiao* (also known as Sichuan pepper, *Pericarpium zanthoxyli*).

Table 1

Class	*Subclass*	*Some notable representative products*
Cured products	Salted products[a]	Salted pork legs
		Nanjing salted ducks
	Cured products	*La Rou*[b]
		Sichuan cured rabbit (*Cha Si Tu*)[c]
Braised and seasoned products	*Jiang Rou*[d]	Beijing *Ching Jiang Rou* (beef/pork)
		Wuxi[e] pork ribs braised in soy sauce
		Hangzhou[f] *Jiang Ya* (whole duck)
		Shanghai five-spice pork
Smoked and roasted products	Smoked products	Chinese bacon
		Smoked pig tongue
		Smoked chicken
	Roasted products	Peking ducks
		Cantonese *Cha Shao*[g] (pork)
Dried products	Wind-dried products	Yunnan[h] *Fung Ji* (wind-dried chicken)
	Meat floss[i] (shredded meat)	Taichang shredded pork/beef
	Barbecued meat slices[j]	
Sausages	Chinese-style sausages[k]	Guangdong-type
		Wuhan-type
		Harbin-type
Hams	Raw hams (*Huo Tui)*	Jinhua hams[l]
		Southern hams
		Northern hams
		Yun hams

[a]Salted products are raw products.

[b]*La Rou* are cured meat (pork, also beef and lamb) products produced in the last month of the Chinese lunar calendar. Guangdong, Sichuan, and Hunan *La Rou* are some of the better-known products. They are normally boneless products; some of the Hunan *La Rou* are bone-in. They are subjected to lengthy natural ageing and dehydration processes in the cold winter months. *La Rou* appears oily and shining on the surface; the fat is golden yellow and translucent, and the meat pinkish-red. Guangdong *Guan Dao Rou* is meat from the pig's hind quarter carved into the shape of a saber, 20 cm long and 1.5 cm thick (imagine the fat as the curved blade and the meat at the back of a saber).

[c]Sichuan province in southwest China is renowned for its cured rabbits. The presentation of the product is unique, in the shape of a silkworm cocoon; thus the Chinese descriptive term *Cha Si*.

[d]*Jiang Rou* are meats marinated in soy sauce. They have a strong soy sauce flavor. In addition to soy sauce, fermented black bean is also used in some products.

[e]Wuxi is a city in eastern China, in Jiangsu province, and is near to Shanghai. The product is known for its salty-sweet taste and delicate aroma.

[f]Hangzhou is the capital city of Jiangsu province in eastern China.

[g]*Cha Shao* literally means 'cooked in forks,' so called because the pork strips are held in fork-like sticks and cooked in flames.

[h]Yunnan province is the most southwest region of China, sharing borders with Myanmar, Laos, and Vietnam.

[i]Meat floss (*Rou Song*), also known as shredded meat, is usually made from pork, beef, or chicken meat. The shredded dried meat is golden in color and has a distinctive flavor and sweet taste. *Rou Song* that come in pork and beef flavors tastes a little like beef jerky. The meat undergoes a process that includes chopping, steaming, frying, and shredding by hand. Soy sauce, sugar, fennel, ginger, and wine are used as seasonings.

[j]Made of either pork or beef. Pork slices are the most popular. Also known as *Rou Gan* in China, and *Bak Kua* in Singapore and Malaysia.

[k]There are more than 30 varieties of traditional Chinese sausages. They are raw, nonfermented products with an a_w in the intermediate-moisture range ($\sim$0.75). According to the degree of sweetness, i.e., the amount of sugar added in the sausage formulation, traditional Chinese-style sausages can be divided geographically into three groups: (1) Harbin-type sausages from northern China province of Heilongjiang, which are characterized by a low sugar content of $\sim$1.4%; (2) Wuhan-type sausages from the central China province of Hubei, which have a moderate sugar level of $\sim$4%; and (3) Guangdong-type sausages from the southern Chinese province of Guangdong, which contain at least 6% sugar, often much more. The Chinese-style pork sausages typically contain large pieces of meat and diced fat ranging from 0.5 to 1 cm in size.

[l]Jinhua ham is a premium meat product that has the excellent quality attributes of Kentucky country ham and Blue Ribbon Virginia ham in the United States. The hams are dry-cured in the winter season. After about 9 months, the hams are carefully shaped to ensure that the leg is straight and the hoof is sickle-shaped. They are then graded according to their appearance and saltiness. The four acclaimed unique properties of Jinhua ham refer to its color, aroma, taste and appearance, and thin skin and fine bones.

Classification of Traditional Chinese Meat Products

TCMPs can be classified according to the animal species, product formulation, processing methods, or product characteristics. Table 1 gives a classification of TCMPs based on the combined criteria of processing methods and product characteristics.

Curing of meat is done by dry rubbing or soaking, or a combination of both. Soaking is a common practice for small cuts where the meat is submerged in brine. A typical brine is composed of crude salt and some flavoring ingredients. As crude salt is normally contaminated with nitrate, no additional nitrate or nitrite is required to initiate the curing reaction.

Braised and seasoned meats are cooked in marinades with soy sauce as the basic ingredient. Cooking is done first on high heat. The heat is then gradually reduced to medium or low. These products have a 'melt-in-the-mouth' characteristic. The different product formulations typically incorporate five different spices, each having a distinctive flavor: star anise, cinnamon, clove, prickly ash, and fennel seeds.

Processing of hams traditionally involves slow fermentation (2–3 months) and long ageing (3–4 months). The total processing time can be up to 9 months. Finished products have a 55–60% yield.

Chinese and Related Cuisines

The Eight Culinary Traditions of China are Anhui, Cantonese, Fujian, Hunan, Jiangsu, Shandong, Szechuan, and Zhejiang cuisines. However, there are also many styles of Chinese cuisine outside China that include Vietnamese, Singaporean, Malaysian, Indonesian, Indian, and American. There are a number of websites that cover these styles. In other words, Chinese cuisine is popular around the world wherever there is ethnic Chinese population. The use of meats such as pork, beef, and chicken were relatively limited in the past and in places like Vietnam and Cambodia, fish is the main protein. American Chinese food typically treats vegetables as a side dish or garnish, whereas traditional cuisines of China emphasizes the use of vegetables. The use of carrots and tomatoes (from the New World) is a latest addition. Native Chinese cuisine makes frequent use of Asian leaf vegetables like *bok choy* and *kai-lan* and puts a greater emphasis on fresh meat and seafood. Stir frying, pan frying, and deep frying tend to be the most common Chinese cooking techniques used in American Chinese cuisine (Figure 5), which are all easily done using a wok. The symptoms of a so-called Chinese restaurant syndrome or 'Chinese food syndrome' have been attributed to a glutamate sensitivity, but carefully controlled scientific studies have not demonstrated such negative effects of glutamate.

Conclusion

TCMPs are representative of the Chinese people's rich cultural and culinary heritage and diversity.

Much before scientific technology was applied in meat processing, the Chinese people had, through intelligent and creative manipulation of product formulations in step with the winter season's favorable weather conditions, produced many of the well-known products such as Jinhua hams and Chinese-style sausages. Hurdle technology in meat processing, elucidated by Leistner in 1985 is an important scientific development in improving the microbiological stability of traditional meat products. It has explained the scientific background for some of the traditional methods used in

Figure 5 A Chinese buffet restaurant in the United States.

China, and Leistner has visited China and further improved the safety of some of the Chinese processing methods.

Further improvement in the quality attributes of TCMPs by application of modern meat science and technology such as the hurdle technology in the industrial scale production will thus benefit the trade in these products globally.

See also: Curing: Brine Curing of Meat. Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Brazil and South America; France. Germany; India and Pakistan; Japan and Korea; Mediterranean; Middle East; North America; Poland. Microbiological Safety of Meat: Hurdle Technology. Smoking: Liquid Smoke (Smoke Condensate) Application. Traditional

Further Reading

Ang, C.Y.W., Liu, K.S., Huang, Y.W. (Eds.), 1999. Asian Foods – Science and Technology. Lancaster, PA: Technomic.

Dong, Y.C., *et al*., 1991. Considerations relevant to the formulation of national standard for the classification and nomenclature of meat products. Meat Research 1994 (4), 3–9. (in Chinese, a quarterly publication of the China Meat Research Center in Beijing).

FAO, 1991. FAO Consultancy Report On Traditional Chinese Meat Products. Geneva: PAO.

Ho, H.F., Koh, B.L., 1984. Processing of some Chinese meat products in Singapore. Proceedings of the 4th SIFST Symposium – Advances in Food Processing, Singapore, pp. 28–39. Singapore: Singapore Institute of Food Science and Technology.

Leistner, L., 1985. Hurdle technology applied to meat products of the shelf stable product and intermediate-moisture food types. In: Simatos, D., Multon, J. L. (Eds.), Properties of Water in Foods in Relation to Quality and Stability. Dordrecht: Martinus Nijhoff, pp. 309–329.

Leistner, L., 1988a. Mould-ripened foods. Fleischwirtschaft International 1988 (1), 26–32.

Leistner, L., 1988b. Shelf-stable oriental meat products. Proceedings of the 34th International Congress of Meat Science and Technology, pp. 470–475. Brisbane, QLD: CSIRO.

Leistner, L., 1990. Fermented and intermediate-moisture products. Proceedings of the 36th International Congress of Meat Science and Technology, vol. III, pp. 842–855. Havana: Food Industry Research Institute, Cuba.

Leistner, L., Gould, G., 2000. Hurdle Technologies – Combination Treatments for Food Stability, Safety and Quality. New York, NY: Kluwer Academic/Plenum.

Savic, Z., Zhang, K.S., Savic, I., 1989. Chinese-style sausages. Fleischwirtschaft International 1989 (1), 35–40.

Relevant Websites

http://en.wikipedia.org/wiki/Chinese_cuisine
http://en.wikipedia.org/wiki/Cantonese_cuisine
http://en.wikipedia.org/wiki/Szechuan_cuisine
http://en.wikipedia.org/wiki/Chinese_sausage
http://en.wikipedia.org/wiki/Vietnamese_cuisine
http://en.wikipedia.org/wiki/Cambodian_cuisine
http://en.wikipedia.org/wiki/Singaporean_cuisine
http://en.wikipedia.org/wiki/Indonesian_cuisine
http://en.wikipedia.org/wiki/Thai_cuisine
http://en.wikipedia.org/wiki/American_Chinese_cuisine
http://www.fao.org/docrep/010/ai407e/ai407e17.htm
http://www.travelchinaguide.com/tour/food/chinese-cooking/meat-dish.htm

France

C Lambel, Ceyrat, France
V Santé-Lhoutellier, INRA, Saint Genès Champanelle, France

Glossary

Appertization It is a thermal processing procedure which sterilizes perishables by heat (110–130 °C).
Back pudding Sausage made with blood.
Charcuterie Etymologically the term refers to 'chairs cuites' (cooked flesh). In its present meaning it represents products from meat processing.
Collagen It is the fibrous protein constituent of bone, cartilage, tendon, and other connective tissue. It is converted into gelatin by boiling.
Dry curing The process involving the use of cure (potassium nitrate or sodium nitrite) mixed with salt followed by a long drying.

Introduction

Many of the meat products have been produced in France for centuries. These products and recipes constitute an important culinary heritage, part of the French gastronomy, and also a significant part of French local and national economy. Meat is a major component of French consumption.

Traditionally, the two most common and popular ways to use meat are stews (e.g., braised meat dishes like beef bourguignon or veal blanquette) and 'charcuteries' (mostly prepared in a delicatessen with pork meat like salami, sausages, cooked ham, dry-cured ham, paté, rillettes, and black pudding). Typical French products and dishes with their characteristics and French names are described in this article.

Braised Meat Courses

In France, before the era of modern breeds and agricultural specialization, the farmers were mostly looking for versatility in the livestock selection. Animals were taking part in field work, giving enough milk fat to make cheese, but were also meat providers at the end of their life. At that time, meat was generally pretty tough and was usually cooked slowly for a few hours and mixed with vegetables. In France, the meat was not roasted until the end of the nineteenth century and not grilled before 1945.

This slow cooking by boiling allows the muscle collagen to turn into gelatin. Gelatin modifies the textural properties of the dish and mainly behaves as a thickener for dishes in a sauce. In fact, in France, any good stew should combine not only bones, fat, and meat but also a good supply of collagen through bone addition.

The source of gelatin is mainly sourced from beef tail, foot, marrow bones, and back ribs.

- Bones: they give taste to the dish and texture to the sauce.
- Fat muscles: they give the taste. Flanken-style ribs (cut of meat taken from the short ribs of beef) as well as the trapezius muscle are often used.
- Lean muscles: give chewing and volume to the dish, but they taste very neutral. Several cuts are used, such as beef chuck, aglet baroness, eye of round steak, and bottom round roast.
- Stringy and sinewy meat pieces: these are gelatinous pieces. They give the moisture and the texture to the sauce. It takes approximately 5 h of cooking for these meat cuts to gelify their collagen and become softer. Several cuts are used, such as beef cheek, shank, and corned beef briskets. These three kinds of pieces in equal proportions will give a balanced and tasteful braised meat dish.

France has accumulated lots of braised meat's recipes with wide varieties of regional expressions.

With Beef/Bull

- Bœuf mironton (mironton of beef): cooked in beef stock and braised in tomato sauce with pickles.
- Bœuf bourguignon: like its name suggests it comes from Burgundy and is slowly cooked in red wine.
- Beef carbonnade: it comes from the north of France and it is braised in dark beer.
- Gardianne de taureau (traditional bull herd leader): from Camargue in the south of France: bull marinated in red wine with onions, bacon, and orange peel and then braised.
- Pot-au-feu (boiled beef): cooked in water with onions, carrots, leeks, and potatoes.
- Bœuf à la ficelle (beef with the string): fillet of beef hung with a string attached to a wooden spoon placed across the pan and cooked in water with turnips, carrots, and leeks.

With Veal

- Blanquette de veau (veal blanquette) (Figure 1): this recipe is the favorite French meal in France; it consists in a veal stew with white sauce. Veal meat used for blanquette is a little expensive and has enough fat in parts (parties), so that the meat does not dry out during the cooking; cuts include height of rib, back of the knee or, breast of veal support, i.e., tendron (middle-cut breast of veal). Veal meats are cooked in a broth made of water, onions, carrots, leeks, garlic, and white wine. Then a sauce is made with a 'roux'

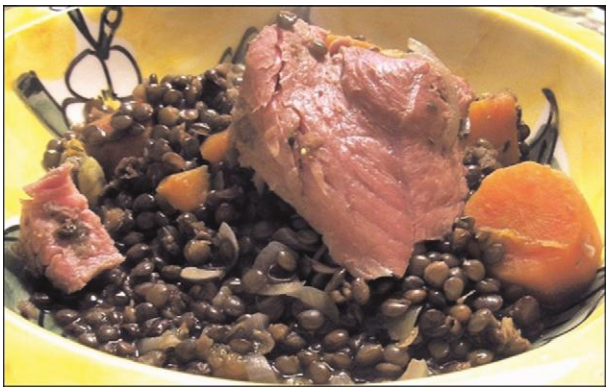

Figure 1 Streaky bacon with lentils.

Figure 2 Veal blanquette.

and in the cooking broth, mushrooms as well as lemon juice are added. The veal is served in the sauce.

- Veal Marengo: diced veal stew with tomatoes, carrots, onions, mushrooms, veal broth, and white wine.

With Pork

- kig ha farz breton: from Brittany, in the west of France: smoked pork breast and foreleg cooked with vegetables in water with a buckwheat flour dough cooked in a canvas bag in the meat broth.
- La potée auvergnate (hotpot): from Auvergne in the center of France: pork breast and foreleg ham cooked for 2 h in water with cabbage, carrots, celery, leeks, onions, and potatoes.
- Petit salé aux lentilles (streaky bacon with lentils) (Figure 2): From Auvergne: pickled pork slowly cooked with lentils in water.

With Other Meat

- Rabbit: civet de lapin aux pruneaux (rabbit stew with prunes): it is slowly cooked with red wine.
- Games: civet de lièvre (hare stew), civet de sanglier (wild boar stew): marinated in red wine (and cognac sometimes) with onions and carrots, then braised slowly in the wine of the marinade.
- Innards: tripes à la mode de Caen (braised tripe Caen's style): braised with carrots in white wine and tripes à la lyonnaise braised with onions in a tomato sauce.

Charcuterie (Delicatessen)

Etymologically, the term refers to 'chairs cuites' (cooked flesh). In its present meaning, it represents products from meat processing. Meat preservation, based initially on salting and smoking, has been deeply connected with the development of 'appertization' combined with cold chain and packaging techniques.

Dry Sausages/Saucisson et Saucisse Sèche

There are 'saucisson' recipes dating back to the Roman Empire. In France, the first appearance of the word 'saucisson' (a word of Italian origin) dates from 1546 in a book by Rabelais and shows how the transalpine cuisine had already penetrated in France.

It is made of animal's gut stuffed with minced meat. The lining is generally composed of two-third of meat and one-third of fat added with salt, sugar, curing agents, and spices. The mix is stuffed into a casing for a mild ripening and drying of 3 months. The basic processing is still the same but mostly mechanized (to respond to mass consumption). During curing, the dry sausage acquires organoleptic qualities due to the physicochemical transformations of myoglobin, carbohydrates, fats, and proteins of the filling.

The most commonly used meat is pork, but there are also 'saucissons' made from bull (e.g., in Camargue in the south of France) or wild boar, poultry, donkey, and pork liver ('figateli' sausage in Corsica).

Being easy to preserve, the dry sausage is a very popular dish in France; it is consumed in all occasions and is served with drinks or for picnics.

Cooked Ham/Jambon de Paris and Dry-Cured Ham/Jambon Sec

These products were invented by the Gauls. Although it was the product of early smoking and salting preservation specialists, the ham recipe was quickly taken over and adapted by other people. The ham is represented in some frescoes and stained glass windows of the Cathedral Notre Dame de Paris. It became the 'star food' of the Middle Ages and the basis of the rural nourishment because it could be preserved easily. Nowadays, in France, two kinds of ham are produced:

- Cooked ham or jambon de Paris (Figure 3): this is the most popular delicatessen type food eaten in France. It represents almost 70% of the processed pork. Basically, it is a pork leg that has been deboned and reshaped, injected with brine, and then cooked in a flavored broth. It is sometimes

Figure 3 Jambon de Paris.

cooked in a cloth sewn around the ham (jambon au torchon). It is commercialized with or without rind.

- Dry-cured ham or jambon sec: many regions of France have their own ways of preparing such a ham. All these regions share a special climate: close to mountain with a draining wind. The quantities of salt and the time of curing vary according to the traditions. The drying adds the final touch and gives the characteristics and quality of cured ham. The ham's weight ranges from 5/6 kg to 9 kg depending on the region. In France, the most renowned hams are Jambon de Bayonne, Jambon d'Auvergne, and Jambon de Savoie. Bayonne ham enjoys EU Protected Geographical Indication (PGI) status. This certification requires professional processors to comply with specifications that provide the consumer with a finished product of optimal quality. Proteolysis in dry-cured ham occurs throughout processing but at different rates and to varying extents depending on salt penetration and water migration. The processing of Bayonne hams, which lasts 9 months, follows the sequence: salting, settling, oven drying, air drying, fat covering, and ripening.

Pâtés/terrines/Rillettes

In the Middle Ages, terrines and pâtés were distinguished by their mode of cooking: terrines were cooked in clay (terre) containers, which explain their name. Pâtés (pies) were baked in dough not necessarily edible at that time. This crust was very thick to protect the stuffing during cooking and transport. Over the years, each province has developed its 'pâté' recipe typical of its land:

- pâté Rennais (de Rennes in the west of France) is traditionally made with pork meat and cooked in the oven.
- In the seventeenth century, the Picardie region was deemed to be well stocked with game. This region is one of the richest regions in France in pâtés and terrines' recipes: Duck pâté d'Amiens, partridge pâté de Montdidier, and also hare and woodcock pâtés.

Nowadays, 'pâtés' are mostly made from pork meat, liver, fat, and lean pieces for the moisture and texture.

Blood Sausage or Black Pudding/White Boudin (Boudin Noir/Boudin Blanc)

Black pudding, of red-brown hue, gets its color from blood from which it is made. The ingredients are put into gut/intestines and cooked. There are as many recipes as regions of France. Pork butchers use more or less spices and add them depending on the region, for example, bread crumbs, Swiss chards, spinach, fresh herbs, flour, eggs, cream, and pig's rind or foot.

Black pudding is one the oldest known deli foods. It was invented in the antiquity by a Greek cook named Aphtonite.

'Boudin' blanc, for its part, is made from white meat (poultry, veal, pork, and pork fat). 'Boudin' (black or white) is still much being appreciated by French people. In 2002, as much as 14 730 tons of black pudding and 6962 tons of white pudding were produced.

Conclusion

The French cuisine offers a huge variety in the names of dishes and in the way they are prepared and these traditions are part of the culinary traditions of French people. The cuisine also is underpinned by the way the meat is produced on the land, especially for each particular agriculture region, the stories surrounding the way the dishes first were created, the traditions accompanying the dish's development, and how the cuisine was modified in the past to take account of seasonality. The two main traditional ways to use the meat in France are meat dishes braised in sauce/stock and 'charcuteries' (delicatessen). The variety of names and variability across the country associated with these aspects of the French meat cuisine are difficult to cover fully. This article provides an overview of the most famous ones.

See also: Curing: Brine Curing of Meat; Dry. Ethnic Meat Products: Mediterranean. Ham Production: Cooked Ham; Dry-Cured Ham. Sausages, Types of: Dry and Semidry. Smoking: Traditional

Further Reading

Daumas, D., 2008. La cuisine des abats. Editions Sud-Ouest, 62 pp.
Dumas, D., Imbertèche, H., 2010. Ris, cœurs, foies & autres abats. Editions Cuisines des Pays de France, 125 pp.
France, B., Vitaux, J., 2008. Dictionnaire du Gastronome. Editions Presses Universitaires de France, 800 pp.
Reynaud, S., 2012. Le livre de la tripe. Editions Marabout, 192 pp.

Germany

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Glossary

Ammerländer Schinken/Knochenschinken/Dielenrauchschinken/Katenschinken Dry-cured ham from the northwestern region of Lower Saxony in Germany.
Badisches Schäufele Cooked, cured, and smoked pork shoulder produced typically in Baden (Germany).
Bayerischer Leberkäse Coarsely or finely chopped emulsion-type meat loaf produced by roasting (Germany).
Braunschweiger Mettwurst Sliceable fermented sausages from a region around the town Braunschweig (Germany).
Eichsfelder Feldgieker Fermented dry-cured and sliceable sausage produced by air drying from a region of Central Germany; veal bladders are used as casings.
Frankfurter Würstchen Cured emulsion-type sausages with sheep casings and a typical smoky flavor and color from Frankfurt am Main (Germany).
Göttinger Feldkieker Fermented dry-cured and sliceable sausage produced by air drying from an area around the town Göttingen (Germany) and using a bladder as casing.
Göttinger Stracke Fermented dry-cured and sliceable sausage produced by air drying from an area around the town Göttingen (Germany).
Greußener Salami Long-term fermented sausages produced in Thuringia (Germany).
Halberstädter Würstchen Cured and intensively smoked emulsion-type sausages with sheep casings from Halberstadt (Germany) mainly sold as conserved products.
Hofer Rindfleischwurst Spreadable fermented and smoked sausages containing beef and backfat from a region near the Bavarian town Hof.
Holsteiner Katenschinken/Katenrauchschinken Dry-cured and cold smoked ham from the federal state of Schleswig-Holstein (Germany).
Kassler Cooked, cured, and smoked pork loin or neck.
Knacker Emulsion-type sausage (Knockwurst) with a typical smoking flavor and color.
Leitsätze für Fleisch und Fleischerzeugnisse des deutschen Lebensmittelbuches Part of the German Food Codex containing guidelines for German meat and meat products.
Münchner Weißwurst Noncured emulsion-type sausages with hog rounds as casings from Munich (Germany).
Nürnberger Rostbratwurst/Nürnberger Bratwürste Noncured emulsion-type sausages in sheep casings (length 7–8 cm) for frying from the area around Nuremberg (Germany).
Pfälzer Leberwurst Spreadable and noncured liver sausages with a typical spicy flavor of marjoram from Rhineland-Palatinate (Germany).
Pfälzer Saumagen Coarse emulsion-type sausage filled in hog stomach from Rhineland-Palatinate (Germany) containing pieces of blanched potatoes and pork, and bacon.
Regensburger Coarsely or finely chopped emulsion-type sausage from the region around the town Regensburg (Germany).
Roter Schwartenmagen Blood sausage with pieces of head pork.
Rügenwalder Teewurst Spreadable fermented sausages containing honey or glucose syrup.
Schwarzwälder Schinken Dry-cured ham with an intensive cold smoking flavor and dark color from the region of Black Forest (Schwarzwald, Germany).
Schwarzwurst Blood sausage.
Stuttgarter Schinkenwurst Coarse emulsion-type sausage.
Teewurst Spreadable fermented sausage produced by a finely or coarsely chopped batter.
Thüringer Leberwurst Spreadable and cured liver sausage with a typical flavor of marjoram from Thuringia (Germany).
Thüringer Rostbratwurst Medium finely comminuted sausage for frying and grilling without nitrite curing salt as well as with an intense marjoram flavor from Thuringia (Germany).
Thüringer Rotwurst Blood sausage with a spicy clove and marjoram flavor from Thuringia (Germany) containing precooked cured cubes of lean meat, heart, or tongue.
Weißer Schwartenmagen Cooked sausages produced by head pork, jowl, and jellied brawn.
Westfälischer Knochenschinken Dry-cured ham produced by a long-term ripening with bone-in and with a mildly smoky and spicy flavor from Westphalia (Germany).
Wiener Cured emulsion-type sausages in sheep casings with a typical smoky flavor and color from Frankfurt am Main (Germany).
Ansbacher Preßsack Cooked sausages produced by cured head meat, jowl, and jellied brawn from the region near the Bavarian town Ansbach (Germany).

Introduction

More than 1500 different kinds of meat products and sausages are produced in Germany. The different types of meat products are defined by the raw materials used, their special quality attributes as well as by the key analytical values. A complete list of products and their specifications can be found in the 'Leitsätze für Fleisch und Fleischerzeugnisse des deutschen Lebensmittelbuches', a section of the German Food Codex containing guidelines for German meat and meat products.

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Some German meat products are traditional ethnic products that are typical of a specific region (Figure 1). An overview of the principal classification of German meat products, together with examples, is provided in Table 1.

When a product acquires a reputation that extends beyond the national borders of the region or country of its origin, it may find itself in competition with similarly named but counterfeit products that pretend to be genuine German sausages. In 1992, the European Union, therefore, established authentication systems such as the 'Protected Designation of Origin,' 'Protected Geographical Indication' (PGI), and 'Traditional Speciality Guaranteed' labels to promote and protect the authenticity of ethnic food products. As an example, ethnic meat products that have been granted PGI protection, or have PGI protection applications pending, are shown in Table 2. These ethnic meat products may also simultaneously be registered as trademarks at the German Patent and Trade Mark Office, with trademarks being linked to the geographical origin of products. However, most of the ethnic meat products in Germany are not protected by such authentication systems. Below, select traditional ethnic products that are of particular popularity in Germany are presented in more detail.

Figure 1 Some typical German meat products: (clockwise from top left) Schwarzwälder Schinken, Pfälzer Leberwurst, Thüringer Rotwurst, Stuttgarter Schinkenwurst, Rügenwalder Teewurst, Nürnberger Rostbratwürste, (down left to right) Münchner Weißwurst, Nürnberger Rostbratwurst, Rügenwalder Teewurst.

Dry-Cured Ham

Schwarzwälder Schinken (Black forest ham)

The process used to produce 'Schwarzwälder Schinken' is shown in Figure 2. The raw material consists of deboned rear pork shanks without or with topside. It is obtained from pigs raised on farms located in the black forest region of Germany dedicated to the production of raw material for 'Schwarzwälder Schinken'. The farms are regulated with respect to feed type, raising conditions, and pig species to ensure a top quality of the raw material. After slaughtering, the raw material is subjected to a quality analysis before use. Parameters that are controlled include pH (<6 to ensure absence of dark, firm and dry – DFD meat), core raw material temperatures of below 4 °C, quality of the cuts as the removal of excess backfat, their weight, and an appropriate ratio of lean meat to set backfat (approximately 20% of lean meat). The pH value in particular is a decisive factor for determining whether the raw material is

Table 1 An overview of the classification of ethnic German meat products

Meat products	*Description*	*Examples*
Dry-cured ham	Salted and cured ham (smoked or air dried)	Schwarzwälder Schinken (Black forest ham), Ammerländer Schinken, Holsteiner Katenschinken, Westfälischer Knochenschinken
Cooked cured meat products	Different cuts (ham, loin or shoulder) of pork, beef, or veal	Kassler (cured and smoked pork loin), Badisches Schäufele (cured and smoked pork shoulder)
Fermented sausages (raw sausages)	Dry cured sausages (sliceable)	Greußener Salami, Göttinger Stracke, Göttinger Feldkieker
	Finely or coarsely minced and cured sausages (spreadable)	Rügenwalder Teewurst, Braunschweiger Mettwurst, Hofer Rindfleischwurst
Cooked sausages (emulsion-type sausages)	Finely chopped (usually heated for consumption)	Frankfurter (frankfurters), Wiener, Knacker (knockwurst), Bayerischer Leberkäse (meat loaf), Halberstädter Würstchen, Münchner Weißwurst
	Coarsely chopped	Regernsburger, Bayerischer Leberkäse (meat loaf), Stuttgarter Schinkenwurst
	Addition of pieces of meat	Pfälzer Saumagen (added with pieces of pork and potatoes)
Cooked sausages	Liver sausages (spreadable)	Thüringer Leberwurst, Pfälzer Leberwurst
	Blood sausages	Thüringer Rotwurst, Pfälzer Blutwurst, Roter Schwartenmagen, Schwarzwurst
	Head meat, jowl (jellied brawn)	Ansbacher Presssack, Weißer Schwartenmagen, Weißer Presssack
Sausages for frying	Fried, broiled, or grilled sausages	Nürnberger Rostbratwurst, Thüringer Bratwurst/Thüringer Rostbratwurst

Table 2 An overview of the protected geographical indication (PGI) of meat-based products in Germany

Dossier number	*Designation*	*Registration date*
DE/PGI/0005/0703	Göttinger Stracke	27/07/2011
DE/PGI/0005/0721	Göttinger Feldkieker	27/07/2011
DE/PGI/0005/0713	Holsteiner Katenschinken/Holsteiner Schinken/Holsteiner Katenrauchschinken/Holsteiner Knochenschinken	31/01/2012
DE/PGI/0005/0722	Hofer Rindfleischwurst	04/02/2011
DE/PGI/0005/00854	Westfälischer Knochenschinken	09/04/2013
DE/PGI/0005/0615	Halberstädter Würstchen	09/10/2010
DE/PGI/0005/0773	Eichsfelder Feldgieker	15/05/2013
DE/PGI/0005/0222	Thüringer Leberwurst	18/12/2003
DE/PGI/0005/0223	Thüringer Rostbratwurst	18/12/2003
DE/PGI/0005/0224	Thüringer Rotwurst	18/12/2003
DE/PGI/0005/0184	Nürnberger Bratwürste; Nürnberger Rostbratwürste	16/07/2003
DE/PGI/0017/1266	Greußener Salami	09/04/1998
DE/PGI/0017/0686	Schwarzwälder Schinken	24/01/1997
DE/PGI/0017/1223	Ammerländer Schinken; Ammerländer Knochenschinken	24/01/1997
DE/PGI/0017/1225	Ammerländer Dielenrauchschinken; Ammerländer Katenschinken	24/01/1997

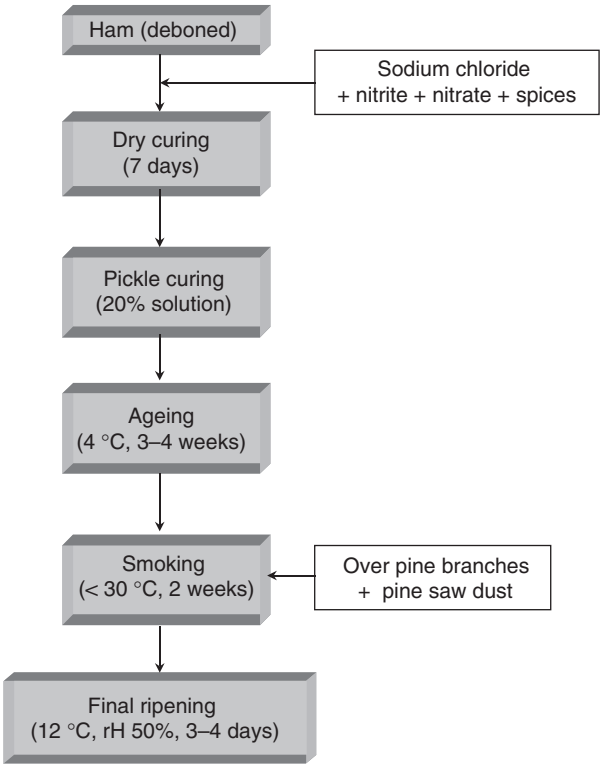

Figure 2 Production process of Schwarzwälder Schinken.

suitable for use in the production of 'Schwarzwälder Schinken' as the surface of hams may become slimy and gooey after drying and the consistency gummy if the pH value of the raw material exceeds 6.0. Another very important factor is to correctly trim the raw material, which in turn determines the ratio between fat and lean meat. The salt and spice mixture used contains nitrite curing salt and/or nitrate and a characteristic spice blend composed of, for example, juniper, whole pepper, garlic, and coriander. Each ham is dry-rubbed with the spice and salt blend, and multiple hams are then stored together in a sealed container. During storage, salt gradually diffuses into the meat. Every second day, hams are turned. Within 3–4 days, a natural brine begins to form and accumulate in the container. As soon as a natural brine has formed, additional brine is added to completely cover hams. The process of brining is completed within 2 weeks. The hams are then removed from the container, washed, and dried. The hams are then laid on a metal grid or hung and allowed to age in a controlled environmental chamber at a temperature of <5 °C and a relative humidity of 60–80% for 2 weeks. During the aging step, moisture is lost, and salt concentration profiles are equilibrated throughout the ham, both key steps to ensure the microbiological safety of hams. After aging, the hams are finally cold smoked for 2–3 weeks at 20–25 °C using pine wood that is the hallmark of the black forest region. The hams are then again aged at a relative humidity of ~50% at 15 °C. After completion of the aging process, hams have lost ~25% of their initial weight. The salt content of the final product must not exceed 15% on a dry matter basis and the ratio between fat and lean meat must not exceed 25% with a water-to-protein ratio of 2.2:1. The final product is characterized by a smoke-covered black surface, a dark-red interior color, a tender texture and an intense smoky and aromatic but not overall salty flavor. In former times, the production of 'Schwarzwälder Schinken' was only possible in the black forest, a forest-dense region in the southwestern part of Germany with general altitudes of approximately 800 m above sea level as the climatic conditions there required for the production of hams were similar to those described above.

Ammerländer Knochenschinken

This special dry-cured ham originated in or near Oldenburg, a town in the northwestern region of Lower Saxony. The raw material from a special variety in this region consists of rear pork shank with topside containing no bone and should weigh ~12 kg. Initially, the raw material is dry cured with nitrite curing salt, sea salt, brown sugar, pepper, juniper, and pimento for a minimum of 10 weeks by manually dry-rubbing the salt and spice mix into the meat once a day. After curing,

the product is hung for approximately 2 weeks for aging. Temperature and humidity conditions during curing and aging should follow standard protocols for ham processing. The hams are then weakly and preferably cold smoked with beech shavings at a high relative humidity for ~10 weeks. A further aging step follows under the above conditions for ~6 weeks. Taken together, the process takes 9 months. The ham has a final water-to-protein ratio of 2.5:1. Owing to the use of beech wood, the surface of products attains a slightly golden color whereas the interior is intensely red; has a very tender texture; and a very lightly smoky and mildly salty flavor.

Westfälischer Knochenschinken

'Westfälischer Knochenschinken' is a highly traditional product that traces its roots back to the twelfth century. In those early times, the production could only be carried out during the cold and moist winter months. The raw material again consists of a whole, bone-in pork hindquarter. In former times, the pigs were fed a special accord diet that was said to yield especially tender and dark red colored pork. The ham is dry-rubbed with a mixture of nitrite curing salt, potassium nitrate and sugar and stored in a closed container with a total curing time of 3–6 weeks to allow for the development of a natural brine. Unlike the production of 'Schwarzwälder Schinken', no additional brine must be added. Once a week during the curing, the hams are turned and resalted with the curing mixture. After 3 weeks, the brine is removed and the hams again left to cure for a period of 3 weeks. Afterwards, they are washed, dried and scrubbed and hung in chambers to allow surface moisture to evaporate and are subsequently smoked. Smoking involves either a long-term cold smoke process with beech wood or an alternating aging process in the open air that is interrupted by the above-mentioned cold smoke process for a total of 5 months. The entire production takes between 6 and 18 months, depending on the sensory quality of the product such as tenderness. The product is sold with the bone being removed. 'Westfälischer Knochenschinken' has a characteristic intensely golden surface and a dark red interior. Its flavor may be described as being mildly smoky and spicy. The texture is very tender.

Fermented Sausages

Greußener salami

This salami is produced in Thuringia, a state on the southeast of Germany, which was subjected to the rule of France in 1897. In contrast to the conventional rapid production of salami (Figure 3), in which starter cultures or glucono-δ-lactone are used to quickly lower the pH value, allowing for a short fermentation time of less than 4 weeks. The manufacturing of the 'Greußener' salami involves a long-term fermentation process that requires a minimum of 5–6 weeks. The raw material for this salami consists of high-quality beef void of sinews and free of fat, and roughly trimmed pork and backfat. The spices used include pepper, garlic and a variety of other spices such as nutmeg. The meat is coarsely ground using a meat grinder and further processed on a bowl chopper. To obtain a well-defined fat distribution in meat without causing the fat to be emulsified, the material should be partially frozen before chopping. After processing on the bowl chopper, the meat batter is filled into cellulose casings at a temperature of −2 °C to −4 °C, hung in a smoking chamber and cold smoked at intervals using beech wood for 4–5 days. Temperature is controlled and the pH is monitored during the smoking

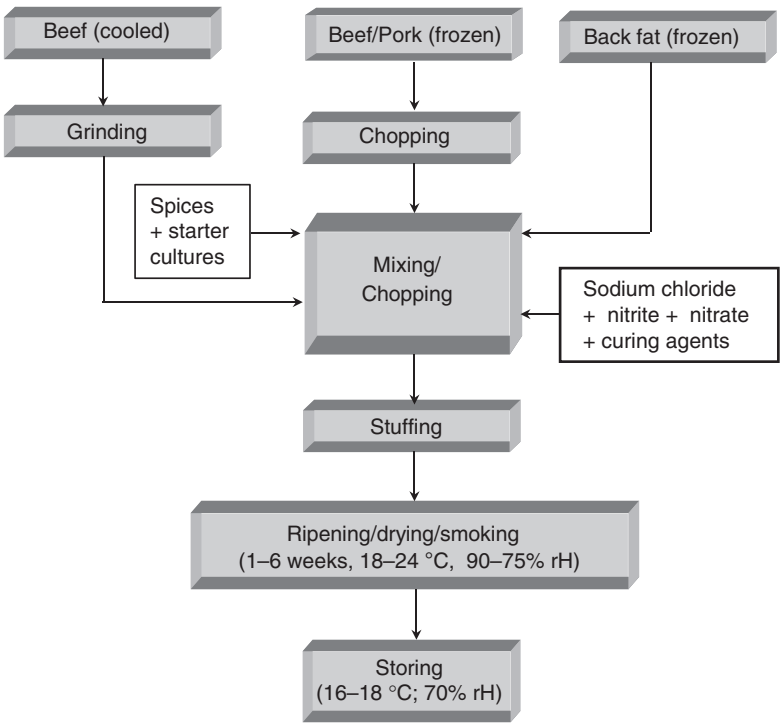

Figure 3 Production process of sliceable fermented sausages (Salami).

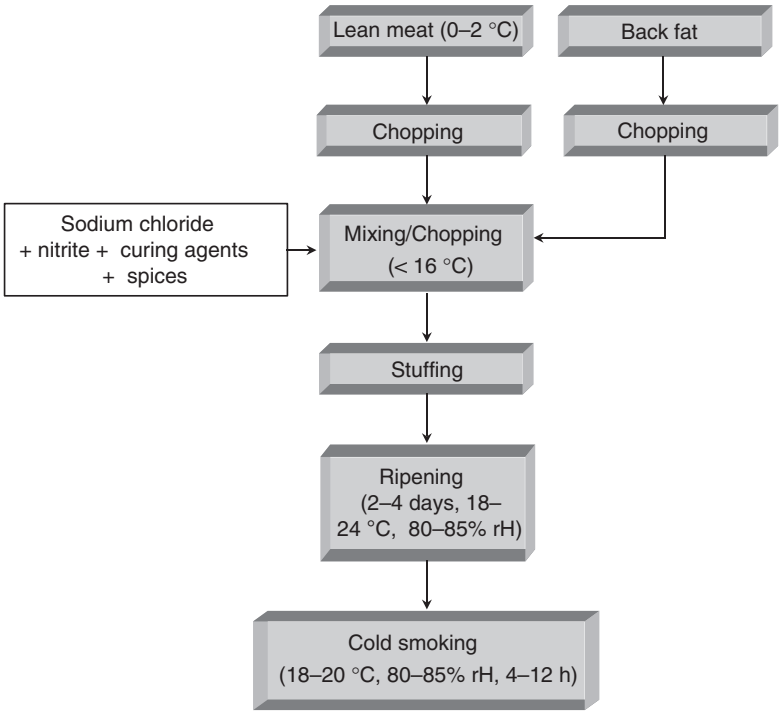

Figure 4 Production process of spreadable fermented sausages (Teewurst).

process. After smoking, the salami is ripened for 4–5 weeks to a final weight of 65–70% of its original weight. This salami has a mild pepper aroma, a stable dark red color and a very firm texture, which allows for the sausage to be easily and thinly sliced.

Rügenwalder Teewurst

In contrast to the production of other spreadable fermented sausages (Figure 4) that belong to the general class of 'Teewürste', the 'Rügenwalder Teewurst' contains honey or glucose syrup. The Rügenwalder Teewurst is made from beef and/or pork with a low content of connective tissue and a fat content of below 45%. Before processing, the raw material is chilled to −1 °C to +2 °C. After mincing and chopping of the lean meat, fat is added. The mixture is then processed on the bowl chopper to attain a finely dispersed (creamy) batter. The batter is filled into a cellulose casing and fermented for approximately 24–48 h at 16–24 °C at a relative humidity of 80–90%. Sausages are then cold smoked at 18 °C, until the products attain an intensely smoked and spicy flavor and a deep red color.

Cooked Sausages

Emulsion-type sausages

Germany is known for its wide variety of sausages but particularly for those that belong to the category of emulsified sausages. Some emulsified sausages are only salted with sodium chloride, such as the 'Gelbwurst', or the 'Münchner Weißwurst', but most sausages such as the 'Lyoner', the 'Halberstädter Würstchen', or the 'Frankfurter' contain nitrite curing salt.

Halberstädter Würstchen

Halberstadt is a town in the central region of Germany and the traditional manufacture process of the 'Halberstädter Würstchen' dates back to more than 100 years. This sausage is a cured emulsion-type-sausage made from finely chopped pork (~45%), beef (~15%), backfat (~15%), and ice (~25%). The batters are filled in thin, tender natural sheep casings (rounds) and are hot smoked for 40–50 min at an average smoking temperature of 60–75 °C. Direct firing with an underfloor furnace fed with beech wood causes the temperature in the smoking chamber in intervals to briefly reach temperatures above 110 °C. This also causes a continual reduction in relative humidity (<25%) to take place. Characteristic features of these sausages are their brown color and an unmistakable, intense smoky flavor. The sausages are mainly sold as a conserved product, for example, in a glass or metal jar.

Frankfurter Würstchen

In Germany, 'Frankfurters' have been known since the thirteenth century and have been protected under the name 'Frankfurter Würstchen' since 1860. The name has been exclusively used for such sausages since 1929. A butcher from Frankfurt, Johann Lahner first manufactured these sausages in Wien and, because of this, they are also known as Wiener or Wiener Würstchen in Germany. Today and, in contrast to the original 'Frankfurters', Wieners are manufactured from pork and beef whereas Frankfurters are made with pork only. They are cured emulsion-type-sausages with a finely chopped batter consisting of ~45% lean and desinewed pork, 25% fat and 30% ice filled in sheep casings (rounds). The sausages have a mildly spicy and smoky flavor and a golden-brown color, a fact that is based on the addition of white pepper and nutmeg

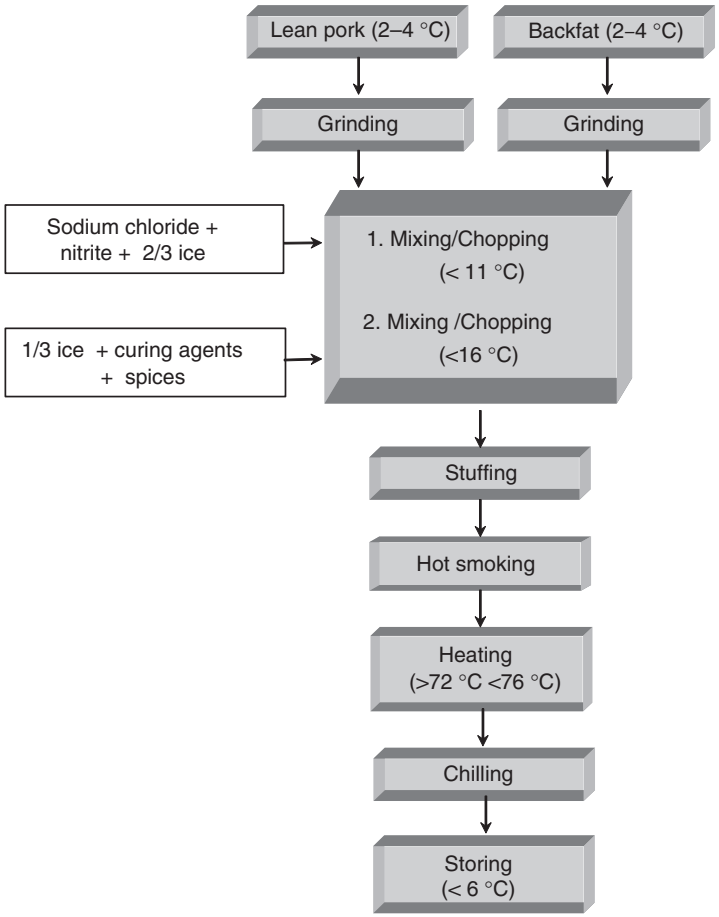

Figure 5 Manufacturing process of Frankfurter Würstchen.

or due to the hot smoking process. After filling, the sausages are first dried at 50 °C and then smoked at 50–60 °C for 45–50 min. In Figure 5, the manufacturing process of Frankfurters is shown.

Münchner Weißwurst

The birth of the 'Münchner Weißwurst' occurred on 22 February 1857, in an inn at the center of Munich, and was the result of an accident. The butcher was planning to prepare an emulsion for an uncured Bratwurst requiring the use of small sheep intestines. However, as sheep casings were not available, he used hog rounds instead. Moreover, for fear of sausages bursting, he cooked the sausage instead of frying them. Original Münchner Weißwurst contained veal, fat, water, and cooked rind from calves head or other connective tissue from calves. Today, this sausage may be made from both veal, beef from heifers, and/or pork and pork jowls. Veal and/or pork, precooked rinds and pork jowls, and fat are ground separately using a 3 mm plate. The chilled lean meat is only coarsely chopped with sodium chloride and additives such as citrates or phosphates. Then, two-thirds of the total amount of ice and the fat are added and chopped to a temperature of ~7 °C. The remaining one-third of ice and a spice blend consisting of onion, pepper, and lemon powder is added, and the mix further chopped to a temperature of 10 °C. The precooked and minced rinds and fresh parsley are added to the emulsion at the very end of the chopping process. The emulsion is then filled into hog rounds, and the sausages are then heated in hot water until they reach a core temperature of 70 °C. They are then chilled in cold water. Overall, the sausage must contain no more than 30% fat and 25% external water. For consumption, the Münchner Weißwurst is best served warm and should be eaten without its casing and additionally with sweet coarsely ground mustard.

Pfälzer Saumagen

'Pfälzer Saumagen' is the hallmark meat products of Rhineland-Palatinate, a state in the Midwest of Germany. The peculiarity of this coarse emulsion-type sausage is the use of a rather high amount (up to 33%) of blanched and cubed potatoes and/or carrots. Defatted pork is coarsely minced. A portion is set aside to be later added to the emulsion. The other portion is emulsified with backfat and ice and typical spices such as marjoram, pepper, nutmeg, and onions are added. A characteristic feature of 'Pfälzer Saumagen' is that the batter is filled into a hog stomach (after having it combined with the above mentioned coarsely minced pork portion) and is then heated. For consumption, Pfälzer Saumagen is best pan-fried and served with Sauerkraut.

Liver Sausages

These spreadable sausages must contain at least 10% liver to be classified as liver sausages. High-quality liver sausages contain at or above 25% pork liver. At contents of 20% liver, no additional emulsifiers are needed because the liver is able to fully stabilize the emulsion. As a first step in the manufacturing of liver sausages, blood vessels and larger bile ducts that may cause a bitter taste are removed from the liver. After rinsing, the liver is finely chopped until it begins to foam. Precooked lean pork belly and fatty pork cuts of the belly or backfat are chopped and then cooled to a temperature of 58 °C, at which time the liver may be added and the batter is further chopped. Temperature before filling of the paste-like mass in casing should not be lower than 40 °C; otherwise solidification may occur making filling impossible and the mechanical strain results in a partial separation of fat and gel.

Thüringer Leberwurst

Thüringer Leberwurst is a liver sausage with a smoky and spicy taste and contains braised onions, pepper, and marjoram from Thuringia. The sausage is normally filled into natural casings or sold in glass jars. The method of production is schematically shown in Figure 6. The principal ingredients are fatty pork cuts, lean pork, and fresh liver. Lean and fatty pork are precooked in hot water, allowed to cool (< 58 °C), and then minced with fresh liver and braised onions using a 3 mm plate. The spices and the nitrite curing salt are then mixed into the mince. Once a homogenous mixture is obtained, the minced mixture is immediately filled into either natural casings, such as hog chitterlings, beef and hog middles, or artificial casings. The sausages are then heated to a core temperature of 75 °C. This temperature is required to inactivate, for example, lactic acid bacteria. Then the sausages are cooled and smoked using beech wood until they assume a characteristic yellow color.

Pfälzer Leberwurst

The manufacturing of this liver sausage is similar to that of Thüringer Leberwurst with the exception of not being cured. The product is more of a home-style sausage often produced during home slaughtering. It consists of a coarsely comminuted liver sausage in the absence of curing salt. It has an intense taste of marjoram. After heating of the pork and fatty pork tissue, broth may be added to the mixture to compensate for cooking losses.

Blood Sausages

High-quality blood sausages such as Thüringer Rotwurst are made from a raw blood and rind containing batter with cube-shaped pieces of precooked lean meat, liver, heart or tongue, and small amounts of backfat. This mixture was heated to 80 °C. Products of lower quality contain fatty pork, for example, pork belly, jowls, and/or backfat.

Thüringer Rotwurst

This sausage is a blood sausage of higher quality because it is made from select raw materials including 55% precooked lean pork with a maximum of 5% visible fat, 25% precooked jowl without rind, 5% pork liver, 7.5% cooked rind, and 7.5% pig blood. Nitrite curing salt and spices such as black pepper, onions, thyme, clove, and marjoram are added. A particular feature of this sausage is that cubes of lean meat must be

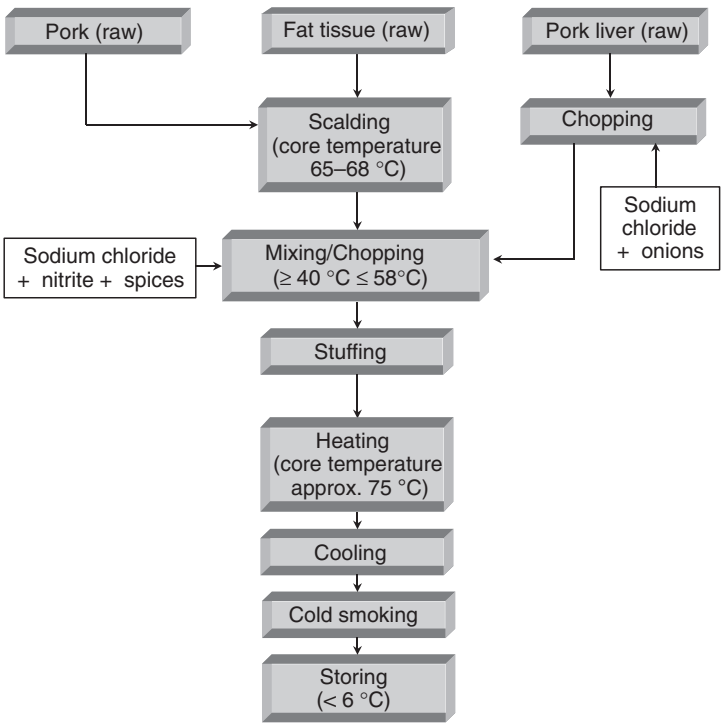

Figure 6 Manufacturing process of Thüringer Leberwurst.

inserted, for example, at least 35%, some of which may be replaced by liver, heart, or tongue. The lean pork and jowl are injected with a cold brine solution, pickled in brine for 12 h, and heated to 80 °C. Cooked rind, onions, and liver are added and minced through a 3 mm plate. Lean meat is scalded. The salt and spices are added and blended into the mixture and blood is finally added. The mixture is filled into hog or beef bungs, caps, stomach, and pig fat ends, and cooked in a water bath for 80 min at 85 °C or filled into glass jars or metal cans.

Special Sausages for Frying

Nürnberger Bratwurst and Nürnberger Rostbratwurst

These small sausages are excellent for frying or grilling. They stem from the area around Nuremberg, a city in Bavaria (the southernmost state of Germany). They were first mentioned in 1462. They do not contain nitrite and are 7–9 cm long with a weight of 20–25 g before heating. The roughly defatted pork is chopped and mixed with salt and spices such as pepper, caraway seed, nutmeg, and marjoram to form a cohesive medium minced batter, which is then filled into small sheep casings. The product is ideally sold raw to be directly grilled or fried. For convenience, they are often sold preheated. A typical method for the consumption of Nürnbergers, particularly in some parts of Franconia, is to heat the sausages in a stock of vinegar, onion, bay leaf, juniper, cloves, and a small amount of salt. In this case, the final products are known as 'Saure Zipfel.'

Thüringer Rostbratwurst

This medium finely comminuted sausage without nitrite curing salt and with a spicy taste is a product that has a centuries-old tradition in Thuringia. The first documented reference is dated back to 1404. The sausage is made from coarsely trimmed pork, pork jowls without rind, and sometimes also trimmed veal or beef. Spices such as pepper, caraway, marjoram, and garlic as well as salt are added to the meat and the mixture is homogenized to a batter that can be filled into a natural small diameter sheep or pig casing. The sausages are linked at approximately 20 cm intervals and cooked at 75 °C for 1 min per millimeter diameter. They are highly aromatic sausages with an intense marjoram flavor and are ideal for grilling.

See also: Curing: Brine Curing of Meat; Dry. Production Procedures. Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Mediterranean; Middle East; North America; Poland. Fermentation. Ham Production: Dry-Cured Ham. Sausage Casings. Sausages, Types of: Dry and Semidry

Further Reading

Deutsche Lebensmittelbuch-Kommission, 2010. In: Deutsches Lebensmittelbuch, Leitsätze für Fleisch und Fleischerzeugnisse, *Bundesanzeiger 134* (*25.7.1975*), *changed* 16.

EEC, 2002a. Publication of an application for registration pursuant to Article 6(2) of Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin, Thüringer Rotwurst National Application No 398 99 006.9, Official Journal C Series (Information and Notices) 114:12–13.

EEC, 2002b. Publication of an application for registration pursuant to Article 6(2) of Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin, Thüringer Leberwurst National Application No 398 99 005.0, Official Journal C Series (Information and Notices) 114:10–11.

EEC, 2002c. Publication of an application for registration pursuant to Article 6(2) of Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin, Thüringer Rostbratwurst National Application No 397 99 006.5, Official Journal C Series (Information and Notices) 114:14–15.

EEC, 2002d. Publication of an application for registration pursuant to Article 6(2) of Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin, Nürnberger Bratwurst/Nürnberger Rostbratwurst National Application No 398 99 002.6, Official Journal C Series (Information and Notices) 63:25–26.

EU, 2010a. Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs, Halberstädter Würstchen EC No: DE-PGI-0005–0615 – 02.07.2007, Official Journal C Series (Information and Notices) 35:9–12.

EU, 2010b. Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs, Hofer Rindfleischwurst EC No: DE-PGI-0005–0722 – 10.10.2008, Official Journal C Series (Information and Notices) 138:37–39.

Fischer, A., 1988. Produktbezogene technologie – herstellung von fleischerzeugnissen. In: Prändel, O., Fischer, A., Schmidhofer, T., Sinell, H.J. (Eds.), Handbuch der Lebensmitteltechnologie. Fleisch. Stuttgart, Germany: Verlag Eugen Ulmer, pp. 488–594.

Fischer, A., 1993. Processing German style: How to make old world products just like the Wurstmachers. Meat and Poultry 38, 28–46.

Pearson A.M. Gillett, T.A., 1996. Sausage formulations In: Processed Meats. (3rd ed.) pp. 242–290, New York, NY: Chapman & Hall.

RAL Deutsches Institut für Gütesicherung und Kennzeichnung e.V., 1972. Schwarzwälder Schinken, Bezeichnung und Kennzeichnung Berlin, Germany: Beuth-Verlag RAL-RG 0102.

RAL Deutsches Institut für Gütesicherung und Kennzeichnung e.V., 1978. Ammerländer Schinken Bezeichnung und Kennzeichnung Berlin, Germany: Beuth-Verlag RAL-RG 0105.

RAL Deutsches Institut für Gütesicherung und Kennzeichnung e.V., 1976. Rügenwalder Teewurst Bezeichnung und Kennzeichnung Berlin,Germany: Beuth-Verlag RAL-RG 0104.

Wahl, W., 2004. Ausgezeichnete deutsche Wurstrezepte/Excellent German sausage recipes, Bad Wörishofen. Germany: Hans Holzmann Verlag.

Relevant Websites

http://www.bmelv.de/SharedDocs/Downloads/Ernaehrung/Lebensmittelbuch/LeitsaetzeFleisch.pdf?__blob=publicationFile
Deutsches Lebensmittelbuch Leitsätze für Fleisch und Fleischerzeugnisse.

http://ec.europa.eu/agriculture/quality/schemes/index_en.htm
European Commission Agriculture and Rural Development.

http://register.dpma.de/DPMAregister/geo/uebersicht
German Patent and Trade Mark Office DPMA Register: Trademarks and Indications of Geographical Origin.

http://www.ral-guetezeichen.de/geographische-herkunfts-gewaehr.html
RAL Deutsches Institut für Gütesicherung und Kennzeichnung e.V.

India and Pakistan

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Glossary

Biryani Indian meat dish made with rice and highly flavored with fried condiments and seasonings.
Dry salted meats The meat is cut into long strips up to 0.5–1 in. in breadth or into small chunks. Cuts are sun dried after adding salt and turmeric powder, which gives a long shelf life, and they are rehydrated before cooking.
Goan Vindaloo Meat preparation from Indian state of Goa using pork, wine, and garlic.
Haleem Type of meat porridge prepared from meat, whole wheat, and lentils along with spices and condiments, which are constantly stirred to make a thick semisolid mass.
Kabab Comminuted meat product made out of minced meat or small-to-large cuts of meat, which is charbroiled to a give smoky flavor.
Kargyong An ethnic sausage-like product from the Himalayan region prepared from yak meat or beef or pork and called yak or lang or faak kargyong, respectively.
Kashmiri wazwan meats Traditional meats from Kashmir including rogan josh, nate-yakhni, tabak manss, aab gosht, rista, and goshtaba prepared by experienced chefs called wazas.
Keema A comminuted meat product traditionally prepared from the low-grade and less expensive cuts of meat, especially of goat or lamb meat.
Kolhapuri mutton Prepared from carefully selected goat or lamb meat, with preferred meat cuts comprising three components: tambada rassa, i.e., red curry, phandhara rassa, i.e., white curry, and mutton sukka, i.e., roasted mutton.
Meat pickle Intermediate-moisture meat product prepared for preserving meat for a long time and making it ready to eat and convenient.
Momo/dumpling Meat fillings of minced pork or any other meat are stuffed in flattened dough made of maida (wheat flour) along with finely chopped onions, ginger, garlic, coriander, and vegetables.
Pish pash/khicheri Meat products made from meat and rice (1:1 ratio for pish pash and 1:2 ratio for khicheri) cooked together along with onions and spices.
Rapka Meat is dried over a traditional home kitchen flame for several weeks by hanging it on a rapka, which is made of bamboo.
Tandoori After marinating the meat product is cooked at high temperature within a tandoor (earthen oven) by way of smoking, grilling, and baking, thereby giving tandoori flavor.
Tikka Boneless meat is cut into small pieces and baked like tandoori in a tandoor following marination in spices and yoghurt.

Introduction

There are several traditional meat products and recipes in the Indian subcontinent, viz. India, Pakistan, Bangladesh, Nepal, Sri Lanka, and other neighboring countries in the region, owing to their similar cultures and the availability of spices. These meat products have percolated down through history and have metamorphosed into spicy ethnic Indian meats. The history of the Indian subcontinent depicts various culinary practices amalgamated into Indian cuisine embodying the cultures of the different settlers. The blend of various aromatic spices with meat and meat products is the basis for ethnic/traditional Indian meats. The diversity of the region has had an impact on the type of meat products available in different parts of the Indian subcontinent and further afield. The meats common to the region are kabab, tikka, biryani, curry, meat pickle, and dry salted meat, among others. The north has tandoori, Kashmiri wazwan meats comprising meats such as rogan josh, nate-yakhni, tabak manss, aab gosht, rista, goshtaba, and shaljam gosht; the south has Hyderabad haleem, Chettinad dishes of Tamil Nadu, and Kerala lamb stew; the west has Kolhapuri mutton and Goan vindaloo; and the east has momo/dumpling, rapka, pish pash, khicheri, kargyong, korma, dopiyaza, etc.

The increasing per capita income in the Indian subcontinent has increased the demand for meat and the traditional/heritage meat products cater to this requirement. The small quantity of the meat produced in the region is processed further into value added and ready-to-eat products as compared to the Western world. Thus, researchers are focusing on standardization of the product profiles and mechanization of ethnic meat product processing. The local knowledge base and information pertaining to the ingredients, local taste preferences, and the history of product formulations passed down through the generations help in the overall improvement of the products. Along with researchers and academicians, the meat industry is also trying to develop these ethnic products for domestic as well as international markets. Undoubtedly, ethnic Indian meat products are quite popular in the western world, especially in the UK and USA. The Indian government is trying to protect these indigenous meat products through the Geographical Indication of Goods (Registration and Protection) Act, 1999, which came into force in September 2003. The quality and distinctiveness of the meat products belonging to particular regions of the country are classified in the fourth schedule of this act under class 29.

The prominent meat products originating from India, Pakistan, and neighboring countries are discussed in this article.

Tandoori

The tandoor is an earthen oven and tandoori is the meat product cooked in the tandoor. The origin of the tandoor is in

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the Thar desert of northwestern India and eastern Pakistan where it is primarily used by the Bhatti tribe. Thus, in India another name for tandoor is Bhatti. Tandoori meats are made out of marinated tender flesh that is deskinned and defatted before being cooked in a special cylindrical clay oven. This oven is used for high-temperature cooking at 450 °C with the help of fired charcoals or wood. This kind of cooking is a combination of smoking, grilling, and baking, giving the meat product a unique tandoori flavor as well as the color, texture, and taste. The most common meat used in tandoori is tender broiler chicken meat with superficial incisions on the body of the cuts or whole chicken. Marination is done for at least 2 h in the blend of spices with ginger-garlic paste and yoghurt and then the meat is smeared with salt and lemon juice for at least 15 min. Tandoori chicken products can have a shelf life of 8 h at 30 °C, 24 h at 3 °C, and 15 days at −15 °C. The antibacterial properties of the spice mix as well as the high temperature in the tandoor help to improve the shelf life of tandoori products.

Tikka

The meaning of tikka is 'bits and pieces' and thus it refers to the meat, mostly boneless chicken, that is cut into small pieces and baked like tandoori in a tandoor following marination in spices and yoghurt. While cooking, butter or oil is frequently brushed over the chicken to ensure that it remains tender and moist and is not overcooked. Tikka can be served as chicken tikka sizzler wherein it is served on a red hot plate along with onions. Tikka sizzlers are also eaten in Afghanistan along with India and Pakistan. Tikka can also be used as an ingredient for preparation of chicken tikka masala. Tikka masala is a gravy dish containing tikka chunks, cream, a blend of Indian spices, and gravy containing sauce and tomatoes. Other than chicken meat, tikka is prepared out of mutton, i.e., lamb meat pieces.

Wazwan Meats

The specialty meat products of the Kashmir Valley are wazwan meats. These are the several kinds of meat products traditionally prepared by experienced chefs called wazas. Rogan josh, nate-yakhni, tabak manss, aab gosht, rista, and goshtaba, among others, are the important products prepared from prerigor hot-boned tender lamb meat. In the series of the meats served in wazwan, the goshtaba is the last one to be served.

Rogan Josh

The name of this red hot lamb meat product is a combination of the words rogan, which means color, i.e., red color, and josh, which means either passion or heat. The mild Kashmiri chilies impart the red color and make it mildly hot, but the real color of the dish is credited to the extract from dried flowers, locally called Marwal. The lamb meat is marinated for 2 h and along with the marinade it is added to the spice mix in oil and cooked over mild heat until it is browned. Then it is stirred while scraping at the bottom of the cooking vessel to develop the characteristic flavor.

Nate-yakhni

In this product the nate, i.e., meat chunks of approximately 5–6 cm in size, are precooked in boiling water for 20 min and then curd, salt, spices, condiments, and ghee are added and boiling is continued for 20 more minutes to obtain the desired consistency called yakhini. This is a popular meat preparation owing to the blend of curd with meat flavor.

Tabak Manss

The rib portion of lamb meat is utilized for this product preparation after chopping it into small pieces and then steam cooking after application of salt and turmeric. The rib bones are then removed and only the finger meat is shallow fried in ghee over mild heat for a long time. The product is very much enjoyed due to its semidry and crispy nature.

Aab Gosht

Aab gosht is a lamb curry cooked in milk. Lamb is boiled in water with salt, ginger-garlic paste, and aniseed powder to make a stock. The milk is then boiled with green cardamom, onions, pepper, and ghee, to which the lamb stock is added. The mixture is then stirred thoroughly until it boils well.

Rista and Goshtaba

Rista and goshtaba are prepared from minced lamb meat that is manually pounded along with fat using a specially designed wooden hammer over a wooden or stone platform. While pounding the meat, salt and ground cardamom are added. Ice cubes are also added so that the meat does not heat while being minced. The meatballs or koftas are prepared manually with the palm of the hand from meat batter and are cooked in the mutton stock gravy. A separate yoghurt sauce is prepared from strained yoghurt, which is cooked by adding water, spices, condiments, salt, and ghee. The koftas are drained out from the stock gravy, added to the yoghurt sauce, and then simmered for a while before the product is ready to serve hot. They mainly differ in their flavors owing to the different formulations used for preparation of the yoghurt sauce. The natural taste, texture, and flavor of these traditional meatballs in yoghurt sauce provide them with an advantage over mechanized minced meat products. Thus, their market potential can be exploited by small-scale industries by packaging them in low-density polyethylene pouches for increased shelf life up to 7 days in refrigerated storage.

Kabab

The invention of kabab is credited to medieval soldiers who used to grill meat on their swords in fires in the open field. The Indian royal houses during the period of the Delhi Sultanate (AD 1206–1526) relished kababs and thereafter it became the most savored meat product in the region. There are many varieties of kabab, which are commonly prepared in India as well as Pakistan. Kababs are comminuted meat products made out of minced meat or small-to-large cuts of meat.

Traditionally, the meat used for kabab is lamb but over the years different types of meats have been used as per local and regional taste. Usually, kababs are charbroiled but nowadays they are also prepared by grilling, roasting, and stewing with the help of modern cooking equipment. Irrespective of the method used for cooking the internal temperature should be 75±2 °C, which can be achieved by charbroiling at 230±2 °C for 3 min or oven roasting at 180±2 °C for 12 min. The higher temperature of charbroiling also helps to lower the cooking loss and thus charbroiled kababs become juicier than oven cooked kababs. Charbroiled kababs have a typical smoky flavor owing to the contact of the charcoal with the meat; they also have a brown appearance, have more yield, and are juicier as compared to oven cooked kababs.

With increasing preference for chicken meat in the region chicken kababs are becoming more popular. Emulsion technology is being harnessed to utilize the spent hen chicken and by-products along with binders such as polyphosphate, maida (wheat four), potato, and soy in combination with mutton for higher yield. A refrigerated shelf life of up to 10 days makes the product a better choice over regular meat. Some researchers have developed a dehydrated chicken kabab mix packed in metalized polyester pouches that has a shelf life of up to 6 months at ambient temperature (27±2 °C); instant kababs can be made from this mix.

There are several variants of kababs found in and around India as well as Pakistan. The kakori and galouti kababs of Uttar Pradesh in India are usually made from goat meat. The kalmi kabab prepared from chicken meat is quite famous in Delhi, but there are several other varieties such as boty, lasoni, reshmi, tikka, tangdi, and bihari that are popular in India. Pakistan has its own varieties of kababs such as seekh, shami, chapli, ban, sajji, etc., that are relished by the local populace.

Biryani

This is a traditional meat product with lamb meat or chicken and rice (usually basmati) as the main ingredients along with fried condiments and seasonings. The rice is partially cooked with bay leaves, green cardamom, black pepper, and cinnamon sticks. The meat blended with partially fried condiments and seasonings is first marinated for two hours and then cooked separately. The meat and rice are then layered on each other and steamed on slow heat, which renders the contrasting flavors of both rice and meat in the resultant dish. The shelf life of biryani is limited but the meat industry is improvising to create ready-to-eat biryani, especially chicken biryani. There are many varieties of biryani found in the Indian subcontinent with slight differences in recipes. They are named locally, for example, Hyderabadi dum biryani, Lucknowi (Awadhi) biryani, Bhatkali, Kacchi, Moradabadi, Calcutta, Kozhikode, Dindigul, Sindhi, Memoni, and Tahari, with slight variations in their ingredients and preparation.

Haleem

Haleem is a type of meat porridge prepared from chicken, goat, or buffalo meat, traditional to India and Pakistan. Hyderabad in south India is especially known for haleem. The meat is boiled with water, whole wheat, and lentils (soaked overnight) along with spices and condiments until it develops a semisolid consistency. The preparation is stirred and the bottom of the pan is 'scrubbed' continuously. The tenderized meat is mashed well and heated slowly over a low flame for a long time to make it semisolid. Once it is ready, crushed fried onions and butter oil are added and it is served with lemons and mint leaves. A meat to wheat ratio of 3:1 is optimum and the product can also be frozen (−18±1 °C) to obtain a shelf life of up to 30 days.

Kolhapuri Mutton

Kolhapur is at the heart of Maharastrian nonvegetarian cuisine culture. The delicious and spicy Kolhapuri mutton is prepared from the carefully selected goat or lamb meat with preferred meat cuts. The Kolhapuri mutton recipe comprises three components: tambada rassa, i.e., red curry; phandhara rassa, i.e., white curry; and mutton sukka, i.e., roasted mutton. The key element in making these three recipes is preparation of the mutton stock.

For mutton stock preparation the meat is cut into medium-size pieces and marinated with salt, turmeric powder, ginger, and garlic paste for 1 h. After marination the meat is cooked in a pressure cooker with sufficient water and kept aside as a stock.

Tambada rassa is a mutton curry, generally spicy, which is prepared by using a special homemade red chili powder that contains ground onions, garlic, and other spices. At the start, a spice mix of cumin seeds, sesame seeds, bay leaves, cinnamon sticks, pepper, cloves, dry red or green chilies, and poppy seeds are sautéd in two spoonfuls of oil. Dry grated coconut and sliced onions are dry roasted until they turn brown. The roasted coconut, onions, and the spice mix are ground in a grinder along with the coriander leaves and some water to make a paste. For preparation of the curry, oil is heated in a vessel and the sliced onions, special homemade chili powder, turmeric powder, grated coconut, and grinded paste along with the water are added. This is boiled and then the mutton stock with a few meat pieces is added for further steaming until the oil is separated. This gives a nice red hot mutton curry to which some lime juice is added for a special taste.

Pandhara rassa preparation requires the same mutton stock. To start, chopped onions, dry grated coconut, sesame seeds, and poppy seeds are ground in a grinder to make a paste. Next the cloves, cinnamon, and black pepper are roasted in pure ghee, to which the ground paste is added along with salt and the mutton stock with a few meat pieces. This is boiled and white coconut milk is added for the typical taste.

Mutton sukka is prepared from all the cooked meat pieces that are left in the mutton stock after preparation of the tambada and phandhara rassa. Initially, chopped onions are roasted in oil until they turn brown, and then salt and the paste of spice mix prepared for tambada rassa are added. Next, all the meat pieces from the stock are added along with the special handmade chili powder and coriander leaves. This mix is stirred for some time and is ready to serve. Though this meat preparation is popular worldwide, it is misinterpreted in many places through the addition of extra quantities of chilies without the special homemade Kolhapuri chili powder.

Goan Vindaloo

Goa was a Portuguese colony and has been influenced by Portuguese culinary practices. Goan vindaloo is an Anglo-Indian meat dish known for its tangy taste and has evolved into a fusion of a Portuguese meat dish and the traditional spices of western India. The name vindaloo is derived from the Portuguese name 'Carne de Vinha d' Alhos' and the speciality of this meat preparation is the usage of wine and garlic. Pork is the meat usually used for vindaloo but other meats such as lamb, chicken, and beef can also be used. In some parts of India potatoes are also added to the meat owing to the misconception that 'aloo' in vindaloo signifies the addition of aloo (i.e., potato in Hindi).

The vindaloo spice mix is prepared by soaking Kashmiri red chilies (which give more of a color than pungency), cloves, cinnamon, cardamom, cumin seeds, mustard seeds, pepper, garlic, ginger and sugar in wine (particularly pheni wine which is made out of cashew fruits), vinegar, and coconut milk for half an hour. After soaking the spice mix is grinded to make a paste. Next, the meat is marinated with salt and vindaloo spice mix for up to four hours. If needed, a meat tenderizer such as papaya paste can be added to the marinade. Onions are fried in oil until they turn brown and the marinated meat is added for cooking. It is cooked over a medium flame until the meat is fully cooked.

Meat Pickles

Meat pickles are the easiest way of preserving meats for long periods of time and making the meats ready-to-eat and convenient. These intermediate-moisture food products made out of different meats are preserved at ambient temperature due to reduced water activity, low pH, and undissociated molecules of added organic acids. Meat pickles are popular in northern and north-eastern India. The meats commonly used for pickling are chevon, pork, chicken, and carabeef, i.e., buffalo meat. Pickle made from chicken gizzard is also eaten indigenously. At ambient temperature the shelf life of the meat pickle can be up to 60 days, but if antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and sodium ascorbate are added and packed in Polyethylene terephthalate bottles it can be several months. The meat is cut into uniform chunks of 2.5–5 cm (1–2 in.) and pressure cooked with salt in a pressure cooker. After cooking the meat chunks are acidified with 2.5% acetic acid (v/v)/vinegar and then deep-fat fried until they turn brown. A smooth fine paste of spice mix with red chilies, tomatoes, onions, coriander, ginger, garlic, cumin, turmeric, lime juice, and yoghurt is heated in oil. The meat chunks are added to the spice mix and blended by gentle cooking on slow heat. They should be heated until the oil is fully released as this oil only gives a preservative effect. Addition of antioxidant to the pickle helps to maintain the flavor and overall acceptability of the product during storage.

Momo/Dumpling

Momo is a traditional meat based delicacy in the north-eastern hill regions of India, especially Sikkim, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Ladakh, and Himachal Pradesh. Momo is said to be a Tibetan origin word and the product is popular in Tibet, Nepal, Bhutan, and China. Meat fillings of minced pork or chicken or goat/lamb, buffalo, or yak meat are stuffed into flattened dough made of maida along with finely chopped onions, ginger, garlic, coriander, and some vegetables. This is shaped into a round pocket or crescent and then steamed for 15–20 min. They may be pan or deep fried after steaming and are generally served with sauce or pickle.

Rapka

Rapka is a type of semidry meat product produced from the meat of mithun and yak. It is popular in the north-eastern hill regions of India for its smoky flavor. The meat is dried over a traditional home kitchen flame for several weeks by hanging it on a rapka, which is made of bamboo. Over the period the meat is semidried by the flames and this imparts a distinct smoky flavor.

Pish Pash and Khicheri

Pish pash and khicheri are traditional and regularly eaten meat dishes of north-eastern India. These two products are made from meat and rice (1:1 ratio for pish pash and 1:2 ratio for khicheri) cooked together along with onions and spices. Vegetables such as cauliflower, beet root, peas, and carrots are also added. They are cooked on a low flame until they become soft and semisolid in consistency. Chicken is the most common meat used but they can be prepared from other meats as well.

Keema

Keema is a comminuted meat product usually prepared from low-grade and less expensive cuts of meat (goat or lamb or any other meat). It is a highly perishable product owing to its comminuted nature, i.e., due to breakdown of the muscle structure and exposure to oxygen. It can be stored for up to 2 days at ambient temperature, but better packaging and/or refrigerated storage along with the usage of hurdle technology helps to extend its shelf life. Hurdle technology for pressure cooked chevon keema by application of humectants such as sugar, isolated soy protein, skimmed milk powder, and sodium chloride; acidulants such as lactic acid; preservatives such as spices, sodium nitrite, and sodium ascorbate; and vacuum packaging increase its refrigerated shelf life by up to 18 days.

Keema can be used for the preparation of curry, kofta (meatballs), and enrobed meat product samosa. Samosa is a traditional Indian fried product for which different vegetarian stuffings are used, but recently minced chicken meat stuffing has been introduced along with other vegetarian stuffs/spices and it is highly preferred in the market.

Dry salted Meat

Dry salted meat can be prepared from both small and large ruminant meats. The meat is cut into long strips up to

12.5–2.5 mm (0.5–1 in.) in breadth or into small chunks. They are sun dried after adding salt and turmeric powder. This gives a long shelf life to these products and they are rehydrated before cooking. In western Maharashtra, particularly in adjoining areas of Kolhapur, the dry salted meat is called 'Chunchunya,' which is oil fried before eating. In Pakistan there is a dried lamb meat called 'Landhi,' which originated in Balochistan province. Sheep are specially fattened for landhi meat, which is eaten during the winter season. In the north-eastern region these dried meats are called Satchu or Suka ko masu or Chartayshya.

Kargyong

Kargyong is an ethnic sausage-like product from the Himalayan region prepared from yak meat or beef or pork and called yak or lang or faak kargyong, respectively. The finely chopped meat and its fat is mixed with salt, garlic, and water; this mixture is stuffed into a natural casing (intestines) called gyuma. After sealing the casing is boiled for 20–30 min and air dried/smoked on bamboo over a kitchen oven for 10–15 days. Similar products called Kheuri (sheep stomach is used as casing) and Chilu (fat is stuffed instead of meat) are prepared in Sikkim. In the Kumaon region there are variants called Geema and Arjia.

There are several other meat products that are prepared with slight variations from the traditional meat preparations already in vogue, e.g., shaljam (meat cooked with turnip) and variants of meat curry similar to Kolhapuri red curry such as korma, chettinad, keralite stew, and dopiyaza. There are many cookbooks that include recently improvised dishes that are mainly based on the original ethnic meat products, and these can be even further modified to meet individual preferences.

Conclusions

Traditional meat products are part of the identity of a local culture and have unique sensory attributes to satisfy the gastronomic desires of the vast majority of a local populace. They have immense potential to generate income and employment for the local people by organized production of these products. At present there are many products that are not scientifically standardized and for which there is no comprehensive information available for their commercial production. The present preparation in batches is time consuming and there is limited shelf life. Thorough knowledge of their formulations, process optimization, commercial production, advanced packaging, and cold chain for distribution could definitely put many potential traditional meats on the world map. Retort pouch processing can be applied to some of the ethnic meat products from the Indian subcontinent such as curries, pickles, biryanis, keema, goshtaba, and haleem to exploit their market potential. Certain products, such as tandoori, owing to their protein-rich and low-fat nature can be marketed as healthy foods. With better know-how and technology, the ethnic/traditional meat products from the Indian subcontinent could become more popular and more widely available worldwide.

See also: Ethnic Meat Products: China and Southeast Asia; Middle East

Further Reading

Anjaneyulu, A.S.R., Thomas, R., Gadekar, Y.P., Lakshmanan, V., Mahapatra, C.M., 2008. Indian traditional meat products: Processing, quality, present scenario and prospects. Indian Food Industry 27 (2), 57–59.

Karthikeyan, J., Kumar, S., Anjaneyulu, A.S.R., Rao, K.H., 2000. Application of hurdle technology for the development of caprine keema and its stability at ambient temperature. Meat Science 54, 9–15.

Kesava Rao, V., Mandal, P.K., Pal, U.K., 1999. Traditional processing of meat and poultry products Proceedings of National Seminar on Processing of Meat, Poultry, and Byproducts for Value Addition. Mysore: Central Food Technological Research Institute, pp. 127–136.

Modi, V.K., Sachindra, N.M., Nagegowda, P., Mahendrakar, N.S., Rao, D.N., 2007. Quality changes during the storage of dehydrated chicken kebab mix. International Journal of Food Science and Technology 42, 827–835.

Padda, G.S., Sharma, N., 1982. Processing and preservation of meat pickle. Indian Food Packer 36 (6), 51–54.

Rai, A.K., Palni, U., Tamang, J.P., 2009. Traditional knowledge of the Himalayan people on production of indigenous meat products. Indian Journal of Traditional Knowledge 8 (1), 104–109.

Rai, A.K., Tamang, J.P., Palni, U., 2010. Microbiological studies of ethnic meat products of the Eastern Himalayas. Meat Science 85 (3), 560–567.

Rao, D.N., 1996. Assessments of physical, chemical, and microbiological attributes in traditional Indian poultry products tandoori and kabab. In: Proceedings of XX World Poultry Congress, pp 57–64. New Delhi: World's Poultry Science Association (India Branch).

Rasheed, M.A., Sudhakar Reddy, K., Kondal Reddy, K., Venkateshwar Rao, L., 2007. Effect of meat and extender ratio on quality of haleem during storage. Journal of Food Science and Technology 44 (4), 430–432.

Samoon, A.H., Sharma, N., 1988. Studies on processing and preservation of Nateyakhni, an indigenous meat product of Kashmir. Indian Journal of Meat Science Technology 1, 32–36.

Relevant Websites

http://www.sizzlingpots.com/recipes/cuisines/indian-recipes/item/76-kolhapuri-mutton.html
Sizzling Pots.

http://en.wikipedia.org/wiki/Chicken_tikka_masala
Wikipedia.

http://en.wikipedia.org/wiki/Wazwan
Wikipedia.

http://www.youtube.com/watch?v=5E3kulJRzGY
You Tube.

Japan and Korea

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Introduction

Japan and Korea share many similarities in environments and cultures in general, which is very understandable when considering the geographical and historical close relationships between these two countries. In terms of eating culture, for example, consumption of three meals a day (mostly including steamed rice, soup, and/or vegetables) using chopsticks and spoons (rather than knife and fork) is typical in both countries. The limited amount of arable land, due to the large areas of mountainous terrain and four distinct weather seasons of both Japan (multi-island) and Korea (peninsula), has contributed to diets based on the consumption of grains and vegetables (and/or seafood) rather than large quantities of meat and/or processed meat products. However, meat consumption in Japan and Korea has been gradually increasing and thus various and unique meat-based diets have developed. The influence of religion on East-Asian cultures (namely, Confucianism and Buddhism) has resulted in a greater emphasis on food preparation and presentation (visual appearance) based on philosophies of harmony within the body and a close commune with nature. Particularly, the historical application of 'yin and yang' in both health and diet has been extended to Korean cuisine. Although there are many similarities in lifestyle between the two countries, each country also has its own distinctive meat-based cuisine.

Japanese Meat-Based Cuisine in General

Japanese cuisine has historically been influenced by both China and Korea. However, such influences have mingled with unique Japanese natural resources and culture contributing to a well-established Japanese cuisine. Japan has a relatively short history of developing meat-based foods in its tradition, compared to other neighboring Asian countries. This is likely due to both the historical influence (as Buddhism prohibited the consumption of meat and fish) and the natural/geographical restriction (with the mountainous terrain limiting large open areas of arable land) in Japan. Thus, the Japanese have developed more of a seafood-based cuisine rather than one based on meat products such as beef, pork, and chicken. One of the most popular Japanese specialties is sushi (fish and rice mixed with rice vinegar called su; Figure 1). Sushi is formed with fish (or other seafood) and rice to make decorative, finger-food sized mounds served with pink, pickled ginger and soy sauce with wasabi (green horseradish-like condiment) for dipping. Various types of sushi have been developed such as Nigirisushi (rice topped with sliced raw fish called sashimi), squid, cooked octopus, crab, shrimp, omelet strips, salmon, sea urchin (uni), or flying fish (tobikko) and makisushi (wrapped in a strip of seaweed; often a roll of sushi rice with cucumber, tuna, mushrooms, or other fillings wrapped in a sheet of seaweed and sliced into individual pieces). The increasing popularity of sushi has 'migrated' into the Westernized style sushi (e.g., California roll) to suit the Western palate, which often includes beef or chicken teriyaki as well.

The Japanese meat-based foods were substantially revised and then were popularized for general consumers after the Meiji restoration in 1867. One particular delicacy is Kobe beef, which refers to beef from the Tajima strain of Wagyu cattle (Japanese black Angus breed) raised in Japan, which is well-recognized for its flavor, tenderness, and juiciness due to high-marbled fat composition within meat (Figure 2). Although Kobe is the main cattle strain known world-wide, there is also Yonezawa (also named Omi) and Matsusaka named after the areas in which they were raised and are equally respected in Japan. The Kobe (or other breeds) beef is often prepared as grilled-steak, steamed in a boiled pot as shabu-shabu or sukiyaki, or even thinly sliced fresh raw as sashimi. Pork is often prepared as the agemono-style dish of deep-fried breaded pork cutlets (called tonkatsu; Figure 3). Chicken is a

Figure 1 Sushi (http://en.wikipedia.org/wiki/Sushi).

Figure 2 Kobe beef in Japan (http://en.wikipedia.org/wiki/File:4_Kobe_Beef,_Kobe_Japan.jpg).

Figure 3 Tonkatsu served with steamed rice and miso soup (http://en.wikipedia.org/wiki/File:Tonkatsu_set_by_zezebono_in_Sapporo,_Hokkaido.jpg).

Figure 4 Tori (chicken) karaage (http://en.wikipedia.org/wiki/File:Tori_karaage_by_clanchou_in_Kanazawa,_Ishikawa.jpg).

Figure 5 Korokke (http://en.wikipedia.org/wiki/File:Korokke.jpg).

Figure 6 Yakitori (http://en.wikipedia.org/wiki/File:Cooking_yakitori.jpg).

favorite as grilled/marinated skewers called yakitori (teriyaki sauce-glazed skewers), and may also be served as sashimi.

Deep-Fried Japanese Meat-Based Dishes

'Karaage' is a deep-fried (agemono) bite-sized meat nugget coated with a mixture of egg, seasoned wheat flour or potato starch, and water before deep frying in a light vegetable oil (Figure 4).

'Tempura' is a very similar dish to Karaage but the ingredients used are mostly seafoods, such as prawns, fish, and/or vegetables.

'Korokke' is a breaded and deep-fried flat patty containing minced meat, seafood, or vegetables with mashed potato or white sauce rolled in wheat flour, eggs, and breadcrumbs (Figure 5). Korokke is originally related to a Portuguese dish, the croquette, which was introduced in the early 1900s.

'Katsu' is another agemono dish of deep-fried breaded chicken cutlets or pork cutlets (tonkatsu; Figure 3). The meat (pork fillet, pork loin, chicken, or fish fillet) for katsu is usually seasoned with salt and pepper, coated lightly in flour, eggs, and bread crumbs before being deep-fried. It is normally served with steamed-rice, miso soup, shredded cabbage, and katsu sauce.

Grilled/Pan-Fried Japanese Meat-Based Dishes

'Yakitori' is grilled/marinated chicken usually cooked on charcoals or gas/electric grill as yakiniku style (Japanese barbecue). The several bite-sized chicken pieces are skewered on a bamboo skewer and often lightly glazed with teriyaki sauce made with mirin (Japanese popular condiment; a kind of rice wine), sake, soy sauce, and sugar (Figure 6).

'Teriyaki' is one type of grilled yakimono dish: a broiled, grilled, or pan-fried meat, fish, or chicken marinated with teriyaki sauce. The meat is dipped in or brushed with the teriyaki sauce several times during cooking. The sauce is made with mirin (Japanese popular condiment; a kind of rice wine), sake, soy sauce, and sugar or honey (and sometimes with ginger, sesame, or garlic) by boiling (reducing) to reach the desired thickness.

Steamed Japanese Meat-Based Dishes

'Shabu-shabu' is a type of nabemono (Japanese hot pot) dish (food that is slowly simmered at the table and one-pot dish; Figure 7) similar to a Mongolian hot pot. 'Sukiyaki' is also very similar in style in that both consist of thinly sliced small pieces of beef and vegetables and served with sukiyaki sauce made from coconut, fermented tofu, tahini, peanut butter, sugar, garlic, lime, and spices. However, shabu-shabu is generally considered to be more savory and less sweet than sukiyaki. A more expensive beef slice cut, such as Kobe beef rib eye, may also be used, but often pork, crab, chicken, duck, or lobster is used. The dish is prepared by simmering a thin slice of meat and pieces of various vegetables in boiling broth in the pot. After the meat is cooked, vegetables are then cooked in the broth and eaten with sauce – noodles, also often cooked in the pot and consumed with the broth at the end.

Korean Meat-Based Cuisine in General

Korea has natural historical associations with both China and Japan, as geographically Korea is located between both countries. Yet, in spite of this, Korea has maintained and developed a distinctive culture (including a special cuisine style) independent of both these countries. One of the most unique characteristics of Korean cuisine is the highly seasoned meal preparation and colorful presentation of dishes. In particular, the mixture and balance of the five tastes (sweet, sour, bitter, hot, and salty) and five colors (white, red, black, green, and yellow) in foods based on the application of 'yin and yang' in both health and diet context, are considered important for serving dishes. Korea is not geographically suitable for raising livestock on an extensive scale (although a significant number of cows, horses, pigs, and chickens are raised) as the mountainous peninsula mostly forms the country's backbone, but hunting and fishing are quite common. Populations once hunted game meat such as deer, wild boar, and pheasants. Some ancient Korean cultures (especially the Goryo dynasty) refrained from the slaughter of cattle and eating meat because of the influence of Buddhism. However, various unique meat-based dishes have been developed since the thirteenth century. Traditional Korean meat-based cuisines can be classified according to the animal species, cooking, or processing methods. However, meats are usually dried as jerky, roasted (gui) on a charcoal grill (bulgogi), steamed (jjim) or boiled for soup (tang), and casseroled (jeongol or sinsullo). For roasting meats, charcoal and gas or electric grills are now used. The use of charcoal is the most traditional cooking method and Koreans especially prefer lump charcoal to the more common pillow-shaped briquettes and hexagonal sawdust briquettes elsewhere. Bulgogi is one of best known Korean barbeque dishes that is marinated with bulgogi sauce including soy sauce, garlic, onions, and sesame oil.

Korean meat products are usually consumed as banchan (side dishes) of the meal and in particular anju (a side dish consumed with alcohol). Among the various Korean meat cuisines, beef and beef variety cuts are especially popular. Cubes, thin slices, or small ribs of marinated beef from Hanwoo (Korean native cattle) and other various cuts are grilled at the table over a small charcoal brazier or gas grill and are one of the most characteristic Korean meat-based dishes (Figure 8). Also, many cuts/portions of pork and chicken are

Figure 7 Shabu shabu (http://en.wikipedia.org/wiki/File:Shabushabu.jpg).

Figure 8 Korean barbeque of Hanwoo beef on charcoal grill and Hanwoo galbigui on gas grill.

used in Korean cuisine in variety of cooking methods such as steaming, stewing, boiling, and smoking.

Grilled/Pan-Fried Korean Meat-Based Dishes

'Gogigui' is a particular method of grilling beef, pork, chicken, or other kinds of meat and by-products. The small size (thin) meat cuts are grilled at the diner's table on a gas or charcoal brazier, which is often built into the table as well (Figure 8) and consumed with dipping either in sesame oil sauce mixed with salt and pepper or with doenjang, a flavorful fermented soybean paste. Along with meat, sliced onions and garlic, mushrooms or pepper are grilled and consumed with lettuce (and other leafy vegetables) by wrapping together all these cooked vegetables, meat, and sauce (called ssam). Steamed rice and doenjang soup (similar to miso soup in Japan) are often served together.

'Bulgogi' (or Neobiani) is one of the most well-known Korean specialties along with kimchi, a spicy fermented cabbage dish heavily seasoned with garlic, onions, and chilli peppers (Figure 9). Bulgogi is usually made from thin slices of loins (or other prime beef cuts) marinated with a mixture of soy sauce, sesame oil, garlic, and pepper to enhance flavor and tenderness. Pork, chicken, and duck meat can also be used for bulgogi marination. Grilling techniques using grid irons or perforated dome griddles that sit on braziers are often used for cooking. Pan-cooking is also common and it produces a delicious and very flavorful broth.

'Samgyepsal gui' consists of thick, fatty slices of pork belly meat (uncured portion used to make bacon). It is grilled on charcoal or pan-plate and Koreans eat it directly from a grill as ssam with side dishes such as various vegetables (lettuce,

Figure 9 Bulgogi served with various banchan (side dishes), leafy vegetables, and sauce (http://en.wikipedia.org/wiki/File:Korean.food-Bulgogi-02.jpg).

Figure 10 Samgyepsal gui (pork belly) and kimchi on charcoal grill.

Figure 12 Dak galbi (marinated diced chicken cuts in a chilli pepper paste) on a hot plate.

Figure 11 Galbi (marinated beef short ribs with a Korean soy sauce) (http://en.wikipedia.org/wiki/File:Korean.food-Galbi-03.jpg).

Figure 13 Raw and barbequed makchang (pork/beef intestine) on charcoal grill.

Figure 14 Jokbal (pig's feet) with fermented shrimp sauce and various vegetables.

Figure 16 Sundae (Korean-style blood sausage) (http://en.wikipedia.org/wiki/File:Korean.food-Sundae-01.jpg).

Figure 15 Bossam (http://en.wikipedia.org/wiki/File:Korean_cuisine-Bossam-01.jpg).

perillar leaves, sliced green chilli peppers, raw garlic, onions, and fermented kimchi). Kimchi is an almost universal accompaniment to Korean dishes. Most Koreans prefer samgyepsal gui to other meats and this makes it a much more expensive cut compared to most other parts of pork meat (Figure 10).

'Galbi' is another famous Korean style barbeque along with bulgogi. Galbi literally means 'rib,' so when it comes to galbi-gui, it indicates cooked ribs, although it is often used without the suffix 'gui' for the marinated-grilled beef (or pork) short ribs (Figure 11). Galbi is normally made with beef ribs (called so galbi; 'so' means beef) marinated in a Korean soy sauce including soy sauce, sesame oil, rice wine, garlic, and pepper. The meat attached to a short rib is uniformly filleted in flat layers and is marinated in the sauce for a period of time. Pork ribs or chicken cuts can be used for galbi as dwaeji galbi ('dwaeji' means pork) or dak galbi ('dak' means chicken), respectively. Just like other gogigui dishes, grilled (or pan-fried) galbi is served with various vegetables (often cooked on the grill together), steamed rice, banchan (side dishes), and doenjang soup. Galbi can also be cooked as galbi-jjim (braised in a sweet soy sauce) or galbi-tang (soup containing pieces of galbi) as galbi-applied dishes. LA galbi is another popular modernized galbi dish and as the name suggests, it originates from the Korean immigrant community in Los Angeles, CA, USA. Basically, it is marinated in a similar way to normal galbi, but the rib is thinly cut across the bone (thus thickness of meat/rib is approximately 0.5 cm).

'Dak galbi' is a popular Korean chicken dish made from marinated diced chicken cuts in a chilli pepper paste (gochujang) with sliced cabbage and sweet potato cooked together on a hot plate. Literally, dak galbi means chicken ribs, but it uses most cuts of the chicken (Figure 12).

'Makchang and gopchang gui' is pork or beef large intestines grilled over charcoals or frying pan. Careful preparation needs to be done to remove unfavorable odors and excessive fat. It is served with dipping sauce made from a mixture of doenjang (Korean fermented bean paste), ground garlic, and chopped scallions (Figure 13).

Figure 17 Korean traditional samgyetang (ginseng chicken soup) served with kimchi.

Figure 18 Seollongtang with steamed rice and kimchi.

Steamed/Soup Type Korean Meat-Based Dishes

'Jokbal' is pig's feet steam-cooked with soy sauce and spices such as ginger, garlic, sugar, and rice wine. Once it is completely cooked, bones are either removed or left attached, and the meat is cut into slices and served cold often accompanied with fermented shrimp sauce (called saeujeot) for dipping or ssamjang (mixed paste condiments with doenjang and gochujang). Jokbal is rich in gelatins and thus gives unique greasiness and flavor. It is often eaten as ssam by wrapping in lettuce with other vegetables by hand (Figure 14).

'Bossam' is a type of typical Korean ssam dish that uses steamed pork (usually shoulder or belly portion cooked in a similar way to jokbal) wrapped in lettuce topped with various leafy vegetables, garlic, onions, and freshly made kimchi often accompanied with ssamjang (Figure 15).

'Sundae' is a type of blood sausage, steamed pig (or cow) intestines stuffed with various contents such as cellophane noodles (called dangmyeon), barley, and pork blood. However, as variations, different kinds of sundae fillings can be stuffed such as rice, kimchi, soybean sprouts, and scallions (Figure 16).

'Samgyetang' is representative of summer cuisine in Korea and also known as ginseng chicken soup. Traditionally, it is made from whole chicken (especially 35-days old chicken), Korean ginseng, dried jujube, whole garlic, and ginger. Owing to the highly nutritious compounds in the chicken soup, Korean people believe it helps to recover the exhausted body in the hot summer (Figure 17).

'Seollongtang' is a beef bone stock; beef leg bones (sometimes ox tail) are boiled/simmered for from several hours to an entire day to extract a nutritious broth containing collagen and minerals. Spring onions, pieces of steamed sliced beef cuts, and noodles are often added in seollongtang to bring more flavor and good taste. Additional seasoning including salt, ground black pepper, red pepper, minced garlic, and chopped spring onions can be added to seollongtang based on personal taste. Kimchi and steamed rice are also served alongside (Figure 18).

Raw Korean Meat-Based Dish

'Yukhoe' is made from raw chopped/ground beef seasoned with various sauces, spices, and other ingredients such as soy

Figure 19 Hanwoo beef yukhoe (marinated raw beef dice) and sashimi.

sauce, sugar, salt, sesame oil, onions, garlic, sesame seeds, black pepper, Korean pear, and a raw egg yolk. Sashimi is served with very fresh raw meat sliced into thin pieces. Owing to safety, only fresh meat can be used and served with sesame oil and salts (**Figure 19**).

See also: Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Brazil and South America; China and Southeast Asia; France; Germany; India and Pakistan; Mediterranean; Middle East; North America; Poland

Further Reading

Hong, J.H., Yoon, E.K., Chung, S.J., *et al*., 2011. Sensory characteristics and cross-cultural consumer acceptability of bulgogi (Korean traditional barbecued beef). Journal of Food Science 76 (5), S306–S313.
Kim, S.H., Oh, S.Y., 1996. Cultural and nutritional aspects of traditional Korean diet. World Reviews Nutrition Diet 79, 109–132.
Korea Foundation, 1997. Korean Cultural Heritage (Traditional Lifestyles), vol. IV. Seoul: Samsung Moohhwa Printing Co.
Mowat, P., 2009. Meat and modernity: Changing perceptions of beef in the making of modern Japan. Cross Sections 5, 41–56.
Nam, K.C., Jo, C., Lee, M., 2010. Meat products and consumption culture in the East. Meat Science 86, 95–102.
Oiso, T., 1976. History of food and diet in Japan. Progress in Food and Nutrition Science 2, 35–48.
Shin, D., 2004. Korean traditional food: Status, prospects and vision for globalization. Extension Bulletin – Food & Fertilizer Technology Center 557, 1–13.

Relevant Websites

http://www.yamasa.org/acjs/network/english/newsletter/things_japanese_29.html
Aichi Center for Japanese Studies.
http://www.krei.re.kr/web/www/home
Korea Rural Economic Institute.
http://asiaenglish.visitkorea.or.kr/ena/index.kto
Korea Tourism Organization.
http://www.nias.go.kr/english/index.nias
National Institute of Animal Science.
http://en.wikipedia.org/wiki/Korean_cuisine
http://en.wikipedia.org/wiki/Japanese_cuisine
http://en.wikipedia.org/wiki/Han-u
http://en.wikipedia.org/wiki/Matsusaka_beef
http://en.wikipedia.org/wiki/Mishima_beef
http://en.wikipedia.org/wiki/Kobe_beef
http://en.wikipedia.org/wiki/Yonezawa_beef
http://en.wikipedia.org/wiki/Kimchi
http://en.wikipedia.org/wiki/Sushi

Mediterranean

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Glossary

Dry curing The typical process involving the use of cure (potassium nitrate or sodium nitrite mixed with salt) followed by long drying.

Drying Products are slowly dehydrated thorough progressive reduction in relative humidity, reaching relevant losses in weight due to water evaporation.

Lipolysis The enzymatic breakdown of lipids with the formation of free fatty acids.

Proteolysis The enzymatic breakdown of proteins with the formation of peptides and free amino acids.

Water activity (a_w) It indicates the availability of water in a food and is defined as the ratio of the equilibrium water vapor pressure over the system and the vapor pressure of pure water at the same temperature.

Introduction

There are many types of meat products in the Mediterranean area that have been produced for centuries. These products exhibit a wide variety of flavors and textures. Today, these products still constitute an important part of the local economy, culture, gastronomic heritage, and tradition. Even though the technology has evolved and become mechanized, the basic processing still relies on traditional manufacturing processes, mostly based on salting and ripening. The products develop intense and characteristic texture and flavor, especially during ripening and drying, where enzymes and microorganisms play an important role. The drying of these products is a common practice due to the privileged Mediterranean climatic conditions. Main types of products and their specific characteristics are described in this article.

Mediterranean Meat Products

Dry-cured ham constitutes one of the main and representative products in Mediterranean countries. Some of the most well known of them are Spanish jamón Ibérico and jamón Serrano, French jambon de Bayonne, Italian prosciutto di Parma and prosciutto San Daniele, Portuguese presunto, and Croatian Istrian dry-cured ham. The production of these hams is based on a careful selection of the raw materials (back legs from specific pig crossbreeds), control of time and processing conditions at key stages like salting (for the penetration of salt and other curing agents), postsalting (to allow salt and curing agents to diffuse through the entire piece), and ripening/drying (for water loss and flavor development). The weight loss may reach 35%. Hams are rigorously controlled at the end of the process for the quality of color, texture, and flavor. Minimum processing time is usually more than 9 months, but for the highest quality such as Iberian hams, the processing time may last up to 24–30 months. The result is a ham with extremely rich flavors that are generated through enzymatic reactions. An Iberian dry-cured ham is shown in Figure 1. These hams are usually consumed raw, cut into thin slices (Figure 2), without further smoking or cooking.

Lacón is a dry-cured product similar to dry-cured ham but produced using the foreleg. It is also salted and dried but the processing time is shorter, usually approximately 4–5 months and is mainly produced in Spain.

Dry-cured loin is an entire piece produced from pork loin. It is cased and rubbed out with salt and spices and dried for 1–2 months until reaching a water content of approximately 50%. Its name is lomo curado in Spain. Another similar product produced from beef in Greece is pastirma.

Culatello is produced in Italy from the large part of the ham near the bottom. It is packaged with the pig's bladder and tied using slip knots in order to keep its nice round shape. Then, culatello is salted and dried like the hams. The

Figure 1 Iberian dry-cured ham (Jamón Ibérico).

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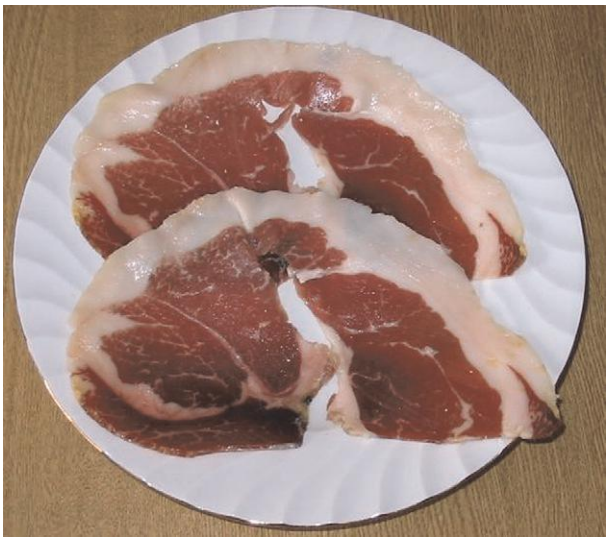

Figure 2 Cross section of Iberian dry-cured ham showing typical marbling.

Figure 3 Typical presentation of sliced chorizo as served in restaurants.

processing is usually between 10 and 14 months. Culatello di Zibello is produced in Parma (Italy).

Coppa is a type of Italian salami made from pork shoulder butt which is salted for 7–10 days and dried for 2–4 weeks. The shape is cylindrical and drying depends on the natural climate conditions in the valley of origin.

Bresaola della Valtellina is a well-known meat product produced in an Italian Alpine valley. Bresaola is made from selected beef cuts from the leg, trimmed of fat, salted, and optionally marinated with additional wine, sugar, and some spices for 7–10 days, stuffed in natural or artificial casings, and dried for more than 4 weeks. The final product has a bright red color and is eaten raw as very thin slices. A similar product, known as cecina, is produced in Spain.

Mortadella Bologna was originated in the sixteenth century in the outskirts of Bologna, where meat was ground in a mortar. Mortadella is produced from pork meat and fat, finely ground and minced, stuffed in natural or artificial casings, and thermally treated to an internal temperature of 68 °C. The final product has a uniform pink color, and white fat spots are evenly distributed through the meat mixture.

Italian salami, like Milano salami, is produced from finely ground pork meat and fat, mixed with salt, sugar, curing agents, and spices, and stuffed into a casing for slow and mild ripening and drying that may take as long as 3–6 months, sometimes even longer. The cross-section is red, with well-defined fat particles having the size of a rice grain. Other products include Hungarian salami, Greek Lefkada salami, and Spanish salami.

French saucisson is produced from coarse ground pork meat and fat, mixed with salt, sugar, curing agents, and spices, and stuffed into casing for a slow and mild ripening and drying. Fermentation occurs at low temperatures, usually <20 °C with slow acid generation and does not fall below pH 5.0. Smoke is not generally applied except for typical sausages in Hungary. The total processing may take up to 6 months, sometimes even longer. The cross-section is dark red, with well-defined large fat particles. Well-known dry fermented sausages include Spanish salchichón and French saucisson d'Alsace.

Spanish chorizo is based on pork and beef with added pork fat, salt, sugar, curing agents, and spices like red pepper, paprika, garlic, and oregano and stuffed in either natural or artificial casings. It is fermented for 1–2 days and ripened and dried for 1–3 months. There are many types of chorizo depending on the diameter, shape, size, sensory characteristics, and geographic origin. In general, it is characterized by a cross-section with an intense red color and uneven distribution of fat (Figure 3).

Most of these Mediterranean typical products have been protected with European Union certifications like the Denomination of Protected Origin (DPO), the Protected Geographical Indication (PGI), or Traditional Speciality Guaranteed (TSG). There are many DPOs in the Mediterranean area. In case of Spain, there are three for Iberian hams: Guijuelo, Dehesa de Extremadura, and Huelva and one for non-Iberian hams, which is denominated Teruel. However, jamón Serrano is Traditional Speciality Guaranteed. Raw materials, processing conditions, and quality of the finished products are rigorously controlled by respective Consortiums or Local Entities who are responsible for achieving the established requirements. For instance, the Serrano Foundation controls all hams produced in Spain which are stamped as Serrano. In a similar way, the Parma consortium controls the hams produced in Parma and stamped as prosciutto di Parma.

See also: Curing: Production Procedures. Drying. Ham Production: Dry-Cured Ham. Sausages, Types of: Dry and Semidry

Further Reading

Frentz, J.-C., 1982. L'Encyclopédie de la Charcuterie. Vesoul, France: Soussana.
Toldrá, F., 2002. Dry-Cured Meat Products. Trumbull, CT: Food & Nutrition Press.

Toldrá, F., 2006a. Biochemistry of fermented meat. In: Hui, Y.H., Nip, W.K., Nollet, M.L., Paliyath, G., Simpson, B.K. (Eds.), Food Biochemistry and Food Processing. Ames, IA: Blackwell Publishing, pp. 641–658.
Toldrá, F., 2006b. Dry-cured ham. In: Hui, Y.H., Castell-Perez, E., Cunha, L.M. *et al.* (Eds.), Handbook of Food Science, Technology and Engineering, vol. 4. Boca Raton, FL: CRC Press, 164-1–164-11.
Toldrá, F., 2007a. Fermented meat production. In: Hui, Y.H., Chandan, R., Clark, S. *et al.* (Eds.), Handbook of Food Product Manufacturing, vol. 2. Hoboken, NJ: John Wiley Interscience, pp. 263–277.
Toldrá, F., 2007b. Ham. In: Hui, Y.H, Chandan, R., Clark, S. (Eds.), Handbook of Food Product Manufacturing, vol. 2. Hoboken, NJ: John Wiley Interscience, pp. 231–247.
Toldrá, F., 2012. Biochemistry of fermented meat. In: Simpson, B.K., Nollet, L.M.L., Toldrá, F., *et al.* (Eds.), Food Biochemistry and Food Processing, second ed. Ames, IA: Wiley-Blackwell, pp. 331–343.
Toldrá, F., Aristoy, M.C., 2010. Dry-cured ham. In: Toldrá, F. (Ed.), Handbook of Meat Processing. Ames, IA: Wiley-Blackwell, pp. 351–362.
Toldrá, F., Mora, L., Flores, M., 2010. Cooked ham. In: Toldrá, F. (Ed.), Handbook of Meat Processing. Ames, IA: Wiley-Blackwell, pp. 301–311.
Toldrá, F., Nip, W.K., Hui, Y.H., 2007. Dry fermented sausages: An overview. In: Toldrá, F., Hui, Y.H., Astiasarán, I. *et al.* (Eds.), Handbook of Fermented Meat and Poultry. Ames, IA: Blackwell Publishing, pp. 321–325.
Toldrá, F., Reig, M., 2007. Sausages. In: Hui, Y.H., Chandan, R., Clark, S. *et al.* (Eds.), Handbook of Food Product Manufacturing, vol. 2. Hoboken, NJ: John Wiley Interscience, pp. 249–262.

Relevant Websites

www.iata.csic.es/IATA/dcie/carn/
Institute of Agrochemistry and Food Technology.
http://www.prosciuttodiparma.com/
Prosciutto di Parma.
www.fundacionserrano.org
Spanish Serrano Ham Foundation.
www1.clermont.inra.fr/tradisausage
Tradisausage.

Middle East

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Glossary

Dawood Basha Minced meat with rice.
Hawawshy bread Minced meat and wheat dough.
Kebabs Grilled, roasted, and stewed dishes of large or small cuts of meat or even ground meat; it may be served on plates.
Masmat A dish containing head meat, stomach, eyes, and tail.
Membar A dish containing the lungs, spleen, large intestine, esophagus, and rice.
Nakanek A seasoned traditional sausage from uncured beef trimmings.
Pastrami A seasoned, dry-cured longissimus.

Introduction

Meat products made from different ingredients are produced to meet consumer demands. In the Middle East, local foods are available in a huge variety derived from culture. Religion also influences the variety and unique flavor differences found in the Middle Eastern foods. Muslims, Christians, Jews, and Arabs have specific preferences for and prohibitions against certain ingredients, such as lamb, fish, beef, or pork. Particular ingredients and preparation techniques are specific to specific religious groups.

The Middle Eastern Meat Products

Owing to religious considerations, the bulk of meat products consumed in the Islamic Middle Eastern area are prepared from cattle (beef and buffalo), mutton, lamb, goat, camel, and sometimes poultry. Because of local habits, horse meat is also used in countries in northwest Africa, such as Tunisia, Algeria, and Morocco.

Lamb and mutton are particularly favored in the Islamic region, where pork consumption is forbidden. These types of meat are seasoned and grilled as kebabs, a popular food in the Arab world. Chicken is a popular everyday food, but quail, pigeon, goose, and duck are also commonly eaten. Christian communities in the Middle East consume a lot of traditional pork products, such as ham, frankfurters, and mortadella.

Sausages

The word sausage is derived from the Latin *salsus*, meaning salted or preserved. Salami is mentioned frequently in the pre-Christian period and may be associated with the Greek city of Salamis in Cyprus. Sausages may be defined as foods prepared from chopped and seasoned meat formed into a symmetrical shape. The origin of sausages dates back to ancient history, because they were mentioned as early as AD 228 by Athenaeus in the *Deipnosophists*, the oldest known cookbook.

Sausage types in the Middle East are grouped according to processing methods. The following groups can be considered to be ethnic products.

- Fresh sausages with the traditional name 'nakanek' are made from uncured beef trimmings. They are delicately seasoned and are very popular. They require refrigerated handling and cooking before consumption.
- Uncooked smoked sausages are usually cured and smoked but not cooked. They require refrigerated handling.
- Fermented and heavily smoked all-beef sausages were originally produced in Lebanon. The fermentation was originally caused by a natural microflora but is now a result of using starter cultures.

The individual sausage varieties in the Middle Eastern countries are distinctive, not only due to the uniqueness of the seasoning but also due to the type and coarseness of mincing the meat constituents as well as the manner in which the sausages are stuffed and processed. In addition to animal tissue, they contain flavors derived from olive oil, garlic, rosemary, black pepper, cardamom seeds, cinnamon, coriander, onion, paprika, nutmeg, ginger, cloves, and a variety of fillers and binders including maize, wheat, oat, and rice flour, which are specific to the Middle East.

Pastrami

Pastrami is popular in the Middle East and is made from the complete muscular longissimus dorsi, which is dry-cured, aged for 7 days in curing tanks, and then air-dried for another 4 days before it is coated with a paste composed of finely powdered Greek paprika and salt. The pastrami is then hung for at least 15 days for completion of the curing before it is sliced just before consumption. The color is dark red.

†Deceased.

Other Meat Products

Other meat products consumed in the Middle East include those made with minced meat and corned beef.

Minced meat with rice (Koftet ros) is usually known as Dawood Basha. It is also mixed with burgol and kobaba. Corned beef is made from beef (approximately 80%), fat (maximum 15%), salt (maximum 3%), and sodium nitrite (maximum 0.005%).

Traditional Meat Dishes

A number of traditional meat dishes are consumed in the Middle Eastern countries. These are described briefly below.

- A sweet dish is made from the lungs, spleen, large intestine, esophagus, and rice (Membar). These products are usually cut and fried in oil. Such meat dishes are popular and traditional in Egypt but are sometimes also eaten in Syria and Turkey.
- Masmat is a dish including the lower part of legs (known as Kawareh), head meat, stomach, eyes (known as Gawaher), and tail (known as Akkawi).
- Blanched feet of buffalo or cattle are traditionally used in Egypt and Syria. The soup created in the process is very nourishing.
- Kobeba is a traditional dish in Lebanon and Syria. It is prepared by mixing beef with an equal amount of broken wheat. It is cooked with tomato and 'kesk' (an Egyptian speciality based on milk and flour).
- Hawawshy bread is a special dish originating from Egypt and is made by mixing minced meat with wheat dough in the shape of bread and baking it in an oven.

See also: Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Brazil and South America; China and Southeast Asia; France; Germany; India and Pakistan; Japan and Korea; Mediterranean; North America; Poland

Further Reading

Campbell, G.P., Cook, P.E., 1995. Fermented Meat. London: Blackie Academic and Professional.
Chizzolini, R., Novelli, E., Zanardi, E., 1980. Oxidation in traditional Mediterranean meat products. Meat Science 49, S87–S99.
Henrickson, R.L., 1978. Meat, Poultry and Seafood Technology. Englewood Cliffs, NJ: Prentice-Hall.
Hughes, C.E., 1953. Kosher Meat Processing. Chicago, IL: Armour Analysis, Armour Livestock Bureau.
Karmas, E., 1976. Processed Meat Technology. London: Noyes Data Corporation.
Lawrie, R.A., 1981. Meat Science, fourth ed. Oxford: Pergamon Press.
Pother, N.N., 1986. Food Science, fourth ed. New York: AVI, Van Nostrand Reinhold.
Varnam, A., Sutherland, J.P., 1995. Meat and Meat Products: Technology, Chemistry and Microbiology. London: Chapman & Hall.

Relevant Websites

http://www.middleeastkitchen.com/meats/meats.html
Middle East Kitchen.
http://en.wikipedia.org/wiki/Arab_cuisine
Wikimedia Foundation Inc.
http://en.wikipedia.org/wiki/Doner_kebab#Saudi_Arabia
Wikimedia Foundation Inc.
http://en.wikipedia.org/wiki/Kebabs
Wikimedia Foundation Inc.
http://en.wikipedia.org/wiki/List_of_kebabs
Wikimedia Foundation Inc.
http://en.wikipedia.org/wiki/Pastrami
Wikimedia Foundation Inc.
http://en.wikipedia.org/wiki/Salami
Wikimedia Foundation Inc.

North America

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Glossary

Barbecue A method of cooking over low heat for long periods of time that involves the application of wood smoke. This is most often done in an oven that uses wood as the primary source of heat. It is to be differentiated from the term 'barbecue' as a synonym for outdoor cookery on a grill.
Bologna A medium to large diameter cooked sausage that is normally used as a sliced luncheon meat.
Jerky A term applied to a thin dried meat that is shelf-stable and ready-to-eat.
Shelf-Stable Meat products that require no refrigeration.
US Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) The regulatory agency of the US government responsible for meat and poultry.

Introduction

The statement that 'necessity is the mother of invention' was never more true than in the development of various ethnic meat products. Local conditions of climate and availability of ingredients plus the need to preserve food for long periods without refrigeration led to the development of meat products that have now become part of our culture. For example, jerky is no longer an item of necessity but has evolved into a popular snack item in the US.

Pemmican

The origin of pemmican is usually attributed to the North American native people. Pemmican is a mixture of dried meats and dried berries or fruits which, by tradition, were pounded together and mixed with melted or partially melted animal fat. It can be compared to modern-day 'energy bars' and served the same purpose, that is a high-energy compact ration that is shelf-stable. Very little, if any, pemmican is produced commercially. There are, however, some commercial derivations of pemmican that have been used in the past as military or camping rations. One of the chief problems in the stability of pemmican and similar products is the high fat content and the tendency to develop oxidative rancidity.

Whole-Muscle Jerky

Whole-muscle jerky can be made from a variety of lean meat, poultry, game and fish sources. It consists of thin strips of lean muscle tissue, usually cut parallel to the muscle fibers. The meat is cured and generally smoked. In the US, jerky is required to reach a moisture:protein ratio (MPR) of 0.75:1 to be labeled as 'jerky'. To be considered shelf-stable (not requiring refrigeration) jerky should be dried to a water activity (A_w) of at least A_w 0.85 and preferably lower.

The meat that is selected for use in jerky is usually less than 10% fat and should be as free from visible connective tissue as possible. It is sliced approximately 5 mm thick parallel to the muscle fibers. The length and width of the strips may vary. To aid in obtaining uniform slices, the meat may be sliced in a partially frozen state. It is cured by applying a dry mixture of curing ingredients and spices. Predominant spices and flavorings include ground black pepper, ground allspice, and garlic powder. Salt content is usually in the range of 3% of the meat weight. A variety of other spices and flavorings can be added depending on consumer preferences. Cayenne pepper is often added to give a spicy flavor. Application of the dry spices and flavorings, curing ingredients, and salt can be achieved effectively by using a tumbler.

Following the application of the salt, cure, and flavoring ingredients, the meat mixture is then held for 24 h at 4 °C to allow the curing ingredients to penetrate the thin strips of meat prior to the drying and smoking process. Following curing, the strips are either hung on combs or laid on racks. Smoke can be applied in a traditional manner or by drenching or atomizing liquid smoke. A variety of wood sources can be used such as fruitwood, mesquite (a very resinous wood that produces a pungent flavor), alder, or any of the traditional hardwoods. This smoking and drying is usually done at a temperature of 60 °C. Care must be taken to prevent 'case hardening', which will ultimately interfere with the correct drying process.

Once the appropriate degree of dryness is reached, the product is ready for packaging and shipment. Jerky may be vacuum-packaged or packaged in a high-oxygen and moisture-transmitting package. Any type of package or container that allows for a high-humidity atmosphere should be avoided, because this will encourage mold growth.

Restructured Jerky

Jerky can be made as a restructured as well as a whole-muscle product. The same lean raw material is first minced through a coarse plate (13 mm) and then mixed with the spices and flavorings, salt, and curing ingredients. It is then reminced through a 3 mm plate. The mixture is extruded in strips approximately 5–9 mm thick and 20 mm wide. The length can be variable, depending on consumer preferences. After extrusion, the strips are laid on perforated metal or plastic screens

for immediate drying and smoking. Drying and smoking procedures are the same as for the whole-muscle product. Packaging procedures are also the same. Restructured jerky lends itself to a continuous processing system.

Another process for forming restructured jerky is to form the meat mixture into a loaf with the desired dimensions of length and width. The loaf is then frozen and subsequently tempered to a convenient slicing temperature. It is then sliced into strips of appropriate thickness. This procedure is less efficient than the preceding one and is only used where small quantities are produced.

Summer Sausage

The term 'summer sausage' is applied to a variety of US semidry sausages similar to the European cervelat sausages. These sausages enjoy their greatest popularity in the midwestern US. The name originated from how this product was first made. Farmers would make sausage in the fall and winter months, and this sausage would be hung in the smokehouse or similar location, and because of fermentation of sugars that were added to the product, the sausage would be safe for consumption during summer months, without refrigeration. This product generally will have a rather low pH, sometimes as low as 4.6. Summer sausage is usually a mixture of beef and pork, although sausages made of beef alone are common. These sausages may be fermented or acidified using various acidulants such as encapsulated acid or glucono-δ-lactone. The US Department of Agriculture Food Safety and Inspection Service requires that summer sausage have an MPR of 3.1:1 or less, a pH of 5.0 or less, as well as fully cooked and smoked to be considered as shelf-stable. For consumer protection, those summer sausages that do not meet shelf-stable requirements should be labeled 'Keep Refrigerated'.

The final mincing size for this product ranges from 3 to 5 mm and the predominant spice used is black pepper along with coriander and mustard. Sometimes whole peppercorns and whole mustard seeds are added and the product may contain garlic. It is smoked or natural smoke flavoring is added.

Fibrous, collagen, natural or laminated casings are used; casing size ranges from 40 to 120 mm. Some of these sausages may be stuffed in a variety of novelty casings ranging from those shaped like American footballs to beer bottles. Summer sausage is often a component of food gift boxes in the US because of its shelf-stability.

Lebanon Bologna

This sausage is unique to the area around the town of Lebanon, PA, USA. It probably has its origin in the European cervelats. It is an all-beef product with a final mincing size of 2–5 mm. Casings are the same as those used for summer sausage. It is fermented to a very low pH (approximately 4.5). The predominant spice used is black pepper. The product is very heavily smoked and has a distinctly 'tangy' and smoky flavor.

Scrapple

Scrapple is a product that originated in eastern Pennsylvania. It consists of a corn (maize) meal that is cooked to a thick gruel or mush to which is added cooked meat and meat by-products. The mixture is then chilled in loaf molds. The chilled loaf is sliced by the consumer and fried. It is usually served as a breakfast item. It should contain 40% meat and meat by-products. Cooked pork head meat is the typical meat source. Characteristic spice flavorings are pepper and sage.

Hamburger or Ground Beef Patties

The ubiquitous hamburger has become a staple of the US fast food industry and a symbol of US culture worldwide. The origins of the 'hamburger' (a ground beef patty) are clouded in conflicting legend. Suffice it to say that the popularity of this fast food staple mushroomed after World War II with McDonalds given the credit for leading the charge.

Depending on regulatory labeling requirements, the patty is made from beef skeletal muscle with restrictions placed on the addition of nonmeat fillers, extenders, or texture modifiers. Final grind size of the meat is usually 3 mm. Conventional wisdom places the fat content at 20%, plus or minus 2% for optimum palatability. There have been attempts to market lower fat versions of the hamburger but these have not attained any great consumer acceptance. Patties may either be marketed as fresh (nonfrozen) or frozen. Patty forming is accomplished using a variety of high speed forming machines. Rapid freezing is mandatory to maximize palatability. Particular attention needs to be paid to microbiological safety with special emphasis on *coliforms*, particularly Shiga Toxin producing *Escherichia coli*'s such as *E. coli 0157:H7*.

Barbecue

This is another particular US phenomenon. Within the US there are wide regional differences in what is considered barbecue. Common to all of the regions is the use of less tender cuts of meat that have been slow cooked at low temperatures over a wood fire or in a wood fired oven or with some other application of smoke. Usually a mixture of spices is applied to the surface of the primal cut and a sauce 'mopped' on the surface during cooking or applied to the finished product after cooking. Characteristic of all true barbecue is the 'smoke ring', a red color that develops in the first few millimeters of the surface as a result of exposure to oxides of nitrogen from the combustion gases of wood. Some of the regional variations of barbecue are: In Kansas City, MO, USA barbecue means pork spare ribs or back ribs served with a thick, sweet, tomato and molasses-based sauce. In Memphis, TN, USA it would be any cut of pork, including ribs that uses a sweeter, tomato-based sauce. As you move south and east it is pork with thinner sauce that is vinegar-based, often with red pepper flakes. A unique version characteristic of Columbia, SC, USA is pork with sweet, vinegar and mustard-based sauce. In Texas, barbecue usually means beef brisket with a spicier sauce similar to the Kansas City version. In addition to pork shoulder cuts, ribs, and beef brisket you may find whole hog, beef ribs, lamb, goat, and poultry.

A variation of barbecue is 'pulled pork' where pork shoulders cuts are cooked at low temperatures until the meat shreds easily. This cooked pork is marketed as a heat-and-eat product covered in an appropriate regional barbecue sauce. There are beef and poultry versions of this product.

Buffalo or Hot Wings

This terminology has nothing to do with the species, water buffalo or the American bison. It is a finger food that originated in the 1960s. According to legend it can be traced to the Anchor Bar in Buffalo, NY, USA (hence the name, Buffalo) and consists of the meatier joints of the chicken wing. The joint proximal to the body, called the 'drumette', is the meatiest and the preferred raw material. The next joint, called the 'flat' is sometimes used. The wing joints are fried and then tossed with a cayenne pepper sauce that usually contains butter and hint of garlic. The wings may be smoked and precooked prior to frying. Boneless versions of the bone-in wings are growing in popularity. Variations of this flavoring are applied to other cuts of meat and poultry. This product has become closely identified with sporting events in the USA. The National Chicken Council estimates that 1.25 billion wings were consumed on Super Bowl XLVI Sunday alone. Recently, an entire food industry has grown up around the Buffalo flavoring and wings have become one of the more valuable parts of the chicken.

Chili Con Carne

Chili con carne (usually referred to simply as chili) consists of coarse ground (6 mm) or diced meat slow cooked with a sauce containing chili peppers and usually tomatoes. The meat is normally beef although pork, lamb, and goat could also be used. Other flavorings such as cumin, onion, garlic, oregano, and even unsweetened chocolate may be added. Beans (navy, pinto, kidney, or black) are often added. The mixture is often sterile canned. Chili con carne is typical of the Southwestern USA and Mexico.

See also: Canning. Chemical and Physical Characteristics of Meat: Chemical Composition; Color and Pigment. Drying. Processing Equipment: Mixing and Cutting Equipment. Sausages, Types of: Dry and Semidry. Slaughter-Line Operation: Poultry. Spoilage, Factors Affecting: Microbiological; Oxidative and Enzymatic

Further Reading

USDA-FSIS, 2005. Standards and Labeling Policy Book. Washington, DC: US Department of Agriculture, Food Safety and Inspection Service. Available at: www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy_Book_082005.pdf (accessed 16.10.13).

Relevant Website

http://meatsci.osu.edu
The Ohio State University.

Poland

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Glossary

Coarse ground Trimmings with the diameter 5–20 mm.
Curing Preserving, usually restricted to preserving with salt, nitrates and/or nitrites and other condiments, using dry or wet (brine) methods.
Meat specialties A wide variety of products that have in common only the fact they are chopped or comminuted meats and edible offals, seasoned, and usually cooked or baked rather than smoked.
Sausages Made from uncured or cured meats, ground, seasoned, stuffed into casings, smoked or un smoked, heated or dried under controlled time–temperature–humidity conditions.
Smoking Applying smoke on meats for color, flavor, and preservation.
Trimmings Pieces removed or cut in the process of trimming, usually the edible lean portions, use for sausages.

Introduction

The oldest written information about processed meat comes from ancient Greece and the Roman Empire, but at present, Central Europe is probably the number one region in the world with respect to consumption and variety of processed meat products. Varied climatic conditions required many methods of meat preservation (salting, curing, marinating, smoking, cooking, and drying) used in different combination with specific types of meat (pork, beef, game, mutton, horse, and poultry), resulting in many different kinds of final products. Every day in the Czech Republic, Germany, and Poland hundreds of different processed meat products are manufactured and their consumption (40–50%) is the highest of the total meat consumption in the world.

Polish Meat Products

In Poland, most of the processed meat products with a very specific taste and aroma that can be considered ethnic are prepared from pork (sometimes with a small addition of other types of meat, i.e., beef, poultry, game, etc.). The pork is obtained from local pig breeds raised under extensive conditions. The meat of such animals has a rather high content of intramuscular fat (marbling).

Polish processed meat can be divided into four groups: smoked meats, sausages, meat specialties, and canned meat. Smoked meats are produced from whole primary cuts of pork. Typical Polish products of this group include Polish smoked ham and Baleron. Sausages are products made from pork trimmings (sometimes with addition of beef, game, or poultry). Krakowska, Kabanosy, Mysliwska, Lisiecka, and Jałowcowa are specific Polish sausages. Meat specialties are products prepared from meat trimmings and offal, with additional cereals (barley grits and/or buckwheat), blood, and seasonings. Native to Poland in this category is Krupnioki. Intensive development of technology and techniques has resulted in similar methods used for meat canning around the world. One of the best-known Polish products in this group, Polish canned ham sold under Krakus brand name, should be specifically mentioned.

Polish smoked ham is produced out of whole pork hind legs (bone-in or bone-out), dry-cured for 20–30 days or by wet method for 10–14 days, smoked in warm smoke, cooked, and smoked again for specific color and taste development.

Polish baleron is produced from pork neck, dry-cured for 24 h, and then immersed in curing brine for another 8–10 days. It is then allowed to drain for 24 h, is stuffed in natural or collagen casings, smoked in warm smoke, cooked, and cooled. The cross section of the final product shows a very dark meat color with marbling. This produces a very specific flavor and juiciness.

Krakowska sucha/krakowska dry/ is produced from pork trimmings (85%), hard pork fat (usually back fat – 10%), and beef trimmings (5%) with the addition of spices (black pepper, nutmeg, and garlic). The meat is dry-cured for 3–5 days, coarse ground and mixed, stuffed into natural or collagen casings of 60 mm diameter, smoked in warm smoke, cooked, and dried for 10–23 days. The final product with its very characteristic flavor has an exceptionally dark color of coarse ground lean and designed meat with small particles of fat evenly distributed over the surface of the cross section.

Polish kabanos is produced from lean and regular pork trimmings (50/50%) coarse ground (no smaller than 8 mm of diameter) and dry-cured for 1 day. Spices are added (black pepper, nutmeg, and caraway) and the mixture is stuffed into natural (sheep) or collagen casings with a diameter no bigger than 22 mm, smoked in warm smoke, baked, and dried for 3–5 days. The final product has very specific texture and flavor.

Mysliwska (Hunter) sausage is produced from cured (dry curing for 2 days) pork trimmings of different content of fat and connective tissue and degree of grinding (class 1 – lean meat trimmings through a 20 mm plate), meat trimmings (class 2 with fat content of approximately 20%) through a 8 mm plate, meat trimmings (class 3 – with some connective tissue) through a 3 mm plate, and then chopped in a cutter with addition of 5% of ice and spices (black pepper, juniper,

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Figure 1 Jalowcowa.

Figure 2 Kabanosy.

Figure 3 Krakowska sucha.

garlic, and sugar). To trimmings class 1 and 2, a marinating solution is added (water, acetic acid, and rapeseed or sunflower oil in ratio of 1:1:1) and mixed with chopped meat of class 3 and stuffed into natural pork casings of diameter no bigger than 22 mm, smoked in warm smoke, baked, and dried for 5–7 days up to 70% of the final yield. The final product has very specific texture and flavor.

Figure 4 Mysliwska.

Lisiecka sausage is produced from cured (dry curing for 2–4 days) pieces of selected ham muscles (excluding semitendinosus and quadriceps femoris) and 15% of 'regular' pork trimmings (50:50 of lean and fat). Meat trimmings are ground with spices (white pepper and garlic) and mixed with pieces of ham muscles and 5% of iced water, then stuffed into natural casing and formed into rounds with diameter of 35–40 cm. Smoking is done in traditional smokehouse using wood of deciduous or fruit trees for 4–5 h. The final product has very specific texture and flavor.

Jałowcowa (Juniper) sausage is produced from pork trimmings and hard pork fat (usually back fat), sometimes with addition of small amount (5%) of beef trimmings, dry-cured, mixed with spices (pepper and juniper), coarse ground, stuffed into natural or collagen casing, smoked in warm smoke, cooked, smoked again in cold smoke, and dried for 3–5 days. The final product also has a very specific flavor, which results from the spices, the types of meat used, and the technological procedure.

Krupnioki is produced from pork dry-cured head meat trimmings, liver, jowls, cereal (i.e., barley grits and/or buckwheat), blood, and spices. The meat trimmings and jowls must be precooked in water and the cereal grits should be precooked in broth (from previous cooking). Then, all the ingredients are coarse ground and mixed with spices (salt, black pepper, marjoram, allspice, and onion), stuffed into natural or collagen casings, smoked in warm smoke, cooked, and cooled.

Kabanosy, Myśliwska, Jałowcowa, and Lisiecka sausages obtained a European Union Certificate of Traditional Speciality Guaranteed Products (Figures 1–4).

The production of these sausages in Poland amounts to approximately 100 000 tons annually. Approximately 40% of these products are being sold to other countries, mainly the UK and US.

None of these products are similar to Polish sausages produced in the US. The closest is Lisiecka but produced using ham muscles.

See also: Ethnic Meat Products: France; Germany; Mediterranean

Further Reading

Dowell, P., Bailey, A., 1992. Wielka księga ingrediencji. Wiedza i Życie. Warszawa, Polska: A Dorling Kindersley Book Original title: The book of ingredients.
Panasik, M., Wojciechowska, M., Zatorski, S., 2003. Kuchnia Polska. Chapter: Mięso. Warszawa, Polska: Świat Książki.
Pisula, A., Dowierciał, R., 1991. Sami Robimy Wędliny. Warszawa, Polska: Pań; stwowe Wydawnictwo Ekonomiczne.
Pisula, A., Pospiech, E., 2011. Mięso – podstawy nauki i technologii. Warsaw: Wydawnictwo SGGW.
Savic Z., Savic, I., 2002. Chapter 1: Prehistory and history of sausage making. Sausage Casings. Vienna, Austria: VICTUS Lebensmittelindustriebedarf Vertriebsgesellschaft m.b.H.
Swatland, H.J., 2010. Meat products and consumption culture in the West. Meat Science 86, 80–85.
Vandendriessche, F., 2008. Meat products in the past, today and in the future. Meat Science 78, 104–113.

Relevant Website

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Meat Science.

EXSANGUINATION

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Glossary

Bleeding/exsanguinations A procedure where the blood vessels in the neck or chest are severed.
Cardiac arrest Stopping heart beat.
Halal slaughter A slaughter procedure that adheres to Islamic teachings where the animal is dispatched by severing the blood vessels of the neck following a prayer.
Kosher The meat produced following Shechita.
Shechita The Jewish method of slaughter carried out by a trained Jewish slaughterman, shochet.
Slaughter The process of exsanguination that ensures the animal cannot recover sensibility.
Sticking Cutting the blood vessels for bleeding.
Stunning The procedure where an animal is rendered unconscious before exsanguination.

Introduction

Exsanguination is the process of severing major blood vessels in order to let sufficient blood out to kill an animal and/or complete the slaughter process. Blood is also let out of the carcass to maintain meat quality and food safety. In cases where head-only preslaughter stunning is used to render an animal reversibly unconscious or religious slaughter without stunning are employed, exsanguination is aimed at killing animals. In this situation, the cause of death would be the loss of blood, insufficient blood supply to the brain resulting in cerebral ischemia and loss of brain function, cardiac shock, and cessation of heart beat (cardiac arrest). However, if a nonreversible stunning method, such as cardiac arrest by an electric current (head and chest application) is used, this could be regarded as a killing method because most of animals would not regain consciousness even if exsanguination was not performed. Nevertheless, cause of death in these animals will often be exsanguination not by the stunning method itself, because blood loss from sticking has a more rapid effect on the brain. Effects of slaughter methods on exsanguination have been reviewed extensively (Figure 1).

Figure 1 Shechita slaughter knife.

Exsanguination Techniques

During slaughter, severance of tissues and blood vessels is carried out using knives of different types with varying thicknesses and lengths. Although ordinary slaughter knives can be used for Halal slaughter (Muslim method), length can vary from 10 to 30 cm, even longer for Shechita (Jewish method) which is applied without stunning (more than 35 cm for cattle). Shapes can also be straight and curved. Jewish slaughtermen use specialized knives called chalaf. Much attention is paid to maintaining these knives whose sharp edges must not have any indentations. Cutting action can be a transverse cut, or partly stabbing and retrograde type.

Two exsanguination methods are commonly used in slaughterhouses. The first method is commonly known as neck cutting and it involves severing two carotid arteries and two jugular veins in the upper neck, often behind the mandibles (jaw bones), and is used to slaughter sheep, rabbits, and poultry. The second method is known as chest sticking and it involves severing brachiocephalic trunk by inserting a knife through the thoracic inlet anterior to the sternum and is used to slaughter cattle and pigs. Chest sticking, also known as thoracic sticking, is carried out on the unconscious animals presented for exsanguinations in a recumbent position on a cradle or conveyor or hoisted on to a overhead rail. As mentioned previously, chest sticking involves severance of the brachiocephalic trunk, a single large vessel emerging from the aorta and leading to common carotid arteries to supply the head with oxygenated blood to the brain and has been shown to be very effective in that brain function is lost rapidly

in comparison with neck cutting due to profuse bleeding. However, during slaughter without stunning, chest sticking is not applied as it is not considered to be practical and is probably against religious rules and tradition. Time to loss of blood and consciousness following slaughter without stunning can vary and depend on species, techniques, number of vessels cut, and physical restrictions on the rate of bleeding.

Pain During Exsanguination

The issue of whether the neck cut is painful has received much controversy and discussion.

There are two different views about the pain during the cut. Those who favor slaughter without stunning on religious grounds think the cut is quick and painless and others argue that varying degrees of severe pain is inevitable. It is claimed that Shechita works as a stunning method and death occurs immediately due to rapid loss of blood. It has been reported that there is no visible reaction from the body and legs of restrained cattle to the neck cut. It is also claimed that neck cutting without stunning with a very sharp knife maintains animal welfare and creates a situation where no pain is felt by the animal due to rapid physiological changes. Similar claims are also made for halal slaughter.

In contrast, other scientists claim that there will be substantial pain involved due to extensive tissue damage. The pain that might be perceived by the animal during the cut and afterwards depends on a number of factors. Under the most successful slaughter conditions, if the incision is performed by a highly skilled slaughterman using a sharp knife, the level of pain inflicted will be the lowest though not totally eliminated. Deviation from this scenario will probably worsen the severity of pain in an exponential manner.

After recent methodological developments related to quantitative analysis of the electroencephalogram (EEG), the experience of pain can now be assessed more precisely. This methodology has been applied during neck cutting in calves and provided evidence for the first time that the act of slaughter is associated with noxious stimulation that would be expected to be perceived as painful.

Exsanguination, Blood Pressure, Cerebral Perfusion, and Loss of Consciousness

Total blood volume is equivalent to 8% of body weight and 18% of cardiac output perfuse the brain. Following effective stunning and slaughter by neck cutting, 40–60% of total blood volume is lost until carcass dressing. Exsanguination results in a dramatic drop in blood pressure leading to a state of shock, and it is also claimed that loss of consciousness occurs rapidly following slaughter without stunning.

In cattle, the time to reach critical levels of blood loss following exsanguination by neck cutting is highly variable and this period can be affected by a number of factors. In addition to ineffective severance of blood vessels, anatomical differences and occlusions of the cut arteries in cattle can lead to recovery episodes in blood pressure. In sheep, however, the rate of loss is much quicker after neck cutting.

The brain of ruminants is perfused with blood from 'rete mirabile,' a vascular network connected to the carotid and vertebral arteries. In cattle, there are extra anastomosis and blood supply to rete mirabile and brain sometimes even after exsanguination, whereas in sheep and goats this is not the case.

Although perfusion is possible, some people claim that the cerebral blood flow after a neck cut would not be sufficient to supply the brain. However, it has also been found that when carotid arteries are cut, the cut end of the arteries sometimes develop aneurysms leading to significant occlusion that impede bleeding efficiency and, as a consequence, prolong the time to onset of isoelectric electrocorticogram (ECoG) in calves. In the same study, it was also found that when carotid artery occlusion occurred, vertebral artery blood flow was maintained, which is the main reason for the delayed onset of unconsciousness in calves slaughtered without stunning.

Sharpness of the knife and performing a complete uninterrupted cut could influence the occurrence of vasoconstriction, clotting, and ballooning also known as carotid occlusion, or aneurysms.

Following slaughter without stunning, it is imperative that consciousness will be lost; however, the time to onset of unconsciousness depends on a number of factors such as the method of restraint, quality of the cut as well as species differences. Time to loss of brain function in cattle has been studied by various researchers who examined electrical activity of the brain using EEG or ECoG, evoked responses as well as animal reactions and reflexes, and found variations. Review of results showed using time to loss of spontaneous brain activity as indicated by an isoelectric or flat EEG/ECoG varied from 10 s to more than a minute; loss of evoked responses from 10 s to 2 min. In regard to sheep, however, similar studies obtained much shorter durations between 2 s and 43 s.

The implications of the above findings are that, following neck cutting, delays in time to loss of consciousness would be serious welfare problems. Certainly any impediment and slowing of blood flow after a cut could raise animal welfare concerns due to delayed loss of consciousness and other poor welfare states originating from the inhalation of blood into trachea.

Blood Loss and Retention

It is of importance to remove as much blood from the carcass as possible during slaughter with or without stunning. It is often claimed that stunning would adversely affect the rate of bleed out and total loss. However, some researchers examined exsanguination and compared stunning and slaughter versus slaughter with no stunning in sheep and cattle. They found no differences in both bleed-out rates and total blood loss at the end of bleed-out time.

Earlier reported studies measured blood hemoglobin content in different muscles as an indicator of bleed out quality. It was found that hemoglobin did not differ in muscles of sheep and calves that were subjected to captive bolt stunning or Shechita. It is claimed that Shechita bleeding and blood loss could still be better after Shechita because of the very sharp knife used and efficacy of cut. Based on existing studies and

available results, it is reasonable to suggest that regardless of whether preslaughter stunning is used or not, blood loss is unlikely to be different.

Carcass and Meat Quality

Stunning and slaughter can have certain effects on carcass and meat quality. These are carcass defects such as hemorrhages, bruising, and broken bones. Blood splash (petechial hemorrhages) in the muscles of sheep can occur due to inappropriate (rough) preslaughter handling, electrical stunning using high voltages, and sometimes due to possible nutritional or unknown factors. These defects and resultant downgrading can occur during slaughter with and without stunning. Prompt and efficient neck cutting following head-only electrical stunning of sheep and lamb minimizes or eliminates any detrimental effect of rising blood pressure on carcass and meat quality, including hemorrhaging or blood splash. Neck cutting in sheep and lambs while the heart is still pumping should result in 75–85% of total possible blood being lost in the first 60 s. As a consequence of prompt exsanguinations following head-only stunning, the carcass would have lost half the circulating blood volume that could be drained from the carcass prior to the onset of clonic convulsive phase. Therefore, blood pressure, under normal circumstances, should not be responsible for hemorrhages.

See also: Human Nutrition: Vegetarianism. Preslaughter Handling: Welfare of Animals. Religious Slaughter. Slaughter-Line Operation: Sheep and Goats. Species of Meat Animals: Cattle; Finfish; Game and Exotic Animals; Pigs; Poultry; Sheep and Goats; Shellfish. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning; Slaughter: Immobilization

Further Reading

Anil, M.H., McKinstry, J.L., Gregory, N.G., Wotton, S.B., Symonds, H., 1995b. Welfare of calves. 2. Increase in vertebral artery blood flow following exsanguination by neck sticking and evaluation of chest sticking as an alternative slaughter method. Meat Science 41, 113–123.

Anil, M.H., McKinstry, J.L., Wotton, S.B., Gregory, N.G., 1995a. Welfare of calves – 1. Investigation into some aspects of calf slaughter. Meat Science 41 (2), 101.

Anil, M.H., Yesildere, T., Aksu, H., *et al*., 2004. Comparison of religious slaughter of sheep with methods that include preslaughter stunning and the lack of differences in exsanguination, packed cell volume and quality parameters. Animal Welfare 13 (4), 387–392.

Anil, M.H., Yesildere, T., Aksu, H., *et al*., 2006. Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: Exsanguination and quality parameters. Animal Welfare 15, 325–330.

Baldwin, B.A., Bell, F.R., 1963a. Blood flow in the carotid and vertebral arteries of the sheep and calf. Journal of Physiology 167, 448–462.

Baldwin, B.A., Bell, F.R., 1963b. The anatomy of the cerebral circulation of the sheep and ox. The dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. Journal of Anatomy 97, 203–215.

Brooks, J., Tracey, I., 2005. From nociception to pain perception: Imaging the spinal and supraspinal pathways. Journal of Anatomy 207, 19–33.

EFSA, 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a Request from the Commission Related to Welfare Aspects of the Main Systems of Stunning and Killing the Main Commercial Species of Animals. Available at: http://www.efsa.europa.eu/de/scdocs/doc/45.pdf (accessed 11.01.13).

Gibson, T.J., Johnson, C.B., Murrell, J.C., *et al*., 2009. Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels. New Zealand Veterinary Journal 57 (2), 84–89.

Grandin, T., Regenstein, J.M., 1994. Religious slaughter and animal welfare: A discussion for meat scientists. Meat Focus International 1994, 115–123. Available at: http://www.grandin.com/ritual/kosher.slaugh.html (accessed 06.01.08).

Gregory, N.G., 2004. Physiology and Behaviour of Animal Suffering Oxford: Blackwell Science. ISBN 0–632-06468-4.

Gregory, N.G., Fielding, H.R., Wenzlawowicz, M.V., Holleben, K.V., 2010. Time to collapse following slaughter without stunning in cattle. Meat Science 85, 66–69.

Gregory, N.G., Shaw, F.D., Whitford, J.C., Patterson-Kane, J.C., 2006. Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. Meat Science 74, 655–657.

Gregory, N.G., Wenzlawowicz, M.V., Alam, R.M., *et al*., 2008. False aneurysms in carotid arteries of cattle and water buffalo during shechita and halal slaughter. Meat Science 79, 285–288.

Gregory, N.G., Wenzlawowicz, M.V., Holleben, K.V., 2009. Blood in the respiratory tract during slaughter with and without stunning in cattle. Meat Science 82, 13–16.

Gregory, N.G., Wotton, S.B., 1984. Time to loss of brain responsiveness following exsanguination in calves. Research in Veterinary Science 37, 141–143.

Haluk, A.M., 2012. Religious slaughter: A current controversial animal welfare issue. Animal Frontiers 2, 64–67. doi:10.2527/af.2012-0051.

Holleben, K.V., Wenzlawowicz, M.V., Gregory, N., *et al*., 2010. Animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary sciences. Dialrel-Deliverable 1, 3. Available at: http://www.dialrel.eu/images/veterinary-concerns.pdf (accessed 06.01.10).

Kallweit, E., Ellendorf, F., Daly, C., Smidt, D., 1989. Physiological reactions during slaughter of cattle and sheep with and without stunning. Deutsche Tierarztliche Wochenschrift 96, 89–92.

Kavaliers, M., 1989. Evolutionary aspects of the neuro-modulation of nociceptive behaviours. American Zoologist 29, 1345–1353.

Kirton, A.H., Bishop, W.H., Mullord, M.M., 1978. Relationship between time of stunning and time of throat cutting and their effect on blood pressure and blood splash in lambs. Meat Science 2, 199–206.

Kirton, A.H., Frazerhurst, L.F., 1983. Effects of normal, light/normal or double stunning on the incidence and severity of blood splash in lambs. Meat Science 8, 1–6.

Kirton, A.H., Frazerhurst, L.F., Woods, E.G., Chrystall, B.B., 1981. Effect of electrical stunning method and cardiac arrest on bleeding efficiency, residual blood and blood splash in lambs. Meat Science 5, 347–353.

Levinger, I.M., 1976. Physiological and general medical aspects of Shechita. In: Munk, M.L., Munk, E. (Eds.), Shechita. Jerusalem: Gur Aryeh Publications, pp. 147–149.

Levinger, I.M., 1995. Shechita in the Light of the Year 2000. Basel, Switzerland: Maskil, L. David.

Newhook, C.J., Blackmore, D.K., 1982a. Electroencephalographic studies of stunning and slaughter of sheep and calves – Part 2: The onset of permanent insensibility in calves during slaughter. Meat Science 6, 295–300.

Newhook, J.C., Blackmore, D.K., 1982b. Electroencephalographic studies of stunning and slaughter of sheep. Meat Science 6, 221–233.

Rosen, S.D., 2004. Physiological insights into Shechita. Veterinary Record 154, 759–765.

Velarde, A., Gispert, M., Diestre, A., Manteca, X., 2003. Effect of electrical stunning on meat and carcass quality in lambs. Meat Science 63, 35–38.

Warriss, P.D., Wilkins, L.J., 1987. Exsanguination of meat animals. Seminar on pre-slaughter stunning of food animals, European Conference Group on the Protection of Farm Animals, 02 June 1987. Brussels: European Commission Report.

Woolf, C.J., 2004. Pain: Moving from symptom control toward mechanism-specific pharmacologic management. Annals of Internal Medicine 140, 441–451.

Relevant Website

http://www.dialrel.eu
DIALREL.

EXTRUSION TECHNOLOGY

PP Lewicki[†]

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Glossary

Coextrusion The extrusion of multiple layers of material simultaneously. It utilizes two or more extruders to melt and deliver a steady volumetric throughput of different viscous plastics to a single extrusion head (die) which will extrude the materials in the desired form.

Cold extrusion The extrusion used mainly to produce food without distortion of its constituents and also to mix, knead, and mold a product in one process.

Die A material-shaping device.

Extruder screw Ingredients that are uniformly pretreated are fed to the extruder screw. Single screw can have different designs; the most popular are as follows: (1) The diameter of the root of the screw increases and the screw pitch decreases along the barrel; the barrel diameter is constant; (2) The diameter of the root of the screw is constant and the screw pitch is either constant or decreases along the barrel; the barrel is tapered. In the twin-screw extruder, the bore of the barrels is shaped as two parallel overlapping cylinders. Two screws rotate in these cylinders and their arrangement can vary depending on the intended product quality. Possible arrangements include counter-rotation and corotation and the engagement of the screws can be full, partial, or none.

Extrusion cooking A method used to texturize starch-based and protein-based materials. Texturization is achieved by damaging the tertiary and quaternary structure of biopolymers, by rearrangement of polymer chains, and by formation of spatial structures that impart particular texture to the final product.

Pressure flow A backward flow, which causes distortion of the helical movement of the mass, which results in an intensive kneading of the dough.

Introduction

The process of extrusion combines several unit operations. Regardless of the purpose of extrusion, mixing, kneading, shaping, and forming are always taking place in the process. Such extrusion is termed cold extrusion. When the process is done at high temperature and high shearing forces, it is called hot extrusion or extrusion cooking.

The design of extruders depends on the mode of operation (cold extruders or extruder-cookers). In general, the extruder consists of a barrel(s) with a screw(s) that conveys the material toward the interchangeable die. Single-screw and twin-screw extruders are manufactured. The barrel(s) is jacketed and, in some designs, the screw can be heated or cooled.

Cold extrusion is used mainly to produce food without distortion of its constituents. It is used to mix, knead, and mold a product in one process. Extrusion cooking is used to create new food properties, especially a specific texture due to porosity, fibrosity, rearrangement of molecular conformation, and structural organization of biopolymers. The creation of new texture by extrusion cooking is the main objective of the process.

[†]Deceased.

Cold Extrusion

Cold extrusion is used to produce pasta, hot dogs, pastry dough, and some confectionery products such as chewing gum and liquorice. The extruder has a deep-flighted screw with a constant pitch and depth that operates at low speed in a smooth barrel. In processing pasta, the screw is divided into two sections or two screws are used one after the other (Figure 1). The feed section has a larger pitch than that in the kneading section. In the feed section, food components are mixed into a homogenous mass and its water content is adjusted to the desired value; in pasta processing, the feed moisture content is 22%. The homogenous mass is then conveyed to the kneading section. The mass follows a helical path down the screw channel. Its velocity varies from lowest at the root of the screw to the highest at the top of the channel. A die restricts mass flow and pressure builds up in the barrel. The pressure difference causes pressure flow in the space between the flights of the screw and the barrel wall. The pressure flow is a backward flow, which causes distortion of the helical movement of the mass (Figure 2), which results in an intensive kneading of the dough. Stretching, mixing, and pressing of the dough generates heat and the temperature of the mass can increase by 15–20 °C. Because each product should be extruded at its optimal temperature, the barrel is cooled with

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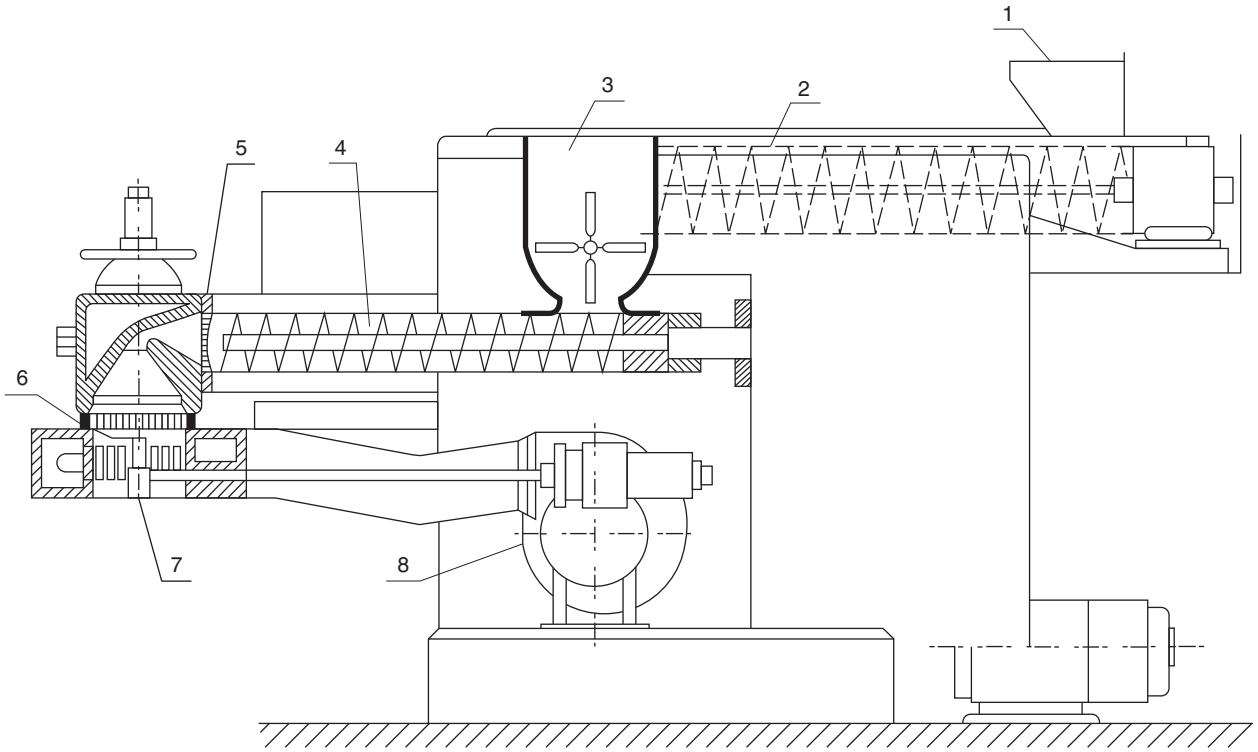

Figure 1 Cold extruder: (1) feed hopper; (2), feed mixer; (3), mixer; (4), kneading section; (5), sieve equilibrating mass flow; (6), die; (7), cutting device; (8), fan. Reproduced with permission from Lewicki P.P. (ed.), Inzynieria Procesowa i Aparatura Przemyslu Spozywczego (Food Processing Engineering and Food Processing Equipment). Warsaw, Poland: Wydawnictwa Naukowo-Techniczne. © 2004 Wydawnictwa Naukowo-Techniczne, Warsaw, Poland.

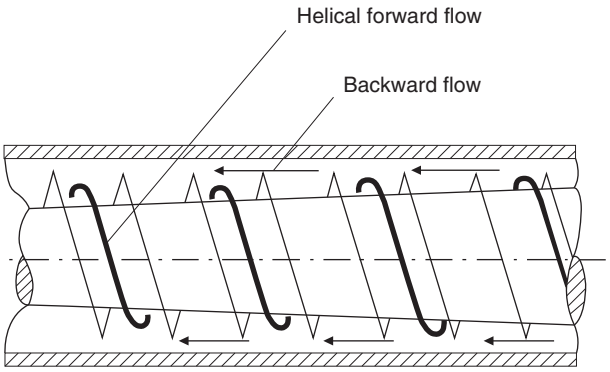

Figure 2 Flow of mass in the extruder barrel.

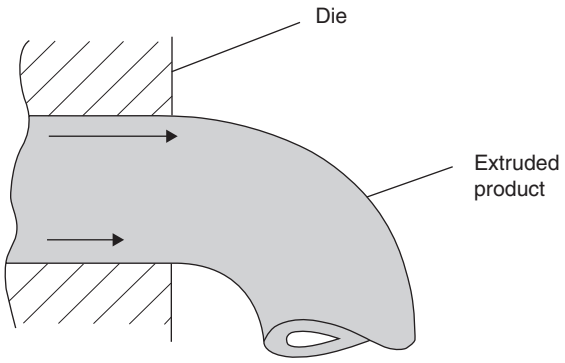

Figure 3 Effect of flow in the die on the shape of the extruded product.

water. The extrusion temperature for pasta should be below 55 °C. Temperatures exceeding the optimal value cause an increase of the pressure in the barrel and results in undesirable changes in food constituents. High pressure at the die increases energy consumption and affects the shape and surface quality of the extruded product.

The mixed and kneaded mass is pressed through the die. The flow through the die must be a plug flow, and shearing forces should be as small as possible. The extruded material should not expand and should keep the shape acquired in the die. Teflon coating of the die inserts gives low friction, so that the extrusion is done with low energy consumption and the surface of the extruded mass is smooth. The shape of the extruded material is determined by die inserts that produce velocity differences in the flowing mass. For example, if one part of the mass flows faster in the die than on other part, the extrudate bends and forms elbow (Figure 3).

Pasta extruders work at pressures of 6–8 MPa with screw speed of 30–40 rev min^{-1} and velocities in the die with Teflon inserts of 65–80 mm s^{-1}. Product moisture content is equal to that of the feed mixture. For efficient operation, the screw must be full of material, so the feed rate is an important variable in cold extrusion.

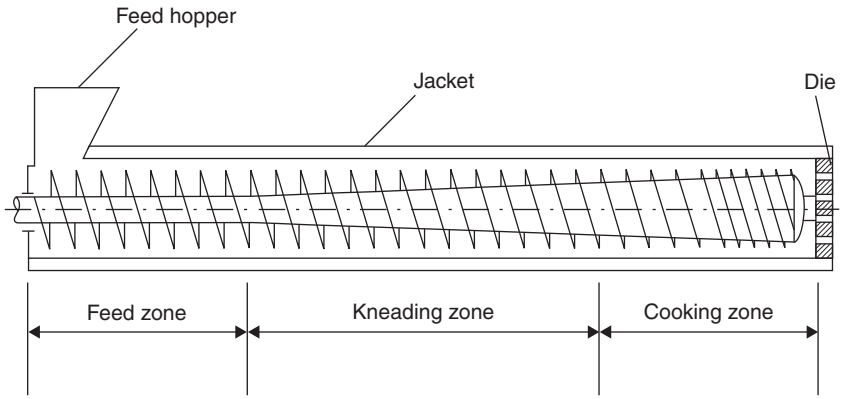

Figure 4 Extruder-cooker.

Extrusion Cooking

Extrusion cooking is widely used to texturize starch-based and protein-based materials. Texturization is achieved by damaging the tertiary and quaternary structure of biopolymers, by rearrangement of polymer chains, and by formation of spatial structures that impart particular texture to the final product.

Single-Screw Extruders

A cooking extruder can be divided into three parts: feeding, kneading, and cooking zones (Figure 4). The feeding zone is a chamber in which granular materials are moistened and heated. It has one or two mixing/conveying screws with large pitch. In the feeding section, materials are mixed, moistened, and heated in order to obtain a somewhat compressed, uniform mass. Shearing is low and the structure of biopolymers is not injured in this section of the extruder.

The uniformly pretreated ingredients are fed to the extrusion screw. This screw can have different designs; the most popular are as follows:

- The diameter of the root of the screw increases and the screw pitch decreases along the barrel; the barrel diameter is constant.
- The diameter of the root of the screw is constant and the screw pitch is either constant or decreases along the barrel; the barrel is tapered.

Both designs make it possible to compress the mass while it is conveyed from the feed zone to the die. To increase friction, the internal wall of the barrel is ribbed or corrugated.

In the kneading zone (the initial part of the extruder screw), the material is compressed, with some shearing, and heated. The heat is generated as a result of viscous dissipation, and further heat can be added through the barrel jackets. The temperature of the kneaded mass at the end of this zone is close to or exceeds 100 °C. The hot, melted, and worked-out mass forms a viscous, plasticized material.

The kneaded mass is further compressed, sheared, and heated in the cooking zone. In this zone, the pressure flow (due to pressure differences in the barrel) is significant and the mass is highly sheared. Heat is generated by dissipation of mechanical energy and the temperature of the mass increases rapidly. Very high shearing forces cause mechanical damage to polymer molecules. The temperature of the mass in the cooking zone, depending on the product, reaches 150–200 °C. The residence time in this zone is less than 5 s. Hot, melted material is discharged through the die. Discharge pressure depends on the product and can be as high as 70 MPa.

Water in the mass has the same temperature as the material, and at the moment of discharge it is superheated with respect to the ambient conditions. To restore equilibrium, water evaporates instantly and provides the most of the force that causes expansion of the product. A porous structure with low density is formed. Most of the water evaporates to the surrounding atmosphere and the water content of the product is much lower than that of the feed. Typical feed moisture is between 11% and 28%, whereas the product moisture content is below 5%.

The design of the die strongly influences the quality of the product. The shape of the die and the extent of expansion determine the shape of the product. The shape of the die also influences the shear rate and strain and, consequently, influences the texture of the finished product. A tapered die opening reduces shearing of the flowing mass and causes damage to macromolecules. It requires a relatively low backpressure. The product is less expanded and has a smooth surface that is more resistant to mechanical stress than the interior. An abrupt change in cross-section of the die opening causes extensive damage to food components and generates a large expansion. A fine porosity and a soft, pithy texture are created. Forcing the mass through the orifice causes storage of viscoelastic energy in the material, which attains rounded shape on relaxation. The surface of the product is irregular and corrugated. Cooling of the die can reduce expansion of the product on discharge.

In general, the conditions of flow of the mass in the die significantly influence the texture of the finished product. The more the mass is sheared in the die, the finer texture is formed, and the product is softer, more porous, and crumbly.

Temperature, shear rate, and strain are the processing parameters that affect the quality of the product in the single-screw extruder. The main variable that affects processing parameters is the moisture content of the feed materials. The moisture content is responsible for the flowability of the mass

and the energy requirement for extrusion. The lower the water content, the greater the energy required to cause the flow. As a result, the mass is strongly sheared, and the dissipation of viscous energy as heat increases the temperature of the dough. More damage is done to macromolecules than at higher moisture contents. Barrels with sectioned jackets make it possible to vary the temperature and control the slip at the barrel wall. At low wall temperatures, the slip is small and the shearing rate increases. The design features also influence the shear rate in the extruder; the design and speed of the screw, and the internal wall pattern of the barrel are the most important considerations.

The main drawback of single-screw extruders is their sensitivity to feed rate, and a complete filling of the screw must be ensured. With partial filling, control of extrusion parameters is not good, and the quality of the product becomes variable.

Twin-Screw Extruders

Twin-screw extruders are widely used in food processing. The bore of the barrels is shaped as two parallel overlapping cylinders. Two screws rotate in these cylinders and their arrangement can vary depending on the intended product quality. Possible arrangements include counter-rotation and corotation and the engagement of the screws can be full, partial, or none. The twin-screw extruder with corotating fully intermeshing screws (Figure 5) is one example of extruder classifications.

An extruder with two nonintermeshing screws works as a single-screw extruder but has a higher output. Mixing, kneading, cooking, and conveying in the intermeshing screws are accompanied by self-cleaning of the screws' surfaces. Corotating screws ensure good mixing and shearing in the whole mass of the product. The mass is not compressed between the screws and the extruder can work at high speeds. Counter-rotating screws are used with materials of low viscosity or materials that slip in single-screw extruders. Hence, oily, sticky, or very wet materials are handled in twin-screw machines. The conveyed material is compressed between the screws and the screws thus work at low speeds.

Corotating, fully intermeshing twin-screw extruders are the most common type in the food industry. The feeding zone is only partly filled with raw material, because twin-screw extruders have a greater ability to convey material down the screw's length. This increased conveyance builds up the pressure at the die and back-flow of the product ensures good filling of the screws. Strong shearing and mixing within the volume of a twin-screw extruder leads to homogeneity of the structure and texture of the product. Kneaded and cooked product leaves the screws and forms a single stream before entering the holes of the die for extrusion.

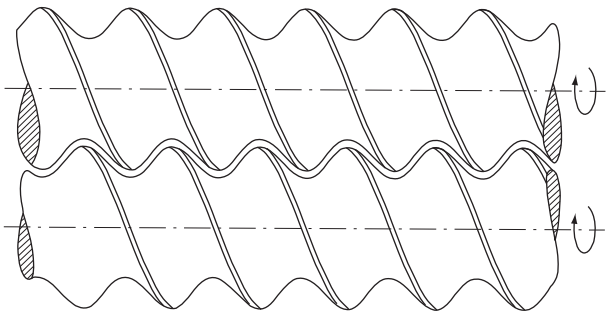

Figure 5 Intermeshing corotating screws.

Coextrusion

Products of composite texture or with filling can be produced by coextrusion. For composite products, two extruders are used or an extruder and a pump may be used. A special die makes it possible to combine two components or to add filling to an outer shell (Figure 6). A pump or extruder continuously feeds material into the extruded outer shell product; the combination is achieved in the die. The water activity should be similar in the extruded outer mass and the filling; otherwise water transfer can occur, and the quality of the product will change during storage.

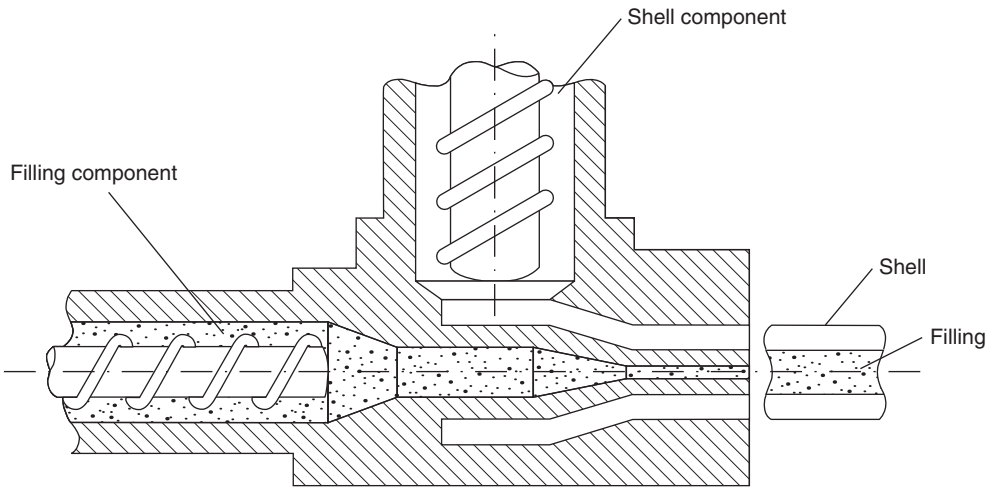

Figure 6 Principle of coextrusion.

Coextrusion is widely used in food production. It is used to produce filled confectionery, filled breakfast cereals, filled snack foods, etc. In meat processing, the process is used in two ways, either to produce filled products or to form an outer skin of collagen dough on sausage products. In some designs, a filled product can be covered with an outer skin in two steps.

Meat products produced by coextrusion include sausages with a central filling (e.g., sausage filled with cheese or apple sauce), bread rolls filled with sausage, filled meat croquettes, filled meat balls, etc. Coextrusion is used to coat a base material with an outer skin. Meat batter is coated with collagen casing, or other hydrocolloids can be used that form a gelled skin. The skin can contain spices or flavors, so coextrusion can become a technique for producing specialty multicomponent foods.

Meat Analogs

High temperatures, large shearing forces and laminar flow in the extruder cause changes in the tertiary and quaternary structure of proteins, align the chains along the streamlines of flow, induce cross-linking and form a layered meat-like structure. Defatted soy flour, concentrate, or isolate is used as raw material. Soy protein, mostly in the native state and with a nitrogen solubility index of 50–70, is the preferred ingredient.

The soy protein is fed into the preconditioner, moistened to 33–45% water on a dry weight basis; the pH is adjusted to the required value and preheated to 80–90 °C. At lower pH values (5.5), the product is chewy, whereas at a higher pH value (8.5), textured protein is tender and rapidly absorbs water. Preconditioned material is fed into the extruder and reaches a temperature of 140–160 °C. Chemical cross-linking occurs, and a layered structure is formed. The more the material is compressed in the extruder and the higher the screw speed, the greater is the degree of cross-linking that occurs, and the more meat-like is the structure. To produce a highly layered structure, side-discharge dies are used for textured vegetable protein. Cooling the mass at the die yields denser, meat-like structures that need longer hydration times. Textured soy protein produced by extrusion cooking forms layered products that absorb approximately three times their weight of water and simulate meat structure.

Textured proteins can be produced at capacities reaching 3 t h^{-1}.

Thermoplastic Starch

Starch mixed with water and some other plasticizers such as glycerin, glucose, or sorbitol and extruded at high temperature forms foil, which application is foreseen as biodegraded packaging material. To increase its mechanical strength, some cellulose fibers, from flax or hemp are added. Addition of some fats increases elasticity of the foil.

The extruded foil is amorphous but during storage acquires some crystallinity. The amount of crystals and the type of crystal structure depends on the processing parameters. The crystallites are formed by amylose helices and their amount increases with increasing humidity of the surrounding air.

The main problem that has been investigated recently, is the instability of properties of the foil under the environmental conditions.

Quality of Extruded Food

Extrusion causes physical and chemical changes in the material undergoing processing. The most pronounced physical change is development of porosity, which at low water contents creates crispness, crunchiness, and a texture typical of extruded products. The cell structure in a porous material is also responsible for its mechanical strength and solubility and ease of biting or breaking. Sounds generated during biting or breaking are depending on the cell structure, the water content, and the ability of the extruded product to absorb water during eating. All the variables responsible for the texture of an extruded product can be manipulated during processing, which means that the quality of the product can be adjusted to the needs and expectations of consumers.

Extrusion can be treated as a high-temperature, short-time process. The chemical changes, therefore, are typical of those resulting from that process. High temperature and increased pressure cause changes in the tertiary and quaternary structure of proteins. Laminar flow and large shearing forces align polymer molecules along the streamlines and form layered structures. Cross-linking by sulfur bridges occurs and the new structure is stabilized by newly formed hydrogen bonds. In general, extrusion does not alter protein digestibility, but at very high temperatures oxidation and destruction of sulfur-containing amino acids can take place. In the presence of sugars, the availability of lysine is decreased in extruded products.

Starch undergoes gelation during extrusion. At high temperatures and in the wet material, some hydrolysis can take place. Moreover, large shearing forces can cause fragmentation of polymer chains and, in consequence, maltose and oligosaccharides are present in extruded products. Gelled starch and the presence of low-molecular-weight carbohydrates make the extruded products easily digested.

Extrusion of materials containing fats and oils forms complexes of those constituents with starch and protein. These complexes are more resistant to oxidation than free fatty acids, and the extruded products are more stable during storage. However, complexes of fats with starch are more resistant to amylolytic enzymes and the content of dietary fiber in extruded product increases.

Vitamins are generally stable under extrusion conditions. Vitamins of the B group decrease by 25%, but retention of thiamin, riboflavin, pyridoxine, niacin, and folic acid in cereals can be as high as 95%. Losses of ascorbic acid and vitamin A can be up to 50%. Vitamin E and β-carotene contents decrease little during extrusion.

Some antinutritional agents occurring in certain raw materials, for example, trypsin inhibitor can be destroyed during extrusion. Consequently, the nutritional value of the product can be improved by extrusion.

During extrusion cooking, a toxic compound – acrylamide is formed. The reagents are asparagine coming from proteins and reducing sugars. The optimum temperature for acrylamide formation is 140–180 °C. The toxicity and cancerogenity of acrylamide was proved in experiments on rats, but doses were very

high in comparison with contents of that compound in foods. In French fries, content of acrylamide is approximately 700 μg kg^{-1}; in potato chips, 550 μg kg^{-1}; in corn flakes, 330 μg kg^{-1}; and in breakfast cereals, 130 μg kg^{-1}. There is no limit set for the content of acrylamide allowable in foods. World Health Organization states that there is no reliably identified threshold for the risk of acrylamide concentration. However, it is recommended to change processing parameters and lower acrylamide contents as much as possible. In 2010, The European Chemical Agency added acrylamide to the list of substances of very high concern.

Economics of Extrusion

Extrusion is a versatile process in which, by changing minor ingredients and the operating conditions, new products with special properties can be produced. Other methods or technologies cannot create the same properties as those of extruded foods. Comparison of traditional methods of breakfast cereal production with production by extrusion shows substantial savings in raw materials, energy, labor, and capital investment. Production of flat bread by extrusion requires an energy input around 0.1 kWh kg^{-1}, whereas traditional technology consumes 10 times more energy. When the extruder is used as a forming machine (in pasta or sausage production), the energy consumption is approximately one-fifth of that in extrusion cooking and is in the range 0.02–0.03 kWh kg^{-1}.

Extrusion is a high-production process that is quite amenable to automation. Low-density cereals can be produced at rates higher than 1 t h^{-1} and expanded pet foods are produced at rates as high as 25 t h^{-1}.

A very important feature of the extrusion technology is the negligible production of waste and effluent. Moreover, waste products of meat processing combined with starch or cellulose by-products can be extruded and produce valuable components of feeding stuffs.

See also: Manure/Waste Management: Waste Management in Europe. Packaging: Technology and Films. Sausage Casings

Further Reading

Fellows, P.J., 2000. Food Processing Technology: Principles and Practice. Cambridge: UK: Woodhead Publishing.
Frame, N.D. (Ed.), 1994. The Technology of Extrusion Cooking. Glasgow: Blackie Academic and Professional.
Gaonkar, A.G. (Ed.), 1995. Food Processing: Recent Developments. New York: Elsevier Science.
Guy, R. (Ed.), 2001. Extrusion Cooking: Technologies and Applications. Cambridge, UK: Woodhead Publishing.
Hayakawa, I. (Ed.), 1992. Food Processing by Ultra High Pressure Twin-screw Extrusion. Basel: Technomic Publishing.
Heldman, D.R., Hartel, R.W., 1997. Principles of Food Processing. London: Chapman and Hall.
Kill, R.C., Turnbull, K. (Eds.), 2001. Pasta and Semolina Technology. Oxford: Blackwell Science.
Kokini, J.L., Ho, C.T., Karwe, M.V. (Eds.), 1992. Food Extrusion Science and Technology. New York: Marcel Dekker.
Lawrie, R. (Ed.), 1991. Developments in Meat Science 5. Barking: Elsevier Science.
Lin, S., Huff, H.E., Hsieh, F., 2000. Texture and chemical characteristics of soy protein meat analog extruded at high moisture. Journal of Food Science 65, 264–269.
Macrae, R., Robinson, R.K., Sadler, M.J. (Eds.), 1993. Encyclopedia of Food Science, Food Technology and Nutrition, vol. 3. London: Academic Press.
Moscicki, L. (Ed.), 2011. Extrusion-Cooking Techniques. Application, Theory and Sustainability. Weinheim: Wiley-VCH.
Riaz, M.N. (Ed.), 2000. Extruders in Food Applications. Lancaster, PA: Technomic Publishing.
Singh, R.K. (Ed.), 1995. Food Process Design and Evaluation. Basel: Technomic Publishing.
Varnam, A.H., Sutherland, J.P., 1995. Meat and Meat Products. Technology, Chemistry and Microbiology. London: Chapman and Hall.
Yoon, K.C., Wang, S.S. (Eds.), 1999. Advances in Extrusion Technology: Aquaculture/Animal Feeds and Foods. Lancaster, PA: Technomic Publishing.

ENCYCLOPEDIA OF MEAT SCIENCES

SECOND EDITION

EDITED BY

MICHAEL DIKEMAN
& CARRICK DEVINE

AP

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

The material in the encyclopedia is not arranged by ordinary alphabetical order, but by alphabetical order according to 97 principal topic areas taken to allow all papers belonging to each principal topic to appear together in the same volume. Within each principal subject, article headings are also arranged alphabetically, except where logic dictates otherwise.

There are four features that help you find the topic in which you are interested:

1. The contents list.
2. Cross-references to other relevant articles within each article.
3. A full subject index.
4. Contributors list.

1 Alphabetical Contents List

The alphabetical contents list, which appears at the front of each volume, lists the entries in the order that they appear in the encyclopedia. It includes both the volume number and the page number of each entry.

2 Cross-References

All of the entries in the encyclopedia have been cross-referenced. The cross-references, which appear at the end of an entry as a See also list, serve four different functions:

i. To draw the reader's attention to related material in other entries.
ii. To indicate material that broadens and extends the scope of the article.
iii. To indicate material that covers a topic in more depth.
iv. To direct readers to other articles by the same author(s).

Example

The following list of cross-references appears at the end of the entry Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants.

> *See also*: Chemical Analysis: Sampling and Statistical Requirements; Standard Methods. Foodborne Zoonoses. Growth of Meat Animals: Metabolic Modifiers. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

3 Index

The index includes page numbers for quick reference to the information you are looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4 Contributors

At the start of each volume there is list of the authors who contributed to that volume.

PREFACE

The Encyclopedia of Meat Sciences, second edition, an extensive revision of the first edition published in 2004, covers all the essential meat topics, ranging from animal production, processing, analytical procedures, and food safety, to final consumption including health issues and nutritional aspects. There are more than 230 articles and these provide a greater breadth of coverage than any existing work on meat science. In addition to publication in print, the Encyclopedia is also available for licensing online that can allow regular updating. The articles are designed to bring a nonexpert up to a level of understanding the interactions among the various disciplines covered in the articles. Most articles are 3000–4000 words long and include a list of Further reading and Websites to expand the content beyond the immediate scope of this work. The Encyclopedia is, therefore, a valuable resource for several levels of education and experience.

The Editors gratefully acknowledge the contributions of the authors of the articles and the Editorial Advisory Board. The board not only proposed subjects to be covered, but also found contributors and then reviewed the articles. The work involved in an Encyclopedia such as this requires an extensive interactive cooperation among the Editors, the Editorial Advisory Board, the contributors, and the publishers, particularly the staff of the Major Reference Works division of Elsevier. The staff included Nancy Maragioglio, Donna de Weerd-Wilson, Anna Gebicka, Cari Owen, Will Bowden-Green, Sam Mahfoudh, Zoey Ayres, and Marise Willis.

The Editors are particularly grateful to Cari, Will, and Sam, who worked very closely with us and who diligently pursued all avenues to obtain contacts with contributors, maneuvered around obstacles, facilitated the day-to-day management, and linked everyone together to meet the deadlines.

Michael Dikeman and Carrick Devine
Editors, August 2014

INTRODUCTION

Meat consumption by hunter–gatherers predated the agricultural revolution. Consumption of meat and fish runs in parallel with human development that is still in process. Humans and animals have now coexisted for thousands of years for their mutual benefit, even though their relationship is changing. Meat does not come from a single, or even a few, animal species, but is derived from a wide variety of species ranging from poultry to pigs, cattle, sheep, goats, and wild game to thousands of species of fish. While many of these species are now intensively farmed, some still coexist with nomadic tribes, whereas, others are raised by families in small village communities, or are even hunted by remnants of hunter–gatherer communities. The second edition of the Encyclopedia of Meat Sciences discusses how the domesticated species evolved; the wide range of harvesting methods for animals, poultry and fish; the historical changes in production, processing and nutritional value, including the beneficial effects of optimum amounts of meat in a diet.

The meat industry is based on obtaining animals, poultry, and fish from pastures, feedlots and specialized intensive production systems, and from extractive industries such as fishing. It is understandable, therefore, that the genetics and management of animals and production systems are prominent in the Encyclopedia. However, the broad field of meat science is much more than harvesting animals and processing meat from them. It includes issues such as preslaughter stress and its effects on meat quality; religious issues; animal welfare; and humane slaughter techniques, all of which are extremely important to ensure that meat quality, cultural issues, and market requirements are harmonized.

Processing methods for the various species are different, but they have all historically developed to ensure, either by conscious design or by experience, that the underlying principles of physiology and biochemistry in the conversion of muscle to meat are optimized. Biochemistry and physiology are extremely important and fundamental disciplines, because they explain how unfortunate, undesirable processing defects such as PSE or cold shortening and toughening can occur and can be avoided. Progress in this area has also enabled significant changes in production and subsequent quality since the first edition of the Encyclopedia of Meat Sciences in 2004.

Understanding these changes requires an appreciation of the structure of carcass tissues, from gross carcass attributes to consideration and understanding of changes at the ultrastructural level. The form and function of muscle tissues, how they change through growth, how they impinge on meat quality, and the way that connective tissue and fat can be major contributors to the final product quality are all covered in these pages. Topics such as cold shortening that can cause meat toughening or inhibition of tenderisation are explained, as well as how procedures such as electrical stimulation evolved to prevent these problems. Assessment of meat quality from measurements such as muscle pH, tenderness prediction through spectral measurements on uncooked meat, color changes on display and storage, and reduction of microbial contamination are critical for many aspects of the meat industry and are also discussed.

There have been many and significant advances in meat animal production based on genetic, nutrition, growth biology, and metabolic modifier research. In regard to meat processing, advances in refrigeration and freezing technology, which is the foundation of perhaps the most important changes ever encountered for food is discussed. Even so, such advances also depend on the way in which microbiology and packaging are integrated to ensure wholesome products with a long shelf life, minimal spoilage, and desirable sensory attributes. However, there are many other ways to preserve food that are also important. Of ever-increasing importance is the topic of food safety, which must receive extensive attention because meat is a perishable product and is critical for a high quality of living and even for human survival. Meat marketing and pricing in all its forms, from wet markets to hotel, restaurant and institutional trade, and transportation are also important. Whole-tissue meat is usually cooked, so, many of the desirable attributes such as flavor development relate to the temperature interactions with various proteins and sugars during cooking. Other cuts are processed in various ways, from smoking to mincing to sausages and the technologies involved are covered.

Not all muscles or cuts of meat are suitable for the same cooking and preparation methods. Therefore, out of necessity, a vast range of highly desirable products has evolved with variations from one ethnic background to another. Other products are merchandized through fast-food restaurants. One can now consume a hamburger in China that is almost identical to that in Chile or in the United States owing to a consistency of product specifications that has become universal. Meat is not only a major source of quality protein and some vitamins and minerals; it often forms the central part of a meal, and is desirable to have the appropriate flavors, aromas, and appearance to conform to the expectations and the way meat is used in various cultures.

This second edition of the Encyclopedia of Meat Sciences also covers controversial health-related aspects of meat consumption and this aspect needs considerably more research. In recent years, the ready availability of meat and other foods has given rise to some health concerns. However, the issues are not always what they seem. The positive and potential negative health-related aspects of meat eating are addressed by experts in dietary and health aspects of meat consumption, but the effect of a single food item should not be considered in isolation.

The wide coverage of topics will ensure that this second edition of the Encyclopedia of Meat Sciences will be an important resource for students or professionals with an interest in meat science or those engaged in the livestock and meat industries. Most of the articles in the second edition are not only a revision of those in first edition but there are additional areas covered. The relatively short nature of the articles makes the Encyclopedia easy and interesting to read.

Michael Dikeman and Carrick Devine
Editors, August 2014

CONTENTS TO VOLUME 2

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This article is a revision of the previous edition article by D Demeyer, F Toldrá, volume 1, pp 467–474.

Glossary

Maillard reaction (French pronunciation: [majaʁ], mah-yar) A form of nonenzymatic browning similar to caramelization. It results from a chemical reaction between an amino acid and a reducing sugar, usually requiring heat.

Matrix-assisted laser desorption/ionization (MALDI) A soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (biopolymers such as DNA, proteins, peptides, and sugars) and large organic molecules (such as polymers, dendrimers, and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. The type of mass spectrometer most widely used with MALDI is the TOF (time-of-flight) mass spectrometer, mainly due to its large mass range.

Metametabolomics Metabolomics represents the systematic study of the metabolome, i.e., the collection of all metabolites in a biological cell, tissue, organ, or organism, which are the end products of cellular processes. The combined analysis of metabolomes of different members of an ecological system is referred to as metametabolomics.

Principal component analysis (PCA) A mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables.

Strecker degradation A chemical reaction that converts an α-amino acid into an aldehyde by an imine intermediate. It is named after Adolph Strecker, a German chemist.

Introduction

The chopping or mincing of meat and fat with curing salt and/or sugar together with spices, herbs, and other plant material, followed by the stuffing of such mixture in casings to form a 'sausage' that is left to ferment and dry, is a practice dating back to centuries before the Common Era. Since these early beginnings, variations in 'meat' species, in nature of ingredients and additives used, and in processing technology have yielded a tremendous variety of 'fermented meat products', almost all differing with site of production. Their shelf life and safety, as well as the specific flavor, texture, and color, are determined by combinations, varying in relative importance, of acidulation brought about by lactic acid production and by the lowering of water activity (a_w) via salt addition (curing) and drying. These effects also trigger enzymatic reactions, by both meat and microbial enzymes, which lead to complex flavor development.

The presence of curing salt and the limited availability of oxygen select for a specific microbiota, consisting mostly of lactic acid bacteria (LAB) and catalase-positive cocci (mainly coagulase-negative staphylococci; CNS), which are adapted to the meat substrate and withstand low water activities and anaerobic conditions. Nowadays, these microorganisms are usually added as starter cultures to standardize the production process. The LAB initiate fermentation that lowers the pH to final values between 4.5 and 5.5, inducing the denaturation of salt-solubilized protein to a gel that can be sliced. An adequate (rapid) reduction of pH, the use of curing salt, and a lowered a_w are the major factors determining both shelf life and safety.

Considerable recent research efforts have generated more detailed knowledge of the interrelated microbiological,

biochemical, chemical, and physical changes taking place during 'meat fermentation', emphasizing two major developments:

- Detailed identification of the diversity within the microbial species involved using methods of molecular genetics, as well as their metabolic features affecting product quality and safety.
- Although industrial and commercial interests favor the development of starter cultures, the importance and variability of enzymes, present in the meat and/or fat raw materials, is now fully recognized.

The many intricate interactions between microorganisms, meat and fat enzymes, and processing conditions determining sausage quality encompass more than the concept of fermentation and are therefore better referred to as 'metabolism.'

The Processing of Fermented Sausages

The industrial production, mainly in continental Europe, from where the technology was transferred to the United States and Australia, referred to as 'ripening,' is separated into two consecutive periods: fermentation followed by drying/maturation. Technologies differ mainly in the length of the total ripening period in relation to that of the fermentation period. High initial rates of lactic acid production resulting from the use of starter cultures that lead to pronounced lactic acid generation and/or high temperatures during fermentation are usually associated with rather short drying periods. The drying period may even be omitted, as in 'summer sausage,' prevalent in parts of the United States and fermented at 38 °C. In Europe, a major distinction can be made between the so-called Northern-type and Mediterranean-type sausages. Northern-type sausages usually contain both beef and pork as raw meats, are ripened for short periods (up to three weeks), and are usually subjected to smoking. Shelf life and safety are mainly due to the fast drop to acid pH (<5.0 after three days), usually at temperatures >24 °C, and to smoking rather than to drying. Mediterranean sausages mostly use only pork and are heavily seasoned, ripened for longer periods (several weeks or even months), mold-ripened, and seldom smoked. Acidulation is slower, usually takes place at lower temperatures (<24 °C), and ends at higher pH values (above pH 5 after three days or more). To compensate for negative effects on shelf life, extensive drying is applied, reducing water activity.

Raw Materials and Additives

Chilled meats (frozen meat tempered to approximately −4 °C) and frozen (<-18 °C) porcine fats after removal of rind (e.g., lard) are most often mixed in a ratio of 2:1. Lard and sausage meat (ham trimmings, jowls, and throats as well as shoulders and bellies) make up 10% and 20%, respectively, of the pork carcass. They are selected mainly on the basis of bacteriological quality, visual fat content, pH (<5.8), and unsaturation ($<12\%$ polyunsaturated fatty acids in fat), as well as oxidation status (minimal peroxide value) of the fat. Widely used ingredients and additives are salt (2–4%) containing sodium nitrite ($NaNO_2$) (added as curing salt containing 0.4–0.6% $NaNO_2$), glucose (0.5–1%), sodium ascorbate or ascorbic acid (0.5–1%), and spices. Nitrite is used because of its antibacterial, color-forming, antioxidant, and flavoring properties. Although the technological and microbiological justification is discussed, part or all of the nitrite is substituted for by potassium nitrate (KNO_3) in Mediterranean-type sausage. Nitrate is then converted into nitrite due to bacterial nitrate reductase activity, present in meat-associated catalase-positive cocci, mostly CNS. Both ascorbate and ascorbic acid are used to improve the stability of the red nitrosylated pigment, an effect closely associated with the prevention of lipid oxidation. Ground pepper (0.2–0.3%) is usually present in all types of sausages. Mediterranean types in particular may contain higher levels (1–3%) of other spices such as paprika and garlic that have been shown to be effective antioxidants, comparable to ascorbate. Additional additives sometimes used for Northern-type sausages are phosphates to improve stability against oxidation and to steer water content; glucono-δ-lactone (GdL, 0.5%) to ascertain fast chemical acidulation with negative effects on flavor development; manganese sulfate (~50 ppm) as cofactor for LAB to stimulate acidification; vegetable proteins (mainly soy isolate), which may also accelerate fermentation; and milk powder or lactose as bulking agent.

Comminution or Chopping

Raw materials and additives, including bacteria (and sometimes yeasts) as starters, are added for mixing and chopping, often under vacuum, in a mincer or cutter. The cutter consists of a set of knives that rotate rapidly ($1–3\times10^3$ rpm), producing a batter in a bowl that rotates slowly (10–20 rpm). The relative speeds of the knives and bowl and the sequence of addition of raw materials and additives determine the fat particle size and are optimized to produce a batter within less than 5 min at temperatures below 2 °C, ensuring minimal damage to the fat tissue.

Stuffing

In most industrial processes, the batter is immediately stuffed under vacuum into natural, semisynthetic (collagen), or synthetic casings that are permeable to water and air, and both ends are clipped. The sausage diameter (e.g., 2–15 cm) is related positively to the relative importance of fermentation (pH) versus drying (a_w) for stability.

Ripening

The sausages, hung in racks, are most often placed in air-conditioned fermentation chambers at high relative humidity (RH) and usually left for ripening in two consecutive stages: fermentation driven by bacterial growth and, mostly after transfer to another chamber, drying for increased stability and development of additional sensory characteristics. Temperature–RH–time combinations during fermentation differ between Northern (20–28 °C/60–90%/62 h) and Mediterranean (5–24 °C/60–90%/100 h) types, whereas drying is carried out under similar conditions (14 °C/78%) for anywhere between 2 weeks (Northern) and several months

(Mediterranean). In the USA, 'summer sausage' production is based on fermentation temperatures exceeding 30 °C with little drying. It has been recommended to have air RH values not more than 5–10% lower than the water activity value of the sausage (expressed as a %) to prevent case hardening, and recommended air speeds are approximately 0.1 m s^{-1}. Controlled fermentation and ripening in air-conditioned surroundings consume considerable amounts of energy, and alternative methods, involving the use of fresh air, have been proposed, inspired by the traditional methods for Mediterranean-type sausages and adapted to local climatic conditions.

Smoking

During the fermentation period, as soon as the red surface color has developed, Northern-type sausages are usually subjected to smoke, generated by controlled combustion of (oak) wood (300–600 °C) to minimize the production of polycyclic hydrocarbons. Smoke contributes to antimicrobial and antioxidant effects, in addition to generating specific flavor and color components, and may be substituted by the use of liquid smoke flavorings. Smoking is hardly used in the production of Mediterranean-type sausages, but is used in Hungarian and Rumanian products, where a light smoking period precedes fermentation.

Microorganisms Involved and the Use of Starter Cultures

In meat fermentation, two main groups of bacteria are considered important: the LAB and CNS. The usually low initial population of LAB in the raw material (3–4 log CFU g^{-1}) becomes dominant during the fermentation step (8–9 log CFU g^{-1}) and is mainly responsible for the safety as well as the flavor and texture of the product through lactic acid production. Some LAB also produce bacteriocins that may contribute to microbial safety, in particular toward *Listeria monocytogenes*. *Lactobacillus sakei*, and to a lesser extent *Lactobacillus curvatus* and/or *Lactobacillus plantarum*, generally constitute the main LAB species. *Staphylococcus carnosus* and *Staphylococcus xylosus* are the main CNS species mainly held responsible for color stabilization, decomposition of peroxides, and contribution to flavor through metabolism of end products of proteolysis and lipolysis. The latter two species are mostly encountered because they are typically applied in starter cultures. Nevertheless, during spontaneous fermentation processes, other species such as *Staphylococcus equorum* and *Staphylococcus saprophyticus* may be just as important. There seems to be considerable variability within CNS with respect to pH sensitivity, overall competitiveness, and flavor generation. Although fermentation may rely on the 'house microbiota' for traditional products, 'starter cultures' have been used since the middle of the twentieth century and are added as frozen or lyophilized cultures ($1–5\times10^{6}$ g^{-1}) for industrial sausages, but also increasingly so for artisan products. Selection of such starters requires knowledge of their potential to outgrow indigenous bacteria in the sausage environment. More importantly, selected strains should not lead to sensory defects or adversary health effects (e.g., biogenic amines), and yield, if possible, additional advantages such as the generation of specific antimicrobial components (e.g., bacteriocins), nutritional properties (e.g., probiotic strains), or exceptional flavor characteristics. This is a difficult but challenging task, considering the large microbial diversity demonstrated in similar types of meat products, also on intraspecies level. Studying the DNA of the sausage microbial ecosystem allows definition of microbial ecology and diversity, whereas RNA analysis and (meta) metabolomics will highlight the microbial populations that are metabolically active, thereby contributing to the fermentation process. The very large numbers of LAB and CNS strains in the bacterial communities developing during sausage fermentation reflect genes determining resilience to the environmental changes within the sausage mixture, involving, for instance, water activity, redox, pH, and oxygen levels, as well as the potential for biofilm formation and cell aggregation. Nevertheless, initial microbial diversity narrows down during the production process, in particular for the LAB where *Lactobacillus sakei* is by far the most competitive species. The specialized 'metabolic repertoire' required has been highlighted within the complete genome sequence of *Lactobacillus sakei* 23 K. This specialized adaptation by *Lactobacillus sakei* has been demonstrated via genome analysis, metabolite kinetics, modeling, and gene expression studies, with respect to its ability to metabolize alternative energy sources such as arginine and nucleosides. Such genome analysis may further allow for new biotechnological approaches to meat fermentation, involving fundamental aspects of the diversity of bacteria adapted to this specific environment, as well as their use as starter cultures involving defined functional properties and absence of potential hazards. Indeed, several commercially available starters have a long history of apparent safe use in industrial meat applications, but recent evidence has stressed that their potential production of toxins and biogenic amines is of major concern. The other and major important criterion in the evaluation of starter strain safety is the presence of transmissible antibiotic resistance markers. The presence of intrinsic resistance and resistance due to mutation of chromosomal genes presents low risk of horizontal dissemination and such strains should be acceptable for consumption. Safety assessment of starter strains should therefore focus on antibiotic profiles and transferable genetic determinants, as acquired resistance by mobile elements may present a risk for public health. In addition, molds (usually *Penicillium* spp.) that may occur on the external surface of Mediterranean sausages may produce mycotoxins. Growth of undesirable molds on the sausage exterior is prevented by pretreatment of the casing with potassium sorbate or pimaricine solutions. Additionally, nonpathogenic molds may be applied as starter cultures to outcompete the potentially hazardous background microbiota.

Sausage Metabolism and pH

During ripening, sausages represent a dynamic matrix, which is affected by actions from both endogenous and microbial enzymes. The most obvious aspect consists of lactic acid production by LAB, mostly originating from the conversion of

endogenous and/or added carbohydrates. LAB produce important amounts of both D(−) and L(+) lactic acid isomers. The resulting pH decrease is counterbalanced by the salt-solubilized and partly hydrolyzed muscle proteins and by ammonia production. In addition, fungal growth may lead to lactic acid consumption and result in pH increase.

Carbohydrates are the main substrate for fermentation but, varying with sausage diameter, respiration also occurs as indicated by oxygen consumption. Moreover, the joint action of bacterial and meat enzymes on sausage protein and lipid fractions represents another important transformation. Paucibacterial meat incubations have demonstrated the importance of acid-activated muscle and fat endogenous proteases and lipases. Myosin and actin are mainly hydrolyzed by the muscle proteinase cathepsin D, activated at acid pH, and producing polypeptides that are further hydrolyzed to small peptides (1–10 kDa) and amino acids by endogenous and microbial peptidylpeptidases and aminopeptidases, respectively. Further deamination and decarboxylation yield small amounts of ammonia and amines, respectively. Provided starter microorganisms do not produce amines, amine production mainly reflects initial bacterial contamination of the raw materials used. Further insight into the proteolytic action of endogenous proteases and the limited contribution of microbial enzymes has recently been provided by proteomic investigations involving identification by Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight (MALDI-TOF) mass spectrometry of peptides separated by two-dimensional (2D) electrophoretic analysis. Likewise, it has been demonstrated that endogenous lipases are almost exclusively responsible for the liberation of free fatty acids during ripening. This lipolysis involves preferential release of polyunsaturated fatty acids, because of the more important phospholipase activity on muscle membrane phospholipids and the specificity of fat cell lipases. It is clear that both free amino acids and free (unsaturated) fatty acids are major precursors for the production of flavor volatiles. This involves bacterial metabolism of, for example, branched chain amino acids, particularly leucine, as well as chemical changes leading to fatty acid oxidation end products and possibly Strecker degradation of amino acids.

Development of Sensory Quality

During the combined, consecutive, and interactive changes that take place during fermentation and drying, the specific color, texture, and flavor characteristics of the different sausage types are developed.

Color

The reddish cured meat color is due to the formation of nitrosylmyoglobin in a complex set of reactions involving the formation of nitrogen oxide (NO) and its subsequent reaction with myoglobin and metmyoglobin, producing red and grayish colors, respectively. The subsequent acid-induced denaturation of the globin moiety ensures the stable red color. Nitrite initially acts as a potent oxidant, producing nitrosylated metmyoglobin on mixing, which has subsequently to be reduced during drying. With nitrate, formation of the nitrosylating NO requires the reduction of added nitrate by catalase-positive cocci (mostly CNS). The latter bacteria are, however, pH-sensitive, which hampers their activity in later periods of Northern-type sausage processing.

Texture and Mouth-Feel

The sausage texture is due to the coagulation of the salt-soluble muscle proteins (mainly myosin) solubilized by chopping. The coagulated gel surrounds fat and meat particles and is stabilized during drying. Unbalanced acidulation induces myosin coagulation and proteolysis hampers texture development, as is the case in attempts to shorten ripening time by the use of freeze-dried meat, encapsulated lactic acid, and glucono-delta-lactone.

Flavor

Flavor, i.e., the combination of taste and aroma, and texture are perhaps the most important sensory characteristic of fermented sausages, as they convince the consumer to repeatedly buy the product.

Volatile compounds that determine aroma (smell or odor) are often considered separately from the nonvolatiles determining taste through receptors on the tongue. Nevertheless, it should be realized that taste and aroma are sensed in an integrated manner. In addition to saltiness, a sour or acid perception is the most relevant taste, in particular in Northern-type sausages. Its intensity is correlated with the presence of D-lactate and acetate. ATP (adenosine triphosphate) metabolites, such as inosine monophosphate (IMP) and hypoxanthine, small peptides (1–10 kDa), and free amino acids contribute to taste as well, mostly of the umami type. Free higher fatty acids are considered to be of less importance.

More than 200 volatile compounds originating from fermented sausages have been identified by gas chromatography and mass spectrometry in the sausage 'head space' and their pattern has been used for product differentiation. The majority are derived from spices and smoking in Mediterranean and Northern-type sausages, respectively. However, only a limited proportion is of sensory relevance for the typical 'fermented sausage' flavor. Some compounds represented by acetic acid, acetaldehyde, diacetyl, and acetoin are derived from carbohydrate metabolism. The main aroma-determining compounds, with the highest odor activity values, are, however, derived from protein metabolism, such as 3-methyl butanal, 3-methyl butanol, and 2-methyl butanal, and are important from the beginning of ripening. Apart from microbial amino acid metabolism, these compounds are also products of the Strecker degradation of amino acids. It has therefore been suggested that Maillard reactions, although normally requiring high temperatures and low water activities, may be of some importance in sausage fermentation. Hexanal, heptanal, and 1-octen-3-ol have also been identified as probable main contributors to the fermented sausage aroma. Lipid-derived compounds are generated mainly at the end of the process by both physicochemical and microbial changes and also include propanal, pentanal, ethyl 3-methyl

butanoate, 3-methyl butanoic acid, 2-methyl propanoic acid, ethyl hexanoate, and nonanal.

Effect of Processing

It has been demonstrated that interrelated processing variables such as the type of starter, vacuum stuffing, temperature, a_w, RH, sausage diameter, and rate of drying affect the relative importance of the metabolic changes described above and thus the final sensory quality of the product. For instance, a rapid drop in pH stimulates the activity of muscle cathepsin D and lysosomal acid lipase, active at acid pH, but inhibits bacterial amino acid metabolism. Reducing the diameter of the sausage and/or increasing the rate of drying may reduce (endo)-proteolytic activity but increases the relative importance of acetate production and respiration of substrates involving oxygen consumption. As a consequence of a more pronounced denaturation, pig muscle proteolytic and lipolytic enzyme activities were shown to be lowered by breed-related higher rates of postmortem muscle pH decline. Effects of animal genetics and production systems on flavor volatiles in cured meat products have been demonstrated as well, as interactions occur between animal species and starter used, affecting quality in salami production. Such findings may, for instance, be related to the existence of porcine gene polymorphisms of cathepsin D. Processing conditions and metabolic changes not only affect the nature of volatiles formed but also conditions determining their liberation during chewing. These include sausage pH, salting-out effects of additives, and the binding of flavor-determining volatiles by sarcoplasmic and other proteins. These findings imply that partial NaCl substitution in meat products by other salts should be considered not only for their salting-out effect but also for their effect on protein-binding ability.

Microbial Stability and Safety

The microbial stability and safety of the sausage is obtained by the introduction of successive 'hurdles' for undesirable microorganisms. The lowering of the redox potential, due to the omission of oxygen by chopping under vacuum, is

Table 1 End products of metabolism in Northern and Mediterranean types of sausages, produced in Belgium[a]

	Northern type sausage (NS)		*Mediterranean type sausage (MS)*	
Dry matter (%)	57 (4)		67 * (3)	
pH	4.8 (1)		5.5 * (2)	
% in DM				
Crude protein	31 (7)		28 * (9)	
Crude fat	61 (7)		81 (8)	
NaCl	5.3 (12)		6.1 (11)	
mmol per 100 g DM				
Lactate	21 (12)		17 * (17)	
Acetate	1.0 (14)		0.86 (19)	
Sugars	0.56 (23)		0.40 * (18)	
mg N per g N				
Peptide α-NH_2-N	30 (13)		27 * (16)	
Free α-NH_2-N	23 (10)		37 * (13)	
Ammonia-N	3 (22)		10 * (18)	
μg BSAeq per mgCP[b]				
Myosin (200 kDa)	18 (30)		25 * (12)	
HMM (150 kDa)	24 (21)		24 (8)	
Actin (46 kDa)	35 (14)		39 (10)	
38 kDa	15 (7)		15 (7)	
mg per g TFA[c]				
Free fatty acids	27 (4)		37 * (11)	
Aroma compounds[d]	(**1**)	(**2**)	(**1**)	(**2**)
Hexanal	123	2836	227	11568
3-Methyl butanal	44	856	32	941
3-Methyl butanol	355	580	315	1015
2-Methyl butanal	7	198	7	225
2-Methyl butanol	22	0	47	0
Ethyl esters	221	0	82	625
Diacetyl+acetoin	3052	311	681	167

[a]Mean value of 25 determinations per type (5 batches and 5 sausagea per batch); values in parentheses are coefficient of variation, i.e., standard deviation as % of mean value.
[b]BSAeq, bovine serum albumin equivalents determined by semiquantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CP, crude protein.
[c]TFA, total fatty acids.
[d]Aroma compounds are given as (**1**) nmol 4-methyl-2-pentanone per kg obtained from headspace analysis; (**2**) ng per kg obtained after steam distillation.
*Significant difference (<0.05) between mean values for NS and MS.
Source: Compiled from data in Demeyer, D., Raemaekers, M., Rizzo, A., *et al.*, 2000. Control of bioflavour and safety in fermented sausages: First results of a European project. Food Research International 33, 171–180.

enhanced by addition of ascorbic acid or ascorbate. This inhibits aerobic bacteria and improves the bactericidal effectiveness of nitrite, an important hurdle. During fermentation, LAB inhibit undesirable bacteria mainly through the production of lactic acid and the associated pH drop but also by production of compounds such as acetic acid, hydrogen peroxide, and, in some cases, bacteriocins. The subsequent drying of the sausage continues the reduction in the water activity, due to addition of salt, to values that prevent growth of spoilage and/or harmful microorganisms (e.g., $a_w < 0.92$ or < 0.94 at pH < 5.0). Correct interaction of all 'hurdles', including the application of smoke in Northern-type production, usually ensures stability and safety. Nevertheless, incidence of pathogens has occasionally been reported, as well as food scares related to fermented sausage consumption.

Modeling the Nature and Dynamics of Ripening

It has been confirmed repeatedly that the safety, shelf life, and sensory quality of fermented sausages are the result of an extremely complex set of interacting microbiological, physical, and (bio)chemical changes during ripening. Some of the complexity of the physical and (bio)chemical characteristics of Northern and Mediterranean types of sausage, as well as their variability and differences, is illustrated in Table 1.

The technological control, evaluation, and standardization of such a complex system of characteristics and changes (involving, for example, the use of nonmeat proteins, spices, altered temperature/RH combinations, sausage diameter and type of casing, and type of starter and carbohydrate) requires comprehensive knowledge of the many interactions involved. Failure to accept this may lead to the loss of traditional flavor of Mediterranean-type sausages, for example, when highly acidifying starter LAB and high amounts of nitrite are used. It is becoming clear that the flavor of Mediterranean-type sausages is associated with a specific pattern of proteolysis, characterized by a lower peptide/free amino acid ratio as well as higher ammonia concentrations (Table 1). This pattern is related to changes in pH, dry matter (DM), and a_w interacting with the type of fermentation as determined by fermentation temperature, length of ripening, starter culture, type of meat used, and sausage diameter. Comprehension of these relations and their use for predictive purposes can be facilitated by the use of simple models. Such models may be analytical or mechanistic:

- Analytical models describe linear or exponential kinetics of time-related changes in sausage metabolite concentrations, microbial characteristics, or sensory quality characteristics.
- Mechanistic models relate weight losses to changes in DM content, in pH, in a_w, and in texture; the kinetics of pH change to lactic acid and ammonia production; and the amounts of end products (lactic acid, acetic acid, ammonia, aroma compounds, etc.) produced and substrate (carbohydrates, oxygen, etc.) metabolized within the framework of biochemical and microbial stoichiometry (Figure 1).

Although such models are over-simplified, they have been supported by experimental data within experimental error and have been used to evaluate the effects of starter culture, sausage diameter, and chopping intensity on the relative importance of

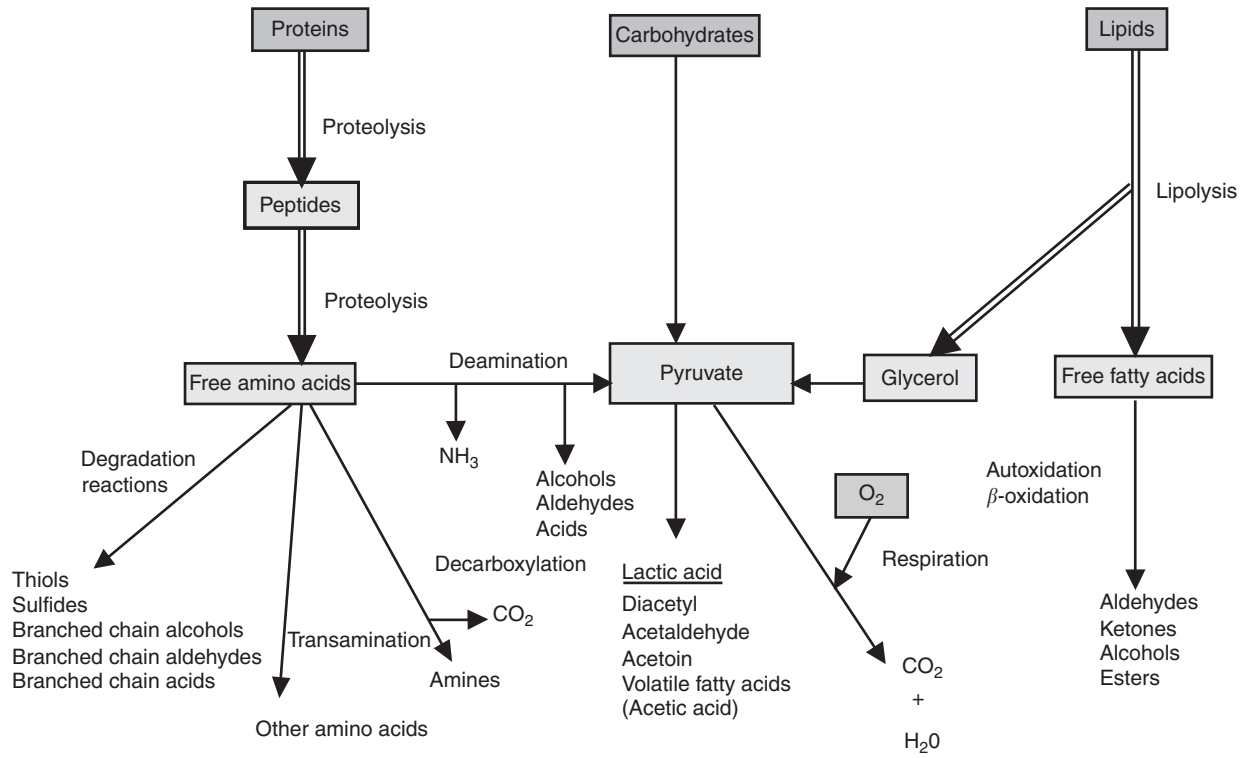

Figure 1 Simplified overview of sausage metabolism. Double lines indicate pathways with a major contribution of muscle and adipose tissue enzymes.

respiration versus carbohydrate and protein fermentation. Evaluation of the effects of technological changes on sensory characteristics and aroma analyses involves more intricate multivariate statistical models such as principal component analysis (PCA). Although there is still a long way to go before control of flavor formation by the use of CNS starters, for example, is adequate, these tools for data analysis have shown the importance of starter species in accelerating flavor formation.

Fermented Sausages as 'Functional Meat Products'

Processed meats have been subject to increasing health concerns from both the consumer and science community. Improving the 'health value' of fermented sausages is a continuing challenge for production technologists. Fermented sausages have, for instance, been reduced in fat content and successfully enriched with orange fiber, short-chain fructo-oligosaccharides, and long-chain *n*-3 polyunsaturated fatty acids. Enrichment in calcium used amounts allowing these sensorial adequate sausages to be considered a 'source of calcium.' The addition of probiotic lactobacilli is actively investigated, including, for example, conjugated linoleic acid-producing strains, but work should include investigation of their survival rate and persistence in the human intestinal tract, as well as their antibiotic resistance. A larger bottleneck is the demonstration of true probiotic effects in humans via dedicated studies. The now widely recognized association of processed (i.e., cured) meat intake with the incidence of colo-rectal cancer should also motivate fermented sausage processing technologists to try and alleviate this problem. In this respect, the use of nitrite may be reconsidered as acceptable no-nitrate/nitrite-added commercial hams, bacons, and frankfurters have been produced. In fact, not only may there exist a causal relationship between colo-rectal cancer incidence and the presence of nitrite and/or the formation of nitrosyl-haem, but nitrite was also shown to prevent the formation of the red pigment Zn-protoporphyrin. This compound has been reported to inhibit hemin-induced DNA damage and colonic cell hyper proliferation, is a major chromophore in dry-cured Iberian ham and in Parma ham cured without nitrite, and may be produced by enzyme treatment to de- or transmetallize metalloporphyrins. It should also be realized that the nature of the fermentation/ripening period of dry-cured meat products may contribute to sausage 'functionality,' as it has been suggested that oligopeptides hydrolyzed by muscle dipeptidyl peptidases can generate angiotensin-I-converting enzyme inhibitory dipeptides (e.g., antihypertensive peptides).

See also: Additives: Functional. Biotechnology in Meat Animal Production: Genetically Modified Organisms in Meat Animal Production. Chemical Analysis for Specific Components: Curing Agents. Chemistry and Physics of Comminuted Products: Spices and Flavorings. Conversion of Muscle to Meat: Glycogen. Cooking of Meat: Maillard Reaction and Browning. Curing: Dry; Physiology of Nitric Oxide. Drying. Ethnic Meat Products: Mediterranean. Functional Foods. Ham Production: Cooked Ham; Dry-Cured Ham. Microbial Contamination: Decontamination of Processed Meat. Microbiological Analysis: DNA Methods. Microbiological Safety of Meat: *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Hurdle Technology; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; Thermotolerant *Campylobacter*, Yeasts and Molds; *Yersinia enterocolitica*. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms; Potential Environmental and Wildlife Sources of Microorganisms in Meat. Modeling in Meat Science: Refrigeration. Processing Equipment: Mixing and Cutting Equipment. Proteomic Technologies and Their Applications in the Meat Industry. Sausage Casings. Sausages, Types of: Dry and Semidry. Smoking: Liquid Smoke (Smoke Condensate) Application; Traditional

Further Reading

Caponio, F., Summo, C., Minervini, F., *et al*., 2006. Characterisation and quality assessment of ripened sausages in relation to the origin of raw material used. European Food Research and Technology 222, 376–379.

Chaillou, S., Daty, M., Baraige, F., *et al*., 2009. Intraspecies genomic diversity and natural population structure of the meat-borne lactic acid bacterium. Lactobacillus sakei Applied and Environmental Microbiology 75, 970–980.

de Ciriano, M.G.I., Larequi, E., Rehecho, S., *et al*., 2010. Selenium, iodine, omega-3 PUFA and natural antioxidant from *Melissa officinalis* L.: A combination of components from healthier dry fermented sausages formulation. Meat Science 85, 274–279.

Demeyer, D., Honikel, K., De Smet, S., 2008. The World Cancer Research Fund report 2007: A challenge for the meat processing industry. Meat Science 80, 953–959.

Demeyer, D., Raemaekers, M., Rizzo, A., *et al*., 2000. Control of bioflavour and safety in fermented sausages: First results of a European project. Food Research International 33, 171–180.

De Vuyst, L., Falony, G., Leroy, F., 2008. Probiotics in fermented sausage. Meat Science 80, 75–78.

Flores, M., Toldrá, F., 2011. Microbial enzymes for improved fermented meats. Trends in Food Science & Technology 22, 81–90.

Leroy, F., Verluyten, J., De Vuyst, L., 2006. Functional meat starter cultures for improved sausage fermentation. International Journal of Food Microbiology 106, 270–285.

Olivares, A., Navarro, J.L., Flores, M., 2009. Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. Food Chemistry 115, 1464–1472.

Pérez-Juan, M., Flores, M., Toldrá, F., 2008. Effect of pork meat proteins on the binding of volatile compounds. Food Chemistry 108, 1226–1233.

Rimaux, T., Vrancken, G., Vuylsteke, B., De Vuyst, L., Leroy, F., 2011. The pentose moiety of adenosine and inosine is an important energy source for the fermented-meat starter culture *Lactobacillus sakei* CTC 494. Applied and Environmental Microbiology 77, 6539–6550.

Sentandreu, M.A., Toldrá, F., 2007. Oligopeptides hydrolysed by muscle dipeptidyl peptidases can generate angiotensin-I converting enzyme inhibitory dipeptides. European Food Research and Technology 224, 785–790.

Skibsted, L.H., 2011. Nitric oxide and quality and safety of muscle based foods. Nitric Oxide – Biology And Chemistry 24, 176–183.

Talon, R., Leroy, S., 2011. Diversity and safety hazards of bacteria involved in meat fermentations. Meat Science 89, 303–309.

Toldrá, F., 2006. The role of muscle enzymes in dry-cured meat products with different drying conditions. Trends in Food Science & Technology 17, 164–168.

FISH INSPECTION

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Glossary

Ciguatera toxin A toxin that causes food poisoning when it becomes concentrated in the flesh of tropical and subtropical predatory fish. The toxin is produced by blooms of dinoflagellates (phytoplankton microorganisms).

Pauly reagent 4-Sulfobenzenediazonium (CAS no. 4332-41-6), a substance forming an intensely colored (therefore, easy to detect) product when attached to amines.

Puffer fish toxin Tetrodotoxin, a powerful (and often fatal) blocker of nerve transmission produced by symbiotic bacteria for use against predatory organisms by the puffer fish (and other members of the Order Tetraodontiformes) and blue-ringed octopuses of the tropical and subtropical genus *Hapalochlaena*.

Rheometer (from Greek, *rhein*, flow) An instrument used to measure the resistance of flesh to physical pressure, which is proportional to freshness.

Scombroid poisoning Illness, including urticaria, caused by the presence of large amounts of histamine in stale (or insufficiently refrigerated) fish. This histamine is produced in large amounts by bacterial action. Symptoms (such as urticaria) resemble an allergy, mimicking the body's natural release of histamine in response to an allergen.

Shellfish toxin A group of toxins found in various kinds of shellfish following contamination from toxin-producing dinoflagellates (cf. Ciguatera toxin).

Torry meter An instrument that measures the dielectrical properties of fish as an objective assessment of freshness (Torry is the name of the research station in Scotland where it was originally developed).

Zero-order kinetics A direct linear relationship where the independent variable has no power function (i.e., x to the power 1; log $1=0$, hence a zero-order logarithmic relationship).

Introduction

Fish not only is a high-protein food but also provides many other essential food elements in a diet. However, the whole process of fish collection, preservation, and storage is fundamentally different than the standard farming practices for terrestrial meat animals, because of the way degradation occurs. This article covers factors affecting fish quality, its measurement, and the inspection process.

The quality of food can be estimated in two ways: food safety and functional quality (Table 1). Safety is judged by whether or not food contains toxic substances, which can be detected by chemical or biological assessment. Functional quality considers food for its commercial value, nutritive value, and good flavor. These can be estimated either by sensory assessment or by instrumental analysis.

Sea foods on the one hand are well known to contain various beneficial components for human health, such as n-3 polyunsaturated fatty acids (e.g., eicosapentaenoic acid and docosahexaenoic acid), taurine, and vitamin D. On the other hand, sea foods often include certain hazardous components from the sea or produced after landing (Table 2). Hazardous components are classified into various types (Table 3). Toxic substances from the sea can be detected only by chemical and biological analysis, although humans can usually avoid them by experience. Hazardous components produced after landing are related to deterioration (freshness loss) of sea food, especially fish. It is, therefore, important to determine the degree of freshness to prevent harm to the consumer.

Table 1 Food quality

Safety-related quality
Proliferation
Food poisoning
Toxins
Pesticides
Prions
Heavy metals
Antibiotics
Functional quality
Freshness
Flavor
Color
Nutrition
Health-maintaining function

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Table 2 Factors compromising the functionality and safety of food

Functionality-undermining factors – Possibly judged by five senses	
Color	
Odor	
Alien substance	Originated in habitat
	Parasitic worms, other organisms, garbage, etc.
	Originated from land
	Insects, garbage, hair, yarn, or glass
	Metal, plastic, etc.
Microorganisms – Fungi and bacteria	
Change in form	
Change in physical properties	
Safety-related factors – Cannot be judged by five senses	
Originally involved in aquatic organisms – Natural toxins	
Originated from biological concentration	
Heavy metals, radioactive elements, and environmental pollutants	
Mixed during processing	
Microorganisms (bacteria and fungi)	
Heavy metals	
Toxins – Histamine	
Lipid peroxides	
Food additives	

Table 3 Hazard factors found in seafood

Biological hazards	Pathogenic bacteria	*Vibrio*, *Escherichia coli*, etc.
	Food-spoiling bacteria	Bacteria that spoil food by producing toxic substances (amines) and unpleasant odors
	Viruses	Norovirus and hepatitis A virus, etc.
	Parasites	*Anisakis*, *Cryptosporidium*, etc.
Chemical hazards	Biological	Shellfish toxin, puffer toxin, ciguatera toxin, mycotoxins, histamine, and heavy metals
	Human origin	Food additives, antibiotics, pesticides, fungicides, and heavy metals Detergents and environmental hormones
Physical hazards		Glass shards, metal, wood, yarn, etc.

Freshness Assessment and its Evaluation

Freshness is assessed by a variety of methods, including sensory, chemical, and physical methods, each with its own merits and demerits (Table 4).

Sensory Method

Subjective assessment of the sensory quality of fish is quick and instrument free and can be used in every situation, but because it is subjective, the results vary according to the assessor. Unfortunately, this method cannot be used for frozen fish. It is possible to standardize sensory assessment data to achieve objectivity and accuracy by training assessors to examine fish. Personal bias is further reduced by using a panel of experts to examine fish and averaging the independent assessments statistically. Such sensory panels can be expensive to set up and run, and their use is justified in only a few instances.

Chemical Method

The chemical method is based on measurement of substances in fish that change after death. There are three main groups of chemicals used for this purpose: adenosine-5′-triphosphate (ATP)-related compounds, nonvolatile biogenic amines (products of decarboxylases), and volatile compounds (products of reduction processes).

Overview of adenosine-5′-triphosphate-related compounds of fish postrigor

Soon after death or cessation of circulation, natural enzymes present in the tissues begin to break down ATP in the muscle to form adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine-5′-monophosphate (IMP), inosine (HxR), and finally hypoxanthine (Hx) (Figure 1). Among the enzymes present is 5′-nucleotidase (5′-Ntase), which catalyses the dephosphorylation of IMP to HxR and is unique due to its lower activity than other enzymes in fish and shellfish muscle in the range −5 to +20 °C and its zero-order kinetic property. This means that IMP will accumulate in early postmortem fish muscle and decrease both linearly and independently of time during storage, at least during the initial stages of spoilage. The monitoring of the decrease of IMP and other nucleotides and the increase of HxR and Hx has thus been used widely for evaluation of fish freshness. The production of HxR and Hx has also been attributed to bacterial enzymes after a long storage period.

Table 4 Methods for fish freshness assessment and its evaluation

Classification	*Concrete assessment method*	*Evaluation*	
		Merit	*Demerit*
Sensory	Outward appearance (color, clearness of the eyeballs, and presence of scratches), hardness (rigor mortis), smell, body shape, and touch	Quick and simple assessment No need for equipment	Subjective analysis Uncertainty Variation among panelists
Chemical	Nucleic acid-related compounds (*K* value), histamine levels, ammonia, and trimethylamine (TMA)	Objective and exact assessment Theoretical data	Relatively time consuming Instruments indispensable
Physical	Hardness (rigor index; RI), texture (rheometer), Torry meter, and impedance	Nondestructive measurement Quick assessment	Need confirmation by chemical analysis Variation with species and body parts
Microbiological	Total count, spoilage bacteria, and pathogenic bacteria	Disease prevention effect	Time consuming No criteria regarding number of bacteria

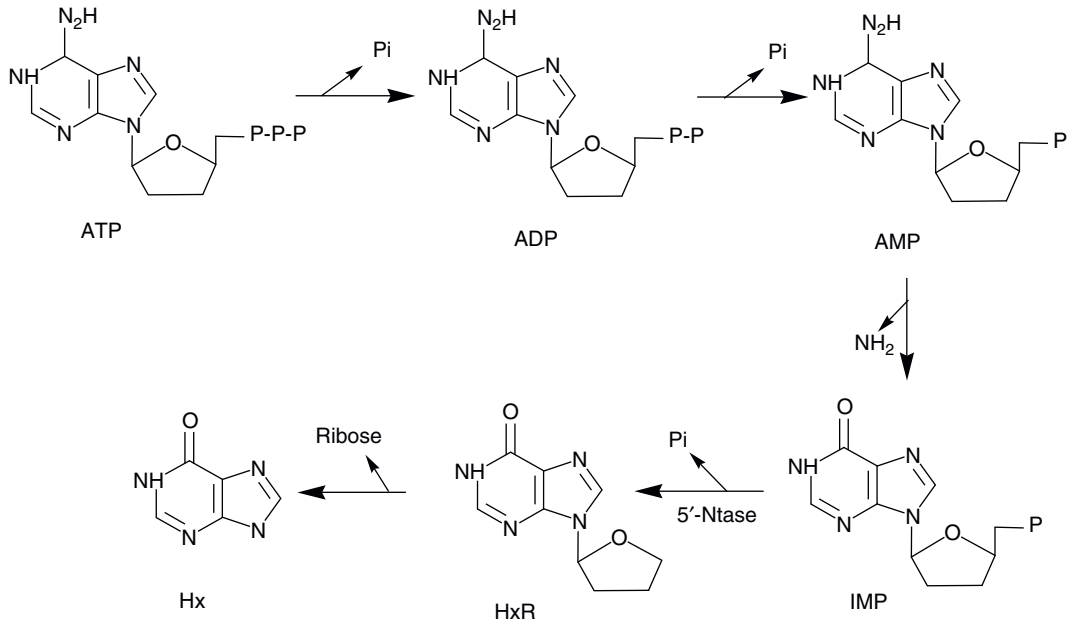

Figure 1 ATP degradation steps in fish flesh.

Measurement of adenosine-5′-triphosphate-related compounds and use for freshness assessment

Various methods for the analysis of ATP-related compounds have been proposed, including ion exchange chromatography, high-performance liquid chromatography (HPLC), enzymatic methods, enzyme colorimetric methods, enzyme-sensor methods, and near-infrared spectroscopy. Among these methods, complete analysis of nucleotide catabolites is possible only by ion exchange chromatography and HPLC. Other methods measure only one or two specific compounds, such as IMP, HxR, or Hx.

Saito *et al.* proposed the *K* value as an index of freshness or spoilage in fish. It is calculated from the values of HxR, Hx, and total nucleotide levels in fish at the time of measurement and is defined according to eqn [1]:

$$K\% = \frac{[\mathrm{HxR} + \mathrm{Hx}] \times 100}{[\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{HxR} + \mathrm{Hx}]} \quad [1]$$

It was observed that in freshly caught fish, the *K* value could be as low as zero, for moderate-quality fish 10–20, values of more than 50 are reached after 10 days of storage on ice, and the highest values, up to 90, were observed in spoiled fish. The *K* value showed excellent agreement with sensory assessment when measured for a number of different species of fish. However, marine mollusks and crustaceans degrade ATP in a different way than that by finned fish, mostly via the adenosine pathway. In spite of this, the *K* value can still be used generally

for freshness assessment, and it is presently considered to be one of the most appropriate indicators of fish freshness. The main drawback of the method is that it requires expensive instrumentation, such as HPLC, and a relatively long analysis time per sample, so the *K* value method has been used mainly in the laboratory and is not used widely on-site in fisheries. Recently, a simpler rapid method for *K* value analysis has been developed on the basis of paper electrophoretic separation and ultraviolet (UV) irradiation.

Measurement of K value by paper electrophoresis: The 'freshness checker'

Sato developed a fast and simple *K* value-measuring instrument named the Freshness Checker (available from QS-Solution). It is based on four steps: preparation of an extract from small pieces of fish (approximately 250 mg) with 600 µl perchloric acid; application of 3 µl extracts onto electrophoresis paper and separation of nucleotides and nucleosides+bases by electrophoresis at 800 V for 5 min; detection of results by UV irradiation and digital imaging of two spots (spot A in the anodal position and spot B in the original position of sample loading; Figure 2); and calculation of *K* value using a computer program (Figure 3). Spot A includes nucleotides, such as ATP, ADP, AMP, and IMP, and spot B includes nucleosides +bases, such as HxR and Hx. The computer program Spot Analyzer calculates the integration ratio of the two spots (spot size and density), according to eqn [2]. In this way, *K* value can be calculated in less than 10 min.

$$K\% = \frac{[\text{Integration value of spot B}] \times 100}{[\text{Integration value of spot A}] + [\text{Integration value of spot B}]} \quad [2]$$

Nondestructive assessment of fish freshness by near-infrared and phosphorus-31 nuclear magnetic resonance

Near-infrared (NIR) spectroscopy is used widely in the agricultural and food processing industries for measuring fat, protein, and moisture in fish, meat, and crops. It is a rapid, sensitive, and nondestructive method for analysis of organic materials with little or no sample preparation. An NIR spectroscopic method has been applied for estimation of Hx and of the *K* value for tuna, mackerel, cod, and salmon. It is noteworthy that NIR spectroscopy gives a prediction correlation of 0.98 with an error of 1.04 days and has proven useful for evaluating fish freshness. The use of partial least squares regression for calibration provides a higher correlation coefficient of prediction for inspection of fish freshness.

Nondestructive phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) is used to evaluate the degree of fish freshness by measuring levels of creatine phosphate (PCr), ATP, and inorganic phosphate (P_i). The ratios of [PCr]/P_i and [ATP]/P_i decrease rapidly and continuously during acceptable stages of deterioration and have been demonstrated as potential indices to estimate fish freshness. The biggest drawback to these methods is the very high expense of the instruments.

Physical Methods

Rigor index

The most dramatic exterior change after death is the development of rigor mortis. This is similar to that in other red meat species but with a different time course, and it is easier to measure because of the way the muscles are arranged. Shortly after death, the muscle is totally relaxed and this limp elastic state continues for some hours; thereafter the muscles become

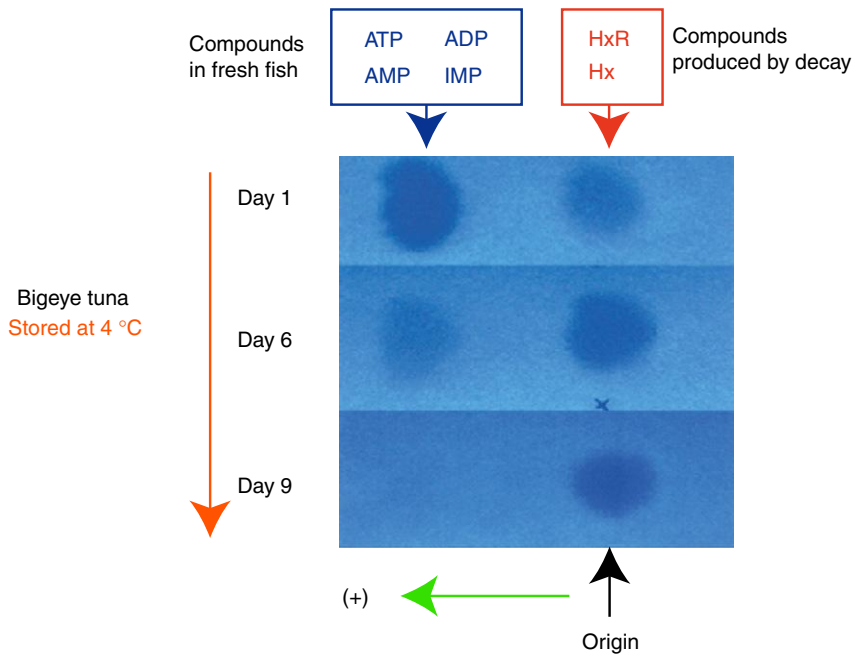

Figure 2 Electrophoretic separation of freshness-related compounds.

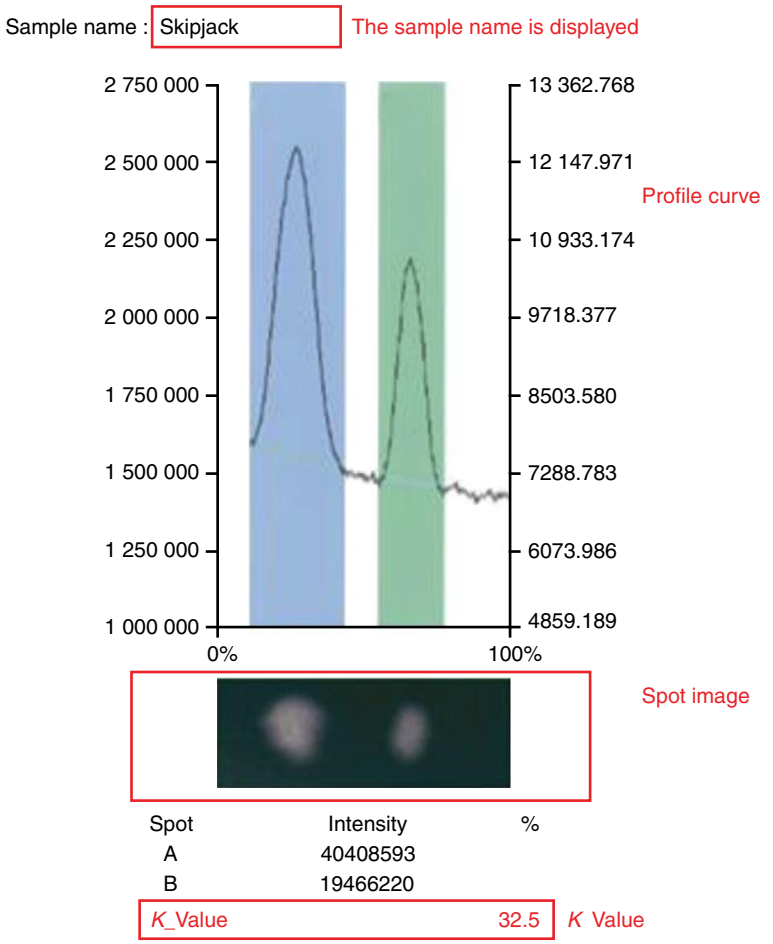

Figure 3 *K* value estimation by Spot Analyzer.

rigid. When fish are hard and stiff, the whole body becomes inflexible and the fish is in a state of rigor mortis. This condition usually lasts for several hours or a few days, and thereafter the rigor resolves. The resolution of rigor mortis, which is mainly due to autolysis, relaxes the muscles again, and they become flaccid but are no longer as elastic as before the onset of rigor. The state of fish body hardness and stiffness is expressed by the rigor index, as shown in Figure 4, and is calculated according to eqn [3].

$$\text{Rigor index} = \frac{L_0 - L}{L_0} \times 100 \qquad [3]$$

The onset, duration, and tension of rigor are influenced by preslaughter stress and physical activity, storage temperature, and the differences between premortem temperature and postmortem storage temperature. Fish use by Japanese consumers can be generally summarized as follows in relation to the state of rigor mortis:

- *Phase 1:* From the moment of slaughter to the end of rigor mortis resolution. The flesh is very fresh and has a good and delicate flavor, with a fresh neutral smell and no fishy odor. Consumers can eat the fish raw as 'sashimi' or 'sushi' and can sometimes enjoy raw fish just after slaughter ('ikizukuri,' live fish cooking). While very fresh, the flesh does not have much 'umami' or very strong taste. Consumers enjoy mainly the texture of the flesh and the delicate specific taste.
- *Phase 2:* After rigor mortis resolution. The fish is still fresh but is eaten after being cooked as a grilled or boiled fish. The flesh has no off-flavors but sometimes a slight fishy smell. The texture is still pleasant.

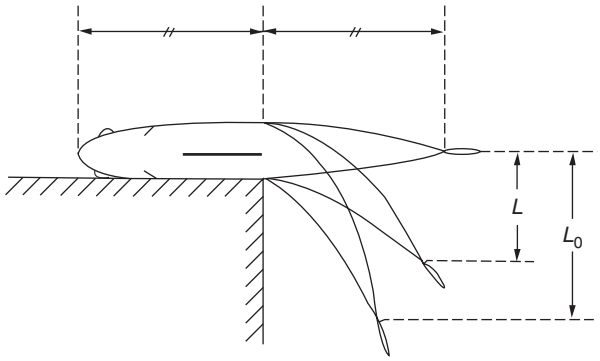

Figure 4 Expression of rigor index. L_0, status just after slaughter; L, status at rigor.

- *Phase 3:* There is sign of spoilage and a range of volatile, unpleasant smelling substances are produced, depending on the fish species. The volatile compounds may be trimethylamine (TMA) or ammonia. TMA has a very characteristic 'fishy' smell. At later stages, unpleasant odors of ammonia, sulfur, and rancidity develop. The texture becomes either soft and watery or tough and dry. This phase is relatively short.
- *Phase 4:* The fish can be characterized as spoiled and putrid.

Texture

It is well known that the hardness of fish flesh decreases gradually after death. This phenomenon is an autolytic change induced by protease activity. There have been attempts to use the textural property for assessment of freshness. Textural properties of fillets from several fresh fish have been measured by a rheometer (Yamaden model 3305, Tokyo) using a flat-ended cylinder at selected time intervals after death. Fish slices (10 mm thick) were measured at right angles to the orientation of the muscle fibers. Regardless of whether rigor had been reached or not, the hardness (breaking strength) of all the tested fish, except for tiger puffer, decreased sharply within 24 h after death and then decreased more gradually, although the values varied markedly between species. These findings suggest that tenderization of fish muscle starts shortly after death, independent of rigor development and regardless of fish species.

The main problems with these methods are sample preparation, the nonuniform structure of fish flesh, and the different orientation of structural elements within the flesh. For example, hardness and shear force values of raw Atlantic salmon (*Salmo salar*) fillets differ along their length: harder toward the posterior.

Electrical sensors

After death, the cellular and microsomal membranes of fish flesh are denatured and damaged. As a result, the ionic strength and electrical properties of the cells and tissues alter. Conductivity changes have been expected to provide a method for measuring postmortem changes or the degree of spoilage, so instruments have been developed to measure electrical conductivity and impedance. Such an instrument is the Torry meter. The data obtained by the Torry meter show good correlation with time after death, independent of the fish species. Electrochemical impedance spectroscopy in the frequency range of 0.1 Hz–100 kHz has been used to measure carp, herring, and sea bass during storage after death at 4, 15, and 25 °C. It is worth noting that this method offers a simple and rapid *in situ* measurement of the onset of spoilage. The phase angle and admittance changes are the best freshness indicators from which the four categories of freshness may be defined for all the species of fish tested.

Safety Assessment

The main hazardous factors related to human life or health found in fish and other sea food are puffer fish toxin, ciguatera toxin, shellfish toxin, histamine, *Vibrio*, and other pathogenic bacteria. The toxins can be detected by routine biological or chemical methods. This article will deal only with histamine analysis and histamine-forming bacteria.

Histamine

Histamine is well known as the substance causing allergy-like food poisoning after ingestion of scombroid fish that are not chilled adequately between harvest and consumption. Histamine intoxication is, therefore, often called scombroid poisoning and is derived from free histidine in fish muscle released by histidine decarboxylase originating from histamine-forming bacteria. The main symptom of food poisoning due to histamine is an itchy rash called urticaria. The worldwide network for harvesting, processing, and distribution of fish and fish products has made scombroid poisoning a global problem. In fact, scombroid poisoning is now the most common form of seafood-borne disease in the US. Mackerel, bonito, and tuna contain high levels of histidine and consequently are susceptible to the accumulation of histamine with subsequent histamine poisoning. The problem with histamine in seafood is quite serious even today, but the details of the production and diffusion of histamine in scombroid fish are well known from the research of Tao *et al*.

As histamine is rather easy to detect and estimate, the histamine content is specified in standard regulations and is used as an index for freshness, quality, and danger of scombroid poisoning. The United States Food and Drug Administration's (FDA's) 'Fish and Fishery Products Hazards and Controls Guide' states that histamine is generally not uniformly distributed in a decomposed fish. If 50 ppm is found in one section, it is possible that other sections may exceed 500 ppm. The FDA has, therefore, specified a safe maximum limit of 50 ppm. The biggest problem is the methodology for histamine determination. When determining histamine in fish, it is very important to separate the histamine completely from the very large amounts of interfering compounds, such as free histidine and carnosine. Therefore, careful and tedious pretreatment is required to avoid contamination with interfering compounds.

Recently, two rapid methods based on an enzyme oxygen sensor assay and enzyme-linked immunosorbent assay have been devised. The first-mentioned method measures the decrease in oxygen concentration in the reaction mixture and uses an antigen–antibody reaction. Both methods can obtain results within 30 min and determine whether a sample contains more or less than 50 ppm histamine, which is the current FDA action level. These tests have several advantages over existing test protocols as they can be evaluated visually. They are also insensitive to salt content, which is a problem with other tests. The drawbacks of these methods include the expensive instruments and high operating costs due to the use of enzyme kits.

A simpler method has been developed on the basis of a one-step derivation of histamine by Pauly reagent and HPLC separation. However, this method requires an expensive instrument and a skilled operator. Recently, an even simpler rapid method has been developed for histamine analysis

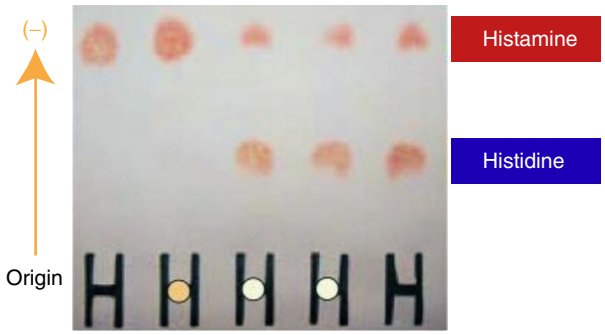

Figure 5 Electrophoretic separation of histamine and histidine.

based on paper electrophoretic separation and Pauly reagent development.

Determination of histamine by paper electrophoresis: The 'Histamine checker'

Sato *et al.* developed a new quick and simple analytical method for histamine determination in fish and other seafood. It consists of sample extraction, adsorption on a paper disc, application of the paper disc on electrophoresis paper, electrophoresis for only 5 min, drying, and color developing by Pauly reagent. Histamine can be satisfactorily detected and completely separated from histidine, carnosine, and other Pauly reagent-positive compounds (Figure 5). This method does not need expensive instrumentation or any tedious pretreatment to eliminate potential interference from other imidazole compounds, such as histidine or carnosine. It can be used to detect histamine in multiple fish and seafood samples simultaneously, detecting as little as 15 ppm histamine, with an absolute minimum detection limit of approximately 10 ng histamine on one spot. The merits of the method are that it is rapid, simple, and can determine several samples simultaneously. It is sold as the Histamine Checker from QS-Solution.

Microbiological Methods

Microbiological methods assess freshness and hygienic quality by measuring the total count of bacteria or sometimes the number of spoilage bacteria or pathogenic bacteria. Total counts vary according to the incubation conditions. The nutrient medium for seawater bacteria requires addition of 2.5% sodium chloride compared with the medium for freshwater bacteria. It is proposed that a tentative total bacterial number for acceptance should be 10 million organisms per gram. However, it should also be noted that there is no correlation between the total count and the presence of bacteria of public health significance. There are many problems related to traditional microbiological examination, such as tedious pretreatment, requirement for skill in execution and interpretation of the results, time-consuming incubation, and so on. To resolve these problems, several new methods have been developed.

Direct count by phase contrast microscopy or fluorescence microscopy is very rapid. The staining of cells with acridine orange and detection by fluorescence microscopy have earned widespread acceptance as the direct epifluorescence filter technique. The main disadvantages with direct counting are sensitivity and inaccuracy, which result from the inclusion of both live and dead bacteria.

Indirect estimation of microorganisms has been proposed, such as measurement of bacterial ATP, deoxyribonucleic acid, carbon dioxide released by bacteria, heat generated by bacteria, conductance, capacitance, and impedance. These methods can provide rapid results, but the main disadvantage is a requirement for efficient separation of bacteria from the fish flesh being tested.

Quick detection and counting of histamine-forming bacteria

Histamine-forming bacteria (HFB) can be detected simply and rapidly by filtration with two-layer membranes and membrane incubation, followed by microscopic observation. Liquid samples, including seawater and seafood, can be assayed on-site using this method. Liquid samples are filtered through a two-layer filtration system, using a pair of circular (25 mm diameter) membrane filters (upper 10 μm mesh, lower 0.2 μm). For seafood samples, the flesh is minced with nine volumes (v/w) of sterilized saline solution under aseptic conditions, kneaded inside a plastic sachet, and then the soluble fraction is filtered by the above method. The membrane filter set is then rinsed with 10 ml sterilized saline solution.

The lower 0.2 μm membrane filter is placed on artificial seawater agar medium (pH 5.8) containing histidine (0.5%), with bromothymol blue (0.04%) as a pH indicator, and incubated at 35 °C (Figure 6). HFB are detected as colonies surrounded by a blue halo on the 0.2 μm membrane filter after 5 h of incubation (Figure 7). The color change in the agar medium can be attributed to an alkaline shift after histamine formation by HFB. Histamine production ability by colonies with a blue halo is confirmed by prolonged incubation in histidine broth medium at 35 °C for 24 h. This is a fairly rapid and simple method for detecting HFB and can be applied to food hygiene systems, such as hazard analysis and critical control point in seafood processing lines.

The colony number can be calculated as follows. With an optical microscope using an 18 mm eyepiece lens and a 10× objective lens, the area of optical view is 2.543 mm^2 (of course, this must be calculated for the particular microscope and lens combination being used). The valid filtration area is 210 mm^2. The average number of colonies (originally single organisms from the food being tested) can be calculated by summing the counts in several fields, and the mean number of colonies per gram of sample can be calculated as in the following eqn [4]:

$$\text{Colonies per g of sample} = \frac{\text{Mean number of colonies} \times 210 \times \text{dilution ratio} \times \text{dilution volume (10 ml)}}{2.543 \times \text{Sample weight (g)} \times \text{filtered volume (1 ml)}} \quad [4]$$

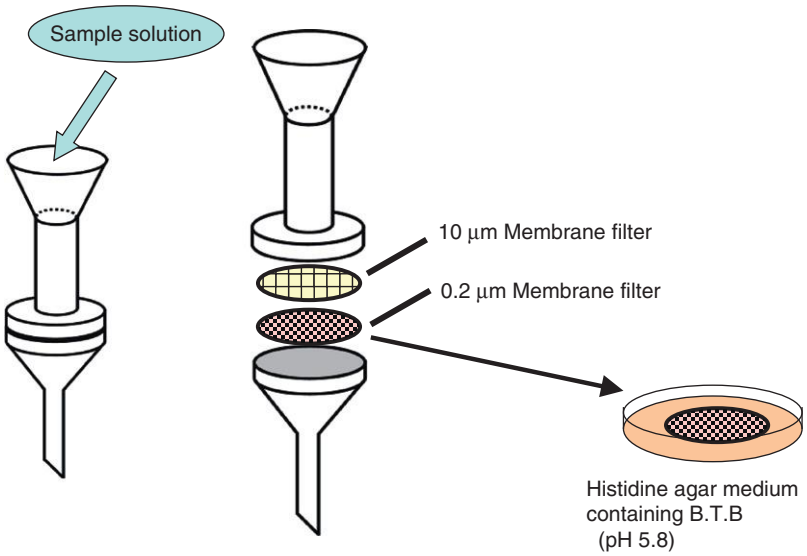

Figure 6 Outline of the detection of histamine-forming bacteria.

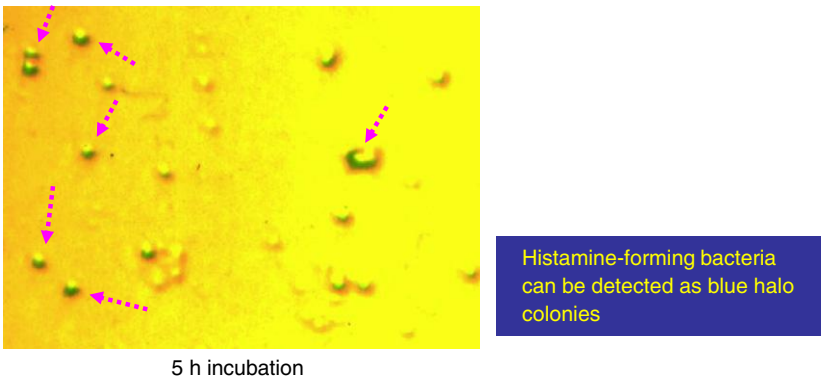

Figure 7 Detection of histamine-forming bacteria by an optical microscope (100×). Reproduced from Tao, Z., Sato, M., Abe, N., Yamaguchi, T., Nakano, T., 2009. Simple and rapid detection of histamine-forming bacteria by differential agar medium. Food Control 20, 903–906.

See also: Chemical Analysis: Physicochemical Analysis Methods; Raw Material Composition Analysis; Sampling and Statistical Requirements. Chemical and Physical Characteristics of Meat: Chemical Composition; Palatability; pH Measurement; Water-Holding Capacity. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Microbiological Analysis: DNA Methods; Indicator Organisms in Meat; Standard Methods. Microbiological Safety of Meat: *Bacillus cereus*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Viruses; Yeasts and Molds. Species of Meat Animals: Finfish; Shellfish. Spoilage, Factors Affecting: Microbiological; Oxidative and Enzymatic. Stunning and Killing of Farmed Fish: How to put It into Practice?

Further Reading

Ando, M., Toyohara, H., Shimizu, Y., Sakaguchi, M., 1991. Post-mortem tenderization of fish muscle proceeds independently of resolution of rigor mortis. Nippon Suisan Gakkaishi 57, 1165–1169.

Bito, M., Yamada, K., Mikumo, Y., Amano, K., 1983. Studies on rigor mortis of fish—I. Difference in the mode of rigor mortis among some varieties of fish by modified Cutting's method. Bulletin of Tokai Regional Fishery Research Laboratory 109, 89–96.

Bremner, H.A., 2002. Understanding the concepts of quality and freshness in fish. In: Bremner, H.A. (Ed.), Safety and Quality Issues in Fish Processing. Boca Raton, FL: Woodhead Publishing Limited and CRC Press, pp. 163–172.

Frank, J.F., 2001. Microbial attachment to food and food contact surfaces. In: Taylor, S. (Ed.) Advances in Food and Nutrition Research 43. San Diego, CA: Academic Press, pp. 319–370.

Furlong, C.E., Soelberg, S.D., Baker, P.E., *et al.*, 2008. Antibody-based assays for toxins in fish and shellfish. In: Proceedings, Joint Annual Meeting of the NSF/NIEHS Oceans and Human Health Centers and the NOAA Oceans and Human Health Initiative 16–18 April, pp. 65–76. Honolulu, Hawaii. Available at: http://www.seattlesensors.com/science.html (accessed 08.09.13).

Gill, T., 2000. Nucleotide-degrading enzymes. In: Haard, N.F., Simpson, B.K. (Eds.), Seafood Enzymes. New York: Marcel Dekker, pp. 37–68.

Gopakumar, K., 2000. Enzymes and enzyme products as quality indices. In: Haard, N.F., Simpson, B.K. (Eds.), Seafood Enzymes. New York: Marcel Dekker, pp. 337–363.

Grigor, J.M., Theaker, B.J., Alasavar, C., O'Hare, W.T., Ali, Z., 2002. Analysis of seafood aroma/odour by electronic nose technology and direct analysis. In: Alasalvar, C., Taylor, T. (Eds.), Seafoods – Quality, Technology and Nutraceutical Applications. Berlin: Springer, pp. 105–1121.

Haard, N., 2002. The role of enzymes in determining seafood colour, flavour and texture. In: Bremner, H.A. (Ed.), Safety and Quality Issues in Fish Processing. Boca Raton, FL: Woodhead Publishing Limited and CRC Press, pp. 220–253.
Huss, H.H., 1995. Quality and changes in fresh fish. FAO Fisheries Technical Papers – T348. Rome: FAO/UN.
Martinsdottir, E., 2002. Quality management of stored fish. In: Bremner, H.A. (Ed.), Safety and Quality Issues in Fish Processing. Boca Raton, FL: Woodhead Publishing Limited and CRC Press, pp. 360–378.
Mavromatis, P., Quantick, P., 2002. Histamine toxicity and scombroid fish poisoning: A review. In: Alasalvar, C., Taylor, T. (Eds.), Seafoods – Quality, Technology and Nutraceutical Applications. Berlin: Springer, pp. 89–103.
Nesvadba, P., 2002. Quality control by instrumental texture measurements. In: Alasalvar, C., Taylor, T. (Eds.), Seafoods – Quality, Technology and Nutraceutical Applications. Berlin: Springer, pp. 43–57.
Niculescu, M., Frebort, II, Nistor C., *et al*., 2000. Redox hydrogel-based amperometric bienzyme electrodes for fish freshness monitoring. Analytical Chemistry 72, 1591–1597.
Nilsen, H., Esaiassen, M., Heia, K., Sigernes, F., 2002. Visible/near-infrared spectroscopy: A new tool for the evaluation of fish freshness? Journal of Food Science 67, 1821–1826.
Niu, J., Lee, J.Y., 2000. A new approach for the determination of fish freshness by electrochemical impedance spectroscopy. Journal of Food Science 65, 780–785.
Pettipher, G.L., Rodrigues, U.M., 1982. Rapid enumeration of microorganisms in foods by the direct epifluorescent filter technique. Applied Environmental Microbiology 44, 809–813.
Robb, D., 2002. The killing of quality: The impact of slaughter procedures on fish flesh. In: Alasalvar, C., Taylor, T. (Eds.), Seafoods – Quality, Technology and Nutraceutical Applications. Berlin: Springer, pp. 7–16.
Saito, T., Arai, K., Matsuyoshi, M., 1959. A new method for estimating the freshness of fish. Nippon Suisan Gakkaishi 24, 749–750.
Sakaguchi, M., Murata, M., Kim, J., 1989. The effects of repeated freeze–thaw cycles on Torry meter readings of carp fillets. Nippon Suisan Gakkaishi 55, 1665.
Sato, M., 2008. Simple and quick measurement of fish and meat freshness by Freshness Checker. Brain Techno News 128, 24–29.
Sato, M, Nakano, T, Takeuchi, M, *et al*., 1995. Specific determination of histamine in fish by high performance liquid chromatography after diazo coupling. Bioscience Biotechnology Biochemistry 59, 1208–1210.
Sato, M., Tao, Z.H., Siozaki, K., *et al*., 2006. A simple and rapid method for the analysis of fish histamine by paper electrophoresis. Fisheries Science 72, 889–892.
Shewan, J.M., Macintosh, R.G., Tucker, C.G., Ehrenberg, A.S.C., 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. Journal of the Science of Food and Agriculture 4, 283–298.
Tao, Z., Sato, M., Abe, N., Yamaguchi, T., Nakano, T., 2009. Simple and rapid detection of histamine-forming bacteria by differential agar medium. Food Control 20, 903–906.
Tao, Z.H., Sato, M., Yamaguchi, T., Nakano, T., 2009. Formation and diffusion mechanism of histamine in the muscle of tuna fish. Food Control 20, 923–926.
US Food and Drug Administration, 1998. Fish and Fishery Products Hazards and Controls Guide, second ed. Rockville, MD: FDA, pp. 1–276.

Relevant Website

http://www.qs-solution.jp/english/index.html
QS-Solution web page.

FOODBORNE ZOONOSES

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Glossary

Foodborne Disease Sickness that is carried or contracted by eating food or water.

Gastroenteritis Inflammation of the lining membrane of the stomach and the intestines characterized especially by nausea, vomiting, diarrhea, and cramps.

Hemolytic uremic syndrome (HUS) A disorder that usually occurs when an infection in the digestive system produces toxic substances that destroy red blood cells causing kidney injury.

Ready-to-eat Food products that are prepared in advance and can be eaten as sold.

Zoonoses Diseases and infections that are naturally transmitted between vertebrate animals and humans.

Introduction

Diseases caused by foodborne agents constitute a worldwide public health concern due to the huge volume of food being produced and consumed throughout the world on a daily basis. In industrialized countries, up to 10% of the human population may annually suffer from foodborne zoonoses, but the real incidence is likely to be much higher. This scenario is probably worse in developing countries. The Centers for Diseases Control and Prevention (CDC-USA) estimates that each year, 1 in 6 Americans (or 48 million people) fall ill, 128 000 are hospitalized, and 3000 die of foodborne diseases. According to data from countries of the European Union, more than 320 000 human cases are confirmed each year of foodborne zoonoses.

Definition

Zoonoses are infections and diseases that are naturally transmissible directly or indirectly, for example via contaminated foodstuffs or from animals to humans. Nevertheless, foodborne zoonotic diseases are caused by consuming food or drinking water contaminated not only by pathogenic (disease-causing) microorganisms such as bacteria and their toxins, viruses, and parasites, but also by prions. The severity of these diseases in humans varies from mild symptoms to life-threatening conditions.

Infective Pathways

There are different transmission routes by which zoonotic agents can be introduced into a food supply chain, especially with regard to the meat processing. The main route is established when an animal becomes contaminated with a pathogen, which may or may not produce clinical signs of disease, and is processed with the food, for example, raw meat cuts. The pathogen is transferred to the product, and if it is consumed without adequate handling or cooking, disease might occur.

Zoonotic Agents Associated with Meat

Meat can be derived from a variety of birds, reptiles, and mammals, especially cattle, swine, sheep, goats, and poultry (chicken, duck, and turkey). Meat animal carcasses and meat cuts can be easily contaminated during processing and, if not properly handled, processed, and properly preserved, they can support the growth of and serve as sources of different spoilage and pathogenic microorganisms.

A variety of sources can contribute to microbial contamination during harvesting, chilling, and cutting processes, when the muscles and viscera of animals are exposed to the environment. Contamination sources include air, water, soil, feces, feed, intestines, processing equipment, utensils, and humans.

Activities involved in the process of animal slaughtering and carcass dressing can result in contamination of the exposed cut surfaces of muscle tissue by both Gram-negative and Gram-positive bacteria, as well as yeasts, molds, and viruses. The most important microorganisms associated with meat are listed in Table 1 and the following sections describe some of the most frequent zoonoses according to European Food Safety Authority (EFSA) and CDC (USA).

Campylobacter

Campylobacter species are pathogens associated with human gastroenteritis in both developed and developing countries. Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp. and the infective dose of these bacteria is generally quite low (less than 100 viable organisms). The species most commonly associated with human infection is *Campylobacter jejuni*, followed by *Campylobacter coli* and *Campylobacter lari*, but other *Campylobacter* species are also known to cause human infection.

The most common disease associated with *C. jejuni* and *C. coli* in humans is an acute inflammatory enteritis following an incubation period of approximately 3 days (ranging from 18 h to 8 days). The disease results in cramping and profuse

Table 1 Zoonotic agents associated with meat contamination and possible occurrence of foodborne zoonoses

Bacteria	*Yeast*	*Molds*[b]	*Virus*	*Other*
Campylobacter	*Candida*	*Aspergillus*	Norovirus	Prions[c]
Salmonella spp.	*Saccharomyces*	*Penicillium*	Hepatitis	
Clostridium perfringens	*Cryptococcus*	*Fusarium*		
Verotoxin-producing *Escherichia coli* (VTEC)	*Pichia*			
Listeria monocytogenes	*Rhodotorula*			
Yersinia enterocolitica	*Trichosporon*			
Clostridium botulinum	*Debaryomyces*			
Bacillus cereus[a]				
Staphylococcus aureus[a]				
Clostridium difficile				
Enterococcus sp.				

[a]Pathogens that produce toxins responsible to cause disease.

[b]Mycotoxin-producing agents responsible to cause foodborne zoonoses.

[c]Infectious protein particles similar to a virus, but lacking nucleic acid that is considered to be the agent responsible for scrapie and other degenerative diseases of the nervous system.

diarrhea accompanied by fever, headache, dizziness, or myalgia. In rare cases, after an infection caused by *Campylobacter* spp., some serious diseases have been reported, for example, Guillain–Barré syndrome (the most common paralytic disease in the USA) and Miller-Fisher syndrome.

This pathogen is widespread, and present in many farm animals; poultry is very susceptible to colonization with high numbers of *Campylobacter*. The average incidences of *Campylobacter* spp. in animals and foods, according to different studies, were approximately 33% for live chickens; 53% for chicken meat; 56% for turkey meat; 32% for geese, ducks and other fowl; 45% for cattle; 6% for beef cuts; and 27% for pork and pork meat.

According to EFSA, campylobacteriosis has remained the most frequently reported zoonotic disease in humans in the European Union (EU) since 2005. Overall, 212 064 confirmed cases of this disease were reported in 2010, which represents an increase of 6.7% compared to 2009. The overall notification rate of human campylobacteriosis was 48.6 per 100 000 population. As in previous years, children under the age of 5 had the highest notification rate (126.8 per 100 000 population). However, the case–fatality rate for human campylobacteriosis was low (0.22%).

According to the last report of the CDC (USA), the estimate was that 6365 cases of acute gastroenteritis due to campylobacteriosis infection occurred in 2010. The overall notification rate of human campylobacteriosis in 2010 was 13.6 per 100 000 population.

Salmonella *spp.*

Salmonella is one the most important species of the Enterobacteriaceae family and this genus is divided into two different species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is subdivided into the subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. Additionally, *Salmonella* is further divided into serotypes using the serotyping scheme developed by Kaufmann and White, for example, *S. enterica* subsp. *enterica* serovar Derby (or *Salmonella* Derby). There are more than 2600 serovars of zoonotic *Salmonella* and the prevalence of the different serovars changes over time.

Salmonella has long been recognized as an important zoonotic pathogen of economic significance in animals and humans. Salmonellosis is usually characterized by the acute onset of fever, abdominal pain, nausea, diarrhea, and sometimes vomiting, after an incubation period of 12–36 h. Symptoms are often mild and most infections are self-limiting, lasting for a few days. However, in some patients, the infection can be more serious and the associated dehydration can be life threatening. When *Salmonella* causes systemic infections, such as septicemia, effective antimicrobials are essential for treatment. Mortality is usually low, and less than 1% of reported *Salmonella* cases have been fatal.

According to EFSA, in 2010, a total of 99 020 confirmed cases of human salmonellosis were reported in the EU. This represents a decrease of 8.8% over the previous year. The EU notification rate for confirmed cases was 21.5 cases per 100 000 population. The case–fatality rate of human salmonellosis in that year was 0.13%. Detection of *Salmonella* was reported from a wide range of foodstuffs, but the majority of data were from various types of meat and meat products. The highest proportions of *Salmonella*-positive units were reported for fresh broiler meat and fresh turkey meat, at average levels of 4.8% and 9.0%, respectively. In fresh pig meat, 0.9% of tested samples were found positive for *Salmonella* and, in the case of fresh bovine meat, 0.2% of sampling units were positive.

The CDC (USA) has estimated that 8256 cases of salmonellosis occurred in 2010. The overall notification rate of human infection due to *Salmonella* in 2010 was 17.6 per 100 000 population with a case–fatality ratio of 0.4%.

Clostridium perfringens

Clostridium perfringens is an important pathogen of human gastrointestinal (GI) tract and can cause food poisoning, antibiotic-associated diarrhea, and sporadic diarrhea. The most important toxin made by this bacterium when in the human GI tract is *Clostridium perfringens* enterotoxin (CPE).

Although ubiquitous in the environment, only a small subpopulation of *C. perfringens* (usually less than 5%) harbors the CPE gene (*cpe*). The number of *C. perfringens* cells

(*cpe*-positive and *cpe*-negative strains) present in most nonoutbreak food samples is generally lower than 10 cfu.

Clostridium perfringens foodborne disease is third in incidence among foodborne illnesses in the United States, causing about 965 958 cases annually. Beef, poultry, gravies, and dried or precooked foods are common sources of *C. perfringens* infections. Deaths are uncommon, but might occur in the elderly, debilitated, or people otherwise predisposed to disease.

The key event for disease is the mishandling of foods from preparation to consumption, observed especially for poultry and beef. Failure to refrigerate after cooking can provide an opportunity for germination of spores and multiplication of vegetative cells. When this food is consumed, vegetative cells sporulate in the gut producing and releasing CPE in the process. The resulting diarrhea and cramping are solely due to the effect of CPE; illness is typically brief and self-limiting with symptoms typically resolved within 24 h.

Escherichia coli

Among the different groups of *Escherichia coli* present in animals and foods, the most important as a zoonosis is the shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC), a group characterized by the production of a shiga-like toxin. Many serogroups of *E. coli* have the potential to cause STEC/VTEC-associated illness, but the predominant serogroups in clinical cases are O45, O26, O91, O103, O111, O121, O145, and O157. STEC/VTEC infection is one of the most important forms of foodborne illness as it can lead to serious and sometimes fatal complications. Acute symptoms are generally self-limiting and include abdominal pain, watery diarrhea, which may become bloody, and sometimes vomiting. Fever is either mild or absent. Additional complications can occur, including hemolytic uremic syndrome (HUS, mainly in children) and thrombocytopenic purpura (mainly in adults). Approximately 5% of STEC/VTEC infections result in HUS, and mortality from HUS is around 10%. Human infection can be acquired through the consumption of contaminated food or water, or by direct transmission from person to person, or from infected animals to humans.

Bovine meat is considered a major source of foodborne STEC/VTEC infections for humans, although other forms of transmission have been identified. In 2010, 8566 bovine meat units were tested in Europe and 0.5% was found to be STEC/VTEC-positive and 0.1% STEC/VTEC O157-positive. The EU disease notification rate in that year was 0.83 per 100 000 population, a slightly higher rate than in 2009 (0.75 per 100 000 population).

Escherichia coli O157:H7 has been studied more than any other serotypes of STEC/VTEC as it was the first recognized causative agent involved in foodborne outbreaks resulting in severe cases of disease and some deaths in children. It appears that STEC/VTEC serogroups other than O157:H7 might be responsible for up to 50% of VTEC illness in both Canada and the United States. The uncertainty about the real incidence of non-O157 STEC/VTEC in foods is due to a lack of routine testing for these organisms, as well as difficulties in finding good markers to identify and distinguish them. *Escherichia coli* O157:H7, however, presents two unique physiological characteristics (non-sorbitol fermenting and β-D-glucuronidase negative) not shared by other STEC/VTEC and this can facilitate its identification.

Listeria monocytogenes

The bacterial genus *Listeria* currently comprises eight species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes*. The risk of contracting listeriosis is high for immunocompromised persons, the elderly, pregnant women, and neonates. Thirteen serotypes of *L. monocytogenes* have been identified, but only three serotypes (1/2a, 1/2b, and 4b) are associated with the majority of outbreak or sporadic cases of listeriosis.

Listeria species are organisms that are widely distributed in the environment, especially in plant matter and soil. The principal reservoirs of *Listeria* are soil, forage, and water; other reservoirs include infected domestic and wild animals. The main route of transmission to both humans and animals is believed to be through consumption of contaminated food or feed. Cooking at temperatures higher than 65 °C destroys *Listeria*, but the bacteria are known to survive under adverse conditions, forming biofilms and multiplying at temperatures as low as 4 °C. Cross-contamination of the product can occur after the killing steps, and *L. monocytogenes* occurrence in ready-to-eat (RTE) foods with a relatively long shelf life is of particular concern.

Listeria monocytogenes infection might result in a wide range of clinical symptoms. Infection can be noninvasive (gastroenteritis, fever) or invasive. The latter can lead to influenza-like symptoms, meningitis, central nervous system damage, and, in pregnant women, fetal infection and/or abortion. Incubation times for invasive infection can be up to 90 days, increasing the difficulty of attributing a particular food vehicle to cases.

Listeria monocytogenes has been recovered from many different foods, and conversely, a variety of different food items such as raw and processed meats, soft cheese, raw milk, hot dogs, seafood, and fresh vegetables and fruits have been linked to both sporadic cases and outbreaks of listeriosis. Pork meat and processed pork products, such as deli meats, have been implicated in *Listeria* outbreaks in European countries during the past decade. *Listeria monocytogenes* is of particular concern in raw, undercooked, or RTE foodstuffs.

Listeriosis cases have been reported mainly in the Northern hemisphere, with very few reports in the Southern hemisphere. A total of 1601 confirmed cases of listeriosis were reported in 2010 by the European Union. As in previous years, elderly persons were especially affected by the disease, with 60.2% of cases occurring in individuals over the age of 65. Overall, a high case–fatality rate of 17.0% was recorded among those cases for which information was available (2009: 16.6%). The EU notification rate was 0.35 per 100 000 population in 2010, which was slightly lower than in 2009 (0.4 per 100 000 population).

The CDC (USA) estimated that 125 cases of listerioses occurred in 2010. The overall notification rate of human listeriosis in 2010 was 0.3 per 100 000 population. However, the case–fatality ratio was approximately 12.8%, the highest among all foodborne zoonoses.

Yersinia enterocolitica

Yersinia enterocolitica is an important foodborne enteropathogen known for causing the disease termed yersiniosis. Clinical manifestations of yersiniosis ranges from mild gastroenteritis to invasive syndromes like terminal ileitis and mesenteric lymphadenitis.

Yersiniosis occurs mostly in young children with symptoms of mild gastroenteritis. However, in elderly persons and in patients with underlying conditions (iron overload, cirrhosis, diabetes, cancer, etc.) systemic forms of the disease are often observed. Symptoms typically develop 4–7 days after exposure and last an average of 1–3 weeks. In older children and adults, right-sided abdominal pain and fever might be the predominant symptoms and can often be confused with appendicitis. Other symptoms such as rash, joint pain, and/or bacteraemia can occur. Infection is most often acquired by eating contaminated food, particularly raw or undercooked pork meat. The bacterium is able to grow below 4 °C making contaminated refrigerated food a probable source of infection.

Even though this species comprises 6 biotypes and nearly 50 serotypes, the most frequently implicated serotype in human disease worldwide is O:3 with almost all strains belonging to biotype 4. Swine are the main reservoir of pathogenic *Y. enterocolitica* strains, harboring them in tonsils and in the oral cavity. The most common route of transmission of yersiniosis is through contaminated water and foods. Data concerning the incidence of *Y. enterocolitica*, and related species in foods, are well documented in many countries throughout the world. In Europe in 2010, 4.2% and 4.1% of pork meat and derived products samples tested positive for *Yersinia* spp. and *Y. enterocolitica*, respectively. Besides that, 6776 confirmed human yersiniosis cases were reported in that region indicating a slight decrease (10%) compared to 2009 ($N=7533$). *Yersinia enterocolitica* was the most common species reported in human cases and was isolated from 91.0% of all confirmed cases.

The CDC (USA) estimated that 159 cases of yersiniosis occurred in 2010. The overall notification rate of human infection due to *Yersinia enterocolitica* in 2010 was 0.3 per 100 000 population.

Norovirus

Viruses are the smallest of all self-replicating organisms, and consist solely of a small segment of nucleic acid encased in a simple protein shell. This definition covers the norovirus and is a good starting point for consideration of viruses of importance for food safety. The family Caliciviridae belongs to the Picornavirus-like superfamily – small, RNA viruses without an envelope. The calicivirus particles measure between 27 and 38 nm and consist of a spherical protein shell and genome of a single strand of approximately 7.6 Kb RNA. The caliciviruses known to infect humans belong to two genera: the genus Norovirus (NoV) and Sapovirus (SaV). The genus NoV is further subdivided into an increasing number of genogroups.

As small and simple organisms, with a small genome, viruses rely to a great extent on the enzymes of the host cell they invade to undergo their replicative cycle. Unlike bacteria, viruses are obligate intracellular species and show tissue tropism, i.e., efficient replication of the human NoVs occurs only in the gastrointestinal tract of humans; replication in other human tissues has not been detected. Although NoV genotypes have been found in beef cattle and swine, none of these animal genotypes have ever been detected in human patients.

Several studies have identified NoV in samples of bovine and swine, demonstrating that NoV strains can be present in livestock. In addition, NoV has also been isolated from retail meat samples, highlighting a possible route for indirect zoonotic transmission of NoVs through the food chain.

NoVs are the major causative agents of classical viral gastroenteritis in all age groups. After a short incubation period of 12–48 h, the disease is characterized by an acute onset of symptoms such as nonbloody diarrhea, vomiting, fever, nausea, weakness, myalgia, headache, and abdominal pain. Hence, the illness is referred to as 'gastric or stomach flu.' A typical NoV infection is self-limiting and treatment focuses on supportive care, prevention, and treatment of dehydration.

A NoV infection often originates from the ingestion of contaminated food or water, or from person-to-person contact. Contamination with small numbers of Nov particles can be enough to cause disease, with as low as 100 particles needed. Infected individuals shed the viruses in stool as well as orally. Aerosolized vomit droplets have also been implicated as a mode of transmission. NoVs (like all viruses) require a living host in order to replicate and are, therefore, incapable of multiplying in food, unlike bacterial pathogens. The CDC (USA) estimated that 23 million cases of acute gastroenteritis due to NoV infection occur every year, which is nearly 60% of all reported foodborne illnesses. Preventing contamination by proper food handling, sanitation, and hygiene is very important in the control of foodborne NoV illness.

Emerging Foodborne Pathogens

Some foodborne diseases are well recognized but considered emerging because they have recently either received more exposure in the news or increased in prevalence. For example, bovine spongiform encephalopathy (BSE), a fatal, transmissible, neurodegenerative disease of cattle, was first discovered in the United Kingdom in 1985. The cause of the disease was traced to an agent related to scrapie in sheep, which contaminated recycled bovine carcasses used to make meat and bone meal additives for cattle feed. In human populations, exposure to the BSE agent (probably via contaminated bovine-based food products) has been strongly linked to the appearance of a new transmissible spongiform encephalopathy of humans called new variant Creutzfeldt–Jakob disease.

Other foodborne pathogens are considered emerging because they are new microorganisms or because the role of food in their transmission has been recognized only recently. Since the description of the genus *Enterococcus* in the 1980s, many taxonomic investigations have resulted in assignment of about 30 species and the two most prominent representatives are *Enterococcus faecium* and *Enterococcus faecalis*. Several species of enterococci can be easily distinguished from other cocci by the ability to grow at 10 and 45 °C, in 6.5% NaCl, in the presence of 40% bile, and at pH 9.6. The enterococci are important in environmental, food, and clinical microbiology because they can be found in the intestines of food animals and can contaminate the meat while slaughtering. In raw meat products (beef and pork cuts), *E. faecalis* and *E. faecium* are the most

predominant species isolated, with mean counts ranging from 10^4 to 10^8 CFU per 100 cm^2.

Enterococcus can not only contaminate raw meats, but they can also be associated with processed meats. Cooking of processed meats might confer a selective advantage on enterococci as these bacteria are known to be among the most thermotolerant of the nonsporulating bacteria. *Enterococcus* are typical opportunistic pathogens and can cause infection in patients that have severe underlying disease or that are immunocompromised. They can cause bacteremia, endocarditis, urinary tract, and other infections. The question whether food strains possess an intrinsic lower pathogenic potential than clinical isolates has still not been fully answered and additional data are needed.

Another emerging foodborne pathogen is the species *Clostridium difficile*, an anaerobic, spore-forming bacterium that can produce toxin A or B on colonization of the gut. Patients at risk for *Clostridium difficile* infection (CDI) subsequently develop diarrhea or, in severe cases, pseudomembranous colitis. Traditionally, elderly and hospitalized patients who had been under antibiotic therapy were considered to be the most vulnerable to CDI. Because food animals can be colonized by *C. difficile*, and the bacteria has been isolated from retail meats, some researchers speculate that *C. difficile* is a food-associated organism and consumption of contaminated meat could be responsible for increased community-associated CDI.

Although the issue of zoonotic transmission of *C. difficile* was raised more than 20 years ago, and the finding of overlapping PCR ribotypes in animals and humans, *C. difficile* isolates have stimulated research in this field and the question of whether zoonotic transmission occurs has not been answered. Circumstantial evidence that *C. difficile* strains from animals were infecting humans (or vice versa) has been reported several times in recent years. Some studies conducted in Europe have persistently reported low prevalence rates, for example in up to 3% of meat samples, in contrast to the United States and Canada, where *C. difficile* is generally reported at much higher rates, for example in up to 42% of meat samples. Further studies are required to provide relevant data on the sources, transmission routes, growth, and survival of *C. difficile* in foods.

There is a question that one must take into account when thinking about foodborne zoonoses: 'Why do foodborne diseases emerge?' New foodborne disease threats occur for a number of reasons including: (1) globalization of the food supply, (2) the inadvertent introduction of pathogens into new geographic areas, (3) travelers, refugees, and immigrants exposed to unfamiliar foodborne hazards while abroad, (4) changes in microorganisms (development of new virulent strains in old pathogens, development of antibiotic resistance or through changes in the ability to survive in adverse environmental conditions), (5) demographic changes in the human population – the population of highly susceptible persons is expanding worldwide because of aging, malnutrition, HIV infections, and other underlying medical conditions, and (6) changes in lifestyle with higher numbers of people eating out (restaurants, canteens, fast food outlets, and street food vendors) or buying preprepared meals.

See also: Animal Health Risk Analysis. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to

Further Reading

Centers for Disease Control and Prevention (CDC), 2011. Vital signs: Incidence and trends of infection with pathogens transmitted commonly through food foodborne diseases active surveillance network, 10 U.S. sites, 1996–2010. Morbidity and Mortality Weekly Report 60, 749–755.

De Wit, M.A., Koopmans, M.P., Van Duynhoven, Y.T., 2003. Risks factors for norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. Emerging Infections and Disease 9, 1563–1570.

Drummond, N., Murphy, B.P., Ringwood, T., *et al.*, 2012. *Yersinia enterocolitica*: A brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. Foodborne Pathogens and Disease 9, 179–188.

European Food Safety Authority, European Centre for Disease Prevention and Control, 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA Journal 10, 260–303.

Fraser, R.W., Williams, N.T., Powell, L.F., Cook, A.J., 2010. Reducing *Campylobacter* and *Salmonella* infection: Two studies of the economic cost and attitude to adoption of on-farm biosecurity measures. Zoonoses and Public Health 57, 109–115.

Gill, A., Gill, C.O., 2010. Non-O157 verotoxigenic *Escherichia coli* and beef: A Canadian perspective. Canadian Journal of Veterinary Research 74, 161–169.

Glatzel, M., Ott, P.M., Linder, T., *et al.*, 2003. Human prion disease: Epidemiology and integrated risk assessment. Lancet Neurology 2, 757–763.

Hammerum, A.M., 2012. Enterococci of animal origin and their significance for public health. Clinical Microbiology and Infection 18, 619–625.

Koopmans, M., Duizer, E., 2004. Foodborne viruses: An emerging problem. International Journal of Food Microbiology 90, 23–41.

Limbago, B., Thompson, A.D., Greene, S.A., *et al.*, 2012. Development of a consensus method for culture of *Clostridium difficile* from meat and its use in a survey of U.S. retail meats. Food Microbiology 32, 448–451.

Motarjemi, Y., Adams, M., 2006. Emerging Foodborne Pathogens, first edition. Washington, DC: CRC Press LLC.

Orsi, R.H., den Bakker, H.C., Wiedmann, M., 2010. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. International Journal of Medical Microbiology 301, 79–96.

Scallan, E., Hoekstra, R.M., Angulo, F.J., *et al.*, 2011. Foodborne illness acquired in the United States – Major pathogens. Emerging Infectious Diseases 17, 7–15.

Songer, J.G., 2010. Clostridia as agents of zoonotic disease. Veterinary Microbiology 140, 399–404.

Songer, J.G., Trinh, H.T., Killgore, G.E., *et al.*, 2009. *Clostridium difficile* in retail meat products. Emerging Infectious Diseases 15, 819–821.

Relevant Websites

http://www.cdc.gov/ncezid/
Centers for Disease Control and Prevention.

http://www.efsa.europa.eu/en/topics/topic/foodbornezoonoticdiseases.htm
The European Food Safety Authority.

http://www.who.int/zoonoses/diseases/foodborne_zoonoses/en/
World Health Organization.

FOREIGN BODIES

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Glossary

Dark field radiology An emerging technology taking advantage of diffraction of X-rays in fibrous matter.
Detectability The size of the smallest foreign body of a given material that is detectable in a specific product with a true probability error of 1%.
Detection limit A true error probability of 1%, which is calculated with a 95% confidence.
Hyperspectral vision A vision-based technology using many narrow spectral bands.
Phase contrast An emerging technology taking advantage of the refraction of X-rays in (soft) matter.

Definition and Implications of Foreign Bodies in Meat – Intrinsic Foreign Bodies

An intrinsic foreign body (FB) originates from the same animal species as the contaminated product does but, by definition, constitutes matter that does not belong in the actual product. Examples of intrinsic FBs (Table 1) could be hair, skin, bone fragments, blood vessels, teeth, or cartilage. Some of these materials could be natural parts of some products but unwanted and subject to complaints in other products. Depending on the definition and quality requirements for a given product, different definitions exist as to which intrinsic materials should be considered FBs.

Contamination with visible amounts of feces, oil, or bile is highly unacceptable to all consumers. Fecal contamination implicates a potential health risk to the consumer and there is zero tolerance to the occurrence of such contamination. Some beef abattoirs detect fecal contamination by fluorescence in UV light from the chlorophyll content of feces. However, the fluorescence method is very costly to establish and effective only when a considerable amount of the feed for slaughtered animals contains substantial amounts of chlorophyll. The method of choice is, therefore, often visual inspection. At high slaughter line speed, visual inspection is problematic. Steam vacuuming of high-risk areas is recommended.

Skin and hair is not acceptable to the consumers. The impact on food safety is generally low, but contamination of cattle carcasses with hair is often followed by dried soil and/or feces, and therefore the zero tolerance principle also includes skin/hair at beef abattoirs. Visual inspection at high slaughter line speed is problematic and, again, steam vacuuming of high-risk areas is recommended.

Teeth, fragments of teeth, and bone fragments can be small and sharp. They can cause harm and unpleasant experiences for the consumer. In high-risk products, detection with X-ray is necessary in order to detect small bone fragments effectively.

The acceptance of cartilage varies and whether or not it is considered critical depends largely on the country or region in which the given product is sold, because food safety is not affected by the occurrence of cartilage. The method of detection is visual inspection.

Blood clots and tissue with visual bruises are generally not considered of importance to the food safety, but several small or one large (more than 15 cm wide) hemorrhage is considered a major quality defect. The method of detection is visual inspection.

Pathological lesions, such as encapsulated abscesses and scar tissue from earlier disease, are generally of relatively low or no importance to food safety. However, they are highly unacceptable to the consumer. Furthermore, some pathological lesions constitute a threat to consumer health. Therefore, the effective detection of pathological lesions is critical. The method is visual inspection.

Table 1 Examples of intrinsic foreign bodies, their general impact on food safety, and the typical method of detection at the production line

Type	*Impact and food safety*	*Detection method*
Feces and bile	High/zero tolerance	Visual inspection
Skin, hair, etc.	High/zero tolerance	Visual inspection
Teeth and bone fragments	High	X-ray
Cartilage	Low	Visual inspection
Blood clots/lesions	Low	Visual inspection
Pathological lesions	High	Visual inspection

Definition and Implications of Foreign Bodies in Meat – Extrinsic Foreign Bodies

An extrinsic FB originates from the environment in which the animal lived, was slaughtered, or from the production line where the meat has been processed and packed.

Examples of extrinsic FBs (Table 2) could be needle parts (due to injections during upbringing), insects, wood, metal, plastic, glass, knives, gloves, and other equipment. The most frequent extrinsic FBs found in meat are forgotten personal equipment or parts of broken machinery, knives, tools,

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Table 2 Examples of extrinsic foreign bodies, their general impact on food safety, and the typical method of detection at the production line

Type	*Impact and food safety*	*Detection method*
Metal/needle parts	High	X-ray/metal detector
Glass	High	X-ray
Concrete/ceramics	High	X-ray
Plastic	Moderate/high	X-ray or inspection
Rubber	Moderate	X-ray
Wood	Moderate/high	Inspection
Soil	Moderate/high	Inspection
Paper	Low	Inspection
Oil from conveyor	Low	Inspection

containers, and other materials in the immediate vicinity of production.

Extrinsic FBs are, in general, highly unacceptable to consumers and often constitute a high risk to consumer health and loss of confidence to the product and manufacturer.

Metal, such as needle parts from syringes, knives, parts of broken knives, or other metal, is highly unacceptable for the consumer and poses a serious risk for injuries. Application of detectable needles with a high content of magnetic alloys is recommended. Metal detectors or X-ray facilities should be used to detect metal parts in meat and meat products.

Presence of glass is critical to consumer health and is very difficult to detect visually when mixed into a product. Therefore, glass should never be present in production and packing facilities. Lamp glass and neon tubes should be repaired outside production hours and all broken glass be collected and secured effectively if accidents happen. Glass can be detected by X-ray.

Concrete and ceramic contamination typically arises from pieces of wall, ceiling, or floor that fall undetected into a product as a result of some kind of accident during production. Concrete and ceramics are unacceptable, pose risks to consumer health, and can be detected by X-ray. However, concrete or ceramics that are broken down to gravel particles may not be detected by X-ray.

Plastic and rubber are used for personal equipment, such as gloves or aprons, containers, tools, conveyor belts, and machine parts during production of meat and meat products. Some types of hard plastic can produce sharp fragments that constitute a risk to consumer health. Transparent types of plastic may even not be visible in the products. Soft plastic or rubber are generally of low or no importance to food safety but are unacceptable to the consumer. Rubber and some types of plastic can be detected by X-ray. However, plastic types that cannot be detected by X-ray are widespread, including polyethylene (PE) aprons that have the comparable X-ray absorption to fat.

Wood is generally only allowed in production facilities where all products are packaged. Wooden fragments are generally of moderate importance to food safety but are unacceptable to the consumer. Wood can be detected only by visual inspection.

Soil is generally of moderate importance to food safety, but soil contains massive amounts of diverse environmental bacterial flora. Further, soil occurring in the vicinity of animals raised for meat is often mixed with fecal matter and should be treated as such. Visual inspection is the only detection method for small soil particles. However, larger lumps of dirt may be detected by X-ray.

Clean paper is of low importance to the food safety but unacceptable to the consumer. Paper in production facilities should be avoided. Paper can be detected by visual inspection.

Oil from the conveyor is a frequent contamination risk. Only oil that is safe to consume should be used. Oil contamination is detected by visual inspection.

How to Assess the Risk of Foreign Bodies in a Production

In general, whether an FB constitutes a food safety risk or just an impairment of quality, the problem should be handled under a processing plant's Hazard Analysis and Critical Control Point (HACCP) or Quality Analysis of Critical Control Points plans, respectively.

Legislation and Customer Demands

In general, legislation states that physical hazards are not allowed to constitute any risk to consumer health. Good manufacturing practice must be observed. Potential risks must be assessed. Where relevant, the risk must be a critical control point. Apart from these general guidelines, the legislation on FBs is quite sparse. However, major retail chains and other major customers often set much more specific requirements including choice of detection technology, occurrence frequency of certain acceptable foreign materials (mainly intrinsic in origin), control/calibration of the required technical solutions, and handling of any contaminated product.

The International Food Standards (IFS Version 6, Jan 2012) include FB management as one of ten Knock Out requirements and correspondingly the British Retail Consortium (BRC Version 6, Jan 2012) includes details on management and handling of (mainly extrinsic) foreign material.

Technologies for Detection of Foreign Bodies in Meat

X-Ray Scanning and Metal Detection

When preventative measures fail, a technical screening system may be appropriate. Many commercial suppliers of screening systems are available. The two main techniques for screening systems are shown in Figure 1.

The metal detectors show good sensitivity to ferromagnetic types of FB and, to some extent, to some other metals like stainless steel and aluminum. X-ray systems are more costly than metal detectors (by approximately 5–10 times) but provide very sensitive screening to a broader range of FB materials like glass, metals, bones, polyvinyl chloride, and stones. Detailed technical descriptions may be found in manufacturers' literature. In this context, focus will be on some general aspects relevant to technical screening systems.

It is important to realize that no single technical solution is applicable for all types of foreign objects. As part of the

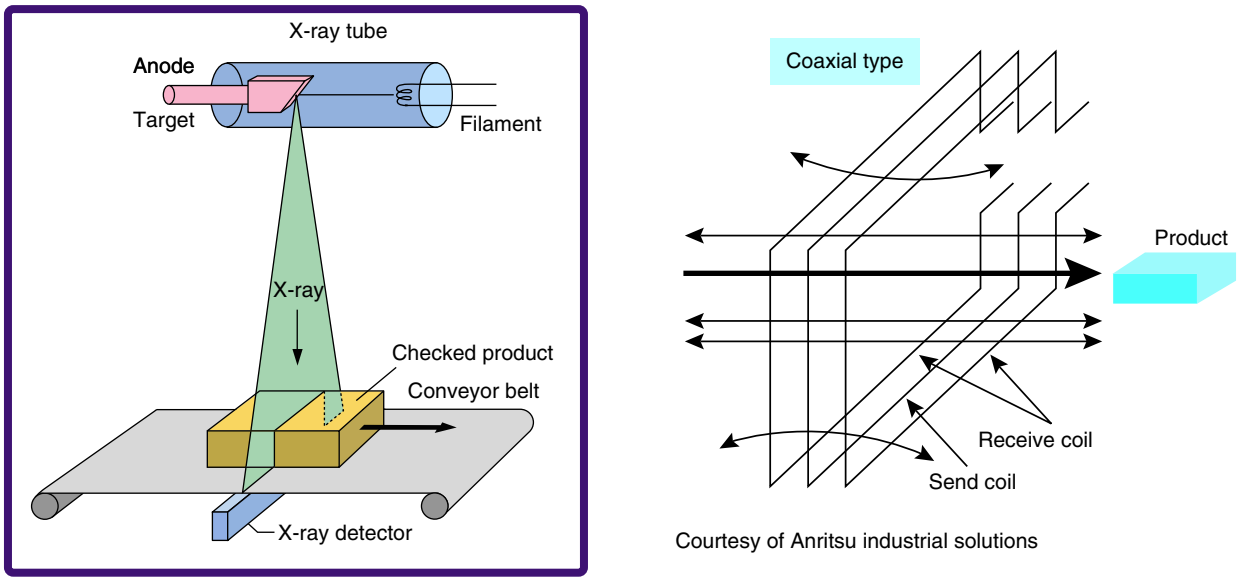


Figure 1 Techniques for screening meat and meat products for foreign objects. To the left, an X-ray system and to the right, the general principles of metal detection, provided with balanced receiver coils.

HACCP analysis, one important task is to identify the hazard level for the relevant types of FB for a specific product. The result of this determination will reveal the primary types of FB. Another important issue is that the customer should agree on the result of the analysis and the agreement should include a level of test for FBs in the product to be delivered. The agreement should include determination of the largest acceptable size of all relevant FBs and how these determinations are made and documented.

A detector signal difference between a 'clean' and a contaminated product is defined as the contrast. All detection technologies rely on a contrast between the meat product and the primary foreign objects in order to identify those objects. Thus, the challenge for the producer is to establish the best contrast situation by proper choice of technology and setup for the primary types of FB.

X-Ray Contrast

The contrast in X-ray systems originates from the difference in absorption of the X-rays by the pure meat product and the FB. As the absorption is depending on sample thickness and tissue composition, the contrast of a given FB varies with each meat product. Thus, it is not possible in general to 'extrapolate' the detection performance from product to product.

The absorption of X-ray energy is proportional to the atomic number and the density of the material. Values for a selection of materials are given in Table 3.

The contrast may be expressed on a scale normally applied in the medical field of computerized tomography (CT) scanning, the Hounsfield scale. This scale is based on the linear attenuation coefficient of X-rays in water. Water is defined to assume a reading of zero Hounsfield units (HU), see below. Another reference point is air, which is defined as a −1000 HU reading. All types of biological tissue and pure elements may then be expressed on the scale (in HU) through the linear attenuation coefficient.

Table 3 Linear attenuation coefficient and Hounsfield value for some materials relevant to meat production. The difference in Hounsfield units (here calculated at 50 keV) reveals the potential contrast of Foreign bodies materials to meat and fat tissue

Material	*HU range*	μ_m *(50 keV)* cm^{-1}
Fat	−180 to −20	0.198
Muscle	20 to 180	0.238
Bone	600 to >2000	0.38
Iron	>60 000	15.417
Acrylic	88	0.247
Polyvinyl chloride	1825	0.641
Teflon	1114	0.480
Polyethylene	−146	0.194
Polystyrene	−72	0.211
Blood	64	0.241
Glass	1970	0.674
Aluminum	3379	0.994
Concrete	2459	0.785
Calcium[a]	5961	1.579
Water	0	0.227

[a]Can exist only as part of various compounds (CaOH, hydroxyapatite, etc.).

The data calculated at 50 keV, shown in Table 3, express the theoretical potential detection of different contaminants when compared with meat and fat attenuation, either expressed in HU or as a linear attenuation coefficient. It may be deduced that poly(methyl methacrylate) PE, and polystyrene (PS) should be avoided in a meat-producing facility, because these polymers have a very subtle contrast to fat and meat tissue, compared with teflon, which is much more detectable.

To estimate the contrast to a small FB, the total attenuation of the radiated beam has to be integrated along the path length

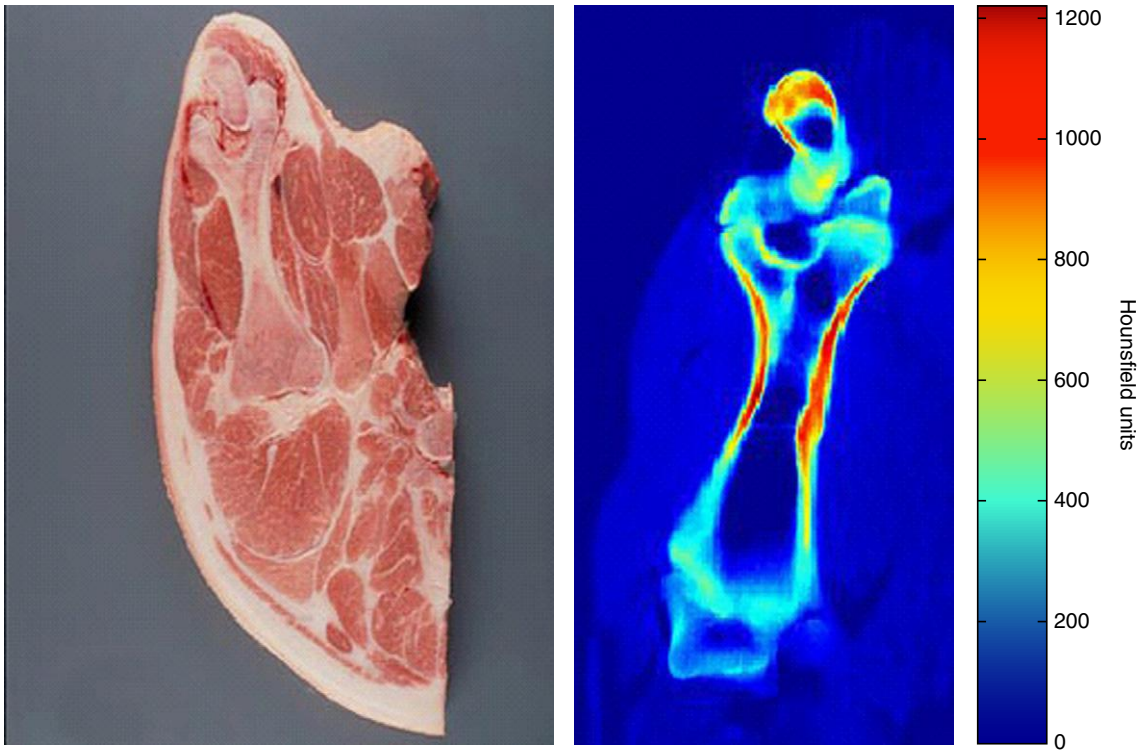


Figure 2 CT scan of porcine bone (humerus) at 120 keV. It is clearly seen that the density expressed in HU varies within the bone, from less than 200 to more than 1000 HU. Please note that negative HU readings are set to zero for reasons of clarity.

through the product using the following formula:

$$\frac{I}{I_0} \cong \exp(-(\mu_m x_m + \mu_{FO} x_{FO}))$$

The emitted intensity I_0 is attenuated to a level I during transition through a thickness x_m (cm) of material with a linear attenuation coefficient of μ_m (exp is the exponential function). For example, 15 cm of pure muscle and a 2-mm thick piece of iron FB in a 50 keV screening system. The meat will reduce the intensity to 2.8% of the emitted radiation. Inclusion of the iron object reduces the intensity further to 0.13% or a contrast ratio of approximately 6.7 dB. This ratio is sufficient for an automatic algorithm to detect in an online system.

Now change the iron object to a piece of glass of the same dimension. The 15 cm of meat leads to the same 2.8% attenuation as previously and the glass reduces the intensity further to 2.45%, leading to a contrast of a little more than 0.5 dB. A contrast of this level is somewhat more challenging to detect using automatic means. If the meat is now substituted by adipose tissue, the two contrast ratios can be recalculated to be 13 and 0.7 dB for iron and glass, respectively. Thus, the contrast between both types of FB and the background material increases when the background material changes from a high attenuating type of tissue, such as meat, to a low attenuating type, such as adipose tissue or fat. When the product thickness decreases, the contrast improves further.

One other important type of FB in meat is bone. Human bone has a reading on the Hounsfield scale in the range 600–1000 HU. This range indicates that bone material is not a fixed material with respect to X-ray attenuation and this principle can be extended to animal bones, even though they are not directly comparable to human. Furthermore, the trabecular part of bone forms a continuous range of density (mineral content and porosity) in the transition between dense cortical bone material and pure cartilage, with HU readings from 600 to 180 (Figures 2 and 3).

Owing to the cutting processes, the trabecular part is a very important FB as the end parts of bones are most frequently present in the raw material of many meat products. This fact presents a severe challenge to conventional X-ray systems. Recently, however, dedicated low-energy X-ray systems, working at 15–25 kV, have demonstrated encouraging results on cartilage detection; an example is shown in Figure 4.

The cost of increased contrast in lower energy ranges is an increase in the total absorption in the meat product, thus low-energy scanning systems are required to have more penetrating X-ray power.

Electromagnetic Contrast

Although it is not as obvious as for X-rays, the concept of contrast in the electromagnetic screening systems is still the difference in detection signal between a pure (clean) product and the signal when placing a FB of a given size into the product.

Metal detectors may assume many preferred embodiments, but all exploit the electric and magnetic properties of FB material.

The general principles are discussed according to the illustration shown in Figure 5.

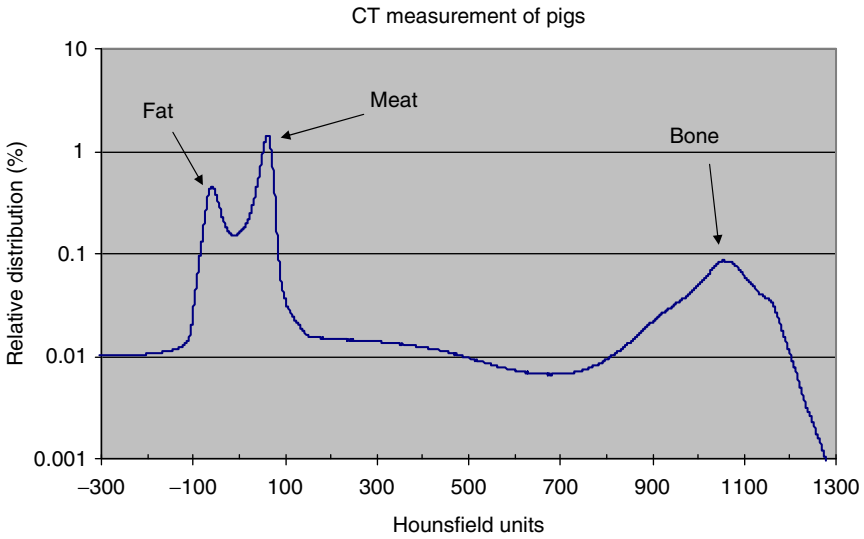


Figure 3 CT measurements of pig carcasses illustrate very clearly the continuous range of HU values between 'bone' and meat tissues. The relative number of voxels (volume-pixels) of each HU value is averaged for more than 100 pigs. The peaks originating from fat, meat, and bone tissue are clearly seen. Please note the log scale of the vertical axis.

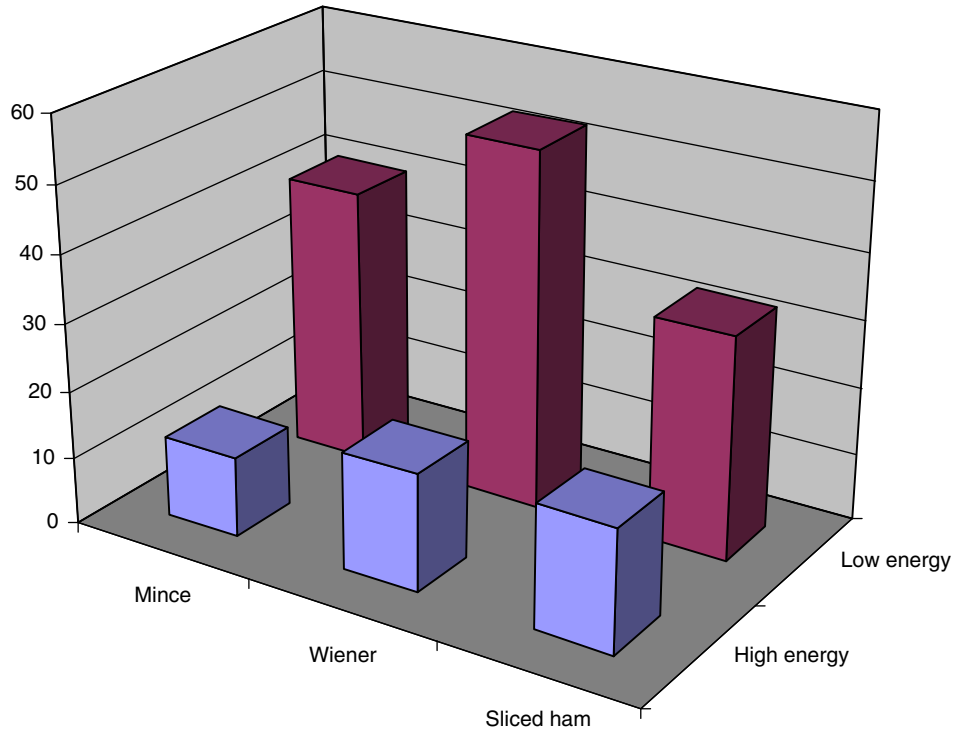


Figure 4 Measuring the contrast between cartilage and three different products (minced beef, Wiener sausages, and sliced ham) shows a considerable increase in contrast when reducing the X-ray energy from high (approximately 45 keV) to low (approximately 25 keV).

An electric coil generates a magnetic field in the aperture of the metal detector. The alternating field is detected by one or more receiver coils that are sensitive to disturbances of the field pattern within the detector aperture. The frequency of the field may assume values from less than 100 kHz to more than 1 MHz.

Most systems are based on a balanced phase-sensitive receiver where the passage of a metallic type of FB will disturb the phase balance between the receiver coils either by magnetic deflection (iron or nickel), which is the dominant effect at low frequencies, or by current absorption (stainless steel or aluminum), which is the dominant effect at frequencies more than 1 MHz.

As the sensitivity of metal detectors relies on generation of a magnetic field within an electric coil, the sensitivity

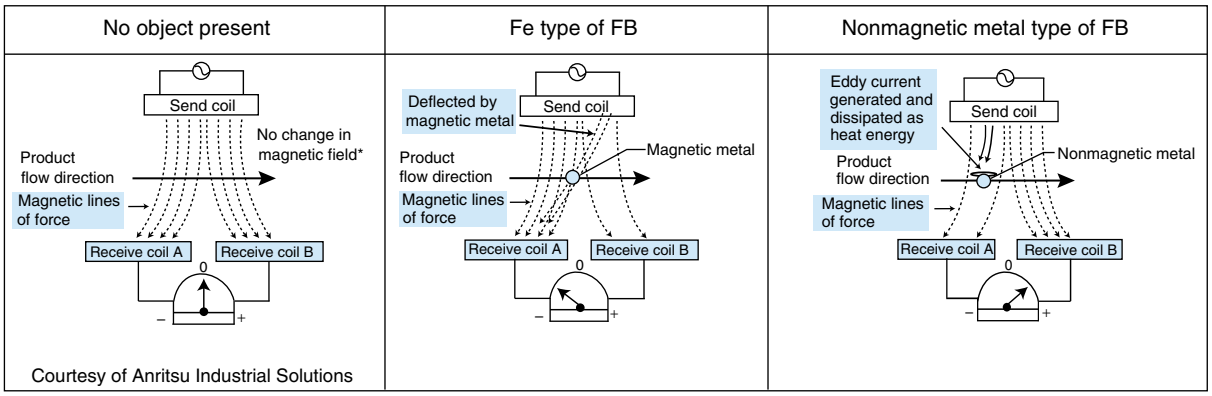


Figure 5 Field deflection in a metal detector, magnetic deflection of Fe types of FB, and current deflection of nonmagnetic types of FB.

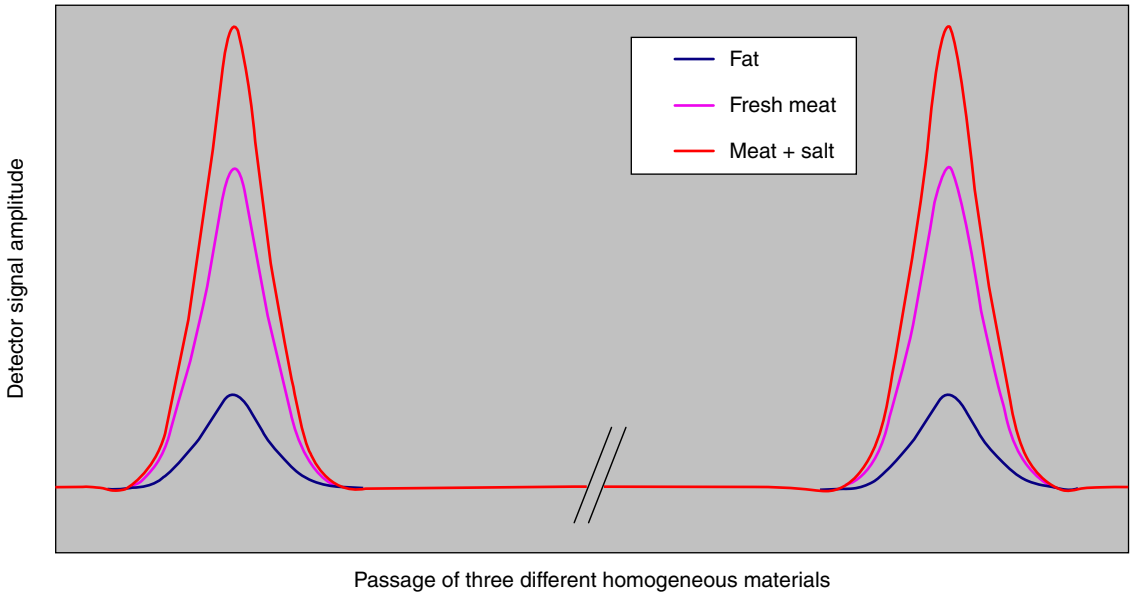


Figure 6 The detector signal amplitude is proportional to the squared receiver signal. All three (long) objects assume the same physical size.

inevitably becomes a function of distance from the FB to the coil. The smaller the distance, the more sensitive the device; in other words, for a specific meat product always choose a metal detector with the smallest aperture (coil) possible. When testing the metal detector, always place the test piece in the center of the opening, where the field is weakest and the sensitivity is at a minimum.

The passage of an ideal homogeneous product through a balanced coil system affects the detector as a function of the material and of its position in the detector aperture. When the product enters the first detector coil, the balance is disturbed. This is also the case when the product leaves the second detector coil. The disturbance generates the output signal of the detector. When the homogeneous product is symmetrically placed in a balanced coil system, the impact is balanced out (Figure 6).

Introducing a metal FB in a specific product changes the detector signal even further. In Figure 7, an idealized situation is shown, with a small spherical piece of iron in the middle of two different long products.

The effect of the FB is the same in both cases, but the product effect is higher for meat than for fat, thus leading to a high contrast in fatty products compared with pure meat products, due to the considerable difference in conductivity. Furthermore, a decrease in temperature to below the freezing point reduces ion mobility, thus leading to a better contrast in metal detectors.

However, the inclusion of salt in the recipe for a meat product increases the conductivity (due to a higher ion concentration) and thus reduces the contrast between the meat and metal FBs. This is an important feature for electromagnetic coil detectors: the contrast is recipe dependant. Fatty products, however, possess a lower conductivity than meat, thus promising an even better contrast to conductive metals.

A heterogeneous product does not produce the idealized output signals shown in Figure 7. A product consisting of a meat and fat mixture generates a weighted average signal between the two constituents, so variation in the meat/fat ratio leads to a different signal in the metal detector. This introduces a risk of rejecting even clean products. Owing to the effect of

the shape of the FB, manufacturers recommend metal spheres as test FBs. But, for real applications with randomly shaped FBs, test experiments should be designed using FBs that the instrument will be required to detect in practice.

Determination of the Specific Detection Limit

Every measurement is subject to a certain small contribution of randomness, also called noise. This is valid for all kinds of measurements, including measurements of X-ray radiation in a FB detector.

The positive or negative random noise contribution will be added to the signal from the FB itself. The noise contribution can, therefore, increase or decrease the signal from the FB according to the sign of the contribution.

When measuring by X-ray radiation, the true value of the measurement can be estimated by repeating the measurement several times under identical measurement conditions. The same method should be used when determining the true value of the detection limit for a given setting of the threshold value in the FB detector. To determine the true value accurately (corresponding to 100% confidence), the measurement must be repeated infinitely many times. Fewer measurements will do, however, if the true value is to be determined with, for example, 95% or 99% confidence.

In principle, the task of determining the true value of the detection limit is impossible, but, in practice, a test can be

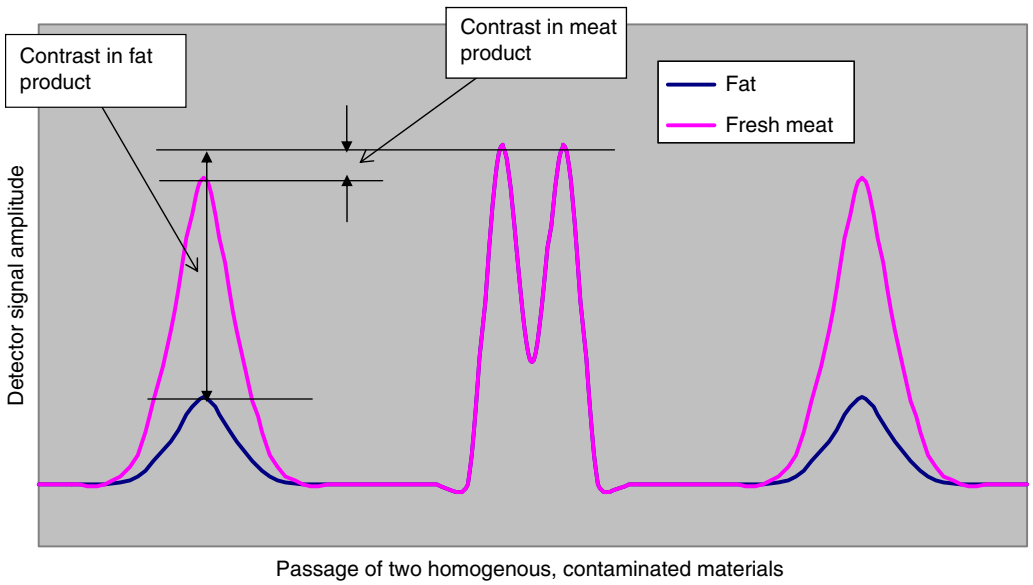


Figure 7 Illustration of the detector signal in the case of a centrally placed contaminant product. When the contaminant is symmetrically placed in the detector aperture, the signal from the two coils is in perfect balance, thus the detector output is reduced.

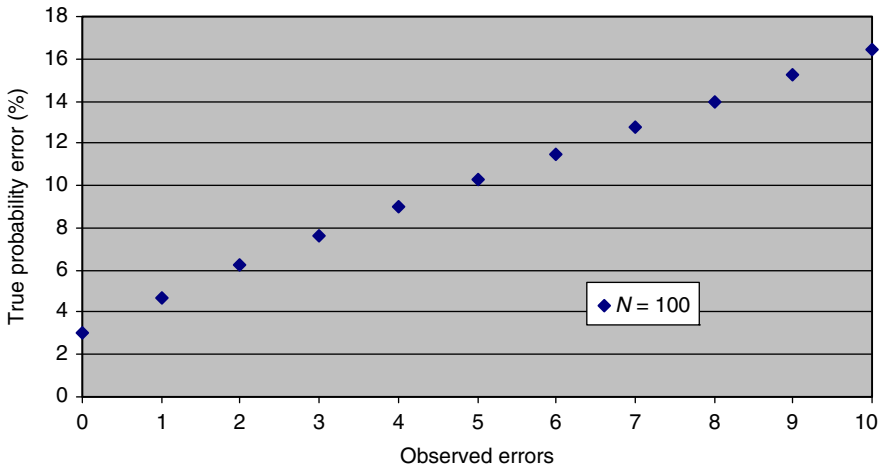


Figure 8 TEP calculated from a test with 100 repetitions. The different number of errors observed are shown on the x-axis and the corresponding TEP. The TEP is calculated with 95% confidence.

made to estimate the true value with good confidence. After setting up the detector, it is the true value that the producer will experience as the production runs through the detector.

As an example of determining the detection limit of an instrument, a metal sphere of Ø 0.5 mm might be placed in a sausage package. With a constant setting of the X-ray equipment, the same packing and metal sphere would be sent through the detector 100 times. The metal sphere will be detected every time, which means that there are no errors. For that reason, it might appear reasonable to assume that the detection limit is less than Ø 0.5 mm, with 100% confidence, and this setting will always detect metal spheres of Ø 0.5 mm and more, during production of this product. However, this is not necessarily true. It is quite possible for the instrument to have a chance of detecting the object that is less than 100% and still detect the object 100 times out of 100 attempts during a test, just as it is possible to toss a coin three times and have it show 'heads' each time – something that one would expect to occur once out of every eight times one made such a trial.

Number of repetitions: $N = 500$

Size (mm)	Number of errors	True error probability
0.2	10	0.034
0.3	3	0.016
0.6	0	0.006
1.0	0	0.006

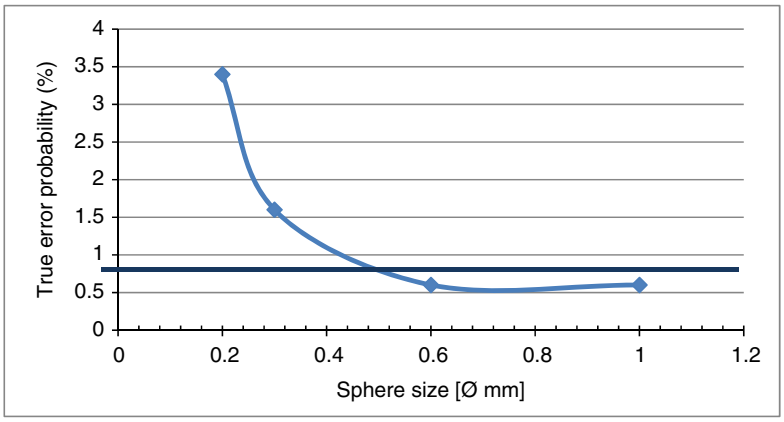
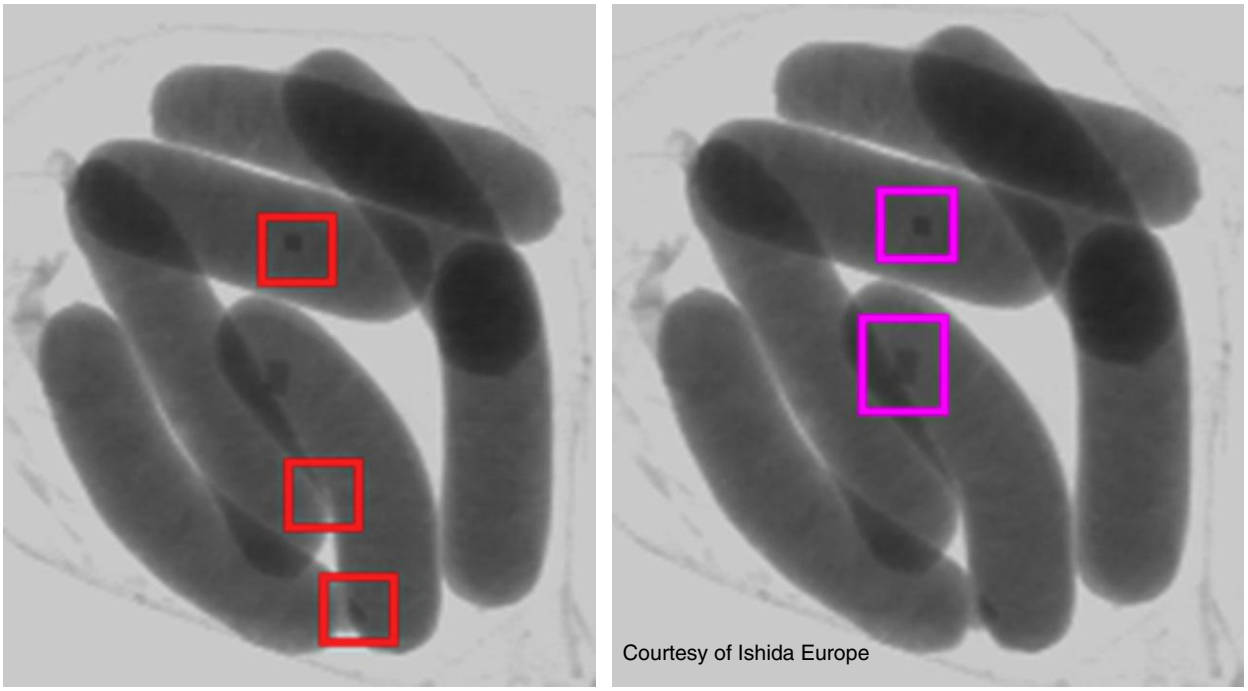


Figure 9 The result from an experiment with four different metal spheres is seen in the table. The experiment is repeated 500 times and the number of missed detections is calculated for each sphere. These numbers result in the TEP seen in the table. Making the diagram of the results leads to an estimate of the detectability of 0.44 mm (sphere) from the intersection of the TEP curve with the 1% line.



(a) (b)

Figure 10 Shown above is an example of the benefit of using dual-energy detection of FBs in randomly oriented sausages. The conventional single energy (a) includes two false positives and a moderate detection performance compared with the dual-energy example (b), reducing the risk of false positives and improving the detection performance at the same time.

In fact, a so-called strength consideration of the 100 repetitions tells that the probability of errors, i.e., the system fails to detect a metal sphere of the size mentioned, is no more than 3%, with 95% confidence. If the measurement can be repeated 200 times without errors, which means that the sphere is found in all 200 cases, it can be said – with the same confidence – that the true error probability (TEP) can be reduced to 1.5%. If the measurement can be repeated 500 or 1000 times – still without errors, and the sphere is detected every time – the TEP must be 0.6% or 0.3%, respectively. However, if the sphere had been missed in some of the tests, then the TEP would be higher than stated above. Figure 8 shows an example of how the true probability for errors is increased with the number of errors observed in a test ($N=100$).

The authors recommend defining the detection limit as the TEP of 1%, stated with a specific confidence of 95%.

According to Figure 7, it can be concluded that 100 repetitions are not enough if the authors wish to express their opinion of the TEP of 1% with 95% confidence, even if no errors are observed. The number necessary can be determined to $N=300$. If this number of repetitions cannot be implemented, the authors can only express their opinion with less confidence.

If there is a wish to determine the detectability (i.e., the size of the smallest sphere detectable with 1% TEP) of a scanning system in greater detail, a test with a range of small spheres must be made. The smallest sphere must be so tiny that it will not be detected in some of the repetitions. An example of such an experiment using 500 repetitions is

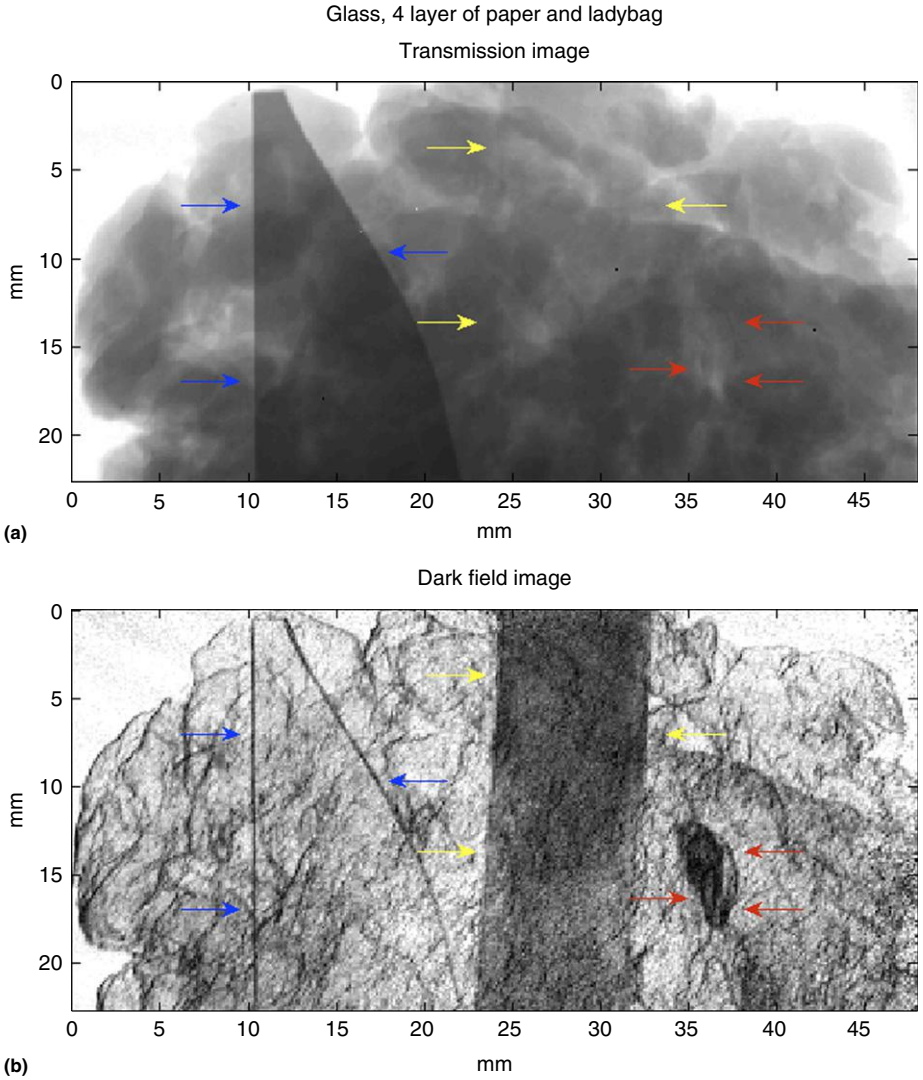


Figure 11 In the figure, the difference between conventional absorption (transmission) and dark field radiology is very obvious. The performance is illustrated using a piece of glass (blue arrows), four sheets of paper (yellow arrows), and an insect (ladybug, *Harmonia axyridis*; red arrows). The fibrous structure of paper and ladybug generates a high contrast dark field radiogram (bottom) compared with the conventional absorption measurement. On the contrary, the glass material has no diffracting inner structure but a considerable attenuation giving high absorption contrast.

shown in Figure 9 below. The table (shown left in Figure 9) shows the observed number of missed test pieces and the corresponding TEP. From the graph, it is deduced that the detectability is 0.44 mm for the setting and product used in the trial.

The same statistical argument as described above is used if tests are to be made to specify the number of false positive FB detections in pure products.

If no false positives are found in 300 representative (pure) products, it can be – with 95% confidence – said that the production of the specific product will experience less than a 1% rate of false positives with the given setting of the X-ray detector.

Finally, the producer must assess the (small) probability that metal FBs will be present in the product. The producer can then use the test results to quantify the reduction in occurrence that could be achieved by using the detecting system.

Emerging Technologies

Hyperspectral vision is also known as multispectral vision and may be considered as a development of conventional color vision based on RGB (red, green, and blue) detection. The new technology provides sensing of the surface reflection of the food product at distinct wavelengths. A few vendors offer hyperspectral vision and more are expected to follow. One of the benefits of this technique, compared with narrow wavelength sensing, is a high sensitivity to foreign materials such as cartilage, fat, and sinews, but detection of thin sheets of plastic like PS and PE is also provided with the new technology. However, one drawback carried over from color vision is that it is restricted to monitoring the surface only.

Dual-energy X-ray is adapted from the medical field of bone density measurements and has been used for some years for fat content determination. This modality is, however, also beneficial for FB detection because it copes with the challenge of product thickness variability. In Figure 11, both thickness and linear attenuation contribute to the total absorption, so by introducing a second measuring energy, two equations for the absorption in the same product are obtained. The two equations may be solved mathematically to give both thickness and average linear density, thus improving the specificity of the detection algorithm. An example is shown in Figure 10, where sausages in random orientation are measured with one energy and with dual energies, respectively. The improvement is noticeable; both a reduction of false positives and better detection contribute to a more versatile and reliable system.

Emerging medical X-ray modalities have recently entered the food scanning industry, providing screening potential with a very high sensitivity to soft and to fibrous materials. This technology explores the full refractive index and not only the absorption difference between materials, so the much more delicate phase term and scattering microstructure contribute to the detection as well as absorption. By using a conventional X-ray source in a grating-based interferometer setup, the X-ray is converted into a spatially coherent instrument able to detect the absorption difference, phase delay, and scattering impact from fibrous structures like insects, wood, and paper. An example comparing the contrast performance of transmission (absorption) with dark field scanning of a minced meat product is given in Figure 11. The improved contrast of fibrous foreign material is clearly seen.

See also: Conversion of Muscle to Meat: Glycogen. Environmental Contaminants. Ham Production: Cooked Ham. Microbial Contamination: Microbial Contamination of Fresh Meat. Microbiological Analysis: Indicator Organisms in Meat. Minced Meats. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Arvanitoyannis, I.S., Tserkezou, P., Varzakas, T., 2006. An update of US food safety, food technology, GM food and water protection and management legislation. International Journal of Food Science and Technology 41, 130–159.
Engel, D.M., Macdonald, D., Nash, C., 2001. Physical hazards. In: Engel, D.M. (Ed.), Managing Food Safety. London: Chadwich House, pp. 144–149.
Lelieveld, H.L.M., 2003. Physical contamination. In: Lelieveld, H.L.M., Mostert, M.A., Holah, J., White, B. (Eds.), Hygiene in Food Processing. Boca Raton, FL/Cambridge, UK: CRC Press/Woodhead Publishing Limited, pp. 62–63, 314–334.
Olsen, A.R., 2003. Ouch! Regulatory perspectives on foreign objects in food manufacturing & distribution. Quality Control, pp. 63–64.
Olsen, A.R., Zimmerman, M.L., 2003. Hard or sharp foreign objects in food. Food Science and Technology 120, 61–67.
Peariso, D., 2006. The importance of preventing foreign material contamination of food products. In: Peariso, D. (Ed.), Preventing Foreign Material Contamination of Food, first ed. Oxford, UK: Blackwell Publishing Ltd., pp. 1–64.
Uys, R., 2003. Back to food safety basics. Food Reviews 49–51.

Relevant Websites

http://dmri-shop.dk/images/steamtools/dokumenter/Tubular-5.pdf
Danish Meat Research Institute.
http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7310.5.htm
FSIS Homepage.
http://www.fsis.usda.gov/regulations_&_policies/fsis_import_reinspection/index.asp
FSIS Homepage.
http://www.foodprocessing.com/ME2/Segments/Publications/Print.asp?Module=publication
Food Processing.
http://vsp.lf.dk/Services/Godkendte%20kanyler.aspx
Pig Research Centre, Denmark.
http://www.ciesnet.com/2-wwedo/2.2-programmes/2.2.foodsafety.gfsi.description.asp
The Consumer Goods Forum.
http://www.fsis.usda.gov/OOPDE/rdada/FRPubs/Docs_02-033N.htm
USDA.
http://www.fsis.usda.gov/News_&_Events/2002_Events/index.asp
USDA.
http://www.fsis.usda.gov/PDF/Food_Defense_Plan.pdf
USDA.
http://www.fsis.usda.gov/Science/Generic_HACCP_Models/index.asp
USDA.
http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/oppde/op/IIM/TOCIIM.htm
USDA.
http://fsis.usda.gov.Fsis:Recalls/
USDA.

FUNCTIONAL FOODS

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Glossary

Angiotensin I-converting enzyme (ACE) inhibitory peptides The peptides having antihypertensive effects and are utilized for functional foods. Such peptides are found in hydrolyzates of many food proteins.

Food protein-derived bioactive peptide Various bioactive peptides have been found from enzymatic hydrolyzates of food proteins (e.g., meat proteins). As representative bioactivities, ACE inhibitory and antioxidative properties have been studied.

FOSHU Foods for specified health use. Foods based on the knowledge concerning the relationship between foods or food components and health that are expected to have certain health benefits and have been licensed to bear labeling claiming that a person using them may expect to obtain that health use through the consumption of these foods.

Functional food Processed foods having disease-preventing and/or health-promoting benefits in addition to their nutritive value.

Functional meat product Meat products with additional physiologically functional properties. Utilization of functional ingredients (e.g., vegetable proteins, fibers, and probiotics) is one approach to develop this kind of products.

Nutraceutical compound Chemicals found as natural components of foods or other ingestible forms that have been determined to be beneficial to the human body.

Prebiotics Nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host.

Probiotics Viable microbial food supplement that beneficially influences the health of the host.

Introduction

Foods are basically evaluated by their nutritional value. In other words, the most important factor for evaluation of foods is the 'primary' function of foods, i.e., the role of standard nutrient components. This primary function is particularly important for people who are suffering from a shortage of food. The 'secondary' function of foods, which is defined as sensory properties such as taste, flavor, appearance, and texture, is also critical for the food industry in many countries.

In addition to these functions, during the past few decades, much attention has been paid to 'tertiary' function of foods. Tertiary function is the role of food components that are expected to prevent diseases by modulating physiological systems (e.g., immune, endocrine, nervous, circulatory, and digestive systems). As examples of tertiary functional properties of foods, anticarcinogenicity, antimutagenecity, antioxidative activity, and antiaging activity have been studied. Owing to increasing concerns about health by consumers, efforts have been made in food industries in many countries to develop new foods with tertiary functions. Such foods having tertiary functions are regarded as 'functional foods.'

Numerous food components showing tertiary functions have been isolated and characterized. For example, many vegetables have been shown to contain a variety of biologically active phytochemicals. There has been an accumulation of scientific findings in recent years regarding the roles of such components in the prevention of diseases. Rapid progress has been made in the development of functional foods based on the results of studies on food components providing positive health benefits other than normal nutritional benefits. There has been extensive research and development in the field of functional foods in the dairy industry. Also, many traditional fermented dairy products have been rediscovered as foods with physiological functionality.

However, studies of tertiary functions of meat and functional meat products have been limited until recently. By increasing or introducing bioactive properties, it should be possible to develop new meat products with potential health benefits. Such meat products would open up a new market in the meat industry. In this article, an overview of functional foods is given and then examples of meat-based functional products are described. The potential benefits of meat components on human health and the development of novel functional products are also discussed.

Overview of Functional Foods

The term 'functional food' was coined in Japan in the early 1980s. Although there is no universal definition of functional food, a typical and simple definition is "processed foods

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Table 1 Representative functional ingredients used for FOSHU products

Ingredients	*Targets*
Dietary fibers	Intestinal disorder
	Cholesterol level
	Blood sugar level
Lactic acid bacteria	Intestinal disorder
Oligosaccharides	Intestinal disorder
Soy proteins	Cholesterol level
Sugar alcohols	Dental caries
Peptides	Mineral absorption
	Blood pressure level
	Cholesterol level
	Triacylglycerol level
Calcium/iron	Mineral level
Polyphenols	Dental caries
	Blood sugar level
Glycocides	Blood pressure level
Sterol esters	Cholesterol level
4-Aminobutanoic acid	Blood pressure level

having disease-preventing and/or health-promoting benefits in addition to their nutritive value." Functional foods overlap with nutraceuticals, medical foods, probiotics, designer foods, pharmafoods, and vitafoods.

Japan is also the first country to have formulated a specific regulatory approval process for functional foods. In 1991, the concept of foods for specified health use (FOSHU) was established. According to the Japanese government, FOSHU are foods based on the knowledge concerning the relationship between foods or food components and health that are expected to have certain health benefits and have been licensed to bear label claims that a person using them may expect to obtain in improved health through the consumption of these foods. Most FOSHU products utilize functional ingredients to help in the maintenance of a healthy human body. Such food ingredients are listed in Table 1. As of March 2012, 990 FOSHU products in 13 categories have been approved in Japan. As a representative example of approved FOSHU products, Ameal-S is a sour milk product containing milk protein-derived antihypertensive peptides (Val Pro-Pro and Ile-Pro-Pro). Daily ingestion of this product is expected to reduce the risk of hypertension. Another example of popular FOSHU products is a yogurt fermented with *Lactobacillus rhamnosus* strain GG. Strain GG, a representative strain of probiotic lactic acid bacteria, has been shown to be effective for intestinal disorders. Products containing the GG strain have been marketed in more than 20 countries under license from a Finnish company.

Regulations for functional foods have not yet been well established in most countries. Also, there are distinct differences in the approach to functional foods between legislators in Japan, the USA, and Europe. For example, in the USA, functional foods are regulated under the same regulatory framework as are conventional foods and dietary supplements. FDA's regulatory scheme does not recognize functional food to be a distinct regulatory category. However, the complexities of developing a functional food to be in compliance with EU food legislation are more considerable.

Functional Meat Products

Meats are rich in essential nutrients, such as proteins, vitamins, and minerals, and also contain other compounds thought to be physiologically functional. Meat could therefore be considered as functional food without any additional processing. Numerous low-fat or fat-free meat products have been developed in many countries, with the USA at the head of the list. Low-salt and sugar-free meat products (e.g., roast ham and sausages) have been developed in Japan. In addition to these 'free' and 'low' type of products, meat products with additional physiologically functional properties have been introduced in some countries. Utilization of functional ingredients is one approach to develop the functional meat products. Such ingredients include vegetable proteins, fibers (e.g., oats, sugar beet, soy beans, and peas), antioxidants, probiotics, and prebiotics (intestinal *Lactobacillus* and *Bifidobacterium*).

Dietary fibers or soy proteins have been used as functional ingredients in FOSHU products in Japan. A pork Vienna-type sausage product that contains indigestible dextrin, a water-soluble dietary fiber made from potato starch, is claimed to have beneficial effects on intestinal disorders. Another example is a pork frankfurter that is a low-fat sausage product containing soy proteins. It is claimed that an acceptable blood cholesterol level can be maintained by consumption of this product. Other than the approved FOSHU products, meat products with additional fibers, proteins, and minerals (e.g., calcium) have also been marketed. Vegetable proteins have been used in meat products for their nutritional and functional value. Soy proteins are typical proteins with health-enhancing activity. They are thought to be effective for preventing cardiovascular diseases, cancer, and osteoporosis. Soy-based ingredients also contain another group of bioactive components, isoflavones. A sausage formulated with a modified potato starch was marketed in the USA. Such dietary fibers contribute to the improvement of intestinal microflora and the reduction of fat intake. Healthier lipid formulation is also a remarkable approach for developing functional meat products.

A group of meat products named Apilight (e.g., pork sausages, hamburger steak, and meat-balls) is unique and profitable for consumers. These are products made from a formulation that excludes ingredients causing allergy symptoms. Although meat is less allergenic than common allergy-induced foods, such as milk, eggs, and soy, meat products (e.g., sausages) often contain vegetable, egg, and/or milk proteins. People with allergies are often affected by allergens in such ingredients. These products have been approved as allergen-free products by the Japanese government. Also, gluten-free and/or lactose-free meat products have been produced in some countries. Possible approaches for functional modification in meat products are summarized in Table 2.

Meat-Based Bioactive Compounds

Nutraceuticals are defined as "chemicals found as natural components of foods or other ingestible forms that have been determined to be beneficial to the human body in preventing or treating one or more diseases or improving physiological performance." Although most nutraceutical compounds have been

Table 2 Possible approaches for functional modification in meat products

Reduction
Fat and cholesterol
Sodium chloride
Sugar
Allergens (vegetable, milk, and egg proteins)
Biogenic amine
Modification
Fatty acid (selection of breeds)
n-6:*n*-3 PUFA (linseed feed)
Addition
Vegetable oils (olive oil)
Fish oils
Conjugated linoleic acid
Plant-based proteins (soy protein)
Peptides (protein hydrolyzated)
Probiotic bacteria
Dietary fibers (apple, pear, and citrus)
Oligosaccharides
Natural extracts
Vitamins (C and E)
Minerals (Ca, Fe, Se, Mg, and Mn)
Plant sterols
Phytate

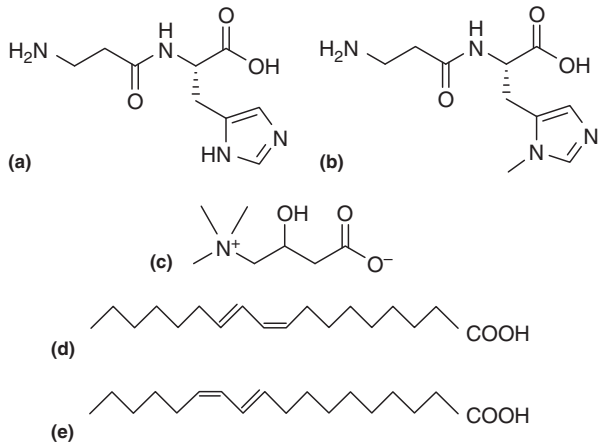

Figure 1 Representative bioactive compounds in meat: (a) carnosine; (b) anserine; (c) L-Carnitine; (d) conjugated linoleic acid (*c*9, *t*11-isomer); and (e) conjugated linoleic acid (*t*10, *c*12-isomer).

found in plants, several attractive meat-based bioactive substances have been studied (e.g., carnosine, anserine, L-carnitine, conjugated linoleic acid (CLA), glutathione, taurine, creatine, coenzyme Q10, choline, balenine, creatinine, lipoic acid, putrescine, spermidine, and spermine). Representative meat-based bioactive substances (Figure 1) are described here. Emphasizing physiological activities originating from meat is one spontaneous approach for developing functional meat products.

Histidyl Dipeptides

Through diet, especially fruits and vegetables, interdiction and neutralization of free radicals have been shown to occur. This beneficial action of food is attributed to the antioxidant potency of various compounds, including ascorbic acid, vitamin E, beta-carotene, and polyphenolic compounds. Antioxidants in fruits and vegetables may decrease the risk of cancer.

Several endogenous antioxidants have been found in skeletal muscle (e.g., tocopherols, ubiquinone, carotenoids, ascorbic acid, glutathione, lipoic acid, uric acid, spermine, carnosine, and anserine). Both carnosine (beta-alanyl-L-histidine; Figure 1(a)) and anserine (N-beta-alanyl-1-methyl-L-histidine; Figure 1(b)) are antioxidative peptides containing histidine. They are the most abundant antioxidatives in meats. The concentrations of carnosine in meat range from 500 mg kg^{-1} in chicken thigh to 2700 mg kg^{-1} in pork shoulder. Anserine is especially abundant in chicken muscle (e.g., 980 mg kg^{-1} in skeletal muscle). Most meat extract products contain carnosine and anserine. These peptides have been reported to play roles in wound healing, recovery from fatigue, and prevention of diseases related to stress. Because anserine is more digestion-resistant than is carnosine, the physiological function of anserine would be more effective than that of carnosine in the human body. For this reason, functional food ingredients with high concentrations of anserine (ca. 98%) purified from fish extracts have been developed.

L-Carnitine

L-Carnitine (beta-hydroxy-gamma-trimethyl amino butyric acid; Figure 1(c)) assists the human body in producing energy and in lowering levels of cholesterol, calcium to improve skeletal strength, and chromium picolinate to help build lean muscle mass. L-Carnitine is abundant in skeletal muscle, especially in beef (e.g., 1300 mg kg^{-1} in the thigh). A fruit juice product containing L-carnitine that is marketed in the USA is advertised as having several beneficial effects, such as maintenance of stamina and fast recovery from fatigue. Also, a product containing much L-carnitine and carnosine, which is used as a functional food ingredient has been marketed in Japan. This product is made from a by-product of corned beef.

Conjugated Linoleic Acid

CLA, which was initially found in cooked beef, is composed of a group of positional and geometrical isomers of octadecadienoic acid (18:2). CLA is most abundant in fat of ruminant animals, because it is converted from linoleic acid by rumen bacteria. After its absorption in ruminant animals, CLA is transported to mammary tissue and muscle. Beef fat contains 3–8 mg CLA g^{-1} fat. The most common CLA isomer found in beef is octadeca-*c*9, *t*11-dienoic acid (Figure 1(d)). Because this fatty acid has anticarcinogenic activity, much interest has been shown in this compound. Commercially available CLA supplements utilized for many studies are usually the mixture of several CLA isomers (e.g., *c*9, *t*11, 41%; *t*10, *c*12, 44%; and *t*9, *t*11/*t*10, *t*12, 7%). Some recent studies claim that *t*10, *c*12-isomer (Figure 1(e)) exhibits stronger physiological activities than *c*9, *t*11-isomer. However, most animal products (e.g., beef and cow's milk) contain only a trace amount of *t*10, *c*12 CLA isomer. The CLA content of foods is increased by heating (cooking and processing). Besides its anticarcinogenic

property, CLA also has antiatherosclerotic, antioxidative, and immunomodulative properties. CLA may also play roles in control of obesity, reduction of the risk of diabetes, and modulation of bone metabolism.

Development of Novel Functional Meat Products

Functional meat products have been developed to some extent. Also, several promising bioactive compounds have been found in meat. Two possible directions of development of novel functional meat products are described here.

Meat Protein-Derived Bioactive Peptides

Many bioactive peptides are generated from food proteins (e.g., milk proteins). As such peptides, antihypertensive, opioid, immunostimulating, antimicrobial, antithrombotic, hypocholesterolemic, antioxidative, and prebiotic activities have been studied. Some of these bioactive peptides are found in hydrolysates of muscle proteins. Angiotensin I-converting enzyme (ACE) plays an important physiological role in the regulation of blood pressure. Several inhibitors of ACE have been found to be effective as antihypertensive pharmaceuticals. Antihypertensive peptides derived from various food proteins have been discovered. Enzymatic hydrolysates of meat proteins have been found to exhibit potent ACE-inhibitory activity and antihypertensive activity in spontaneously hypertensive rats. Peptides, named myopentapeptides A (Met-Asn-Pro-Pro-Lys) and B (Ile-Thr-Thr-Asn-Pro), were identified in the enzymatic hydrolyzate of porcine myosin. Because several food products, such as sour milk and soup products, containing antihypertensive peptides have been marketed for hypertensives, the hydrolysates of meat protein and their corresponding bioactive peptides might be utilized for functional foods.

Even in antihypertensive peptides, it has been found that antioxidative and prebiotic peptides are generated from meat proteins by enzymatic digestion (Table 3). These bioactive peptides might exist in fermented meat products, because meat proteins are hydrolyzed by muscle and bacterial enzymes during fermentation and storage. Fermented meat products, as well as fermented dairy products, might be rediscovered as functional foods. It is also possible that ingestion of meat proteins will contribute to the maintenance of human health, because digestive enzymes such as pepsin could generate bioactive peptides from meat proteins.

Table 3 Examples of bioactive peptides from meat (miscle) proteins

Bioactivity	*Sequence*[a]	*Muscle source*
Antihypertensive	LKP	Chicken
Angiotensin I-converting enzyme inhibitory	LAP	Chicken
	VWI	Porcine
	ITTNP	Porcine
	MNPPK	Porcine
	FQKPKR	Chicken
	VLAQYK	Bovine
	IVGRPRMQG	Chicken
	RMLGQTPTK	Porcine
Antioxidative	VW	Porcine
	DLYA	Porcine
	SLYA	Porcine
	DLQEKLE	Porcine
Prebiotic	ELM	Porcine

[a]The one-letter amino acid codes were used.

Probiotics, Prebiotics, and Synbiotics

Although starter lactic acid bacteria are widely used for producing fermented meat products, unlike in dairy products, little attention has been given to therapeutic activities of these bacteria in meat products. The main approach for improving the beneficial physiological properties of dairy products has been the development of 'probiotic lines' of traditional fermented products. Thus, it seems possible to develop meat products that are beneficial for health by using probiotic bacteria. Probiotic foods have been defined as "viable microbial food supplement that beneficially influences the health of the host." Also, probiotic foods are regarded to be functional if they have been satisfactorily demonstrated to beneficially affect one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being or reduction of risk of disease.

In 1998, a German producer launched a salami product containing three intestinal strains (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium* sp.). This product is claimed to have health benefits and is thought to be the first probiotic-like salami product to be marketed. Shortly after, a Japanese producer began to market a new meat-spread product fermented with probiotic bacteria (*Lactobacillus rhamnosus* FERM P-15120). *L. rhamnosus* FERM P-15120 has been screened from the collection of human intestinal lactobacilli for meat fermentation. However, further scientific studies on the relationship between ingestion of such products and human health are needed.

In the food industry, much interest has also been shown in the utilization of prebiotics, which are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host." Oligosaccharides and dietary fibers are representative prebiotic substances used for processed foods. Such prebiotics have been utilized for several meat products. A mixture of probiotics and prebiotics, which is called synbiotics, is utilized for many foods, such as fermented dairy products. Because meat products with probiotics, prebiotics, and synbiotics have a great future potential, it is expected that increasing interest will be shown in basic research and potential applications for developing new functional meat products.

Conclusions and Future Prospects

Possible strategies for developing healthier meat and meat products, including functional foods, are listed in Table 4.

Table 4 Strategies for developing healthier meat and meat products

Modification of carcass composition
Manupilation of meat raw materials
Reformulation of meat products
Reduction of fat content
Modification of the fatty acid profile
Reduction of cholesterol
Reduction of calories
Reduction of sodium content
Reduction of nitrites
Incorporation of functional ingredients

Although functional meat products were described mainly from the viewpoint of food processing in this article, all aspects of animal production and product processing should be considered for developing healthier products. Through manipulation of animal feed, the composition of animal products can be improved. The effects of feed supplements, such as vitamin E, on meat properties (e.g., color, lipid stability, and sensory quality) have been characterized extensively. However, there has not been sufficient study on their effects on health benefits of fresh meat for humans. Several studies have shown that feeding conditions of animals affect the contents of bioactive components, such as L-carnitine and CLA, in animal products. Further such efforts are expected for creating differentiated meat and meat products with potential human health benefits. However, there are still some difficulties in marketing functional meat products: functional meat products are unconventional, and consumers in many countries do not recognize meat and meat products as healthy foods, unlike milk and dairy products. Thus, further studies are needed to demonstrate the benefits of meat and meat components for human health. Along with accumulation of scientific data, there is an urgent need to inform consumers of the exact functional value of meats.

See also: Additives: Functional. Chemical Analysis for Specific Components: Micronutrients and Other Minor Meat Components. Fermentation. Human Nutrition: Macronutrients in Meat; Meat and Human Diet: Facts and Myths; Micronutrients in Meat; Nutraceuticals

Further Reading

Ansorena, D., Astiasarán, I., 2007. Functional meat products. In: Toldrá, F. (Ed.), Handbook of Fermented Meat and Poultry. Hoboken, NJ: Wiley-Blackwell, pp. 257–266.

Arihara, K., 2006a. Functional properties of bioactive peptides derived from meat proteins. In: Nollet, L.M.L., Toldrá, F. (Eds.), Advanced Technologies for Meat Processing. Boca Raton, FL: CRC Press, pp. 245–274.

Arihara, K., 2006b. Strategies for designing novel functional meat products. Meat Science 74, 219–229.

Arihara, K., Ohata, M., 2008. Bioactive compounds in meat. In: Toldrá, F. (Ed.), Meat Biotechnology. New York: Springer, pp. 231–249.

Arihara, K., Ohata, M., 2011. Functional meat products. In: Saarela, M. (Ed.), Functional Foods: Concept to Products, second ed. Cambridge: Woodhead Publishing, pp. 512–533.

Arihara, K., Ohata, M., 2011. Probiotics and prebiotics in meat products. In: Kerry, J.F., Kerry, J.P. (Eds.), Processed Meats: Improving Safety, Nutrition and Quality. Cambridge: Woodhead Publishing, pp. 403–417.

De Vuyst, L., Falony, G., Leroy, F., 2008. Probiotics in fermented sausages. Meat Science 80, 75–78.

Farnworth, E.R., 2008. Handbook of Fermented Functional Foods, second ed. Boca Raton, FL: CRC Press.

Fernández-Ginés, J.M., Fernández-López, J., Sayas-Barberá, E., Pérez-Alvarez, J.A., 2005. Meat products as functional foods: A review. Journal of Food Science 70, R37–R43.

Jiménez-Colmenero, F., 2007. Functional foods based on meat products. In: Hui, Y. H. (Ed.), Handbook of Food Products Manufacturing – Principles, Bakery, Beverages, Cereals, Cheese, Confectionary, Fats, Fruits, and Functional Foods. Hoboken, NJ: John Wiley & Sons, pp. 989–1015.

Jiménez-Colmenero, F., Reig, M., Toldrá, F., 2006. New approaches for the development of functional meat products. In: Nollet, L.M.L., Toldrá, F. (Eds.), Advanced Technologies for Meat Processing. Boca Raton, FL: CRC Press, pp. 275–308.

Kröckel, L., 2006. Use of probiotic bacteria in meat products. Fleischwirtschaft 86, 109–113.

Leroy, F., Falony, G., De Vuyst, L., 2008. Latest developments in probiotics. In: Toldrá, F. (Ed.), Meat Biotechnology. New York: Springer, pp. 217–229.

Saarela, M., 2011. Functional Foods: Concept to Products, second ed. Cambridge: Woodhead Publishing.

Schmid, A., 2010. Bioactive substances in meat and meat products. Fleischwirtschaft International 25, 127–133.

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GENOME PROJECTS

Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry

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Glossary

a* value Color axis from the Commission Internationale de L'eclairage color space describing redness (+ a*) and green (− a*).

Cooking loss Proportional change in weight of meat after cooking.

Marbling Colloquial term applied to intramuscular fat due to it resembling the flecks of white in marble.

Pearson correlation (r) Coefficient indicating linearity of relationship between two parameters.

Warner–Bratzler shear force The force required to cut cooked meat perpendicular to the muscle fiber direction.

Introduction

Genomics holds great promise for application in the meat industry in terms of managing eating quality. Since the late 1960s, genetics, and specifically crossbreeding, have been used to increase animal growth rate, carcass size, and carcass leanness; however, genomics now offers promise of selection of genotype within the genetic line. Most genetic improvement within the meat animal species has focused on increasing growth rate and feed efficiency, which produced an increase in the proportion of glycolytic muscle fibers in skeletal muscles. The increase in this muscle fiber type was associated with increased cooked meat toughness ('decreased cooked meat tenderness' would be preferred) as well as decreased fatness and this selection practice reduced consumer acceptability of meats. Recent genetic selection has looked at selecting for tenderness and marbling in an effort to increase the eating quality of meat from chickens, and cattle and pigs in particular.

Meat Tenderness

Meat tenderness is the most important factor affecting consumer satisfaction and, despite the factors affecting it being well understood, meat tenderness is difficult to predict or control at processing. Chilling rate within coolers can vary dramatically with location within the chiller and the response to electrical stimulation in beef processing can vary with carcass size and fatness and electrical stimulation parameters. Selection of meat animals for tenderness might reduce the effect of the postmortem environment and can potentially distribute an economic advantage to producers who select livestock for the trait.

Beef

Initial investigations focused on the activity of the proteolytic enzymes of the calpain (CAPN) family and their inhibitor calpastatin (CAST) in the muscles of Brahman cattle (*Bos indicus* spp.) because beef from the Brahman cattle is much tougher than that of cattle *Bos taurus* spp. CAPN and CAST were targeted for study because they retain the proteolytic and the inhibitory activity, respectively, in the skeletal muscle postmortem; and increased CAPN and decreased CAST activities were associated with increased cooked beef tenderness. The genetic study of CAST activity has indicated that it is heritable, with the greatest heritability observed in *B. indicus* cattle ($h^2 = 0.45 + 0.17$), and genotypic correlations between it

and cooked meat tenderness as measured by Warner–Bratzler shear force are approximately $r=0.70$ in Brahman cattle. Phenotypic correlations between CAST and Warner–Bratzler shear force have been lower than the genotypic correlations, however, indicating that the 'environmental' factors such as those affecting the carcass early postmortem are still significant in determining cooked meat toughness. These environmental influences on shear force decrease the heritability of shear force ($h^2=0.29$, 7 days postmortem, Brahman cattle), making it difficult to use as a selection criterion itself (Dikeman*et al.*, 2005 reported h^2 of 0.40 at 14 days).

Bovine meat quality marker panels are commercially available and include single-nucleotide polymorphisms (SNPs) for both CAPN and CAST. The GeneSTAR® marker panel available from Pfizer Animal Health™ includes SNPs in three genes: two SNPs in CAPN1, one in CAST, and one in thyroglobulin (TG5), an anonymous quality gene. The SNPs are identified as CAPN1 (316-T2) for CAPN1 (4751-T3). A second company, Igenity®, also markets a tenderness marker panel TenderGENE® that contains the same SNPs for CAPN1 316-T2 and 4751-T3 as well as a second polymorphism in CAST described by University of Guelph researchers (UoG-CAST). The preferred homozygous genotype for UoG-CAST was associated with a 35% decrease in the proportion of tough beef steaks at 7 days postmortem and was associated with carcasses that had an increased fat yield ($1.44+0.56\%$) and a decreased bone yield. Sires have been identified that have genotypes for tender beef as well as decreased back fat thickness; therefore, sufficient genetic variation exists to potentially select for cattle that produce tender beef without a reduction in carcass lean yield.

Commercially available bovine marker panels contain multiple SNPs for CAPN and CAST because the effects of their haplotypes are additive. If animals have favorable mutations in all of the SNPs in CAPN and CAST, there is a potential for a decrease in cooked beef toughness of up to 1 kg of Warner–Bratzler shear force, a difference that would be detected by most consumers. The applicability of these marker panels varies with subspecies, with the μ-CAPN 316-T2 and CAST markers best suited to selection for tenderness in purebred and crossbred *B. taurus* populations, whereas the other μ-CAPN SNP is applicable to both *B. taurus* and *B. indicus* cattle.

There might be significant commercial advantage in using such a panel for marker-assisted selection for meat quality because the frequency of the preferred alleles might be relatively low. For example, in one population studied, approximately 64% of 550 heads were not homozygous for the favorable alleles for CAPN and only 32% had one of the favorable alleles for this polymorphism. This polymorphism is also associated with the greatest reduction in shear force; however, as noted above, combining the three markers can yield the greatest improvement. Hence, there is a lot of improvement possible from increasing the frequency of the 'tender' alleles (depending on the starting frequencies).

Although these markers have been commercialized and are being used for marker-assisted selection of breeding stock, CAST and CAPN markers explain at most 25% of beef tenderness variation. This means there is potential to identify additional markers and to increase the value of such approaches. One interesting gene is DNAJA1, which was identified by functional genomic studies. Initial results suggest that reduced expression of DNAJA1, whose product is involved in apoptosis, accounts for 63% of beef tenderness (although this is likely to be an overestimate as is usually the case for small research studies). The mechanism by which DNAJA1 downregulation would improve meat tenderness is unclear. One possibility is that its downregulation in the muscles of cattle that produce tender beef might lead to muscle cells in the live animal being prone to apoptosis and thus there might be a requisite increase in proteolytic activity related to cell turnover. It might also lead to muscle cells entering apoptosis and necrosis earlier postmortem than cattle without this allele of DNAJA1, which would initiate proteolysis of sarcomeric muscle proteins while the carcass is warm, thus enhancing proteolytic enzyme activity and potentially meat tenderness. The implications for other traits need to be explored. For example, such an effect might impact the health of these animals or their feed efficiency, given the possibility of increased cell turnover, which might increase the basal metabolic requirements and the residual feed intake.

Changes in toughness have also been observed with the bovine F94L variant mutation for myostatin, the muscle regulatory factor controlling muscle fiber proliferation during embryo myogenesis, which produces increased loin muscle area in affected cattle (myostatin mutations are the basis for 'double muscling' in some breeds such as the Belgian Blue (BB)). Homozygous cattle for this gene variant appear to have increased cooked meat tenderness as indicated by a lower peak shear force of approximately 15% at 1 day postmortem and of 12% at 26 days postmortem compared to heterozygous cattle. However, heterozygous cattle exhibit little advantage over nonvariant cattle, with reduction in peak shear force values of only 4% and 2% at 1 and 26 days postmortem, respectively. (The extensive Germplasm Evaluation Project at the US Meat Animal Research Center, does not support the BB heterozygotes being slightly more tender; 5.91 kg for BB vs. 5.07 kg for Angus at 7 days, and 4.89 kg for BB vs. 4.08 kg for Angus at 14 days postmortem when compared at a constant age. The differences were greater at constant marbling and constant fat thickness). Further research is warranted on this variant so that its impact on production performance and meat quality can be used to advantage in the cattle industry.

Pork

Testing for well-known mutations such as the ryanodine receptor 1 mutation (RYR1) is usually conducted so that pigs with this mutation are not included in research or breeding populations because this mutation has a dominating effect on reducing muscle pH, increasing muscle deposition, and lowering meat quality. Even so, the effect on meat quality is essentially recessive, with pork from heterozygote animals being close to normal pork. The PKRAG3 gene mutation is approached similarly, as it causes comparable reductions in pork eating quality by rendering adenosine monophosphate-activated protein kinase (AMPK) insensitive to intracellular AMP levels causing abnormally high sequestration of glycogen

in the muscle. PKRAG3 mutations that negatively affect pork eating quality are numerous, however, with multiple missense substitutions described for this gene. The largest effect is a dominant mutation (RN) associated with the Hampshire breed, although this allele has been found in other breeds. The mutation was originally identified because of its large impact on the yield of processed ham using a particular method from the French 'Rendement Napole.' Interestingly, the effect is countered by the presence of processing aids such as polyphosphates, so that this can be thought of as a genotype x treatment effect. It also explains why the impact was more important in Europe where there was a trend away from using additives in food production. However, it illustrates how these types of effects (e.g., chilling regimen, carcass stimulation, etc.) need to be taken into account when studying the genomics of meat quality.

Porcine markers for melanocortin 4 receptor (MC4R, Sus scrofa chromosome 1 (SSC1)), leptin receptor (LEPR, SSC6), cathepsin Z (CTSZ, SSC17), CAST (SSC2), tissue necrosis factor (TNF, SSC7), heat shock protein 70.2 (HSP70.2, SSC), myostatin (MSTN), and adenosine monophosphate deaminase 1 (AMPD1, SSC4) have been found that are associated with pork meat quality. Many of the markers are associated with more than one meat quality characteristic or measurement, with differences in additive and dominance effects among the traits. For example, CAST has been associated with Warner–Bratzler shear force, cooking loss, and sensory juiciness, chewiness, and firmness. All of these effects need to be taken into account when considering the value of the markers (see Section Genome-Wide Association Studies). Transcriptome analysis correlating ribonucleic acid (RNA) concentration for different genes to pork quality traits has shown that cooked loin shear force was related to the activation of complement proteins usually promoted by ischemia. This result suggests that preapoptotic homeostatic mechanisms of the muscle cell might encourage sarcomere protein proteolysis either directly or indirectly and concur with observations made in bovine muscle that increased beef tenderness is related to the rapid onset of apoptosis/necrosis.

At the time of writing this, there is no marker panel commercially available to assist with carcass and meat quality trait selection for pigs. The PorcineSNP60 BeadChip is available for genotyping pigs from populations where meat quality is known and it contains approximately 60 000 SNPs, but its cost is prohibitive for use in general herd selection. The BeadChip presently is useful as a research tool, and research using it is now beginning to identify SNPs associated with various aspects of meat and carcass quality. Despite no commercial marker panel existing for the selection of meat quality in pigs, reports have been published showing the use of sets of markers to improve aspects of meat quality in value chains. In pork, pH and color at 24 h postmortem are related to several different aspects of meat quality, including consumer acceptability, color, and water-holding capacity or drip in the retail case. A number of gene variants have been shown to have a significant effect on these traits. The best known example is the Halothane gene (RYR1 or calcium release channel 1 – CRC1: see above) associated with pale, soft, exudative (PSE) meat. Panels of markers have been used to select for reduced drip in case-ready product in the retail case. Boars are selected based on these markers to improve the water-holding capacity of the product in retail stores. Selection for improved pork quality has also focused on increasing pork intramuscular fat (see Section Marbling) as it is associated with increased sensory acceptability when present at levels less than about 3.5% wet weight. Within pork genetic line the relationship has not been as clear, so further work within and between genetic lines will be needed to elucidate the interaction between pork marbling and genetics.

Poultry

As for pork, a series of problems have been identified in poultry meat. For example, muscle myopathies, PSE meat, and the reduced cohesiveness of meat can be poultry meat quality problems. Studies have shown that the heritability for breast meat quality traits such as ultimate pH, color, drip loss, and shear-force are low to moderately heritable (h^2=0.06–0.31). Again, as in pigs, the rate of decrease in pH postmortem and ultimate pH of the meat have been shown to be the key factors of chicken meat quality; lower pH leads to paler, more exudative, and tougher breast meat. As for the other species, the amount and type of fat in the carcass and muscle is of interest for quality and especially flavor and succulence. Not surprisingly, quantitative trait loci (QTL) for all meat quality traits have been identified for chicken and turkey. The availability of genomic tools means that these QTL are now available for dissection for the development of deoxyribonucleic acid marker panels to improve the quality of poultry meat.

Marbling

SNPs have been sought for marbling of the loin muscle in both cattle and pigs because cattle carcass value is contingent upon loin marbling, whereas pork loin eating quality is strongly associated with increased marbling. Enhancement of intramuscular fat has been complicated by the discovery that different genes appear to drive fat deposition in different carcass areas and muscles. For example, in pigs, a QTL on SSC7 is related to intramuscular fat deposition in the gluteus medius muscle (a muscle of the ham), but this does not appear to influence the intramuscular fat within the longissimus thoracis et lumborum muscle of the loin. Importantly, the correlation with back fat is less than 1 (typically x in pigs and y in cattle). This means that there is great potential to use genetic markers to help select for increased marbling without increasing the exterior fat, which is undesirable in terms of today's consumers.

Muscle Fiber Biochemistry

The effects of muscle fiber type on pork meat quality are well known and well described as the metabolic characteristics of the fiber types are critical to the characteristics of meat. Differences in the percentage of specific fiber types exist both among muscles of the same animal and among breeds. Genomic tools have been developed to quantify fiber types in muscle samples. Selecting for rapidly growing, heavily muscled animals led to an indirect selection for white muscle fibers, which rely specifically on glycolysis, the Krebs cycle, and the electron transport chain for generation of energy. With this

fiber type, glycogen stores are high and the decline of muscle pH within the early postmortem period is rapid, lowering the pH dramatically while the carcass and muscles are warm. The combination of low pH and high muscle temperature produces meat with significant denaturation, so that the meat has a low water-holding capacity, a pale color and is soft, characteristics similar to that of the PSE condition produced by other mutations. The decline of intramuscular pH in muscles that are primarily composed of white fibers is also extensive, with ultimate pH values often being below pH 5.5. This is also because the metabolism of the white muscle fibers favors glycolysis. Muscles that consist of primarily white fibers also tend to have little intramuscular fat. The reduction of intramuscular fat in pork led to reduced eating quality and acceptability of pork, and, therefore, breeding strategies in the pig industry have focused within the last 10 years on increasing the proportion of intramuscular fat or 'marbling' in pork. This selection indirectly increased the proportion of red oxidative muscle fibers that have a metabolism focused on lipolysis and fatty acid utilization. With the shift in muscle energy metabolism toward fatty acid utilization, early postmortem pH decline slowed and the ultimate pH increased to a value at or above pH 5.5. With the interest in increasing intramuscular fat, the peroxisome proliferator-activated receptor-gamma coactivator-1α gene has become of interest in the selection of pigs for increased intramuscular fat as this gene is involved in energy and fat metabolism.

In the porcine loin, transcriptome analysis indicated that cooking loss, a* (redness) value, and pH at 45 min postmortem were affected by variation in the transcription of genes associated with the onset of apoptosis, glucose metabolism, and cat-ion channel activity, respectively. How expression of these genes specifically affects each meat quality characteristic is yet to be examined; however, early onset of apoptosis may lead to increased cooking loss through increased protein denaturation due to extensive proteolysis. Moreover, the redness of pork (a* value) may be affected by the transcription of glycolytic enzymes because decreased glycolytic enzyme transcription can be associated with reduced oxidative muscle fiber population, which has increased myoglobin concentrations.

Candidate Genes

Initial research following the identification of the Halothane gene was based on the so-called candidate gene studies, where information on gene function, for example, from biochemical studies, enabled researchers to look for gene variants in the genes coding these proteins and ask if they explained variation in meat quality. The CAPNs and CAST are good examples of this approach. Although information can transfer well across species (CAST has been shown to impact tenderness in several species including sheep as well as cattle and pigs), this approach is sometimes called comparative genomics. Even so, this approach has been criticized over the years as being like a 'needle in a haystack,' however, it has generated some very interesting results. The main drawback is that it depends on 'what is known,' whereas genetic approaches, such as QTL mapping, or its modern equivalent called 'genome-wide association studies' (GWAS; see Section Genome-Wide Association Studies), allow the researcher to find novel genes. The RN gene was an example, as this subunit of PRKAG was first identified through such studies on pigs. Once a region of the genome has been identified, researchers can now conduct fine-mapping or resequencing to identify variations that might explain the trait variation. This then takes researchers back to candidate gene studies using the latest bioinformatic approaches to exploit information on genomes and gene networks (see Section Gene Expression Analysis).

Genome-Wide Association Studies

With the advent of high-throughput sequencing methods, it has become relatively straightforward to generate large numbers of SNPs for populations of interest and to drive forward GWAS for all species. These panels are available at 50 000 and 600–800 000 for cattle, and 60 000 for pigs and chickens. Different designs are used from the classical F2 cross between different lines to within population studies using commercial genotypes. Associations between the traits of interest and many regions of the genome are found for all traits studied. These studies require large numbers of observations, typically more than a thousand. In the case of meat quality, the traits most commonly measured are ultimate pH and color, as well as drip loss and water-holding capacity. In the case of beef and pork, shear force is also commonly measured. It is also possible to include sensory traits. Performance data are also collected on the same animals allowing the identification of negative associations between the traits to be identified. This is a very important aspect of commercial implementation of these tools. Breeders and producers need to understand the net value of marker effects in their population and system. It does not necessarily help if improved tenderness comes at a large cost in terms of lost growth rate or feed efficiency. Fortunately, markers can help select for the desired outcome of improved meat quality at an affordable production cost.

Gene Expression Analysis

Another approach for the identification of genes and gene products involved in meat quality is to study gene expression in muscle tissues. This is the transcription of DNA into messenger RNA (mRNA), which is then translated into proteins, for example, enzymes (CAPNs) and structural proteins (myosins, titin, etc.) that the authors are interested in with respect to meat quality. The hypothesis is that the phenotype of the muscle is related to the genes being expressed in the tissue, and that differences in expression between muscles of different quality will explain some of that difference. The timing of sampling is very important as expression varies over time; in general, muscle is sampled as soon as possible postmortem. Alternatively, some studies have looked at embryonic muscle because muscle fiber type is determined at this stage of development. It is also possible that the important genes are expressed externally, such as hormones that lead to changes in muscle function or performance – these genes would not be identified by expression analysis of muscle samples, although their impact on muscle gene expression will be observed.

Ultimately, genes that are expressed differentially between muscles or muscle states (i.e., PSE vs. dark, firm, dry muscle) become candidate genes for further studies. The genes are sequenced from interesting species of animals to identify genetic variants that are then used in association studies. This can be done with individual genes, or panels of genes up to high density SNP sets of 10 000, 60 000, or even 700–800 000 as now being done in cattle.

Next-generation sequencing technologies are now being used to generate much greater depth of information for gene expression studies (RNAseq) and are beginning to replace microarray studies. The latter use known genes (or sequences) and are, therefore, more targeted, whereas RNA-seq provides much more power to look at gene variants (such as alternate splicing of mRNA) and will rapidly become the preferred method for such approaches as it allows researchers to explore the unknown (see Section Candidate Genes). This is an exciting development and it is also possible to analyze samples for other RNA species such as microRNA, which are expressed in different tissues and might explain variation b among muscles as well as among animals.

Linkage Disequilibrium

This term describes the association between DNA markers and genes. It is important as it determines the utility of DNA markers in different populations and situations. Essentially, markers (or genes) that are more often inherited together than would be expected by chance are in linkage disequilibrium, or more simply they are linked such that the analysis of one can predict the state of the other. This means that in some cases it is not necessary to identify the underlying causative gene (or even the causative mutation – sometimes called the quantitative trait nucleotide). If a DNA marker can be shown to be associated with the trait in a population, then it can be used to help select for that trait. Most commonly, this is now achieved by combining the genomic information with the phenotypic information to produce a genomic estimated breeding value (gebv). The utility depends, at least in part, on the distance between the marker (acting as a signpost) and the causative gene. However, population history and genetic background as well as other factors also play a role in this. For example, though markers can usually do a good job of predicting a trait within a population, their impact is often very disappointing across populations. This has made implementation of marker-assisted selection relatively difficult in cattle compared with pigs and poultry. However, a difference in the structure of the beef industry compared with the others is also a major factor. The identification of the causative mutations or use of denser marker panels, so that the distance between markers and the causative genes are smaller should help. Even so, one of the hurdles remains access to the phenotypic data on which to train or discover the associations between the markers and the traits of interest.

Proteomics

Increasingly, proteomics is being used to investigate the interaction between genomics and environment so that the actual expression of proteins is verified in the physical context of the animal and its genetics. Protein expression has been characterized in both antemortem and postmortem muscle in order to relate the animal's physiological state to that of meat and its subsequent meat quality. The investigation as to how protein expression or degradation affects meat quality is still nascent, but results realized to date portend the power of proteomic methods when coupled with genomics.

Much of the work in meat quality proteomics began to appear in the literature around 2005, and few proteins were identified, most likely because high-throughput protein identification systems were just becoming available. Moreover at that time, the potential for intellectual property development from these studies was still being assessed and understood, so most studies focused on the success of the application of proteomic techniques rather than the actual impact of protein differences on meat quality. Subsequent studies identified that phosphorylation of AMPK at threonine-172 was associated with increased glycogen accumulation and decreased intramuscular fat in the longissimus muscle of beef steer carcasses. In addition, the study of bovine proteins in postmortem muscle indicated that increased detection of proteins from the inner and outer membranes of the mitochondria was indicative of increased beef tenderness, suggesting that early or extensive apoptosis in early postmortem muscle might be conducive to the tenderization of beef. Proteomic studies offer the potential for direct protein markers that might predict meat quality or, like gene expression studies, information that can be used to identify DNA markers that can be used to improve quality.

Traceability

Having generated products with improved meat quality, there might be a need to be able to 'identity preserve' such products to help protect their value, helping give consumers confidence in products and preventing other lower quality products substituting for them. DNA traceability offers a useful way to help verify the origin or quality of the products. A sample of meat, even from a cooked product, for example, in a restaurant, can be traced back through the production system, back to the farm, and even individual animal, as the DNA of an animal is unique (except for twins or clones) and available for analysis long after the product has been separated from physical traceability, such as the radio frequency identification tags.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. Boar Taint: Biological Causes and Practical Means to Alleviate It. Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Adipose Tissue; Chemical Composition; Color and Pigment; Palatability; pH Measurement; Protein Functionality; Water-Holding Capacity. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycogen; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Double-Muscled Animals. Human Nutrition: Macronutrients in Meat; Meat and Human Diet:

Facts and Myths; Micronutrients in Meat. **Measurement of Meat Quality:** Measurements of Water-holding Capacity and Color: Objective and Subjective. **Proteomic Technologies and Their Applications in the Meat Industry. Species of Meat Animals:** Cattle; Pigs; Poultry; Sheep and Goats. **Tenderizing Mechanisms:** Enzymatic

Further Reading

Aslam, M.L., Bastiaansen, J.W.M., Crooijmans, R.P.M.A., Vereijken, A., Groenen, M. A.M., 2011. Whole genome QTL mapping for growth, meat quality and breast meat yield traits in turkey. BMC Genetics 12, 61. Available at: http://www.biomedcentral.com/1471–2156/12/61 (accessed 19.01.14).

Behrends, S.M., Miller, R.K., Rouquette, Jr., F.M., *et al*., 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. Meat Science 81, 433–438.

Bernard, C., Cassar-Malek, I., Le Cunff, M., *et al*., 2007. New indicators of beef sensory quality revealed by expression of specific genes. Journal of Agricultural and Food Chemistry 55, 5229–5237.

Cherel, P., Herault, F., Vincent, A., Le Roy, P., Damon, M., 2012. Genetic variability of transcript abundance in pig skeletal muscle at slaughter: Relationships with meat quality traits. Journal of Animal Science 90, 699–708.

Ciobanu, D.C., Lonergan, S.M., Huff-Lonergan, E.J., 2011. Genetics of meat quality and carcass traits. In: Rothschild, M.F., Ruvinsky, A. (Eds.), The Genetics of the Pig. Wallingford: CABI, pp. 355–389.

Dikeman, M.E., Pollak, E.J., Zhang, Z., *et al*., 2005. Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds, and heritabilities and expected progeny differences for Warner–Bratzler shear force in three beef cattle breeds. Journal of Animal Science 83, 2461–2467.

Fernandez, X., Monin, G., Talmant, A., Mourot, J., Lebret, B., 1999. Influence of intramuscular fat content on the quality of pig meat – 2. Consumer acceptability of m. longissimus lumborum. Meat Science 53, 67–72.

Font-i-Furnols, M., Tous, N., Esteve-Garcia, E., Gispert, M., 2012. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptablity of pork with different intramuscular fat content. Meat Science 91, 448–453.

Fortin, A., Robertson, W.M., Tong, A.K.W., 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. Meat Science 69, 297–305.

Juszczuk-Kubiak, E., Flisikowski, K., Wicińska, K., Poloszynowicz, J., Rosochacki, S., 2009. Identification of the new polymorphisms in the promoter region of the CAST gene in cattle. Meat Science 82, 278–283.

Lines, D.S., Pitchford, W.S., Kruk, Z.A., Bottema, C.D.K., 2009. Limousin myostatin F94L variant affects semitendinosus tenderness. Meat Science 81, 126–131.

Maltin, C., Plastow, G., 2004. Functional genomics and proteomics in relation to muscle tissue. In: te Pas, M.F.W., Everts, M.E., Haagsman, H.P. (Eds.), Muscle Development in Livestock. Wallingford, UK: CAB International, pp. 267–296.

Quintanilla, R., Pena, R.N., Gallardo, D., *et al*., 2011. Porcine intramuscular fat content and composition are regulated by quantitative trait loci with muscle-specific effects. Journal of Animal Science 89, 2963–2971.

Rincker, P.J., Killefer, J., Ellis, M., Brewer, M.S., McKeith, F.K., 2008. Intramuscular fat content has little influence on the eating quality of fresh pork loin chops. Journal of Animal Science 86, 730–737.

Schenkel, F.S., Miller, S.P., Jiang, Z., *et al*., 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits in beef cattle. Journal of Animal Science 84, 291–299.

Smith, T., Domingue, J.D., Paschal, J.C., *et al*., 2007. Genetic parameters for growth and carcass traits of Brahman steers. Journal of Animal Science 85, 1377–1384.

Underwood, K.R., Tong, J., Zhu, M.J., *et al*., 2007. Relationship between kinase phosphorylation, muscle fibre typing, and glycogen accumulation in longissimus muscle of beef cattle with high and low intramuscular fat. Journal of Agricultural and Food Chemistry 55, 9698–9703.

Van Eenennaam, A.L., Li, J., Thallman, R.M., *et al*., 2007. Validation of commercial DNA tests for quantitative beef quality traits. Journal of Animal Science 85, 891–900.

Wheeler, T.L., Shackelford, S.D., Casas, E., *et al*., 2001. The effects of Piedmontese inheritance and myostatin genotype on the palatability of longissimus thoracis, gluteus medius, semimembranosus, and biceps femoris. Journal of Animal Science 79, 3069–3074.

White, S.N., Casas, E., Wheeler, T.L., *et al*., 2005. A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. Journal of Animal Science 83, 2001–2008.

Relevant Websites

http://www.livestockgentec.com/
Livestock Gentec.

http://www.afdp.ualberta.ca/
University of Alberta.

http://www.afns.ualberta.ca/
University of Alberta.

GROWTH OF MEAT ANIMALS

Contents

Adipose Tissue Development

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Glossary

Adipocytes Fat cells.
Anabolic lipid metabolism The synthesis of fatty acids or fats.
Brown adipose tissue A tissue that is rich in mitochondria and is involved in thermogenesis.
Catabolic lipid metabolism The breakdown of fats or oxidation of fatty acids.
Leptin A hormone secreted by adipocytes involving regulation of body composition.
Lipogenesis The synthesis of fats in adipocytes and liver cells.
Lipolysis The breakdown of a triacylglycerol into fatty acids and glycerol for further metabolism.
Thermogenesis The generation of heat.
Uncoupling protein 1 The protein involved in thermogenesis.
White adipose tissue A tissue that is both a storage site of excessive energy and a dynamic endocrine organ.

Introduction

Adipose tissue (AT) comprises the largest energy depot and provides energetic fuel for mammals. The major physiological role of AT is to store energy and to mobilize it depending on metabolic needs. Insulation is another important function of AT, particularly subcutaneous AT. Adipocytes or fat cells are the primary AT cell types. Blood vessels, nerves, connective tissues, and other cell types also reside in AT. There are two types of AT – brown and white, which are distinct in cell structure and functionality. Brown AT (BAT) confers the ability for nonshivering thermogenesis. White AT (WAT) is the common site to store excess energy in the form of triglycerides (triacylglycerol or TG). Both BAT and WAT are controlled by endocrine and neural molecules to maintain the proper shape and size under normal health conditions. Therefore, understanding the underlying molecular mechanisms regulating AT development provides important implications to improve meat quality and ameliorate human diseases, such as obesity and diabetes.

Brown Adipose Tissue

Physiologically, BAT, which is rich in mitochondria, plays an important role in dissipating heat from chemical reactions (thermogenesis). Most mammalian newborns and hibernating mammals have BAT and it regresses during the first weeks postpartum. In newborn ruminants, most of the AT is BAT, with only small amounts of WAT. Newborn lambs and calves retain highly active BAT after birth when the need for thermogenesis is high. Adipocytes in BAT of most species store lipid in multiple small droplets instead of one large droplet, as seen in WAT. An exception is brown adipocytes of newborn calves that contain a large central lipid vacuole and only a few peripheral lipid inclusions. The total amount of BAT is approximately 1.5–2.0% of body weight of newborn calves. Recently, the identification of small amounts of BAT in human adults suggests that this BAT may be a therapeutic target site for obesity and diabetes because of its high capacity to perform adaptive thermogenesis to dissipate excessive fat storage through heat production.

Master Regulator of Adaptive Thermogenesis in Brown Adipose Tissue

Uncoupling protein 1 (UCP1) is abundantly expressed in the inner mitochondrial membrane of mammalian BAT. In BAT, fatty acids (FA) from lipolysis (degradation) of BAT TG or from lipolysis in WAT with the released FA delivered from the plasma are substrates for FA oxidation and heat production. In mitochondria, electrons from oxidative metabolism travel along an electron gradient (electron transport) to terminally combine with oxygen. Energy dissipated in the gradient is trapped in the synthesis of adenosine triphosphate (ATP), the primary energy storage molecule. UCP1 uncouples O_2 consumption from ATP synthesis to short-circuit the mitochondrial process and generate heat.

Thyroid hormone and the neurotransmitter, norepinephrine, released from the sympathetic nervous system activate the β-adrenergic receptor signaling pathway to activate triacylglycerol lipase. The lipase converts TG into free FA that activate UCP1. Although the role of UCP1 in BAT mitochondrial function is clear, the physiological role of UCP1 in BAT thermogenesis was gradually discovered and evidenced through recent studies using UCP1-ablated mice. Absence of UCP1 in mice does not influence the development of BAT and the basal respiration of brown adipocytes. However, brown adipocytes from UCP1-ablated mice are unable to elevate heat production in response to noradrenergic stimulation. High fat feeding increases thermogenesis and thus UCP1 is required. Cold-induced nonshivering thermogenesis and inhibition of body weight gain in response to injected leptin both require UCP1. Activation of rodent adrenergic receptors through injection of norepinephrine or a β_3-adrenergic receptor agonist requires the presence of UCP1 to stimulate thermogenesis under thermoneutral (30 °C) and cold (5 °C) conditions. The 129 Sv mouse strain, well known for antiobesity characteristics, requires UCP1 to exert the obese-resistant properties under high fat feeding. In conclusion, the absence of UCP1 does not influence the development and differentiation of mammalian BAT but influences cold/noradrenergic-stimulated thermogenesis and also the ability to increase energy expenditure during high fat feeding.

Molecular Mechanisms Regulating Brown Adipose Tissue Development

The molecular mechanism controlling BAT development has not been fully revealed. Generally, BAT and WAT share some adipogenic transcription factors to induce differentiation. In the early differentiation process of BAT, CCAAT/enhancer-binding proteins β (C/EBPβ) and C/EBPδ are induced and contribute to the expression of peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPα. PPARγ is the key driver of fat cell differentiation, whereas C/EBPα coordinates with PPARγ in the enhancement of adipogenesis. PPARγ coactivator 1-α (PGC1α) is more highly expressed in BAT than in WAT and increases the thermogenic program of FA oxidation, mitochondrial biogenesis, oxygen consumption, and UCP1 expression during cold acclimation. PPARα agonists also induce UCP1 expression. Overexpressing FOXC2 activates BAT development and the β-adrenergic signaling pathway to counteract diet-induced obesity. However, PGC1α, PPARα, and FOXC2 are not master regulators to determine the specific commitment to a brown adipocyte fate.

A common origin, previously assumed, for brown and white adipocytes has recently been challenged. Currently, BAT has been shown to be more closely related to skeletal muscle than WAT because both brown adipocytes and muscle express some of the myogenic factors, such as myogenic factor 5 (Myf5). A zinc finger transcription factor (PRDM16) is selectively expressed in brown versus white adipocytes and contributes to control the differentiation of brown adipocytes. Activating PRDM16 in WAT or white progenitor cells induces the switch to the phenotype of brown fat, including elevation of PGC1α, UCP1, and uncoupled respiration. Depletion of PRDM16 in BAT yields a nearly total loss of the characteristics of brown adipocytes. Other striking results demonstrated that PRDM16 controls a switch between skeletal myoblasts and brown adipocytes. Loss of PRDM16 in BAT precursors results in their differentiation into skeletal muscle. Conversely, ectopic expression of PRDM16 in myoblasts converts them into brown adipocytes. These results imply that white and brown adipocytes are derived from different cell lineages. BAT and skeletal muscle can both be derived from the same Myf5-positive precursor cells, whereas WAT adipocytes are not. PRDM16 complexes with C/EBPβ to induce PPARγ expression and this coactivation can convert naïve fibroblasts into brown adipocytes. These results suggest that intervention in this signaling pathway may be used to manipulate the proportion of WAT, BAT, and muscle to benefit meat production and to obliterate human diseases.

White Adipose Tissue

Originally, WAT was conceived as a stationary storage site for excessive energy. Today, WAT is recognized as a dynamic endocrine organ with active anabolic and catabolic metabolism maintaining an animal's energy balance. Moreover, adipocytes can secrete numerous adipokines that exert paracrine or endocrine functions. These adipokines, including leptin, adiponectin, visfatin, omentin, resistin, retinol-binding protein 4, and inflammatory mediators such as tumor necrosis factor-α, interleukin-6, and interleukin-10, regulate food intake and energy expenditure and mobilization. The most well-known adipokine is leptin, which reduces feed intake through inhibition of appetite in the hypothalamus of the brain. Dysregulation of leptin is closely associated with insulin resistance, obesity, and diabetes.

White Adipose Tissue Development in Newborn Mammals

At birth, most mammalian newborns have little depot fat and develop WAT rapidly with increasing TG biosynthesis. The developed WAT serves as an energy storage organ when energy intake exceeds requirements and also provides energy to other tissues when nutrients are scarce. Thermoinsulation is another role of WAT, particularly subcutaneous AT deposited under the skin. Perirenal WAT is deposited near the kidneys. Omental and mesenteric WAT are deposited in the mesenteries

suspending the gut. Perigonadal WAT, such as periovarian or periuterine, depots in females or the epididymal depot in males is around the reproductive organs. Intermuscular WAT is between skeletal muscles and intramuscular WAT is inside individual muscles. The proportion of WAT deposited in the various depots is species specific. In beef cattle and particularly in pigs the subcutaneous depot is large. Dairy cows have large mesenteric depots. Growth of WAT consists of three processes: hyperplasia, differentiation, and hypertrophy. Hyperplasia or the increase in cell number occurs in undifferentiated precursor cells (preadipocytes) but not in differentiated adipocytes. The differentiation process involves an initial increase in the transcription factors C/EBPβ and C/EBPδ followed by an increase in PPARγ and finally C/EBPα. The promoter region of a number of genes characteristic of the adipocyte phenotype contains response elements that specifically bind and are activated by PPARγ or C/EBPα. Hypertrophy is the increase in size of already differentiated adipocytes. The process involves synthesis and deposition of TG in the adipocyte. Preadipocytes are derived from embryonic mesoderm and maintained in the fibroblast-like shape until differentiation is initiated when the cells are presented with appropriate stimuli.

Hyperplasia

The increased number of preadipocytes can occur both in WAT and *in vitro* system. The stromal vascular fraction containing preadipocytes can be obtained by collagenase digestion from WAT. Preadipocytes have been successfully isolated from cattle, dogs, humans, pigs, rodents, and sheep. In the common culture medium of Dulbecco's Modified Eagle Medium/F12 with 10% fetal bovine serum, primary preadipocytes can proliferate rapidly and proceed to differentiation in cell culture dish. Several clonal preadipocyte cell lines have also been established from rodents. The most common one is 3T3-L1. This cell line and *in vitro* culture system provides researchers with convenient tools to understand adipocyte biology, such as adipocyte differentiation and accumulation of TG in lipid droplets. Recently, determining the ^{14}C content incorporated into deoxyribonucleic acid in adipocytes is a new technique developed to evaluate the age of fat cells in the body. The results from the technique show that there are approximately 10% of adipocytes renewed each year and the turnover rate of adipocytes is increased with obesity.

Differentiation

Primary preadipocytes presented with appropriate stimuli differentiate into adipocytes. Preadipocytes from different species or clonal cell lines require diverse factors. Generally, three major factors or hormones are indispensible in adipocyte differentiation – insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX). Several other factors are also required for differentiation in some species, such as transferrin, thyroid hormone, and the PPARγ ligand thiazolidinedione. Treatment of preadipocytes with dexamethasone activates C/EBPβ. IBMX functions as a phosphodiesterase inhibitor to raise intracellular cAMP levels in order to increase expression of C/EBPδ. Activation of C/EBPβ and δ in preadipocytes reenters the cell cycle and undergoes several rounds of mitosis, known as mitotic clonal expansion. C/EBPβ and δ further induce the transcription of C/EBPα and PPARγ. On this stage, adipocytes with activation of C/EBPα and PPARγ promote adipogenesis and adipocyte-specific target genes, such as fatty acid-binding protein (aP2) and adiponectin, known as terminal differentiation markers. Insulin activates PI3-kinase/Akt signaling pathway to promote PPARγ activity and increase adipocyte differentiation.

Hypertrophy

During the adipocyte differentiation, activation of adipogenesis increases TG biosynthesis. Mature adipocytes have the capacity to store TG in lipid droplets with increase of its size. Lipid droplets are conceived as a dynamic cellular organelle specialized in the storage of neutral lipids, such as sterol esters and TG to prevent lipotoxicity of unesterified lipids. Lipid droplets comprise a neutral lipid core surrounded by a phospholipid monolayer made up of free cholesterol and several proteins, such as perilipin, diacylglycerol acyltransferase 2 (DGAT2), and caveolin. There are high activities of mobilizing lipids from lipids droplets (lipolysis) or synthesizing newly formed TG into storage (lipogenesis) on feeding or excessive energy state in mammals.

Adipocytes have multiple small lipid droplets in early phase and then they fuse into a large lipid droplet accompanied with accretion of TG storage. Lipid droplets occupy majority of cell volume of adipocyte with signet-ring morphology (Figure 1). TG accounts for more than 90% of the mass of AT in the later stages of growth (depending on the species, depot, and diets). Adipocyte size is largely varied in different depots and growing periods. Generally, adipocyte size in newborn pigs is approximately 20 μm in diameter and increase to more than 200 μm in older pigs. Bovine adipocytes can increase to 500 μm in diameter.

Anabolic Lipid Metabolism

De Novo Lipogenesis

When dietary energy is in excess of the requirements, the energy from dietary lipids and nonlipid precursors can be converted into lipids and stored in AT. *De novo* lipogenesis (DNL) is the metabolic process that converts nonlipid carbon precursors into lipids (Figure 2). Glucose is the primary carbon source in nonruminant mammals, whereas acetate is the primary carbon source in ruminant mammals. Glucose is converted to pyruvate in the glycolytic pathway and then to citrate in mitochondria. Citrate transported to the cytoplasm yields cytosolic acetyl-CoA. Acetyl-CoA is carboxylated to malonyl-CoA by acetyl-CoA carboxylase, the first key enzyme in DNL. Malonyl-CoA is then used as a two-carbon donor for the complex multienzyme fatty acid synthase. A series of sequential reactions – decarboxylation, reduction, and dehydration – lead to the synthesis of the 16-C saturated fatty acid palmitate. Production of longer chain FA and polyunsaturated FA requires elongase and desaturase enzymes, such as stearoyl-CoA desaturase 1. Amino acids can be transaminated or

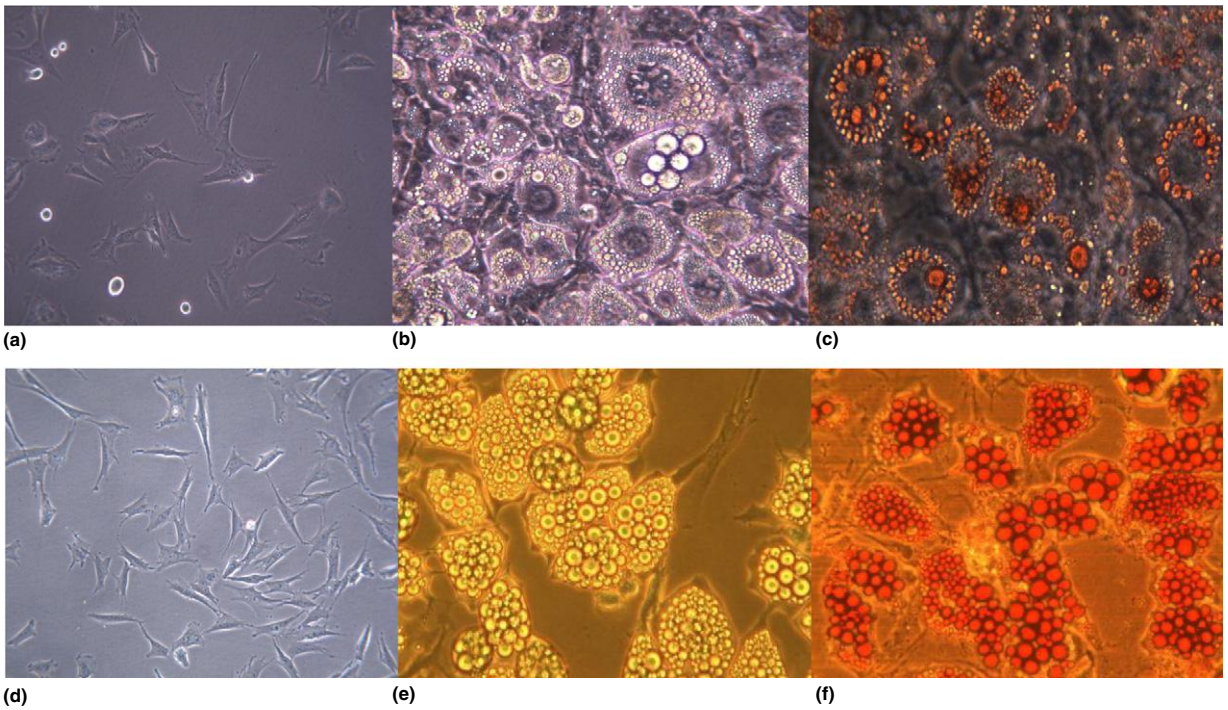

Figure 1 Morphological changes of 3T3-L1 mouse cell line (a–c) and pig primary preadipocytes isolated from stromal vascular fraction (d–f) during adipocyte differentiation. 3T3-L1 and pig primary preadipocytes maintain the fribroblast-like shape (a and d). On differention process with appropriate stimuli, preadipocytes begin to synthesize and accumulate triglycerides in lipid droplets and differentiate into adipocytes (b and e). Adipocyte genes, such as fatty acid-binding protein 4 (aP2) and adiponectin, are highly expressed in mature adipocytes. Lipid dropletes are stained and characterized by Oil red O staining (c and f).

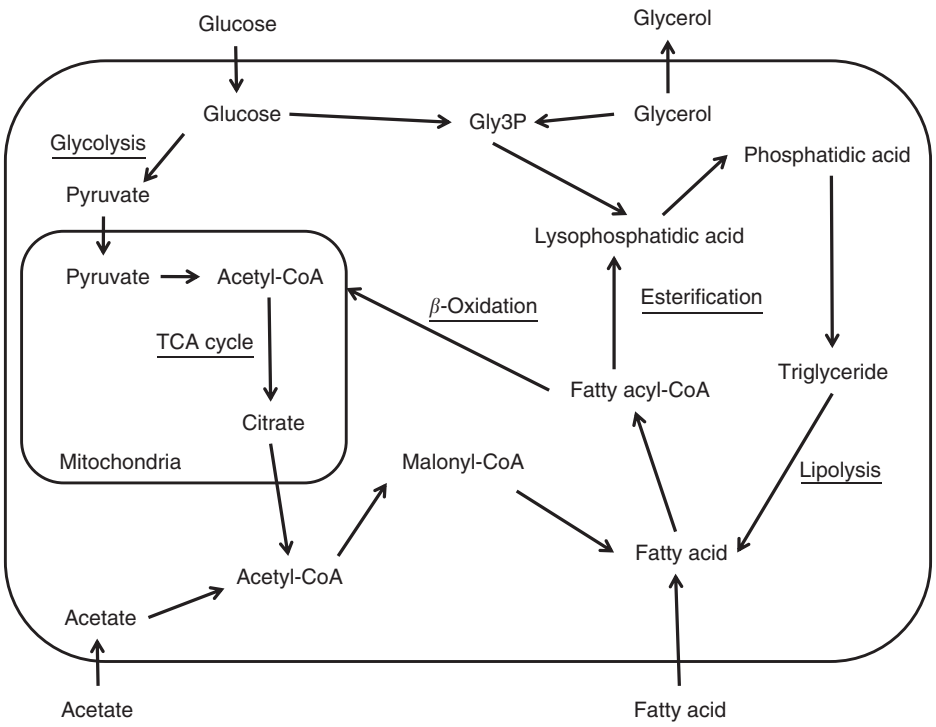

Figure 2 Outline of metabolic pathways in adipocytes.

deaminated to carbon compounds and acetate, the main product of bacterial fermentation in the rumen of ruminant mammals, which may also contribute carbon for fatty acid synthesis.

Two tissues are mainly responsible for DNL in mammals – liver and WAT. The relative contribution of these tissues is different for different species. In humans, the liver is the major site for DNL, whereas in pigs, adipocytes are the main site and in rodents both tissues are involved in DNL. In ruminants, the liver is the major site before ruminal development, but after development WAT is the major site. Suckling mammals have a diet rich in fat so there is little DNL until after weaning. However, lipoprotein lipase (LPL) must be available after birth to supply FA to the adipocyte from the circulating lipoprotein triacylglycerol. Lipolysis is also necessary for the newborn mammals to respond to periods of dietary insufficiency and to cold stress; the capacity for lipolysis is increased rapidly after birth.

Lipoprotein Lipase

FA are detergent molecules and thus toxic to cells. To avoid toxicity, FA are bound to albumin or esterified into complex lipids like phospholipids or TG. In plasma, the TG is part of various lipoproteins, particularly the chylomicrons containing exogenous dietary lipids and very low-density lipoproteins (VLDL) secreted from the liver. These two lipoproteins constitute more than 90% of blood FA. Elevated free FA in plasma contribute to metabolic diseases, such as obesity or diabetes. Ruminants produce VLDL but not chylomicrons. Uptake of FA from the plasma into adipocytes requires the enzyme LPL. LPL is produced and exported by the WAT and also by other peripheral tissues, including BAT, skeletal muscle, cardiac muscle, and mammary gland that utilize FA for storage or energy. LPL after synthesis and translocation resides on the endothelial lumen of capillaries, where it is active in hydrolysis of TG-rich lipoprotein to release free FA, 2-monoacylglycerols, and lipoprotein remnants. LPL activity is regulated in different physiological conditions (fed, fasting, and exercise). During the postprandial period, insulin and glucose upregulate LPL activity in WAT or other tissues to cleave the circulating TG into FA that are absorbed by the adipocyte or other tissue cells. FA that are not oxidized for energy are mostly esterified into phospholipid and TG.

FA hydrolyzed from lipoprotein by LPL or dissociated from albumin enter adipocytes by diffusion or by the facilitated transporter system including an FA translocase (CD36), an FA transport protein, and a membrane-bound FA-binding protein. FA inside adipocytes are bound to cytosolic transport proteins, cytosolic FA-binding proteins, and acyl-CoA-binding protein, which binds FA-CoA. FA-CoA are then esterified or oxidized. Excessive energy from carbohydrate, amino acid carbon skeletons, or lipids are converted to TG and then stored in cytosolic lipid droplets. An important intermediate is glycerol-3-phosphate (Gly3P), which forms the backbone on which the FA are esterified. Gly3P can be derived from glycerol, glycolysis, or glyceroneogenesis. Glycerol uptake from the plasma and phosphorylation by glycerol kinase is quite low in WAT. An FA-CoA is esterified to Gly3P by glycerol-3-phosphate acyltransferase to form lysophosphatidic acid (LPA). Another FA-CoA is esterifed to LPA by 1-acylglycerol-3-phosphate acyltransferase to yield phosphatidic acid (PA). The phosphate is removed to form diacylglycerol (DG) and finally a third FA-CoA is esterified to DG by diglyceride acyltransferase to form TG for storage in lipid droplets. Many tissues have the capability to synthesize TG, but AT is the major site for TG storage. The liver is another major site for for TG synthesis with the TG largely becoming part of the VLDL lipoproteins. To sum up, the anabolic processes of WAT involve activation of LPL, uptake of FA from capillary into adipocytes, activation of DNL (in many species), and promotion of FA esterification to TG.

Catabolic Lipid Metabolism

Catabolic and anabolic metabolism are well-controlled and sophisticated processes. The catabolic pathway, termed lipolysis, hydrolyzes TG with the release of FA (Figure 2). Three lipase enzymes are involved in the hydrolysis of TG. The initial step activates the rate-limiting enzyme adipose triglyceride lipase (ATGL), which removes an FA from TG to produce DG. ATGL has 10-fold more specificity for TG than for DG. ATGL is highly expressed in WAT and BAT. Its expression increases as adipocyte differentiation progresses and overexpression of ATGL increases basal lipolysis. Inhibition of ATGL reduces hormone-stimulated lipolysis, suggesting that ATGL is crucial in the basal and hormone-stimulated lipolysis. Hormone-sensitive lipase (HSL) removes another FA, primarily from DG, but the substrate specificity is lower with hydrolysis of TG, monoacylglycerol, cholesteryl esters, and retinyl esters. The third lipase, monoacylglycerol lipase, is a hormone-independent lipase responsible for the removal of the last FA from TG.

Lipolysis is controlled in different energy states, primarily by insulin to inhibit and by β-adrenergic agonists, adrenaline (epinephrine) and noradrenaline (norepinephrine), to stimulate. The catecholamines, noradrenaline and adrenaline activate lipolysis through β-adrenergic receptor signaling coupled to G-protein activation. This signaling activates adenylyl cyclase, an enzyme that converts ATP to cyclic AMP, which then activates cAMP-dependent protein kinase A (PKA). PKA then phosphorylates HSL and perilipin A, the most abundant protein associated with lipid droplets in adipocytes. The phosphorylation of perilipins and HSL is necessary for the dispersal of the lipid droplets resulting in full lipolytic activity. Lipolysis is stimulated during fasting to provide energy to adipocytes and other tissues. ATGL is also regulated by nutritional status (increased during fasting and reduced during feeding or by insulin).

In sum, AT is currently recognized not only as a stationary site for storage of excess energy or as a site to mobilize lipids for utilization in other tissues but also as a dynamic organ with comprehensive control of lipolysis and lipogenesis under feeding or fasting states. Understanding the underlying molecular mechanisms regulating AT development and fat deposition becomes a prominent issue in treating human metabolic diseases and improving animal production. The appropriate increase of intramuscular AT depot especially

provides a desirable flavor for consumers in the production of high-quality meats.

See also: Chemical and Physical Characteristics of Meat: Adipose Tissue. Growth of Meat Animals: Growth Patterns; Metabolic Modifiers. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists

Further Reading

Brasaemle, D.L., 2007. Thematic review series: Adipocyte biology. The perilipin family of structural lipid droplet proteins: Stabilization of lipid droplets and control of lipolysis. Journal of Lipid Research 48, 2547–2559.

Dinel, A.L., Kolditz, C., Langin, D., 2010. Metabolism of fatty acids in adipocytes. In: Christen, Y., Clément, K., Spiegelman, B.M. (Eds.), Novel Insights into Adipose Cell Functions. Berlin, Heidelberg: Springer-Verlag, pp. 21–43.

Farese, Jr., R.V., Walther, T.C., 2009. Lipid droplets finally get a little R-E-S-P-E-C-T. Cell 139, 855–860.

Farmer, S.R., 2006. Transcriptional control of adipocyte formation. Cell Metabolism 4, 263–273.

Gerrard, D., Grant, A., 2003. Principles of Animal Growth and Development. Dubuque, IA: Kendall Hunt.

Kajimura, S., Seale, P., Spiegelman, B.M., 2010. Transcriptional control of brown fat development. Cell Metabolism 11, 257–262.

Lefterova, M.I., Lazar, M.A., 2009. New developments in adipogenesis. Trends in Endocrinology & Metabolism 20, 107–114.

Mersmann, H.J., 1991. Regulation of adipose tissue metabolism and accretion in mammals raised for meat production. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals, Advances in Meat Research. London: Elsevier Applied Science, pp. 135–168.

Scanes, C.G., 2003. Biology of Growth of Domestic Animals. Ames, IA: Iowa State University Press.

Sethi, J.K., Vidal-Puig, A.J., 2007. Thematic review series: Adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. Journal of Lipid Research 48, 1253–1262.

Smith, S.B., 1995. Substrate utilization in ruminant adipose tissues. In: Smith, S.B., Smith, D.R. (Eds.), The Biology of Fat in Meat Animals. Champaign, IL: American Society of Animal Science, pp. 166–188.

Smith, S.B., Smith, D.R., 1995. The Biology of Fat in Meat Animals. Champaign, IL: American Society of Animal Science.

Spiegelman, B.M., 2010. Transcriptional regulation of brown and white adipogenesis. In: Christen, Y., Clément, K., Spiegelman, B.M. (Eds.), Novel Insights into Adipose Cell Functions. Berlin, Heidelberg: Springer-Verlag.

Walther, T.C., Farese, Jr., R.V., 2009. The life of lipid droplets. Biochimica et Biophysica Acta 1791, 459–466.

Relevant Website

http://en.wikipedia.org/wiki/Adipose_tissue

Endocrinology

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Glossary

Chondrogenesis The process by which cartilage is formed and developed.

Chorionic binucleated cells Cells located in the placental layer called the chorion; it is suggested that the normal function of mature chorionic binucleate cells at all the stages of bovine and ovine pregnancy is migration into the uterine epithelium to release its granules and subsequent condensation to a cell remnant, which is phagocytosed by the chorionic epithelium.

Cotyledon A lobule of a mammalian placenta.

Glucocorticoids A class of steroid hormones that bind to the glucocorticoid receptor, which is present in almost every vertebrate animal cell. These hormones help the body respond to stress and to environmental change and guide fundamental processes associated with converting sugar, fat, and protein stores to usable energy; inhibiting swelling and inflammation; and suppressing immune responses.

Lactogenic Inducing lactation, the onset of milk synthesis, and secretion, and includes all of the changes in the mammary epithelium necessary to go from the undifferentiated mammary gland in early pregnancy to full lactation sometime after parturition.

Proteomic Relating to the structure, function, and interactions of the proteins produced by the genes of a particular cell, tissue, or organism.

Somatogenic Originating in or relating to the cells of the body.

Somatotroph A cell of the anterior pituitary that produces somatotropin (growth hormone).

Somatotrophic Having a stimulating effect on body growth.

Transcription factor A protein or molecule that binds to specific DNA sequences or regions, thereby controlling the flow (or transcription) of information from DNA to form specific messenger RNAs.

Introduction

Growth of meat-producing animals is a complex, interactive set of processes initiated at or just after conception that can have effects until death. The expansion of genomic and proteomic techniques and studies has resulted in the discovery of numerous new factors that control the growth and carcass composition of livestock. The endocrinology of growth encompasses the biosynthesis, storage, chemistry, biochemical and physiological function of hormones, and the cells of the endocrine glands and tissues that secrete them. Hormones are secreted directly into the blood stream rather than into a duct system and have a variety of functions or actions on target organs or tissues. One hormone might affect more than one organ or tissue, and conversely, one organ or tissue might be affected by more than one hormone. Hormones and growth factors acting on the same cell type as those in which they are produced are said to have autocrine actions. Hormones and growth factors that act on other cell types in the same tissue or organ in which they are produced are said to have paracrine actions. This brief review will focus on the main hormones and growth factors, with an emphasis on growth of muscle and fat, the main constituents of meat. Commercial growth promotants are covered elsewhere and are not included in this article.

The article is divided into two sections: hormones and factors controlling embryonic and fetal growth and hormones and factors controlling postnatal growth. A number of the hormones and growth factors are active on growth before and after parturition, although in some cases they have different functions. This article will deal in greater depth with particular endocrine factors at the developmental time considered to be most important.

Embryonic and Fetal Growth

Regulation of embryonic and fetal growth is directly related to tissue differentiation and organogenesis. Imprinting of genes has been strongly implicated in fetal and placental growth. An example relevant to meat production is the imprinting of the insulin-like growth factor II (IGF-II) gene. A change in imprinting of the IGF-II gene resulting from embryo culture used in *in vitro* systems can result in the large calf or lamb syndrome. It is also reasonable to suspect that nuclear–cytoplasmic interactions within a cell, which can be altered by external growth factors around conception, can influence subsequent growth and development. These early effects on growth might be achieved by altering the degree of methylation of growth-related genetic loci, which are under the control of growth factors, which in turn are under in utero environmental influences. After conception, the growth of the embryo depends on cell hyperplasia and hypertrophy, differentiation, and also on cell survival or death by programmed cell death, called apoptosis. These cellular systems can be controlled by extracellular growth factors, although the response to a specific growth factor is to some extent dependent on the physiological state of the cell. An example is insulin-like growth factor I (IGF-I), which can stimulate, through the type I IGF receptor, both hypertrophy and hyperplasia via the phosphoinositide-3 kinase (PI-3) kinase or mitogen-activated

protein kinase pathway, or cell survival through the PI-3 Akt/Bad signaling pathway. Apoptosis – programmed cell death – is also controlled by extracellular factors, such as morphogens like Sonic Hedgehog.

Morphogens are factors that act at a distance from their site of secretion, are dose-dependent, and induce specific cell type development. Transcription factors such as Bicoid and Hunchback also act like morphogens before cellularization by producing specific concentration gradients that determine the orientation of the embryo by controlling the anterior–posterior and dorso–ventral developmental gradients. In lambs, retinoic acid activates the expression of bone morphogenic protein 2 and Sonic Hedgehog, and these two factors in concert control directly or indirectly the anatomical differentiation and growth of limbs. Maturation of organs and tissues does not occur simultaneously, but progresses along the anterior–posterior/distal–proximal axes displaying 'early to late' gradients of development in the embryo. As already mentioned, patterning signals are probably set up before the first cell divisions. These gradients are then maintained by subsequent morphogens produced by different cell types. The development of tissues and organs along these gradients can be modified by nutrient availability through nutritionally sensitive hormones and growth factors, although the developmental sequence remains unchanged.

Undernutrition of sheep at different periods of gestation leads to disproportionate growth of various organs, such as the heart and liver. This is not, as has often been assumed, limited to a particular stage of gestation. Endocrine changes reflect the original intention programming for a future postnatal lifestyle of the animal, if conclusions from epidemiological studies on starved human populations are accepted. Therefore, altered body proportions at birth might reflect complex metabolic adaptations as well as an altered hormonal environment that influence postnatal patterns of growth. Insults or negative influences during gestation that cause significant developmental changes in fetal growth and in organs and tissues are now considered to program permanent changes in the structure and function of the endocrine systems. Some changes resulting from early fetal manipulations are considered to cause permanent endocrine changes that are thought to predispose the mature animal to adipose cell hyperplasia, obesity, and endocrinological problems such as type II diabetes.

Myogenesis

The growth of a specific tissue such as muscle is dependent on endocrine, paracrine, and autocrine factors. Muscle cells – myoblasts during fetal development – are initially formed from the segmental mesodermal somites. A number of morphogens from surrounding tissues induce myogenic differentiation; examples include Wnt-1 and PAX3. Wnt-signaled transduction is crucial for maintaining the balance between proliferation and differentiation throughout embryogenesis and postnatal life. PAX3 is a gene that belongs to the paired box (PAX) family of transcription factors. This gene was formerly known as 'splotch' and has been identified with ear, eye, and facial development. PAX3 is also responsible for controlling the migration of myogenic cells into the limb buds to form the muscles of the limbs.

Insulin-Like Growth Factors

The determination of the myogenic progenitor (precursor) cells and their subsequent proliferation to form myoblasts, which fuse to form myotubes, and continue to fuse with existing myotubes to form muscle fibers, is controlled by myogenic regulatory factors. The expression of the myogenic regulatory factors is in turn controlled by other positive and negative growth factors. IGF-I and IGF-II both increase when myoblast proliferation is increasing during muscle development in cattle, swine, and sheep. Recently, a local muscle IGF-I variant, mechano growth factor (MGF), has been associated with stretched muscle. The role of MGF during muscle development is at present uncertain. However, IGF-II is the dominant IGF during fetal development, and IGF-II messenger ribonucleic acid (mRNA) has been shown to increase when myogenic progenitor cells are proliferating, migrating, and fusing in regenerating muscle. Regeneration often appears to follow similar developmental pathways as found in developing tissues. IGF-I, which is also expressed during fetal muscle development, might also be involved in these early stages of muscle fiber formation. However, growth hormone (GH), the pituitary hormone that controls the majority of circulating IGF-I, is not necessarily involved in muscle growth during gestation: mice overexpressing GH do not show increased muscle growth at birth. However, in pigs, GH treatment during certain developmental stages during gestation has been reported to have different long-term effects on muscle development. If GH is administered to pregnant sows early in gestation, at a time when myoblast proliferation is at a maximum, the number of secondary myotubes in fetal muscles results in more muscle fibers in piglets at birth. Administration of GH in midgestation delays muscle maturation, whereas administration in late gestation results in an increase in muscle fiber size.

Myostatin

Unlike IGF-I, myostatin, a member of the transforming growth factor (TGF)-β superfamily, is a negative regulator of muscle cell proliferation. Mutations in the myostatin gene result in double-muscled cattle, a condition that results from a doubling of the secondary myotubes during fetal muscle development and an increase in total number of muscle fibers, but not an increase in muscle fiber size (fiber hypertrophy) at birth. The myogenic regulatory factors have been linked with myostatin during secondary myotube formation in cattle, indicating that myostatin has a coordinating role in the terminal differentiation and fusion of myoblasts to form secondary myotubes. It has been shown in tissue culture that myostatin stimulates expression of P21, which prevents the phosphorylation of retinoblastoma (prb), a transcription regulatory protein, allowing prb to bind to the E_2F transcription factor and so blocking myoblast proliferation. Myostatin, like other TGFs, is produced as a precursor protein that is cleaved before secretion to yield the *N*-terminal associated peptide (LAP) and the *C*-terminal processed peptide. The pro-peptide has been

shown to antagonize the biological activity of myostatin, by binding to myostatin and preventing myostatin binding to its receptors. Follistatin, which inhibits follicle-stimulating hormone, has also been shown to be capable of binding to myostatin and inhibiting its actions. Mutations in the propeptide region of myostatin in mice generate the compact hypermuscular mouse, which increases muscle mass postnatally and particularly in the hind-quarter muscles. In general, these results show that myostatin can inhibit muscle growth in various ways during fetal development.

Adipogenesis

Adipogenesis, the formation and growth of adipose tissue (fat) during fetal development, is controlled by local paracine/autocrine factors, which are themselves controlled by the overall hormonal status of the fetus and the mother. The effects of changes in these hormones or factors are dependent on the stage of development, with the proliferation of preadipocytes, adipocyte differentiation and formation of mature lipid-filled adipocytes, all being sensitive to changes in the endocrine system of the fetus.

Insulin

Insulin, which controls glucose supply to and/or uptake by tissues within the fetus and the mother, seems to be a major controller of fetal adipose tissue lipogenesis (lipid synthesis); experimentally induced diabetes in pregnant sows increases fetal adipogenesis and lipogenesis. Fetal hypophysectomy and replacement therapy have revealed that a number of fetal hormones controlled by the pituitary are important in adipocyte hyperplasia and lipogenesis. Fetal GH treatment decreases the lipogenesis associated with hypophysectomy and antagonizes thyroxine-stimulated lipogenesis. Thyroxine, which is under the control of thyroid-stimulating hormone, appears to control both adipocyte hyperplasia and fat deposition in the fetus. Glucocorticoids, from the adrenal gland, are among the major hormones controlling adipocyte development and differentiation. This appears to be dependent not only on the hormone concentrations in the blood, but also on the number of adipocyte glucocorticoid receptors (GRs). In pigs, the number of GRs is low at 50 days of gestation and increases to a maximum at 105 of gestation. Birth occurs at about 114 days of gestation. The number of GRs in adipocytes can be manipulated by insulin, which may indicate that glucocorticoids are a major factor in the action of insulin on fat deposition in the fetus.

Tumor necrosis factor-α regulates adipose cell differentiation by decreasing the peroxisome proliferator activated receptors (PPARα). Members of the TGF-β superfamily have been shown to block adipocyte differentiation. Myostatin, a member of that family, has been shown to inhibit pre-adipocyte differentiation by controlling PPARα. Thus, myostatin, as previously discussed, not only inhibits pre-adipocyte differentiation, but also inhibits myoblast hyperplasia, and as a result, reduces muscle fiber number. This clearly indicates a close, coordinated physiological link between muscle and fat growth during fetal development.

Placental Growth Factors

Fetal growth is dependent not only on fetal hormones and growth factors but also on the balance of nutrients between the mother and the fetus. There may be a trade-off between the growth of the fetus and the mother's future rebreeding potential, depending on plane of nutrition of the mother. The placenta is the interface between the mother and the fetus. The placenta is not a passive organ: it secretes an array of hormones and growth factors that regulate placental growth and fetal growth, and maintains a homeostatic balance between mother and fetus. This includes control of nutrients passed on to the fetus from the mother. The placenta also protects the fetus from infections as well as preparing the mother for parturition and lactation.

The placenta secretes placental growth hormone (PGH) and placental lactogen (PL), which probably have a common ancestry. PGH has high homology with pituitary GH and can bind to both somatogenic and lactogenic receptors, but it is a weaker lactogen than pituitary GH. PGH is the dominant maternal GH during the later stages of pregnancy and down-regulates maternal pituitary GH. Although the placenta expresses growth hormone-releasing hormone (GHRH) and the hypothalamic GH inhibitor somatostatin, and somatotropin release-inhibiting factor (SRIF), these do not appear to be major controllers of placental GH. PGH appears to be regulated by glucose, which controls maternal IGF-I. The effect of maternal treatment with GH on fetal growth is dependent on the gestational age and species.

PL has both lactogenic and somatotrophic activity, although the lactogenic effects are more prominent. This is an example of a placental hormone having a significant indirect effect on postnatal growth by affecting mammary development and future milk production, and as a result, preweaning growth of her progeny. There are species differences; PL is found in ruminants but it has not been found in pigs. PL is mainly restricted to the maternal circulation and appears to exert a metabolic effect that stimulates maternal lipolysis, and thereby increasing maternal insulin, which causes insulin resistance and inhibition of gluconeogenesis. This makes more glucose available for the growth and development of the fetus. PL may also increase the flow of amino acids to the fetus by restricting maternal utilization. In sheep, PL is produced by chorionic binucleated cells of the placenta and is found in both the maternal and the fetal circulation. Maternal ovine PL (OPL) is first detected at 40–60 days of gestation and reaches its peak at 120–140 days. Recent studies from Israel have shown that ewes immunized against OPL have higher birth weights of lambs resulting from multiple births, and all immunized ewes produced more milk than nonimmunized ewes. The antibody response is an immuno-potentiating effect not an antibody-inhibiting effect.

The roles that placental GHRH and SRIF play in controlling fetal growth are unclear. The effect of SRIF on fetal growth has been tested by direct injections of SRIF into the placental cotyledons of cows: fetal GH was reduced. Injections of GHRH and thyroid-stimulating hormone increased fetal GH but not PLs. SRIF injected into the mother showed only a rebound effect when the infusion stopped; an effect on the fetus was not seen.

Administering SRIF directly to the fetus suppresses fetal GH but not placental GH or PL. Maternal immunization against SRIF has been shown to affect fetal growth: passive immunization increased birth weight; active immunization showed an increase in fetal protein in pigs and increased litter size, but had no overall increase in birth weight. In lambs, there was no effect on birth weight, but postnatal growth was increased. The results from maternal SRIF immunization are variable, and if the effects of SRIF are to be fully understood, the SRIF treatments will have to be administered to specific sites to determine the local effects.

In summary, the endocrinology of fetal growth is complex and highly interactive, with the same or similar hormones from the maternal pituitary, placenta, and fetus often having different functions and these different functions themselves often being dependent on gestational age. This interactive complex set of maternal–fetal hormone and growth factor effects has not been the main focus of growth endocrinologists, yet it is the foundation from which postnatal growth and endocrine control of growth are derived. Recent human epidemiological studies have resulted in the formulation of the Barker hypothesis (fetal origin of disease). This claims that nutritional deprivation of a fetus during critical developmental periods results in the metabolic/endocrinological balance of the fetus being reset so that postnatal metabolism, physiology, and endocrinology are permanently changed. These changes become maladaptive if the nutritional levels differ between the fetus and the adult. The change results in obesity, type II diabetes, hypertension, and so on. This hypothesis, although emanating from human studies, may have direct applications to management systems of meat animal production.

Postnatal Growth

Growth Hormone

Postnatal growth, although no longer dependent on maternal/placental nutrient supply, is still dependent in mammals on the mother's milk supply, which is under the control of GH and prolactin. Postnatal growth after weaning is controlled by appetite, feed conversion efficiency, and repartitioning of nutrients to various tissues. Appetite and nutrient repartitioning are under endocrine control.

The somatotrophic axis, centered around GH synthesis and secretion, is the most important cascade of hormones controlling postnatal growth and is related to the appearance of somatotrophic receptors in the liver during postnatal development in lambs, calves, and pigs. GH, as indicated by its name, is considered to be the most important pituitary hormone associated with postnatal growth. GH has direct anti-insulin effects on fat by decreasing lipogenesis and increasing lipolysis and on carbohydrates by increasing blood sugar. GH has indirect effects through IGF-I, which controls skeletal growth by stimulating chondrogenesis and muscle growth by stimulating satellite cell division and fusion with existing muscle fibers causing hypertrophy. GH also interacts with other hormones, such as cortisol, to produce synergistic effects that enhance the individual diabetogenic and lipolytic effects of GH and cortisol, whereas cortisol can block some of the anabolic effects of GH.

The main effects of GH on growth have been considered to be indirect. The classical somatomedin hypothesis proposed that GH acted on the liver to increase synthesis of IGF-I, with hepatic IGF-I mediating those anabolic effects. This was initially challenged by the idea that there also existed local tissue IGF-I produced in response to GH. The somatomedin hypothesis has been revisited recently because, when hepatic IGF-I was deleted by genetic engineering, circulating IGF-I levels fell to 25% of normal, but there was no immediate effect on growth. These experiments challenged the concept that circulating hepatic IGF-I is crucial for normal GH-controlled postnatal growth.

GH has been administered to meat-producing animals to stimulate lean growth. Dose–response studies in which growing pigs were administered from 50 to 200 micrograms of recombinant porcine GH per kg live weight per day from 25 to 90 kg body weight contained 28–38% more muscle mass and from 40% to 80% less adipose tissue mass. Studies in which GH or GHRH were administered daily also improved growth rate, efficiency, and carcass composition of growing lambs and pigs. In some studies in which overfat lambs were treated with GH, a heavyweight lean lamb could be produced with 6 weeks of GH treatment. Similar effects have been produced in experiments in which growing cattle were administered GH. This indicates that GH treatment not only stimulates growth but repartitions nutrients from fat to muscle by reducing lipogenesis, increasing muscle protein synthesis, as well as stimulating lipolysis to varying degrees.

Growth Hormone-Releasing Hormone

GH itself is controlled by the hypothalamic factors GHRH and somatostatin (SRIF). GHRH stimulates GH secretion through a G-protein-coupled receptor that initially increases cyclic adenosine monophosphate (cAMP), which stimulates GH secretion. A reduction in GHRH leads to a marked decrease of normal postnatal growth, as well as obesity, which is associated with hyperphagia. Daily administration of GHRH increases growth rate, improves feed efficiency and improves carcass composition in growing lambs and pigs.

Somatostatin

Somatostatin (SRIF) is found not only in the hypothalamus but also in the gut. SRIF inhibits the release of GH from the pituitary somatotrophs, as well as inhibiting a number of gastrointestinal and pancreatic hormones. SRIF acts through several G-protein-coupled somatostatin receptors. In the pituitary somatotrophs, SRIF decreases cAMP, inhibits Ca^{2+} channels and activates K^{+} channels. These effects inhibit both the basal and the stimulated release of GH.

Insulin

Insulin, secreted from the pancreas, in addition to its primary role of regulation of carbohydrate homeostasis, also stimulates growth. Insulin deficiency results in poor growth pre- and

postnatally. This suggests that insulin has a role in somatic growth that might be permissive and controls the uptake and utilization of carbohydrates and amino acids. In pigs, a lack of insulin inhibits normal growth by up to 50%, but in other studies administration of insulin did not increase muscle growth or live weight gain of well nourished pigs. In addition to controlling blood glucose, insulin also controls some aspects of amino acid transport into tissues, such as muscle, and inhibits protein degradation, so increasing protein deposition. Carcass fatness is also controlled, to some extent, by insulin, with a reported positive correlation between plasma insulin concentrations and carcass fatness. GH administration to growing pigs reduces adipose tissue/cell sensitivity to insulin, thereby reducing lipogenesis and carcass fat content. In abnormal clinical situations, such as hyperinsulinaemia, insulin may stimulate growth through the type I IGF receptor, as well as acting through the insulin receptor.

Pubertal Growth

Growth in both females and males increases around puberty – the 'pubertal growth spurt.' This growth spurt occurs at different ages between sexes and has different effects on overall growth and growth of muscle and fat. Sex differences in growth rate and carcass composition have been associated with testicular androgens (testosterone, dihydrotestosterone, and androstenonedione) in the male, and with ovarian hormones (estrogens and progestogens) in the female.

Male hormones

In the male, testosterone and dihydrotestosterone are the main testicular hormones that control growth and increase lean muscle mass. Testosterone appears to have two modes of action. Its direct action on striated muscles is through specific intracellular receptors. Testosterone circulates in blood bound to sex steroid binding proteins that assist the entry of testosterone into the cell. The indirect effect of androgens on lean muscle growth is thought to be in association with GH. When androgens were administered to hypophysectomized rats, the animals failed to grow and required GH to achieve a normal growth response to testosterone. However, although the natural testicular androgen testosterone might promote lean growth by requiring GH for maximum stimulation, synthetic androgens, such as trenbolone acetate (TBA), stimulate growth by binding directly to muscle fibers and their androgen receptors, although details of the mechanism of action are not clear. Treatment of feedlot cattle with a combined implant of estradiol and TBA increases muscle satellite cell number, increases expression of muscle IGF-I mRNA and increases circulating levels of IGF-I. Treatment of bovine satellite cell cultures with either estradiol or TBA increases expression of IGF-I mRNA, increases rates of proliferation and protein synthesis, and decreases rates of protein degradation.

Female hormones

The female hormone estrogen has been shown to stimulate growth in sheep and cattle, with a greater response found in older lambs and castrated male cattle. High doses of estradiol, however, inhibit growth and antagonize the actions of GH. This clearly indicates that the growth-promoting effects of estrogens are dose-dependent; as a consequence, when estrogen-based growth promotants are given to livestock on high-phytoestrogen pastures, very limited growth promotion is observed.

The effects of estrogens on growth are thought to be mediated in part through an increase in GH, although as mentioned in the preceding section, the direct effects on bovine satellite cells in culture have been shown to be mediated at least in part through the classical estradiol receptor, estrogen receptor-alpha (ESR-1), the IGF-I receptor, and the G protein-coupled estrogen receptor (GPER-1). Therefore, direct actions on the IGF-I axes independent of GH have been identified, and estrogens do have direct muscle satellite cell and tissue effects. Muscle growth might be directly stimulated by estrogens by direct binding to estrogen and androgen muscle receptors. It is now clear that a complex interaction of numerous pathways and receptors is involved in anabolic steroid-enhanced bovine muscle growth.

Progesterone is another ovarian hormone that has been shown to stimulate growth. Progesterone is naturally produced by the corpus luteum under control of luteinizing hormone. Progesterone might bind directly to muscle through the androgen receptor, so having a direct effect on lean growth. The actions of the synthetic progestagen, melengestrol acetate, however, might be indirect through blocking ovulation, so increasing ovarian estrogen production from immature ovarian follicles, which then stimulates growth.

Anabolic agents, mainly based on androgens or estrogens, are routinely used in the North American cattle industry during the finishing of steers on feedlots. These products have been commercially used for over 40 years, with new combinations developed recently, and will not be dealt with here beyond the information provided above.

Glucocorticoids

Glucocorticoids produced by the cortex of the adrenal gland have effects opposite to those of insulin. They decrease the uptake of glucose, so increasing blood sugar levels, and increase protein breakdown and lipolysis. Although these effects are growth-inhibiting rather than growth-stimulating, corticosteroids at physiological concentrations are necessary if the efficient stimulation of GH secretion is to be achieved by GHRH. The use of glucocorticoids such as dexamethasone to treat inflammation impairs growth by reducing the local tissue stimulation of IGF-I by GH by reducing both the IGF-type 1 receptor and GH receptor.

Catecholamines

Catecholamines, such as adrenaline (epinephrine), produced and secreted by the adrenal medulla and noradrenaline (norepinephrine), released by specific types of nerve endings, are under the control of the sympathetic nervous system. These hormones are normally released under acute stress or excitement, such as fear, and are important in the regulation of heart rate, blood flow, and metabolic adaptation to provide more energy to critical tissues. However, a number of synthetic

catecholamines, including ractopamine hydrochloride and zilpaterol hydrochloride, are used as muscle growth promotants in livestock.

These feed additives are absorbed into the blood and act on the β-adrenergic receptors of muscle and other tissues. These anabolic actions do not appear to involve IGFs. Studies conducted with growing steers demonstrated that continuous infusion of small amounts of cimaterol into the hind limb directly causes increases in amino acid uptake, increased protein synthesis and muscle growth without significant changes in GH, insulin, IGF-1, or thyroid hormones.

Recently Identified Factors Controlling Growth

With the advent of genomics and proteomics a number of new factors that control tissue growth have been identified. Two such factors that are produced by adipose tissue are leptin and adiponectin.

Leptin

Leptin is produced by adipocytes, although its receptors are found in numerous tissues including the brain. Leptin appears to be involved in the central control of energy balance regulation, achieved by controlling appetite and fat deposition though the manipulation of or feedback to neuropeptide Y in the brain, which controls appetite. Leptin also has local effects on peripheral insulin resistance. Resistance to leptin leads to obesity, which can be the result of defects in the leptin receptor and/or in downstream signaling or in the leptin carrier protein.

Adiponectin

Adiponectin is a protein that is only produced in adipose tissue but which circulates at relatively high concentrations. Adiponectin enhances insulin sensitivity and glucose tolerance and appears to increase free fatty acid oxidation in muscle. Adiponectin, like leptin, appears to be involved in regulation of energy homeostasis, which indirectly controls the repartitioning and utilization of nutrients.

Myostatin

Myostatin, already discussed with reference to muscle fiber formation during fetal development, has a role in both muscle and fat growth in the adult. Myostatin has been shown to control cell division of myoblasts during muscle fiber formation during fetal development and growth. The extension of the time period during which this occurs, leading to daughter myoblast fusing into the muscle fiber, allows ongoing muscle fiber formation. Myostatin can, therefore, reduce or block the potential for postnatal muscle growth if it appears sooner or is present at higher levels when myotube formation is occurring. Removal of myostatin has been shown to lead to partial suppression of fat accumulation and abnormal metabolism of glucose.

Summary

The endocrine regulation of growth can occur in two main ways. The first is via hormones acting through receptors and growth factors that are directly implicated in growth overall and in repartitioning of nutrients, bringing about differential tissue/organ growth. These hormones and factors are often involved in nutrient homeostasis, and therefore can be altered by level of nutrient intake. The second is an endocrine dysfunction that causes a clinical imbalance and reduced growth (i.e., reduced GH synthesis and secretion). In addition, growth appears to be sensitive to many clinical illnesses. This article has focused on some of the hormones and growth factors involved in growth, especially the growth of muscle and fat. Known GHs/factors are now very numerous and undoubtedly more will be identified. It is apparent that hormones and growth factors are highly interactive (i.e., GH markedly reduces the sensitivity of adipose tissue to insulin and steroid hormones act through several receptor pathways), and control nutritional homeostasis as well as specific growth and development of tissues and organs.

Growth factors are an integral part of growth and development from the first cell divisions after conception to the mature adult. Postnatal growth is dependent not only on the hormones and growth factors during that growth phase, but on growth and development from conception. This is most clearly seen in the determination of muscle fiber numbers that occurs before birth.

The future for growth and endocrinology in meat science is the identification of the early growth factors that determine the future growth and carcass composition of the animal, which can be used as selection markers, or for generating management strategies or genetically engineered animals.

See also: Growth of Meat Animals: Adipose Tissue Development; Growth Patterns; Metabolic Modifiers; Muscle; Physiology. Muscle Fiber Types and Meat Quality

Further Reading

Arnold, H., Della-Fera, M.A., Baile, C.A., 2001. Review of myostatin history, physiology and applications. International Archives of Biosciences 1, 1014–1022.

Bass, J.J., Sharma, M., Oldham, J., Kambadur, R., 1999. Growth factors controlling muscle development. Domestic Animal Endocrinology 17, 191–197.

Bell, A.E., Bauman, D.E., Beermann, D.H., Harrell, R.J., 1998. Nutrition, development, and efficacy of growth modifiers in livestock species. Journal of Nutrition 128, 360s–363s.

Butler, A.A., Yakar, S., LeRoith, D., 2002. Insulin-like growth factor-1: Compartmentalization within the somatotrophic axis. News in Physiological Sciences 17 (2), 82–85.

Dayton, W.R., White, M.E., 2014. Role of satellite cells in anabolic steroid-induced muscle growth I in feedlot steers. Journal of Animal Science 92, 30–38. doi:10.2527/jas2013-7077.

Etherton, T., Bauman, D.E., 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiological Reviews 78, 745–761.

Fowden, A.L., 1993. Insulin deficiency: Effects on fetal growth and development. Journal of Paediatrics and Child Health 29, 6–11.

Gluckman, P.D., Pinal, C.S., 2003. Regulation of fetal growth by the somatotrophic axis. Journal of Nutrition 133, 171s–174s.

Harding, J.E., 2001. The nutritional basis of the fetal origins of adult disease. International Journal of Epidemiology 30, 15–23.
Houseknecht, K.L., Baile, C.L., Matteri, R.L., Spurlock, M.E., 1998. The biology of leptin: A review. Journal of Animal Science 76, 1405–1420.
Kambadur, R., Sharma, M., Smith, T., Bass, J.J., 1997. Mutations in myostatin (GDF8) in double-muscled Belgium Blue and Piedomontese cattle. Genome Research 7, 910–915.
Moore, T., Reik, W., 1996. Genetic conflict in early development: Parental imprinting in normal and abnormal growth. Reviews of Reproduction 1, 73–77.
Vincent, J.-P., Briscoe, J., 2001. Morphogens. Current Biology 11 (21), 851–854.

Growth Patterns

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Glossary

Allometric growth The growth rate of a tissue or organ in relation to its whole.
Callipyge sheep A natural genetic mutation that produces extremely muscled hindquarters in sheep.
Double muscling An inherited condition in cattle, characterized by hyperplasia (increase in number) and, to a lesser extent, hypertrophy (enlargement) of muscle fibers.
Intermuscular fat The fat located in the seam between muscles.
Intramuscular fat The fat interspersed between muscle fibers within the muscle.
Myostatin A secreted growth differentiation factor protein that is a member of the transforming growth factor beta protein family that inhibits muscle differentiation and growth in the process known as myogenesis.

Introduction

Growth in meat animals is often described as an increase in the size (weight or dimensions) of the animal. An animal can increase in weight with the accumulation of fat (lipid), but this is not considered true growth. The growth of animals can be monitored in several ways, including measurements of length, width, girth, and weight. Generally, live weight has been the most important and most commonly measured parameter, probably because of the ease in obtaining weight information and the importance of weight in marketing animals for use as meat. If weight is recorded over time, it is possible to construct a graph that illustrates the growth pattern of an animal; the growth curve or line will be 'sigmoid,' so called because of its resemblance to the shape of the letter S (Figure 1). The zero time of the curve is in most instances the point of conception, with the curve reaching a plateau when the animal reaches mature size. Mature size can be defined as the point at which the weight change with time is only slight and there is little or no change in the fat content of the animal.

Most animals have very similar growth patterns, varying only in terms of the time it takes to reach mature size. For example, a mouse will reach mature size in several weeks, but an elephant may take 50 years to reach mature size. Because of differences in mature size and growth rate, it is difficult to compare between species and even within breeds of the same species. To make a more relevant comparison, growth is expressed as a percentage or fraction of gain of the present size of the animal instead of absolute gain. Fractional growth rates of organs and tissues vary from conception to maturity. This variation in growth is referred to as differential growth. Because the growth of each organ and tissue occurs at different times, this differential growth will be exhibited by changes in shape and proportions of parts of the animal.

The driving force behind expression of differential or proportional growth in an animal species is evolution. The 'function' of the animal kingdom is to survive and reproduce to sustain the species. As animals grow they will develop organs and tissues that are most critical for life before those organs and tissues that are a lower priority for survival. For example, at birth the brain, heart, and liver are more fully developed than bone, skeletal muscle, or fat. Understanding this fact helps to comprehend the patterns in which animals grow. Granted, in the past century, humans have altered animals to fit their needs for food, power, and fibers, but these changes are minor when compared to those of a millennia of evolutionary pressure.

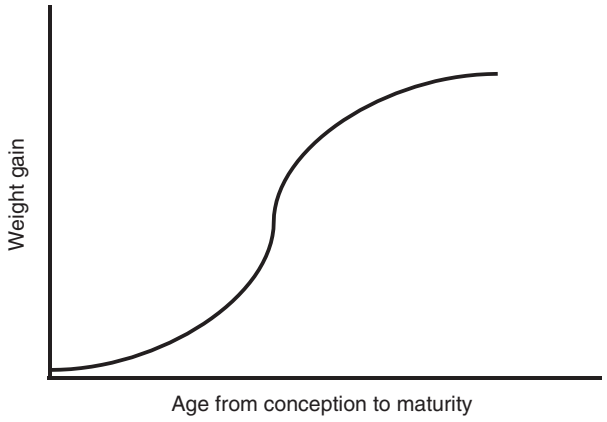

Figure 1 Illustration of the cumulative growth based on weight gain of an animal from conception to maturity.

Measuring Patterns of Growth

There is vast body of literature documenting growth patterns of tissues in animals. Two researchers who contributed to this knowledge are Sir John Hammond and the zoologist Julian Huxley. Both provided the basis of understanding proportional growth. Huxley found that proportional growth could be measured as the weight of a tissue, an organ, or a subdivision, such as an individual muscle, in relation to the whole animal or tissue. This methodology became known as growth allometry and has been used by researchers to examine growth patterns in tissues such as muscle, fat, and bone. Huxley was able to develop a mathematical method to detect the changes in growth impetus of various tissues in relationship to the whole animal or tissue. It is possible to compare

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the relative growth of a unit of the whole animal or tissue on a logarithmic scale using eqn [1]

$$y = bx^k \quad [1]$$

where y is the weight of the tissue, x is the weight of the body, b represents the y intercept, and k is the slope or growth impetus.

This can also be expressed in terms of logarithms as eqn [2]

$$\log y = \log b + k \log x \quad [2]$$

The slope of the resulting regression (k) is called the allometric growth ratio. If $k=1$, then the unit of body being compared to the whole is growing at the same rate and is referred to as having average growth impetus. If $k>1$, then the unit of the body is growing faster than the whole and is referred to as having high growth impetus. If $k<1$, the unit of the body is growing slower than the whole, commonly referred to as low growth impetus. Using allometry, it is possible to compare the growth of various tissues in relationship to the whole body throughout the lifespan of the animal.

Organ and Tissue Growth Patterns

Comparative patterns by which organs and tissues develop follow the principle that the tissues or organs most essential for survival develop first. The relative order of growth of different tissues is similar for all species of farm animals. As illustrated in Figure 2, nervous tissue is the first to develop, followed by bone, muscle, and fat. During the growth and development of the embryo, the first noticeable tissue is the notochord. This develops in a process called neurulation and will eventually become the nervous system. The nervous system begins very early to direct the development of the other tissues in the body. For example, neurulation in the pig is complete by 15 days after fertilization. At approximately 35 days the cerebral hemispheres of the brain have achieved a relatively high degree of development and the midbrain is large and overhangs the developing cerebellum. Examining the allometric growth curve of the brain, it is observed that k is less than 1 throughout the postnatal life of the animal. The reason for this is most likely that the accelerated growth prenatally is critical for proper brain function at birth for survival.

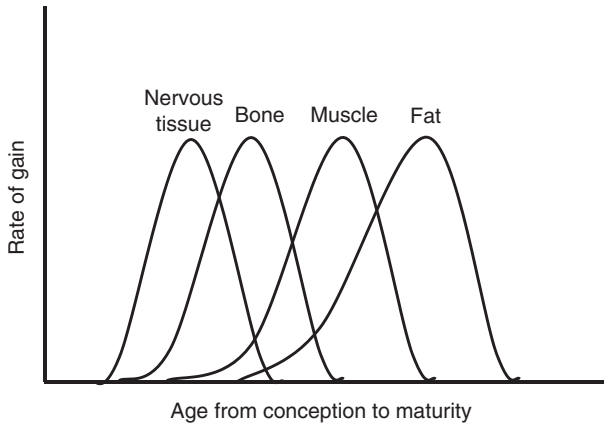

Figure 2 Growth rate of nervous tissue, bone, muscle, and fat in the animal body as a function of age from conception to maturity.

Patterns of Carcass Growth

One of the primary reasons for raising livestock is for meat production. Muscle, fat, and bone are the major components of a dressed carcass derived at harvest. A quality carcass is characterized by a large amount of lean muscle, a minimal amount of bone, and an adequate amount of fat. Figure 3(a) illustrates the percentages of tissues in the carcass as the animal grows. Muscle needs to be functional at birth therefore it comprises a high percentage of the body at birth, and as fat increases then muscle percentages decrease. At birth most animals have limited fat depots but fat becomes a major factor in carcass composition as the animal matures. Bone begins to develop in prenatal life to allow the animal to function at birth but has very little effect on carcass composition during postnatal growth.

Carcass composition can vary due to gender, frame size, and several genetic conditions. In general, intact male cattle will have an increased muscle mass and less fat than females and steers (Figure 3(b)). Animals that reach mature weights earlier have an increased fat percent (Figure 3(c)). However, if later maturing animals are allowed to grow they will reach similar composition but at a greater weight. Cattle with the genetic condition of double muscling will have increased percent muscle with a subsequent decrease of fat and a slight decrease in percent bone (Figure 3(d)).

Patterns of Bone Growth

Growth of bone determines the ultimate length of individual muscles and therefore is a major determinant of muscle growth. Obviously, as bones increase in length, muscles will also grow. For example, as the limb of an animal lengthens, it stimulates the longitudinal growth of muscle; not until bone lengthening slows is there a substantial increase in the radial growth of muscle.

There is a tendency for the distal extremities or appendicular skeleton of the animal to complete the growth cycle first, followed by the proximal and axial parts. Early observations showed that the growth pattern of animals follows a definitive sequence, i.e., metacarpals, radius–ulna, and humerus in the fore limb; and metatarsals, tibia–fibula, and femur in the hind limb. The only bone in the appendicular skeleton lagging in development is the scapula. The bones of the head generally develop early, which can be observed by the increase in head size compared to the rest of the body in young animals. The possible reason for this is to protect the brain and also to provide the animal with the ability to obtain food that is essential for survival and growth. The pattern in which bones and limbs develop is referred to as 'centripetal' growth or center-seeking growth which is characterized by the early development of bone at the extremities, with waves of growth moving to the center of the body. Besides growth beginning at the extremities and moving to the center of the animal, there are waves of growth moving along the vertebral column from

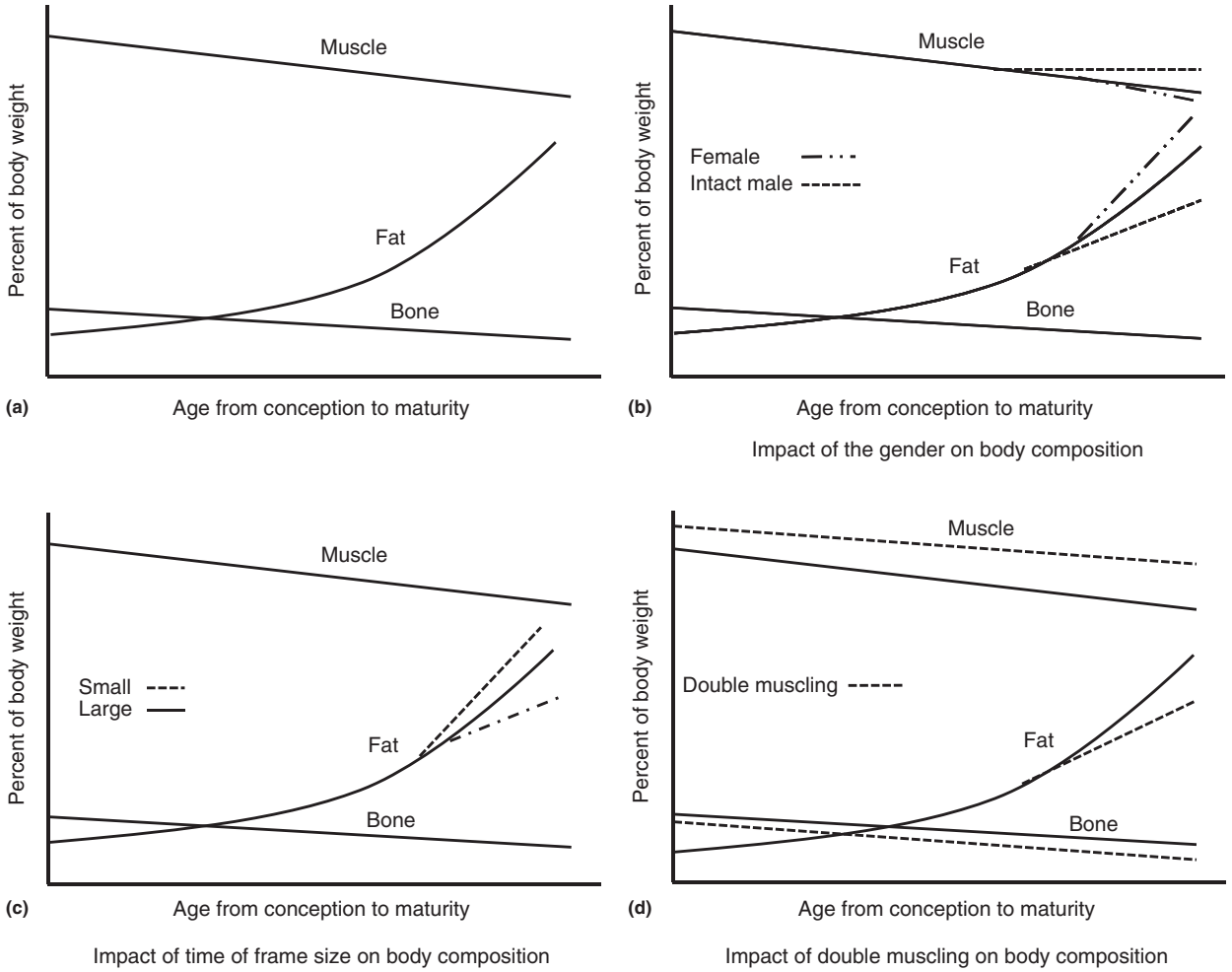

Figure 3 (a) Relative changes as a percentage of body weight of muscle, fat, and bone as a function of age from conception to maturity. (b) Relative changes as a percentage of body weight of muscle, fat, and bone as a function of age with the variation due to gender. (c) Relative changes as a percentage of body weight of muscle, fat, and bone as a function of age with the variation due to frame size. (d) Relative changes as a percentage of body weight of muscle, fat, and bone as a function of age with the impact of the double muscling genotype.

the posterior to the anterior of the animal. This can be observed by the ossification of the bone in the sacral area first; as the animal matures, the cartilage located on the distal portion of the thoracic spinous processes ossifies beginning on the most posterior vertebrae and proceeding to the anterior vertebrae.

Growth of bone slows relative to the body during the postnatal growth period. This is particularly true for bones in the distal limbs, which as explained earlier, may be due to the survival needs for early prenatal bone development.

Patterns of Muscle Growth

Many researchers have examined the growth patterns of muscle in livestock species to determine whether they could be altered to increase the amounts of muscles of higher value. This has met with limited success but, in the process, a vast amount of knowledge on growth patterns has been obtained. Most data were obtained primarily from postnatal animals, and little work has been done in understanding the growth patterns of muscle in prenatal animals. Some muscles may have already exhibited an increase growth impetus, but it is not observed in most studies.

The concept of functional demand, which is muscles develop based on their need, may help in understanding the pattern of muscle development. For example, if a newborn animal is to survive, it must be mobile so that it can stay close to its dam and nurse. To accomplish these two functions, it must have well-developed muscles in the distal portion of its limbs and the jaw. Muscles that perform these functions have been called 'early-developing' muscles because they have completed their development before birth. Other muscles that have minimal function at birth have completed only a small part of their total development at birth. Muscles of the abdominal wall would be classified in this category. As a calf begins to consume roughage and the rumen develops the muscles of the abdominal wall develop to support the gut and its contents.

Muscles can be divided into categories of low, average, or high growth impetus. These muscles are referred to as having a monophasic growth pattern. Other muscles may exhibit a

changing pattern during the time growth is monitored. For example, growth impetus may be low then change to average. These muscles are said to have a diphasic growth impetus.

The low and low-average muscles are very critical for movement of the animal. These muscles probably had a high impetus prenatally but this is difficult to determine. As mentioned earlier, these muscles are located in the distal portion of the limb and the deep muscles of the thicker part of the body. They comprise approximately 33% of the total musculature at birth but decline to approximately 25% in the animal at market weight. It is interesting to note that these muscles lose the least amount of weight during periods of body weight loss.

Muscles classified with an average growth impetus may be expected to perform uniform function throughout the life of the animal. Most of these muscles are located in the proximal part of the fore limb. These muscles provide support for a newborn during suckling and will support the adult animal when the center of gravity of the animal shifts to the fore limb.

Muscles classified as having a high growth impetus might be expected to grow more rapidly to meet functional demands placed on them, such as increased weight of the animal. Most muscles in this group are termed weight-supporting, such as the serratus ventralis, which provides a direct line of force for the weight of the thoracic portion of the trunk of the animal to the fore limb. Two muscles in the abdominal wall, the internal oblique and the rectus oblique, play a role in supporting the additional weight of the abdominal contents.

Also included in muscles that have high growth impetus are muscles of the hind limb and loin. These muscles are rather light at birth, which is fortuitous because this eases the birthing process. The development of the hind limb muscle postnatally is important for the maximum locomotive performance of the animal. In males there is an increase in muscles of the shoulder and neck during puberty. These muscles assist in survival and reproduction, more especially reproductive competitiveness.

Patterns of Fat Depot Deposition

Fat does not have the same functional activity as muscle or bone, yet it is very important in the survival of the animal. Without some type of energy store, the animal would not be able to survive. As mentioned earlier, a newborn piglet has only 2% body fat and, unless nourishment is provided shortly after birth, it will not survive. Fat clearly is the most variable tissue in the body in terms of percentage of body weight. Not only does the amount of body fat vary, but also the partitioning between depots within the body changes during growth.

The accumulation of fat in the various depots of the body generally follows a constant pattern, with the perirenal fat being the first deposited. Perirenal fat can be observed in the newborn animal, and even if the animal is severely restricted in energy a residual amount of fat is present.

Intermuscular fat, which is located between muscles and subcutaneous fat, located between the body and skin, follows perirenal fat, with intramuscular fat (located within muscles) being the final fat depot to develop. To illustrate this, growth allometry research in pigs revealed that mesentery and cavity fat increased faster than body weight the first 40 days of life, indicating early development. From 60 to 120 days there is a rapid increase in subcutaneous fat indicating development later. Similar growth patterns have been observed in cattle. When nutrients are restricted, the first depot to reduce in size is the depot that was deposited last, i.e., there would be a loss in intramuscular fat followed by subcutaneous and intermuscular fat respectively.

There are differences between species in relative proportions of subcutaneous versus intermuscular fat. For example, pigs have a greater proportion of total fat as subcutaneous fat compared to cattle. In cattle the pattern of deposition of subcutaneous fat shows that fat deposition is most rapid in the ventral portion of the animal during the fattening stage, with the most rapid location in the flank area, followed by the brisket. Early in the finishing phase, there is an increase of fattening in the loin area, but this diminishes later. The area showing the slowest fattening is in the round and chuck region. A possible reason for the reduced fat deposition in the moving limbs is that the mechanical movement of the limbs precludes the deposition of fat.

Gender Differences in Growth Patterns of Body Constituents

Gender of animals has a profound effect on muscle and fat growth patterns. This can be observed in the differences between males, females, and castrated animals. Most of the research in this area has focused on cattle because these animals are generally marketed after they have gone through puberty. The most apparent growth pattern difference that can be observed is, as a bull matures sexually, there is an increased development of the muscle in the neck and thoracic region. This probably serves two purposes: it provides support as the center of gravity shifts to the forelimbs of the animal, and it also allows an increase in the animal profile to provide for more success in the competition for and breeding of cows. This shift in muscle growth may also be explained by the observed reduction in the percentage of muscle in the posterior or pelvic limb of bulls compared to steers. In reality, muscles in the pelvic area of both animals are the same but, in bulls, the muscles are a lower percentage of the total muscle mass. There are no differences in growth patterns observed between steers and heifers in the thoracic and pelvic limbs.

Several studies have indicated there is a slight increase in the allometric growth of the abdominal muscles of heifers. These slight differences may be due to weight changes in heifers, where change in function is observed with the need for additional support for weight gain during pregnancy. Abdominal fat also increases, possibly to provide energy for fetal growth and subsequent lactation.

Fat deposition is also slightly different between genders. The most observable difference is that heifers increase in fatness sooner, followed by steers and then bulls. This difference may be readily observed if animals are slaughtered at constant weights. With the difference in the rate of fattening, the time at which animals are marketed for slaughter is important. Heifers fatten sooner and will produce lighter carcasses when compared to steers or bulls.

The amount and location of intermuscular fat is slightly different between genders. In a comparison of the amount of intermusclar fat in the forequarter, keeping the amount of intermuscular fat in the hindquarter constant, it is observed that heifers and steers have more fat in this depot than bulls. The difference in the growth patterns of the muscles in the neck and thorax between males and females is also observed in pigs. Intact males have larger muscles in the neck and thorax than do females. Gender effects on fat deposition are also observed in pigs. Several studies have shown boars have less total fat than females, whereas castrated males have more fat than females. There were also differences in the distribution of fat, with boars having lower amounts of total subcutaneous fat and higher amounts of intermuscular fat than females. One area in which boars have slightly higher amounts of subcutaneous fat is the neck and thorax region.

Genetic Variation in Growth Patterns of Carcass Components

Researchers have examined whether genetic selection can alter the growth patterns of carcass constituents. In most instances, it is difficult to identify differences in growth patterns of components of the typical carcass. For example, no differences have been identified in the growth impetus of bone due to genetic selection. There are differences in the weight of bone in large-framed versus small-framed cattle, but the development follows a very similar pattern in both.

In most livestock no differences have been observed in the relative distribution of muscle growth patterns among parts of the body. When comparing muscle growth patterns of cattle with widely diverse genetic backgrounds, there is striking similarity in the relative weight of individual muscles and in the various groups of muscles. This has led scientists to conclude that the muscles considered to be the expensive portion of the carcass (proximal muscles of the pelvic limb and muscles surrounding the spinal column), irrespective of the breed, constitute 56% of the muscle in a carcass.

There are some genetic differences among fat deposition patterns. The most obvious is observed between cattle from dairy versus beef breeds. It is widely accepted that dairy breeds have a larger percentage of total fat as perineal fat and less subcutaneous fat, and a slightly greater propensity for increased intramuscular fat whereas beef breeds have a higher percentage of subcutaneous fat. Differences are also observed when comparing *Bos taurus* and *Bos indicus* breeds. *Bos taurus* breeds generally have a higher amount of subcutaneous fat than *B. indicus* breeds. This difference is theorized to be caused by the differences in the thermal environments in which they have evolved. There are differences in fat content within the *B. taurus* breeds, with British breeds generally being fatter than Continental breeds when animals are slaughtered at the same weight. Several studies have shown the amount of subcutaneous fat can be altered in pigs when they are compared at the same weight or age.

Recently researchers have identified two genotypes that have a profound impact on muscle growth. Double muscling, a condition mentioned earlier is the result of a mutation of the myostatin gene which stimulates an increase in muscle mass. Double muscled cattle generally exhibit a higher muscle to live weight ratio, a high proportion of muscle in the carcass, higher muscle to bone and muscle to fat ratio compared to cattle selected for 'traditional meat characteristics.' The callipyge genotype in lambs is unique in that it causes a differential growth with an increased growth of muscles in the pelvic and torso regions of the animal. This condition does not manifest itself until several weeks after birth, however, at slaughter, lambs with the callipyge gene have heavier hind limb muscles with no differences observed in the muscle of the shoulder.

Nutrition Effects on Growth Patterns

In most instances, nutrient restriction slows the growth of muscles, but they compensate when adequate nutrition is restored. Consequently, because of this fact, it has been difficult to alter the distribution of muscles within the animal body. The tissue most affected by nutrient restriction is adipose tissue. As mentioned earlier in the Section Measuring Patterns of Growth, the loss of fat depots follows a pattern similar to fat deposition, but in reverse. For example, if cattle are restricted in feed, the depot that will lose the greatest total amount of weight will be the subcutaneous depot, followed by intermuscular fat, and then kidney fat. When cattle are placed on a recovery diet after energy restriction, the intermuscular fat depots have priority over the subcutaneous depots. The response of depletion and recovery is the same, irrespective of age.

See also: Carcass Composition, Muscle Structure, and Contraction. Classification of Carcasses: Beef Carcass Classification and Grading; Pig Carcass Classification. Double-Muscled Animals. Growth of Meat Animals: Adipose Tissue Development; Endocrinology; Metabolic Modifiers; Muscle; Physiology. Muscle Fiber Types and Meat Quality. Physical Measurements: Other Physical Measurements. Species of Meat Animals: Cattle; Pigs; Sheep and Goats

Further Reading

Allen, C.E., Beitz, D.C., Cramer, D.A., Kauffman, R.G., 1976. Biology of Fat in Meat Animals. Madison, WI: University of Wisconsin. NC Regional Research Publication No. 234.

Berg, R.T., 1976. New Concepts in Cattle Growth. Sydney: Sydney University Press.

Berg, R.T., Anderson, R.B., Liboriussen, T., 1978a. Growth of bovine tissues. 2. Genetic influences on muscle growth and distribution in young bulls. Animal Production 27, 51–61.

Berg, R.T., Anderson, R.B., Liboriussen, T., 1978b. Growth of bovine tissues. 3. Genetic influences on patterns of fat growth and distribution in young bulls. Animal Production 27, 63–69.

Berg, R.T., Walters, L.E., 1983. The meat animal: Changes and challenges. Journal of Animal Science 57, 133–146.

Davies, A.S., 1974a. A comparison of tissue development in pietrain and large white pigs from birth to 64 kg live weight. 1. Growth changes in carcass composition. Animal Production 19, 367–376.

Davies, A.S., 1974b. A comparison of tissue development in pietrain and large white pigs from birth to 64 kg live weight. 2. Growth changes in muscle distribution. Animal Production 19, 377–387.

Dickerson, G.E., 1985. Potential uses of genetic variation in components of animal growth. Current concepts of animal growth vol. 1. Journal of Animal Science 61, 104–117.
Gerrard, D.E., Grant, A.L., 2003. Principles of Animal Growth and Development. Dubuque, IA: Kendall/Hunt.
Lawrence, T.L.J., Fowler, V.R., 1998. Growth of Farm Animals. New York: CAB International.
Pearson, A.M., Dutson, T.R., 1991. Growth Regulation in Farm Animals. New York: Elsevier Science.
Swatland, H.J., 1994. Structure and Development of Meat Animals and Poultry. Lancaster, PA: Technomic, pp. 425–493.

Relevant Websites

bovine.unl.edu
Bovine Myology.
porcine.unl.edu
Porcine Myology.
http://en.wikipedia.org/wiki/Meat#Growth_and_development_of_meat_animals

Metabolic Modifiers

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Glossary

Anabolic A class of hormones such as anabolic steroids (technically known as anabolic–androgenic steroids) that are drugs with a steroid ring system with similar effects of testosterone. They stimulate protein synthesis and muscle growth, and insulin and have androgenic and virilising properties.

Androgenic/androgen Androgenic is the broad term for any natural or synthetic compound (usually steroid hormones) that stimulates or controls the development and maintenance of male characteristics by binding to androgen receptors.

Beta-adrenergic agonists (BAA) β1- and β2-agonists are the primary agonists studied, which act on beta-adrenergic receptors. A β_1-agonist is ractopamine; a β2-agonist is clenbuterol and zilpaterol hydrochloride.

Estradiol A sex hormone about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect. Estradiol may be presented as E_2 or as estradiol benzoate.

Estrogens/estrogenic The primary female sex hormones. Natural estrogens are steroid hormones, whereas some synthetic ones are nonsteroidal.

Genomic/nongenomic steroid actions The genomic pathway of steroid actions involve hormone binding to cytosolic receptors and subsequent modulation of gene expression followed by protein synthesis. Nongenomic steroid actions involve alternative pathways that do not act on the genome.

Ractopamine A drug used as a feed additive to promote leanness in animals raised for meat. Pharmacologically, it is a β_1-agonist.

Testosterone Manufactured as testosterone propionate.

Trenbolone A steroid supplied as trenbolone acetate. It is used to increase muscle growth and appetite.

Zilpaterol Zilpaterol hydrochloride primarily is a β_2-adrenergic agonist.

General Effects of Metabolic Modifiers

Steroid Hormones in Animal Production

Steroid implants are used in nearly all beef cattle in North America and are commonly used in most intensive production systems worldwide. In general, implants increase feed consumption and skeletal muscle protein deposition, resulting in improved performance, increased production efficiency, and greater lean mass. Steroids have minimal effects on total carcass fat content but may have some depot-specific effects on fat deposition. Bone density and thickness may be increased by steroid implants and long-bone length can be increased or decreased, depending on the dosage of the steroid and the sex and maturity of the animal at exposure.

Several compounds have been approved for use as implants and different dosages are available for each of the compounds. Efficacy can range from modest growth enhancement, with minimal effects on quality grade, to large increases in growth and efficiency, often with reductions in quality grade (Figure 1). Typical use of implants will increase weight gain by 18–25%, improve efficiency of feed utilization by 12–18%, and increase carcass weight by 9–35 kg, depending on the length of the feeding period.

Steroid hormones are not used in food production in the European Union (EU), and importation of meat from animals that have received the products is banned in EU countries.

Classification of compounds

Steroid compounds used in food animal production include androgens, estrogens and progestins. Commonly used compounds can be classified as naturally occurring or synthetic as described below.

- Estrogens
  Naturally occurring: estradiol
  Synthetic: zeranol
- Androgens
  Naturally occurring: testosterone
  Synthetic: trenbolone
- Progestins
  Naturally occurring: progesterone
  Synthetic: melengestrol acetate (MGA)

Estradiol may be presented as E_2 or as estradiol benzoate, which is approximately 71% estradiol by weight. Trenbolone (TBOH) is manufactured as trenbolone acetate (TBA), which is approximately 80% TBOH by weight. Testosterone is manufactured as testosterone propionate.

Estradiol is more anabolic than zeranol, meaning that a dose of estradiol will stimulate growth more than the same quantity of zeranol. Because of this, zeranol-containing products contain more active compound (by weight) than estradiol-containing products, yet have less anabolic activity. Estradiol is also more estrogenic than zeranol. TBOH is much

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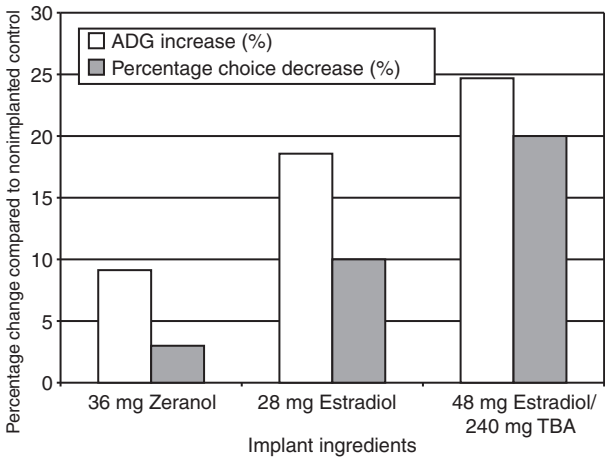

Figure 1 Typical results from commonly used feedlot implant programs. Adapted from Anderson, P.T., 2000. Mechanisms by which metabolic modifiers alter growth rate and carcass composition of meat animals. Proceedings of the 53rd Annual Reciprocal Meat Conference, pp. 31–35. Savoy, IL: American Meat Science Association.

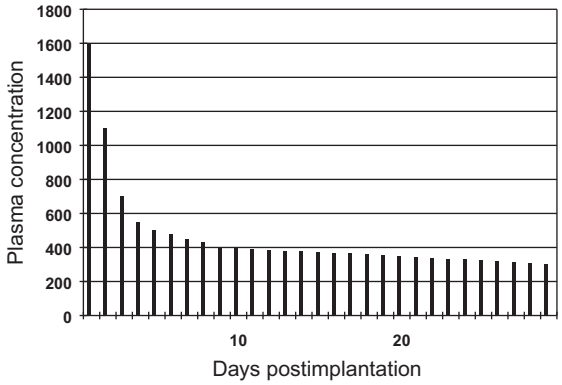

Figure 2 Idealized steroid concentration following implantation.

more anabolic than testosterone and only slightly more androgenic. Some products for heifers contain testosterone, but it is impractical to produce a steer product with enough testosterone to be economically favorable for the producer, so TBA is the more significant androgen in animal production. A significant difference between TBOH and testosterone is that TBOH cannot be aromatized (converted to estradiol).

Delivery to target cell types

Most steroids are administered as subcutaneous implants, placed in the middle third of the ear. Implant products are either compressed pellets, with a high concentration of compound and a small portion of inert ingredients such as lactose or cholesterol, or silastic rods impregnated with estradiol. Estrogenic implants range in dosage from 10 to 72 mg, TBA-containing products range from 40 to 200 mg. Many products contain both an androgen and an estrogen. MGA, an orally active steroid, is sold as a feed additive but is not sold in implant form. MGA dosage ranges from 0.25 to 0.50 mg per head per day.

Dissolution from compressed pellet implants is a first-order function, with payout (release of compound from the implant) diminishing as the physical size of the implant decreases. These products dissolve completely over time, typically in the range of 100–150 days. Use of coating materials to delay release of drug from a portion of the pellets in a dose and thereby extend the effective life of the implant is a recent innovation.

Payout from silastic products approaches zero order with products designed to payout for approximately 100, 200, or 400 days, depending on the product chosen. The silastic does not dissolve and remains in the ear indefinitely. After implantation, the product enters local circulation around the implant site. Organic components, such as the benzoic acid in estradiol benzoate are cleaved as soon as the product enters the bloodstream. The insoluble steroid binds reversibly to specific carrier proteins (steroid-binding globulins) for delivery to target cell types.

Plasma concentrations of exogenous steroid will look like the idealized data in Figure 2. There is a biphasic release of compound from the product that results in high blood levels for 24–48 h postimplantation, followed by a slow decline in concentration over the remaining life of the implant, usually 50–150 days. The initial high concentration of compound in the blood may be due to several factors: the physical insult of implanting causes increased blood flow to the implant site; the product may have some adherent compound that dissolves rapidly; and the animal likely has limited ability to clear the compound from the bloodstream at first exposure. Following this initial period, the compound is absorbed according to the physical characteristics of the implant and the blood volume and other characteristics of the animal. Circulating hormone levels will decrease over time as the implant is depleted. This reduction in circulating hormones accompanies a reduction in growth stimulus, suggesting a threshold level of circulating steroid required to maintain maximal performance.

If TBA is implanted in combination with E_2, the circulating E_2 levels can be maintained for approximately 100 days; however, implantation with E_2 alone resulted in baseline E_2 levels after 60 days postimplantation. Once E_2 enters the circulation it is cleared rapidly. E_2 has a rather short half-life in circulation. Infused E_2 has a half-life of approximately 8–42 min. High circulating E_2 following implantation is most likely the result of a transient high release rate from the implant rather than a slow clearance rate from plasma. However, the animal can adapt to high levels of E_2 exposure. Increasing the dose of E_2 had no significant effect on circulating E_2 in heifers implanted with either one or two E_2 implants, indicating that clearance rate had been altered.

The pattern of circulating TBOH is similar to that of E_2. Administration of a single TBA implant (300 mg) resulted in elevated plasma TBOH (over 900 pg ml^{-1}) in heifers the day following implantation. The circulating levels then gradually decreased to 400 pg ml^{-1} on day 90 postimplantation. In bulls, circulating TBOH concentrations increased to approximately 1000 pg ml^{-1}, were sustained at that level until week 8, and then began to decline until week 11 when the bulls were reimplanted and the circulating TBOH rose again.

Circulating TBOH concentrations are affected by the presence of other steroids. Circulating TBOH levels in

TBA + E_2-implanted steers were twice as high as those in steers implanted with TBA alone. Additionally, serum TBOH levels have been shown to be higher, especially the first few days after implantation, in implanted heifers fed MGA compared to implanted heifers not fed MGA. Apparent differences in circulating TBOH levels in the presence of other steroids may be due to E_2 or MGA competition with hepatic TBOH metabolism. Furthermore, it has been suggested the half-life of one steroid is often influenced by simultaneous administration of another steroid. Finally, synergism between TBOH and E_2 most likely is more important than individual serum hormone concentrations.

Circulating hormone concentrations are not valid for assessment of duration of implant product payout and such values are often misinterpreted. The circulating level is a function of the difference between two rates – the rate at which compound enters the animal's bloodstream, and the rate at which it is cleared by the liver or kidneys – rather than simply of the payout from the implant. The minimum effective concentration is not known for all animal types and production situations, so assessing the concentration in the blood does not provide sufficient information to assess the function of the implant. Performance is not highly correlated with circulating steroid concentration. The correlation between weight gain and serum TBOH concentration has been reported to be no greater than +0.29. Other studies have reported little to no relationship between circulating steroid hormone levels and rate and efficiency of gain in animals treated with steroid hormones. In growing bulls, circulating steroid concentration had little correlation with growth rate.

Receptors for estrogens, androgens, and progestogens are located in most cell types, but in vastly different proportions. The concentration and binding affinity of these receptors affect the ability of the steroid to elicit a response in that cell type.

Genomic steroid actions

Steroid hormone receptor proteins act as transcription factors. These receptors recognize specific *cis*-acting deoxyribonucleic acid (DNA) sequences, referred to as hormone response elements (HREs), on target genes. The HREs are located on the 5′ promoter region of hormone-responsive genes. A general schematic of classical genomic steroid action is as follows:

1. Steroid hormones, which are lipophilic, gain entry into a target cell by simple diffusion.
2. Receptors are often associated with other cytosolic proteins such as chaperone and heat-shock proteins that help to stabilize the receptor.
3. Once the steroid binds to the receptor, these heat-shock proteins dissociate.
4. The above transformation results in increased affinity of the receptor for the HRE.
5. Some receptors are found in the cytosol and translocate to the nucleus after ligand binding (glucocorticoid and mineralocorticoid receptors), whereas others (estrogen, androgen, and progesterone receptors) are located in the nuclear region.
6. Following ligand binding, the ligand–receptor complex binds to palindromic DNA sequences in the promoter regions of hormone-responsive genes.
7. Binding of the ligand-activated receptor to HRE on hormone-responsive genes either initiates or upregulates transcription or can cause a downregulation of transcription.

Nongenomic steroid actions

The above sequence is often referred to as genomic steroid action because gene transcription must be upregulated or downregulated as a result of hormone action. This process can take many hours after initial exposure to the steroid.

Recently, nongenomic mechanisms of steroid hormones have been investigated. A nongenomic steroid action is a rapid intracellular response caused by steroids that is inconsistent with the classic genomic model. These changes can occur within seconds to minutes following steroid administration and are insensitive to transcription and translation inhibitors, suggesting that this signaling pathway may involve a classical second-messenger cascade such as phospholipase C, cAMP/cGMP changes, protein kinase C, etc. Nongenomic effects may be mediated by a receptor type other than the classic steroid hormone receptor since anti-steroid molecules – such as RU 486, a potent anti-glucocorticoid and anti-progesterone – do not block these nongenomic effects.

Steroid excretion

When implants are administered in the approved location (subcutaneous; middle third of ear), the risk of detectable residue levels that could be harmful to human health is negligible. Circulating steroid levels in implanted ruminants are influenced by the release rate of the steroid from the implant and the metabolic clearance rate of the steroid in the animal's body. Metabolism of both estrogens and androgens occurs in the liver through a series of hydroxylation and reduction steps. The liver is also the site of steroid conjugation. Once conjugated, the metabolites are water-soluble and can be excreted through the kidneys and eliminated from the body in the urine. In ruminants, it appears that a significant (45%) amount of steroids, specifically diethylstilbestrol, can be eliminated through the feces.

Once released from the implant, steroid hormones have a short half-life in the circulation. After administration of an intravenous bolus of E_2 to steers, the estimated half-lives were 1 min and 20 min in the fast and slow clearance pools, respectively. These data suggest that steroids, specifically E_2, are metabolized very rapidly once released from the implant.

Effects of steroids in food-producing animals

Steroid implants influence growth through two general mechanisms: effects on feed consumption, and effects on tissue growth not specifically related to nutrient intake. In growing cattle, estrogenic implants increase feed consumption by 6–8%. Stimulation of feed consumption is dose-dependent, continues for the effective life of the implant, and has been observed within 24 h of implantation. The increased caloric intake accounts for approximately one-half of the increased growth when estrogenic implants are used.

TBA dose titration studies indicate that TBA causes a 1–2% decrease in feed consumption in feedlot cattle. When estrogens and TBA are used in combination, feed consumption is increased. Pasture studies have not been designed to test the

effects of implants on consumption of forage or carrying capacity of pastures.

Effects of steroids on skeletal muscle

Muscle tissue contains both androgen and estrogen receptors, but the concentration of these receptors in muscle is often 1000 times less than in reproductive tissues. However, the relative binding affinities for the androgen receptor in skeletal muscle and prostate are identical. Androgen receptors in muscle tissue have been characterized in several species including rat, porcine, bovine, ovine, and human. Similarly, estrogen receptors in muscle tissue have also been characterized in rat and bovine.

The level of circulating steroid has been reported to be an important determinant in the amount of unoccupied steroid receptors. In sheep, implantation with TBA appeared to reduce the number of detectable androgen receptors as compared to those in nonimplanted lambs. TBA implantation decreased the binding affinity of cytosolic androgen receptors. Implantation of calves with TBA + E_2 reduced the number of free estrogen receptors approximately 83% as compared to nonimplanted calves. Upregulation or downregulation of steroid receptors may occur via gene transcription. Androgen withdrawal, via castration, for 4 days resulted in 1.5-fold to 3-fold increase in androgen receptor mRNA in the rat prostate. Testosterone propionate injections in castrated rats 24 h before tissue removal reduced androgen receptor mRNA to the level of intact males.

Steroids have both direct and indirect effects on muscle growth. In the case of estrogens, the direct effects are thought to be secondary to indirect effects mediated by changes in other hormone profiles. The primary effect of estrogens is through an altered somatotrophic axis. estrogens increase pituitary size and increase the proportion of somatotrophs in the pituitary. The pituitary is also more responsive to somatotropin releasing factor (SRF). Production of insulin-like growth factor-1 (IGF-1) is increased and both somatotropin (ST) and IGF-1 binding characteristics are altered. These changes work together to produce higher circulating ST, a more efficacious release pattern, and a more responsive muscle, resulting in stimulus of muscle growth.

Increased ST does not explain all of the effects of estradiol. Exogenous ST has been shown to increase growth of estradiol-implanted cattle, and estradiol and SRF are additive in affecting circulating metabolites and growth factors. Effects of estradiol and ST are nearly additive when energy consumption is restricted to the level of cattle without estradiol implants. Other hormones such as insulin and thyroid hormones are also altered, supporting the increased muscle growth.

The direct effects of androgens are significant. TBOH works directly on the muscle cell to stimulate muscle protein synthesis and deposition. The specific gene products that respond to TBOH have not been fully characterized.

Androgens have significant indirect effects as well, primarily through altered glucocorticoids. Circulating cortisol is reduced in TBA-implanted steers, and both cortisol binding and response to adrenocorticotrophic hormone (ACTH) are also diminished. Androgens also produce an altered ST profile (higher, more frequent peaks and lower troughs). These changes make the circulating hormone profile of TBA-implanted steers more like that of bulls, and result in increased muscle deposition.

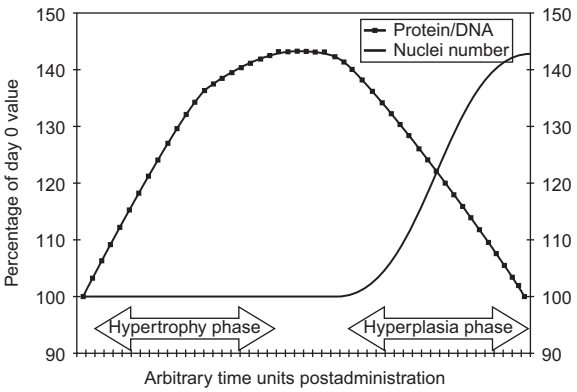

Figure 3 Idealized effects of TBA + E implantation on hypertrophy and hyperplasia of skeletal muscle.

The effects listed above can be observed within days after implantation. Early muscle growth stimulus is primarily hypertrophic in nature, which has been shown by depressed DNA–protein ratios (Figure 3). Prolonged exposure (weeks) to combined estrogenic–androgenic implants produces hyperplasia (increase in satellite cell nuclei) as well. In this case, the quantity of muscle protein is increased but normal DNA–protein ratios are observed, indicating that proliferation of satellite cells results in increased quantity of DNA in the muscle. Cell culture studies have shown that the mitogenic activity of sera from implanted steers is increased, providing support for the idea that implants initially increase hypertrophy and ultimately increase hyperplasia to support increased muscle mass.

Combined TBA + E_2 implants increase carcass protein by approximately 10% compared to nonimplanted steers. Much of this increase occurs for the first 40 days postimplantation (Figure 4). Increases in circulating and locally produced IGF-1 have been reported during this period of rapid muscle growth in TBA + E_2-implanted steers. Because IGF-1 is known to be a potent stimulator of both proliferation and differentiation of satellite cells, locally produced IGF-1 could act through autocrine or paracrine mechanisms to promote the proliferation and differentiation of muscle satellite cells, thus enhancing skeletal muscle hypertrophy. In fact, satellite cells isolated from the semimembranosus muscle of TBA + E_2-implanted steers after 35 days of steroid exposure exhibited a shorter lag phase and began proliferating sooner when placed in culture than cells from nonimplanted steers. TBA + E_2 either directly or indirectly activates quiescent satellite cells *in vivo* or maintains them in a proliferative state, supporting muscle hypertrophy.

Effects of steroids on adipose tissue

Steroid hormones are not thought to have significant direct effects on adipose tissue. Estrogen receptors are present in low concentrations and the presence of androgen receptors in adipose tissue has not been demonstrated. Indirect effects are due in part to altered ST, and increased energy consumption, which would enhance fat deposition. The net effect is that fat

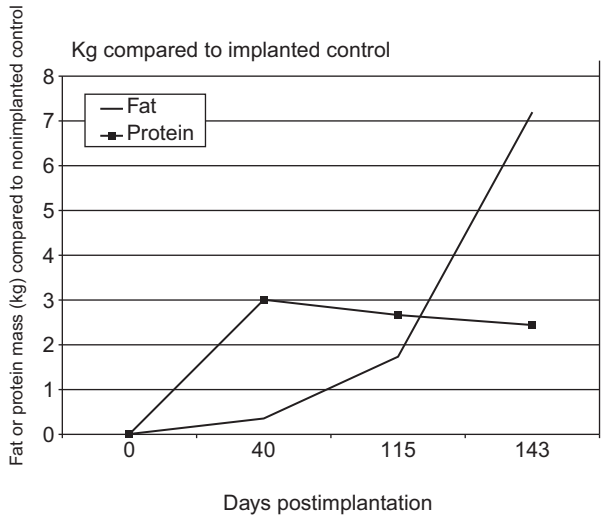

Figure 4 Changes in carcass protein and fat accumulation over time between carcasses obtained from implanted steers (120 mg TBA and 24 mg E_2), compared to carcasses from nonimplanted steers. Adapted with permission from Johnson, B.J., Anderson, P.T., Meiske, J.C., Dayton, W.R., 1996. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. Journal of Animal Sciences 74 (2), 363–371.

deposition rate in food-producing animals is not greatly altered by exogenous steroids. Carcasses from treated cattle may be leaner, but this is due to increased quantity (and concentration) of muscle, not to decreased quantity of fat.

There may be some fat depot specificity because many studies have shown reduced intramuscular fat (marbling) in implanted cattle, especially males. This could be a true effect of the steroids but it could also be an artifact. Most studies have harvested implanted and nonimplanted cattle at similar time end points. In these studies, chemical composition usually differs between treatments, with carcasses from implanted animals leaner, so marbling would be expected to differ as well. Investigators who chose end points other than time have typically observed less marbling reduction.

Effects of steroids on bone growth

Sex steroids are vitally involved in control of skeletal growth. Adult levels of sex steroids are required for a pubertal growth spurt (velocity) to occur in both males and females. Prolonged exposure to these adult steroid levels results in closure of the epiphyseal growth plate (cessation). Thus, steroids can be positive (velocity) or negative (cessation) in bone growth. As a practical matter in animal agriculture, the effects of implants on bone growth are modest. Limited work has shown that long-bone growth of females can be increased by exogenous estradiol, either preweaning or postweaning. Theoretically, excess steroid levels could limit bone growth by hastening growth plate closure, but there is no research to prove that this has occurred and steroid levels required are likely greater than those used in production agriculture. Exogenous steroids appear to have little effect on bone growth of steers.

Melengestrol acetate

MGA suppresses estrus in cycling females and the resulting behavioral change is favorable for animal performance. In addition, MGA induces a hyper-estrogenic state in the static ovary, which in some ways mimics the use of an estrogenic implant. Some studies have shown estrogenic implants and MGA to have additive effects, but others have not. Differences in these results may be based on dosage or animal characteristics such as maturity or genetic potential for growth. MGA effects are additive with those of TBA, and MGA appears to ameliorate negative effects of TBA on marbling in some production situations.

Practical considerations of steroid use

Because dosage and duration of implant products are not ideal for some production situations, cattle may be implanted more than once during the feeding period. Use of these reimplant programs typically improves performance compared to use of a single implant, but may further reduce quality grade (Figure 1). Implants may increase sexual or aggressive behavior of confined cattle. To properly design implant programs, dosage, duration, and desired outcome of the production situation must be considered. MGA may also be situation-specific, because a mature ovary is the primary target tissue, so immature or spayed heifers may not respond.

Because implants are administered in a nonsterile surgical procedure conducted under field conditions, abscessation of the implant site is common. Even skilled personnel using excellent technique and sanitation will observe abscesses in 3–5% of implant sites. Under poor conditions, rate of abscessation can be much higher. Data from quality assurance surveys conducted by implant manufacturers indicate that approximately 1% of implants are expelled owing to pressure from the abscess resulting in substantial missed economic opportunity. Proper technique and sanitation can minimize these problems, but there is a risk of infection after implantation regardless of sanitation. Implant products are available with a pellet of tylosin tartrate added as a local antibacterial to minimize implant site infections. Owing to improved health of the implant site, inclusion of the tylosin pellet results in increased weight gain compared to implants without tylosin, even with excellent sanitary technique and a low rate of abscessation.

Beta-Adrenergic Agonists in Animal Production

Beta-adrenergic agonists (BAA) are phenethanolamine compounds approved for use in food animal production in several countries. In the United States, ractopamine is used in both swine and cattle production to improve growth rate and efficiency of lean-tissue deposition. Zilpaterol was approved for use in cattle production in the United States in 2007 after extensive study and both ractopamine and zilpaterol had significant market uptake. Sale of zilpaterol in the United States was suspended in 2013 because of reports by beef processors of hoof problems of cattle in holding pens. The FDA has determined that zilpaterol use is safe for cattle and consumers and zilpaterol remains available in other countries. Other BAA, such as clenbuterol and cimaterol, have been widely used as

Table 1 Typical responses to beta-adrenergic agonists in food-producing animals

	Ruminants	*Swine*
Average daily gain	+10–30%	+0–10%
Feed conversion efficiency	+15–30%	+5–15%
Carcass fatness	−5–30%	−10–15%

Source: Adapted with permission from Anderson, P.T., 2000. Mechanisms by which metabolic modifiers alter growth rate and carcass composition of meat animals. Proceedings of the 53rd Annual Reciprocal Meat Conference, pp. 31–35. Savoy, IL: American Meat Science Association.

research compounds but are not approved for use in food animals.

BAA are orally active and are presented as feed additives, in contrast to steroid hormones, which have minimal oral activity (except MGA). BAAs are dosed in ppm, mg per head per day, or $g\,t^{-1}$. Typically, BAAs are administered only to animals consuming a high-energy (finishing phase) diet at the end of the finishing period during the final 3–6 weeks of production. Zilpaterol is typically withdrawn for three or more days prior to slaughter, whereas ractopamine requires no withdrawal. Typical effects of BAAs in food-producing animals are shown in Table 1.

BAA can be generally classified as β_1 or β_2, on the basis of their receptor affinity. Ractopamine is a selective β_1-agonist, while zilpaterol and other widely studied compounds are β_2-agonists. BAA are used to improve live performance and carcass leanness of cattle, sheep, swine, or poultry. There are species differences in responses to the various compounds (Table 1) and both dosage and duration of treatment affect response. In ruminants, feeding β_2-agonists for 20 days can produce effects on live weight gain and feed conversion efficiency that are as great as the effects produced by 150–200 days of steroid implant exposure, with greater increases in carcass leanness. Both β_1- and β_2-agonists affect carcass leanness and muscularity in cattle but β_2-agonists are more efficacious.

Mechanism of Beta-Adrenergic Agonists Action

Use of BAA increase carcass leanness by increasing the quantity of muscle mass and by decreasing the quantity of fat. These compounds are often called partitioning agents because they appear to divert dietary energy from fat deposition toward muscle deposition. In nonruminants, use of BAA results in mobilization of energy already stored as fat for use in muscle growth and in cattle, zilpaterol does the same. In this case, they may be called repartitioning agents. BAA can dramatically increase protein deposition and cause net fat deposition to cease or become negative, even in rapidly growing cattle consuming high-energy diets. There are indications that zilpaterol use in cattle can mobilize nutrients from non-carcass tissues for deposition in the carcass. This partially explains why zilpaterol use results in greater carcass weight gain than live weight gain and a much larger increase in the proportion of live weight that is carcass than ractopamine. Effects of BAA are realized in administration periods as brief as 20 days.

Table 2 Comparative effects of β_1- and β_2-adrenergic agonists in ruminants

	Agonist type	
	β_1	β_2
Protein synthesis	++	+
Protein degradation	NS	–
Net protein deposition	+	++
Lipogenesis	–	–
Lipolysis	NS	++
Net fat deposition	–	–
Muscle mass	+	++
Muscle cross-sectional area	+	++
Muscle fiber number	NS	NS
External fat thickness	NS	–

Abbreviation: NS, not significant.

BAA directly affect both adipose and skeletal muscle tissue. BAA actions are mediated by a class of membrane-spanning receptors called beta-adrenergic receptors (β-AR), of which three subtypes exist: β_1-AR, β_2-AR, and β_3-AR. A fourth type, β_4-AR has been proposed but not confirmed and may be an artifact. These receptors function by binding to G_s proteins. Following coupling to G_s proteins, adenyl cyclase is activated, which converts ATP to cAMP. Cyclic AMP in turn regulates activity of protein kinase A. In skeletal muscle, β_2-agonists increase muscle mass by increasing protein synthesis and decreasing protein degradation, whereas β_1-agonists increase synthesis but do not affect degradation, except when very high doses are fed (Table 2). The β_1-agonist ractopamine has been shown to increase the abundance of skeletal muscle-specific mRNAs in experiments both *in vivo* and *in vitro*. Administration of β_2-agonists increases muscle mass by 8–40% with later maturing muscles affected the most. Longissmus muscle cross-sectional area is increased by 11–39%. Increased muscle fiber cross-sectional area accounts for these differences, with the greatest increase in Type II fibers and some studies indicating that the proportion of Type II fibers may be increased as well.

These changes in muscle hypertrophy occur with no apparent change in DNA content of muscle, implying that muscle satellite cells are not activated to fuse into the existing fibers to support this rapid increase in muscle hypertrophy. The lack of DNA accretion in existing muscle fibers limits the ability to sustain muscle hypertrophy, long term. Administration of β_1- or β_2-agonists reduces circulating and local IGF-1 as well as IGF-1 mRNA in skeletal muscle, which could explain the lack of effect on DNA, as IGF-1 is a stimulator of satellite cell proliferation. BAA-induced stimulation of muscle hypertrophy could be enhanced if BAA dampening of IGF-1 could be impeded.

In adipose tissue, activation of the β-AR and the subsequent intracellular signaling cascades result in activation of hormone-sensitive lipase, which facilitates lipolysis. Furthermore, receptor activation has inhibitory effects on enzymes responsible for *de novo* synthesis of fatty acids and triglycerides. Specifically, several key steps in the conversion of glucose to triglycerides are inhibited by the activation of β-AR. The net

result is that this energy substrate is then available for deposition in other tissue such as skeletal muscle, a result which could be called repartitioning.

Practical Considerations for Beta-Adrenergic Agonists Use

β-AR are found in all cell types studied but their proportion varies widely so it is the relative and absolute proportion that determines effects on specific tissues, rather than simply presence or absence. For example, bovine skeletal muscle contains β_2-AR in much higher concentration than β_1-AR, typically 10X or more. This is one reason that β_2-agonists are more efficacious in the bovine. Comparative effects of β_1- and β_2-agonists are shown in Table 2.

The intracellular mechanisms that control protein turnover *in vivo* are in some ways similar to postmortem changes which improve beef tenderness. Use of β_2-agonists, such as zilpaterol reduce tenderness in some situations, likely because the reduction in turnover that increases skeletal muscle growth alters postmortem changes as well. For example, administration of an experimental β_2-agonist, L-644,969, increased calpastatin in skeletal muscle. Calpastatin is an specific inhibitor of calpains and thus reduces tenderness by diminishing postmortem protein degradation. At high doses, the β_1-agonist ractopamine binds to β_2-AR as well. This cross-reactivity could mimic the effects of administration of a β_2-agonist, explaining both increased efficacy of ractopamine at higher doses and negative effects on tenderness that have been noted at these doses.

Effects of BAA appear to be related to animal maturity, with younger animals less responsive. For example, fetal bovine skeletal muscle cells have minimal β-AR present, although they do have β_2-AR mRNA. Comparative growth effects in young versus old rats and calves versus yearling cattle, indicate greater production responses in the older animals. In yearling cattle, as days on feed increase, β_1-AR mRNA decreases in skeletal muscle, whereas β_2-AR mRNA increases. This progression was not noted in younger calf-fed cattle.

It appears that β-AR in growing animals can become slightly refractory to prolonged (days) exposure to BAA. This should not be surprising, given the close structural similarity of BAA to epinephrine, which produces rapid effects but is cleared from circulation quickly as well. A practical means to supply growing animals with ever-increasing doses of BAA through the administration period could enhance productivity.

Comparative Efficacy of Steroids and Beta-Adrenergic Agonists

Since there are no restrictions on use of steroid implants and BAA in the same animals, questions will arise about the absolute and comparative efficacy of these two classes of growth promotants used concomitantly. In theory, steroid implants and BAA should be additive in stimulating lean-tissue growth in beef cattle due to their distinct mechanisms of action. Limited research has confirmed this with each class of compounds eliciting a typical response in the presence or absence of the other.

Earlier research evaluating the effects of bovine somatotropin (bST) and steroids revealed additive effects of these two growth promotants on weight gain and protein deposition in beef cattle. The conclusion that each had distinct mechanisms of action in stimulating growth was surprising, because E_2 use increases circulating ST levels. In fact, research suggests that E_2 and bST were not additive in the effects on circulating ST. Taken together, these data suggested that the growth-promoting effects of estrogens were not fully mediated by the increase in ST secretion.

Steroid implants stimulate skeletal muscle growth through increases in muscle hypertrophy as well as through activation of muscle satellite cells to provide the critical DNA to sustain muscle hypertrophy. It appears that locally produced IGF-1 can mediate many of these effects. Since BAA do not increase the DNA content of skeletal muscle, administration of a steroid implant 60–90 days prior to feeding BAA should have additive effects on lean-tissue deposition in beef cattle because of the different mechanisms of action. However, some research has shown that clenbuterol (a β_2-agonist) increased local expression of IGF-1 in skeletal muscle of rats. If the effects are the same in beef cattle, there might be some overlap in potential mechanism of action in which the two classes of compounds would not be completely additive.

Effects of Growth Promotants on Palatability

The effects of steroid implants on the palatability of the end product have been studied, but results are not conclusive. In most studies, implants did not significantly affect tenderness, juiciness, or flavor. Negative effects on tenderness were observed in some studies in which higher dose implant products were used. Labels on some higher dose implants state that if tenderness is of primary importance that they should not be used late in the finishing period. These negative effects can be as great as 0.6–0.8 kg increase in shear force. Interactions exist between steroid dosage and animal type. For example, negative effects on tenderness are more likely to be observed when multiple doses of androgens are administered, especially in more muscular breeds of cattle. In most other production situations, the effects of implants on tenderness are either nonsignificant or not of practical importance.

The effects of BAA on tenderness depend on the compound studied. Ractopamine has not affected tenderness of beef unless fed at the highest dose studied (300+ mg per day) for at least 28 days. In contrast, zilpaterol, which is more efficacious, typically does increase toughness when electrical stimulation and adequate aging (at least 21 days) are not used.

In an extensive study involving five universities and Merck Animal Health, involving both beef cattle and Holstein cattle that were fed zilpaterol for 20 days as part of a typical finishing diet, tenderness of several muscles was reduced unless a minimum of 21 days of wet aging was used. Feeding zilpaterol for more than 20 days resulted in toughening of all muscles, particularly the *gluteus medius* muscle from heifers, even with 21 days of aging. In 2013, some beef processing plants observed hoof damage of zilpaterol-fed cattle while those cattle were in holding pens prior to harvest. While FDA reiterated the

safety of the product, Merck Animal Health suspended marketing of the product until further studies are conducted.

Summary

Metabolic modifiers, including steroid hormones and BAA, increase production and improve efficiency of food-producing animals. This results in economic benefit to livestock producers and influences the relative price competitiveness of protein sources for consumers, favorably influencing market share for those species in which they are used. Consumers benefit from reduced costs and, in some cases, improved nutrient content of the meat. Additionally, fewer resources are required for production of meat and environmental impact is lessened. Most consumers accept product from animals produced with prudent use of metabolic modifiers. The variety of compounds and dosage choices available to producers allows for specific 'targeted outcome' programs to fit the production and marketing objectives of individual operations. The next decade of research will likely focus on production aspects of concurrent use of steroids with BAAs and the influence of these products on consumer acceptance of meat.

See also: Chemical and Physical Characteristics of Meat: Palatability. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists; Red Meat Animals. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Anderson, P.T., 1991. Trenbolone acetate as a growth promotant. Compendium on Continuing Education for the Practising Veterinarian 13, 1179–1191.

Anderson, P.T., 2000. Mechanisms by which metabolic modifiers alter growth rate and carcass composition of meat animals. Proceedings of the 53rd Annual Reciprocal Meat Conference, pp. 31–35. Savoy, IL: American Meat Science Association.

Baxa, T.J., Hutcheson, J.P., Miller, M.F., *et al*., 2010. Additive effects of a steroidal implant and zilpaterol hudrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers. Journal of Animal Science 88, 330–337.

Claus, H.L., Dikeman, M.E., Murray, L., *et al*., 2010. Effects of supplementing feedlot steers and heifers with zilpaterol hydrochloride on Warner-Bratzler shear force interrelationships of steer and heifer longissimus lumborum and heifer triceps brachii and gluteus medius muscles aged for 7, 14, and 21 d. Meat Science 85, 347–355.

Gunderson, J.A., Hunt, M.C., Houser, T.A., *et al*., 2009. Feeding zilpaterol hydrochloride to calf-fed Holsteins has minimal effect on semimembranosus steak color. Journal of Animal Science 87, 3751–3763.

Hancock, D.L., Wagner, J.F., Anderson, D.B., 1991. Effects of oestrogens and androgens on animal growth. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals, vol. 7. Cambridge, MA: CAB International, pp. 255–297.

Hilton, G.G., Montgomery, J.L., Krehbiel, C.R., *et al*., 2009. Effects of feeding zilpaterol hydrochloride with and without monensin and tylosin on carcass cutability and meat palatability of beef steers. Journal of Animal Science 87, 1394–1406.

Holmer, S.F., Fernandez-Duenas, D.M., Scramlin, S.M., *et al*., 2009. The effect of zilpaterol hydrochloride on meat quality of calf-fed Holstein steers. Journal of Animal Science 87, 3730–3738.

Johnson, B.J., Halstead, N., White, M.E., *et al*., 1998. Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. Journal of Animal Science 76, 2779–2786.

Lawrence, T.E., Gasch, C.A., Hutcheson, J.P., Hodgen, J.M., 2011. Zilpaterol improves feeding performance and fabrication yield of concentrate finished cull cows. Journal of Animal Science 89, 2170–2175.

Mersmann, H.J., 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. Journal of Animal Science 76, 160–172.

Mills, S.E., 2002. Biological basis of ractopamine response. Journal of Animal Science 80, E28–E32.

Moody, D.E., Hancock, D.L., Anderson, D.B., 2000. Phenethanolamine repartitioning agents. Farm Animal Metabolism and Nutrition 65–95.

Owens, F.N., Gill, D., Dolezal, G., Morgan, B., Horn, G., 1997. Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle. Stillwater, OK: Oklahoma State University, Oklahoma Agricultural Experimental Station, pp. 957–961.

Preston, R.L., 1999. Hormone containing growth promoting implants in farmed livestock. Advanced Drug Delivery Reviews 38, 123–138.

Tucker, H.A., Merkel, R.A., 1987. Applications of hormones in the metabolic regulation of growth and lactation in ruminants. Federation Proceedings 46, 300–306.

Relevant Websites

www.meatscience.org
American Meat Science Association.

www.elanco.com
Elanco Animal Health.

www.revalor.com
Merck Animal Health.

www.zilmax.com
Merck Animal Health.

www.zoetis.com
Zoetis Animal Health.

Muscle

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Glossary

Fasciculus Bundle of myofibers.
Hyperplasia An increase in number of cells.
Hypertrophy An increase in size of cells.
Intrafascicularly terminating myofibers End in fasciculus, do not reach both ends of muscle.
Mesoderm The embryonic layer from which muscle is derived.
Myoblast Single cell-forming muscle.
Myofiber Muscle fiber.
Myotube The immature myofiber formed from fused myoblasts.
Secondary fiber An alternate form of immature myofiber.
Somite A block of mesoderm.

Introduction

Principles of muscle growth in meat animals help us understand how meat yield and quality are interrelated. Meat is derived from muscles that once pulled on parts of the skeleton for body movements in locomotion, respiration, and chewing. Myofibers make up skeletal muscles, and often are many centimeters in length but only a small fraction of a millimeter in diameter. Each myofiber is a single cell with many nuclei.

Regulation of myofiber numbers is largely genetic and prenatal, provided the fetus is well nourished. Later, animal nutrition and exercise have a strong effect on the longitudinal and radial growth of myofibers, adding to their length and thickness. Myofibers adapted for repetitive activity tend to have aerobic metabolism, needing abundant oxygen from the blood stream, and are supported by large numbers of mitochondria and lipid droplets. Mitochondria release energy whereas lipid droplets store energy. Lipid droplets enhance the taste and juiciness of meat. Other types of myofibers are specialized for strong, rapid contraction but are easily fatigued. These myofibers tend to be anaerobic, using stored carbohydrates (glycogen) if blood vessels cannot supply enough oxygen. Anaerobic myofibers bulging with contractile myofibrils are particularly important in rapid muscle growth.

Rapid muscle growth and profitable meat production are often achieved by rapid growth of anaerobic myofibers, but these are not the ones with the best tenderness, juiciness, and taste. Conversely, traditional breeds may produce high-quality meat but only slowly and uneconomically. Thus, how commercial pressures to increase meat yield may detract from meat quality is seen.

A few pointers might be useful for readers unfamiliar with histological terminology. The prefixes myo- and sarco- both indicate muscle. Myo- tends to be an optional prefix used if there is a risk of confusing myofibers (i.e., muscle fibers) with some other types of fibers, such as connective tissue fibers. The suffix -blast indicates a cell that forms something. Thus, a myoblast makes myofibers, wheras a fibroblast makes connective tissue fibers. But the mechanisms involved differ radically. Myofibers are very large cells made by the fusion of small cells (myoblasts). Connective tissue fibers are cables of protein assembled outside cells (fibroblast) exuding the formative materials.

The suffix 'mere' indicates a part. Thus, a sarcomere is a small contractile unit along the length of a muscle. Chains of sarcomeres form myofibrils within myofibers. Note the diminutive ending indicating a fibril is much smaller than a myofiber. The sarcomeres along the hundreds of myofibrils inside a myofiber are neatly in line with each other. The alternating dark (myosin) and light (actin) portions of sarcomeres result in whole myofiber appearing to be transversely striated.

To complete this hierarchy of somewhat confusing terms, they are listed from largest to smallest: (1) myofasciculus – visible by eye, a bundle of myofibers; (2) myofiber – visible with a microscope, a very large cell; (3) myofibril – a contractile organelle composed of chains of sarcomeres, and located inside a myofiber; and (4) myofilament – a protein filament, part of a sarcomere. The prefix 'myo' can be dropped from any of the above terms if not needed.

Allometry

Differences in growth rate (allometry) occur among various muscles. Along the vertebral axis, a wave of growth proceeds from head to tail, starting prenatally. In the limbs, growth waves proceed from the distal extremities proximally toward the trunk of the body. Vertebral and hindlimb growth waves, having passed along the loin and up the hindlimb, eventually combine in the rump (pelvic region). Thus, the most valuable cuts of beef, pork, and lamb tend to develop late.

Muscle allometry is measured using Huxley's equation, $y = bx^k$, where k is the allometric growth ratio, the slope of a log:log plot. The log of a muscle's weight is plotted against the log of body weight. If $k > 1$, the muscle is growing faster than the remainder of the body, and vice versa. If $k = 1$, individual muscle yield remains constant as the body grows. Sometimes the log:log plot may give a curve rather than a straight monophasic line, thus showing k is changing. In these cases, the curve might be divided at a suitable point and treated as a biphasic system.

It is important to match the units of measurement on both axes, weight with weight, or length with length. When comparing results from different studies, the term on the x-axis must be the same to make a valid comparison. The allometric

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equation can be used to analyze carcass conformation without knowing animal age. Allometric analysis can reveal subtle but important changes in muscle and body growth, such as the late growth of throat muscles to counterbalance the elastic ligament suspending the bovine head, or the late growth of abdominal muscles to cope with the bulging rumen.

Myogenesis in Embryonic Development

Mesoderm

Mesoderm is the middle of the three layers forming an embryo. Most of the meat on commercial carcasses originates from somites. Somites are blocks of mesodermal cells along left and right sides of the developing spinal cord. Adipose and connective tissues also originate from mesoderm. The two other embryonic layers are ectoderm (giving epidermis and nervous system) and endoderm (giving digestive glands and gut). Muscles developing from blocks of somitic mesoderm can be invaded by connective tissues from elsewhere in the embryo (from somatic mesoderm – in sheets, not blocks). Invading connective tissues provide vital guidance in establishing the arrangement of myofibers.

Myoblasts and Myotubes

Myofibers have a far greater volume than other body cells because of their great length. A single nucleus cannot service such a large volume of cytoplasm, so myofibers have many nuclei (i.e., they are multinucleated). Theodore Schwann, who in 1839 first proposed that animal bodies are composed of countless cells, also discovered how the multinucleated state of myofibers originates. Multinucleated myofibers are formed by the fusion of mononucleated myoblast cells, often via an intermediate stage – the myotube. A myotube has an axial core of sarcoplasm (muscle cytoplasm) surrounded by a tubular arrangement of myofibrils (contractile organelles). The first myofibers pass through a myotube stage, whereas myofibers formed later in prenatal development directly acquire a central axis of myofibrils and peripheral nuclei (as in most mature myofibers). Myofibers passing through a myotube stage may be called primary myotubes or myofibers, to distinguish them from secondary myofibers missing the myotube stage.

New nuclei are formed by mitosis. New deoxyribonucleic acid (DNA) copies existing DNA, followed by separation of two daughter nuclei. Completion of mitotic activity in mesodermal stem cells and commitment to myogenesis are controlled by the MyoD family of gene-regulating proteins (MyoD, Myogenin, myf5, and MRF4). Before commitment to become myoblasts, dividing mesodermal cells later to form muscle may be called premyoblasts (Figure 1(a)). Only after commitment can they be called myoblasts. This helps us remember that only the cells leading up to the myoblast are capable of mitosis. Myoblasts are not capable of mitosis.

The brand new myoblasts, having given up mitosis and turned to the synthesis of ribonucleic acid (RNA) instead of DNA, now exhibit many of their new proteins. They develop two long cytoplasmic extensions (Figure 1(b)). Their cytoplasm with high levels of RNA is basophilic (readily stained with basic dyes). Myoblasts explore their environment using the tips of their cytoplasmic extensions. If they contact suitable partners, myoblasts fuse together to form a multinucleated cell (Figure 1(c)). Fusion is mediated by cyclic adenosine monophosphate and protein phosphorylation. Transverse striations develop as new myofilaments self-assemble into sarcomeres and as myofibrils grow and proliferate. Between the myofibrils are many mitochondria. Tapered or fusiform structures (Figure 1(c)) occur where myotubes grow by incorporating new myoblasts.

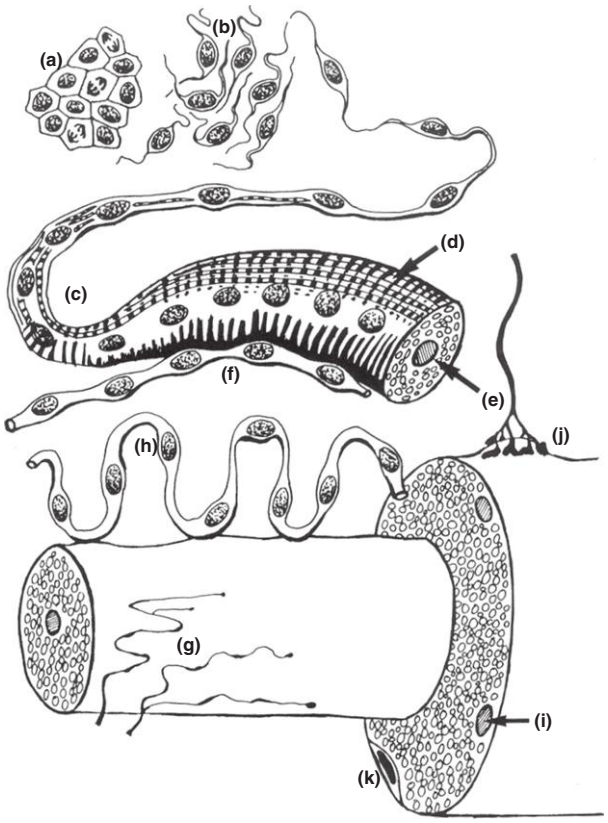

Figure 1 A composite sketch of various stages of myogenesis and muscle growth. (a) Mitotic cells, (b) myoblasts, (c) fused myoblasts forming a multinucleate future myofiber with contractile myofibrils, (d) addition of new myofibrils under the plasma membrane to form a hollow tube of myofibrils, (e) nuclei in the axis of the myotube, (f) fusion of myoblasts temporarily located on an original primary myotube to form a new secondary myofiber, (g) initial innervation of a myotube, (h) secondary myofiber thrown into snake-like folds by contraction of the myotube on which it is located, (i) true myofiber nucleus, (j) en plaque neuromuscular junction, and (k) satellite cell pressed into the surface of the myofiber but not fused with it.

Secondary Myofibers

Successive generations of myoblasts accumulate in developing muscle. The later myoblasts cling by pseudopodia pushed into the surfaces of existing myotubes. Instead of fusing with the myotube, these new myoblasts fuse with each other to form a secondary myofiber (Figure 1(f)). By this time, myotubes are innervated by axons invading from the spinal cord (Figure 1(g)). Innervation causes slow

contractions. Myotube contractions accelerate the side-by-side contact and fusion of myoblasts. Once secondary myofibers develop their own myofibrils, their uncontracted stiffness causes secondary myofibers to form snake-like folds along their supporting myotube (Figure 1(h)). Secondary myofibers then separate from the myotubes, freeing spaces on myotube surfaces for other secondary myofibers to form.

In summary: (1) myotubes function as contractile templates accelerating development of secondary myofibers, (2) myotubes establish myofiber arrangement within a muscle, and (3) myotubes are located centrally within bundles of myofibers. Only approximately 20% of myofibers originate from myotubes.

Degeneration

Cellular degeneration often occurs during myogenesis. Nuclei can be disrupted and myofibrils appear to be fragmenting rather than assembling. Perhaps this is what happens to myofibers failing to attach to connective tissue and, therefore, unable to develop tension when stimulated to contract.

Innervation

All surviving myofibers are innervated by terminal axons of motor neurons. Starting in the spinal cord, axons grow into developing muscles, partly by following connective tissues stretched by skeletal elongation, and partly by chemical attraction. Initial neuromuscular junctions are diffuse and irregular. On fast-contracting myofibers, the neuromuscular junction consolidates to a compact en plaque junction (Figure 1(j)). Junctions on slow-contracting myofibers show less consolidation, especially on poultry slow myofibers.

Prenatal muscle contraction is essential for the complete formation of secondary myofibers. Factors inhibiting prenatal muscle movements (such as plant toxins or loss of amniotic fluid) cause hypoplastic muscles (reduced myofiber numbers). Prenatal muscle movements also are essential for the shaping of articular cartilages on joint surfaces. The development of motor neurons, myofibers, and articular joints is completely integrated. A defect in one system affects the other two, often resulting in conditions such as congenital articular rigidity (arthrogryposis).

Double-Muscling – Myofiber Hyperplasia

What beef producers usually call 'double muscling' is caused by myofiber hyperplasia (an increased real number of myofibers) originating from an increased number of myotubes. Developing animals have a system to inhibit excessive muscle development controlled by a protein, myostatin (GDF8). This inhibitor itself is inhibited by another protein, follistatin. Thus, a defective gene for myostatin or high levels of follistatin will both act to increase muscle mass. Myofiber hyperplasia is widespread in the bodies of double-muscled animals. But the first three somites of the embryo, which give rise to muscles rotating the eye-ball, do not develop extra myofibers, although muscles growing into the head region later in development may become hyperplastic, such as the enlarged tongue muscle (macroglossia). This is because trunk and limb muscles differ genetically from extraocular muscles, especially in their Pax3 and Pax7 genes.

Double muscling is quite common in some beef breeds. In sheep it is rare. Myofiber hyperplasia might have occurred in the ancestral stock of broad-breasted turkeys. In cattle, double muscling is inherited as a simple Mendelian trait with incomplete dominance. Double-muscled cattle have a generalized deficiency of connective tissue, including a paucity of adipose cells. Thus, their meat is lean and relatively tender (unless cold-shortened). Obstacles to commercial exploitation of double-muscling include deleterious pleiotropic effects on male and female reproductive systems, difficult natural copulation, difficult calving, and structural problems.

Postnatal Development

Hyperplasia versus Hypertrophy

Whereas myofiber hyperplasia (increase in myofiber number) dominates prenatal development, postnatal development is dominated by myofiber hypertrophy (increase in size). Hypertrophy occurs in two dimensions – radial and longitudinal. At birth, the axial nuclei of myotubes move to a peripheral position beneath the plasma membrane (Figure 1(i)), although poultry myofibers sometimes retain axial nuclei.

Satellite Cells

Stem cells or residual premyoblasts capable of mitosis remain on the surfaces of myofibers as very small satellite cells. Endomysial connective tissue sheaths trap them below the basement membranes of myofibers (Figure 1(k)). Satellite cells have very little cytoplasm and the plasma membranes separating them from myofibers are seldom seen by light microscopy. For many years, therefore, it was difficult to explain the origin of increasing numbers of myofiber nuclei as myofibers grew in length and diameter.

When myofibers are growing or regenerating after injury, satellite cells divide by mitosis controlled by IGF-1. Typically, one daughter cell remains as a satellite cell capable of further mitosis whereas the other fuses into the myofiber to provide a new nucleus no longer capable of mitosis but switched on for producing RNA for muscle protein synthesis. Most dividing cells reveal themselves by showing chromosomes, but chromosomes cannot easily be seen when satellite cells undergo mitosis – simply because the chromosomes have no space to separate in such a small cell. Why such a complex system? Most likely to protect the sources of new nuclei in a damaged myofiber – which is a very unusual giant, multinucleated cell.

Histochemistry

The concentric arrangement of secondary myofibers around myotubes mirrors the distribution of fast-contracting, anaerobic myofibers (=white, Type II, or αW) around slow-contracting, aerobic myofibers (=red, Type I, or βR). This is

seen clearly in pork, but can require statistical testing to demonstrate in other species where myofiber arrangement changes during growth. In general, myotubes typically become slow-contracting, aerobic myofibers whereas secondary myofibers become fast-contracting, anaerobic myofibers. However, few muscles maintain the 20:80% ratio of myotubes to secondary myofibers when they become specialized for different activity patterns, and most muscles show transformations from one myofiber type to another as they grow. Thus, myofiber-type ratio is a snap-shot at a particular age and position within a muscle.

The last secondary myofibers formed prenatally are grouped around their primary myotubes, but are beneath older secondary myofibers. The last secondary myofibers typically become dual purpose myofibers capable of fast-contraction and strong aerobic activity. They may transform to become fast-contracting, anaerobic myofibers or to become slow-contracting, aerobic myofibers. The direction of the change depends on muscle activity patterns. Sometimes, myotubes start as slow-contracting, aerobic myofibers but make a major transformation to become fast-contracting, anaerobic myofibers. For example, chick breast muscle starts with many myotubes but finishes with nearly all white myofibers.

Body weight increases as a cubic function of linear body dimensions, while muscle strength increases only as a squared function (muscle cross-sectional area). Hence, postural muscles working against gravity as animals grow heavier might adapt by myofiber-type transformations from fast-contracting to slow-contracting, and (or) from anaerobic to aerobic. This adaptability of skeletal muscle has been shown in countless experiments. The type of innervation of a myofiber determines its balance of MyoD versus myogenin, its contractile properties and its predominant energy source (aerobic vs. anaerobic). When experimentally cross-reinnervated, a myofiber tends to follow the dictates of its new axon. Patterns of activity are particularly important, mediated by calcium ions acting on genes in the myofiber nucleus.

Tapered Myofibers

Myofibers are grouped into bundles (fasciculi) by perimysial connective tissue. In short fasciculi, myofibers can extend from one end of the fasciculus to the other. But in long fasciculi, many individual myofibers are much shorter than the length of the whole fasciculus and have a tapered ending anchored in connective tissue. Tapered myofibers may be called intrafascicularly terminating myofibers.

Within a myofiber, the Z-lines of adjacent myofibrils are linked laterally by a meshwork of desmin filaments and are attached to the plasma membrane by proteins such as vinculin and dystrophin. Outside the myofiber, the plasma membrane is covered by a basement membrane in which are embedded finely branched reticular fibers (Type III collagen) of the endomysium. When a tapered myofiber contracts, the tension it generates is transferred to the fibrous framework of the whole muscle. This integrates the all or nothing contraction of an individual motor unit (myofibers innervated by one motor neuron) into a smooth response by the whole muscle.

In transverse sections, tapered endings appear as small-diameter myofibers, although they can easily be missed by light microscopy. Detection of tapered myofibers is enhanced by delineating each myofiber with a silver stain. Tapered endings can confound attempts to measure the radial dimensions of myofibers in transverse sections. Furthermore, when histochemical myofiber-type ratios are determined during growth or adaptation of a muscle to a particular pattern of activity, a change in ratio has two possible explanations. First, the innervation of a myofiber might have changed its histochemistry. Alternatively, there might have been a change in the relative lengths of myofiber types, with one type growing more rapidly than another.

Several relays of tapered myofibers might extend along the length of a long fasciculus. Furthermore, several relays of fasciculi might extend along the length of a whole muscle. At no point along such a muscle will all its myofibers appear in the same plane. Thus, it is important to distinguish between the real number of myofibers in a muscle (obtained by total muscle dissection in nitric acid to loosen connective tissue) and the apparent myofiber number (obtained by sampling from muscle transverse sections).

Radial Growth of Myofibers

Skeletal myofibers are prismatic (with sides flattened by compression), but are generally treated as cylindrical in growth studies. Caution is needed when attempting to measure a mean radius or diameter from a prismatic structure, especially when it has not been sectioned perpendicularly. This is why cross sectional area is more reliable than diameter as an indicator of radial growth. Myofibers often grow >0.1 mm in mean diameter.

Within myofibers, individual myofibrils grow radially by the peripheral incorporation of new myofilaments. This lengthens the diffusion pathway along which calcium ions are released or re-sequestered by the sarcoplasmic reticulum for the control of muscle contraction. A buildup of calcium ions in the axis of myofibrils might initiate proteolysis by calpains. Continued activity can then cause myofibrils to shear longitudinally like a split log, giving an increase in the apparent number of myofibrils. Thus, the radial growth of myofibers occurs with an increase in the apparent number of myofibrils.

Radial growth of myofibers lengthens the oxygen diffusion pathway from capillaries on the myofiber surface inwards to the axis of myofibers. There is a concern this might alter myofiber metabolism when radial growth is pushed too far. Differences in myoglobin concentrations between histochemical myofiber types make this a complex problem. Mapping of a mitochondrial enzyme, succinate dehydrogenase (SDH), shows where oxygen is abundant. Strong aerobic activity in myofibers is usually peripheral, near to the capillary source of oxygen on myofiber surfaces. Fast-contracting, anaerobic myofibers exhibit the greatest radial growth. Their axes become devoid of SDH and, especially in pigs, can store massive amounts of glycogen, accompanied by phosphorylase activity. It is suspected but not proven this might contribute to rapid or extended postmortem glycogenolysis leading to pale, soft, exudative pork.

Fast-contracting muscles generally show positive allometric growth of their fast-contracting myofibers, whereas slow-contracting or postural muscles generally show positive allometric growth of their slow-contracting myofibers.

Longitudinal Growth

Longitudinal growth of myofibers occurs by the formation of new sarcomeres at the ends of the myofibrils where they attach to the inner surface of the plasma membrane. Myotendon junctions (between myofibers and collagen fibers) have numerous invaginations allowing myofibrils to attach laterally to the plasma membrane. Tension generated by a contracting myofibril is transferred laterally through the Z line, desmin, and vinculin to the myofiber beyond. This lateral transfer of tension is of fundamental importance because it allows new filaments to be added parallel to those already formed. If it were not for this lateral transfer, incorporation of new filaments in series with those already formed would be extremely difficult, like attempting to add an extra link to a chain already under tension. Thus, the length of myofibrils can be increased by the lateral addition of new sarcomeres without the end of the myofibril detaching from the plasma membrane.

With the progressive formation of new sarcomeres, the older ones are behind the new ones. The process of adding a new sarcomere might take as little as 20 min in a rapidly growing muscle. This activity matches the high level of polyribosomal RNA at the ends of myofibers, which often are more basophilic than the remainder of the myofibers.

The arrangement of myofibers in a muscle determines where increments from longitudinal myofiber growth are added. In the longissimus dorsi, for example, myofibers are at approximately 45° to the vertebral axis and longitudinal myofiber growth contributes to the depth of the longissimus dorsi. Longissimus dorsi width, however, is the product of apparent myofiber number multiplied by the radial growth of myofibers.

Changes in Myofiber Numbers

Most, if not all, myofibers are formed prenatally. Postnatal muscle growth is accomplished solely by radial and longitudinal hypertrophy to give thicker and longer myofibers, respectively. However, myofiber numbers in a standardized plane half way along a muscle might sometimes appear to increase postnatally. This is most likely caused by the longitudinal growth of intrafascicularly terminating myofibers (tapered myofibers not reaching the end of their muscle). Toward the end of commercial growth in cattle, some muscles show a reverse effect, with considerable reductions in apparent myofiber numbers. Thus, when the number of myofibers at a particular point along the length of a muscle are counted, only those running through this point can be counted. Many other myofibers not reaching the point (i.e., the tapered, intrafascicularly terminating myofibers) are missed. Fast growth of tapered myofibers might increase the apparent number of myofibers, whereas slow growth of tapered myofibers might decrease the apparent number of myofibers.

Endocrinology

Somatotropic hormone (STH) added to myoblasts *in vitro* has little effect. But STH stimulates liver cell mitosis. If myoblasts are cultured together with liver cells, myoblasts then respond to STH. Thus it was discovered that STH activates mitosis by causing the liver to produce a polypeptide hormone, known for a while as somatomedin but now called insulin-like growth factor (IGF). IGF has two forms (IGF-1 and IGF-2), and each form has a specific cell surface receptor. Acting in the opposite direction to IGF is transforming growth factor-β. This inhibits differentiation of myoblasts and their subsequent fusion to form multinucleated myotubes. Fibroblast growth factor is a heparin-binding polypeptide. It stimulates proliferation of premyoblasts and inhibits their differentiation to become myoblasts.

Amino acid uptake by myofibers is enhanced when myofibers contract or are stretched. Cortisol blocks amino acid incorporation into myofibrillar proteins. When muscles are growing: (1) STH and insulin facilitate movement of amino acids to the polyribosomes assembling muscle proteins, (2) steroid hormones enhance messenger RNA activity, and (3) thyroid hormones facilitate DNA accumulation and formation of RNA. Stimulation of the immune system, however, can cause the release of monokines and reduce muscle growth.

Further Reading

Bismuth, K., Relaix, F., 2010. Genetic regulation of skeletal muscle development. Experimental Cell Research 316, 3081–3086.

Buonanno, A., Fields, R.D., 1999. Gene regulation by patterned electrical activity during neural and skeletal muscle development. Current Opinion in Neurobiology 9, 110–120.

Swatland, H.J., 1994. Structure and Development of Meat Animals and Poultry. Lancaster, PA: Technomic Publishing.

Relevant Website

http://www.aps.uoguelph.ca/~swatland/gasman.html
University of Guelph.

Physiology

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Glossary

Allometric growth Growth of a portion of an organism relative to or in comparison with growth of the whole organism.
Basal lamina The membrane that surrounds a cell membrane and defines the outer boundary of a cell.
de novo An expression meaning 'anew.'
Glycogen The storage form of carbohydrate, a polysaccharide consisting of branched-chain connected glucose molecules.
Hypophysectomy Removal of the pituitary gland.
Metabolism Chemical reactions or changes in living cells by which energy is provided.
Postprandial Following a meal.
Thyroidectomy Removal of the thyroid gland.

Introduction

The physiology of growth of meat animals has been actively studied for more than a century, and new, more detailed information is gathered each year as new methods are developed to study the physiological processes regulating the muscle, bone, and adipose tissue growth. Although nearly all aspects of physiology are important to growth, the focus of this article is on endocrine (hormonal) influences on growth. When nutrition is adequate and other physiological functions are normal, endocrine influences on skeletal muscle, adipose tissue, and bone are critical determinants of rate of growth and composition of the entire meat animal. These three tissues plus the supportive connective tissue (tendons, ligaments, and cartilage) comprise the 'carcass' derived at harvest, from which meat and meat products are produced. Growth of the skeletal muscle, adipose tissue, and bone is of primary importance because these tissues represent approximately 46–54%, 25–31%, and 12–15%, respectively, of the total weight of meat animal carcasses at typical market weights.

The goal of this article is to describe the important aspects of the physiology of and hormonal influence on the skeletal muscle, adipose tissue, and bone growth and to discuss briefly how nutritional modulation of growth is mediated through the endocrine/growth factor axis. The nervous system also plays an important role in hormonal regulation of growth, and an explanation of these interactions will be presented. Emphasis is placed on endocrine regulation of growth and development after birth of the animal. The number of muscle cells (muscle fibers) is 'set' at birth of meat animals, so the growth of muscle occurs primarily through an increase in cell size (length and radial area). The growth of adipose tissue, however, reflects the summation of effects of increasing cell number and increasing cell size after birth. In normal adipose tissue growth, increases in cell size contribute more than increases in cell number. Growth of the long bones in the skeleton involves a complex pattern of cell division, synthesis of cartilage-like material, and remodeling of that material and replacement with more calcified matrix to increase length, width, and thickness. Focus on the growth phase following birth is also justified because this is when functional hormone-receptor populations increase rapidly, and maturation of the gonads during adolescence adds new hormonal influences coinciding with the onset of puberty and influence patterns of tissue growth.

Growth Physiology Concepts

The physiology of growth of meat animals involves a complex of hormone and growth factor influences on the metabolic processes in cells responsible for growth of those tissues. These influences are regulated by the rate of synthesis and secretion of hormones and growth factors; the number of receptors present in a tissue; and the age, gender, genotype, and nutritional status of animals. Some hormones act quickly and stay in the circulating bloodstream for short times (short half-life); others act over longer periods, largely because they stay in the blood for a longer time (long half-life). Although hormone action is similar in most animals, subtle variations in the hormonal control of growth and metabolism are found among different mammalian and avian species. The concepts of a chemical messenger initiating a response in a cell and the use of the term 'hormone' to describe that messenger were stated in 1905 by Starling. The concept of activation through a receptor or in a cell was first put forward in 1906 by Langley. Today, the complexity of hormonal interactions across tissues and the means by which hormones coordinate and exert their influences on growth is still unraveling.

The major endocrine gland secretions that provide chronic positive influences on growth include insulin, thyroid hormone, somatotropin (ST, growth hormone), the female hormones known as estrogens, the male hormones known as androgens, and the glucocorticoids. Several other hormones are involved in the regulation of synthesis and secretion of these 'growth-supporting' hormones, the regulation of energy or mineral balance, and the sensitivity of cells to metabolic hormones or growth factors. For example, the glucocorticoids produced by the middle layer of the adrenal gland are critical in modulating response to stress (i.e., heat and cold stress) and increasing the sensitivity of cells to other hormones. The catecholamine adrenaline (epinephrine) is produced by the inner part of the adrenal gland, the medulla, and regulates the heart

rate and blood flow in an acute fashion (in seconds). However, when transient nutrient intake deficiencies occur, adrenaline stimulates the release of glucose from stores of glycogen in muscle and the liver or stimulates the release of fatty acids from stored lipid in adipose cells to provide a source of energy to support essential or basal metabolism and highest-priority growth processes.

Age effects

The relative growth rates of the skeletal muscle, adipose tissue, and bone change with time in the growing animal, resulting in changes in the percentage of live weight represented by each tissue from birth to market weight or mature size. This is called allometric growth, and it results from changes in the priorities for use of nutrients by these tissues that occur from birth to market weight or maturity. When the plane of nutrition is high (adequate or excess amounts of nutrients being consumed), rates of bone and skeletal muscle growth are relatively high at birth and slowly decline toward achievement of sexual maturity or market weight. In contrast, adipose tissue growth is very slow for several weeks after birth, but accelerates at an increasing rate with increasing age and body weight, achieving exponential rates of growth as the animal approaches or exceeds normal market weight. Although muscle cell number (fiber number) is already at maximum by birth in meat animals, adipose cell number increases manyfold between birth and market weight or mature size through differentiation (or recruitment) of new or dormant cells. Accordingly, the capacity for adipose tissue growth from birth to mature size is greater than that of muscle, but we do not yet know which hormones and growth factors determine how many fat (adipose) cells an animal will have during the different phases of growth. Excess levels of fuel energy consumption increased the rate of lipid synthesis and storage in adipose cells. Insulin is the major hormone that is facilitating these processes.

Gender effects

Gender influences involve maturation of the gonads during adolescence, which leads to increased synthesis and secretion of the primary female hormone estradiol, and the primary male hormone testosterone. Androgen secretion is high in the fetus, declines after birth, and then increases dramatically at puberty, further complicating the dynamic patterns of muscle growth that are also influenced by other hormones. This coordinated action of hormones creates age-related, gender-specific differences in growth patterns of the bone, skeletal muscle, and adipose tissue that result in carcass composition differences. Carcasses from intact males contain larger muscles, a higher percentage of muscle and lower percentage of fat than those from females, and carcasses from castrated males tend to fall between the two. One exception to this general rule is that castrated male swine (barrows) contain more fat on average than females (gilts) at the same age or weight. Gender influences also determine differences in mature size. Estrogenic hormones are known to initiate slowing and cessation of bone growth, leading to smaller skeletal stature and smaller mature size and weight of females as compared with intact or castrated males. The physiology of bone growth in cattle is interesting because castration of intact males (bulls) to produce steers allows continued bone growth to occur, and steers over 2 or 3 years of age are significantly larger and will achieve a heavier mature weight than bulls or normal females. Castration of males in all meat animals causes lower testosterone concentrations in the blood during growth, and this leads to fatter pigs, cattle, and lambs when compared with their intact male counterparts.

Genetic effects

Experimental work has demonstrated that meat animals can be selected for increased mature size, greater muscularity, and lesser amounts of carcass fat at a similar weight. Increased mature size usually results from selection for greater growth rates and leaner animals at a typical harvest weight. Animals can also be selected for increased muscularity and decreased fat, independently or in combination, at a given weight. This selection process causes a reduction in the percentage of energy consumed being converted to stored fat, and these animals are considered less physiologically mature than the unselected cohorts. They will grow to achieve the same percentage of fat in the body or carcass, but that is achieved at higher body weights. Environmental conditions such as heat and cold, nutrient availability, and the desired carcass or meat composition all provide the rationale for selection criteria used in the production of meat animals. The genetic potential for growth determines the animal's nutrient requirements for maximum growth rate.

Growth is influenced by a multitude of genes, and an interesting one is the myostatin gene. Myostatin controls muscle fiber formation during fetal muscle growth. The timing and extent of increased myostatin present during fetal development determines when muscle fiber formation stops. A condition exists in a few breeds of cattle, called the 'double-muscled' condition, in which muscles are much larger (up to 40–50%) than the same muscles in most other breeds or compared with normal animals of the same breed. Most muscles in 'double-muscled' cattle contain up to 40% more muscle fibers before birth and beyond. The application of molecular techniques has demonstrated that the 'double-muscled' phenomenon is caused by small changes in the deoxyribose nucleic acid (DNA) base-pair sequence that lead to functional changes in the gene that produces myostatin. These changes lead to a reduced production of myostatin during fetal development, and this leads to greater rates of muscle fiber formation and higher muscle fiber number. As the muscle fibers are not larger, a greater muscle fiber number is responsible for faster muscle growth after birth leading to larger muscles.

Metabolic effects

One hormone known to be critically important in partitioning of nutrient use for growth among the main tissues is the growth hormone or ST. ST stimulates bone and skeletal muscle growth, but it reduces or inhibits adipose tissue growth. Rates of synthesis and secretion of ST decline from birth to market weight, in parallel with decreasing rates of muscle and bone growth and accelerating rates of adipose tissue growth. Circulating concentrations of ST also respond to changes in the level of energy and protein nutrition, altering the sensitivity of cells to insulin to help direct use of nutrients in an appropriate fashion. Elevation of ST in the circulation redirects nutrients toward increased muscle and bone growth and decreased

Table 1 Effects of somatotropin (growth hormone) on animal tissues, hormone regulation, and metabolism in growing animals

Tissue	*Physiological effect*
Adipose tissue	Decreased sensitivity to insulin and decreased glucose uptake
	Decreased lipid synthesis
	Decrease in adipose tissue mass (all depots)
	Decreased glucose oxidation
	Increased lipolysis if animal is in negative energy balance
Skeletal muscle	Increased protein synthesis
	Increased protein accumulation in muscle
	Increased muscle growth rate and muscle mass
Bone	Increased synthesis of bone matrix
	Increased bone growth rate
Liver	Increase in liver growth rate and liver mass
	Increased glucose output
	Decreased ability of insulin to inhibit gluconeogenesis
Intestine	Increased absorption of calcium and phosphorus absorption
	No change in intestine weight
Metabolic indices	Decreased circulating concentration of blood urea nitrogen
	Decreased amino acid oxidation
	Decreased glucose clearance from blood
	Decreased glucose oxidation
	Increased synthesis and secretion of insulin-like growth factor-1 (IGF-1)
	Increased circulating concentration of IGF-1-binding protein (IGFBP-3)

adipose tissue growth. A summary of the effects on metabolic activities in various tissues and on overall metabolism is presented in Table 1. Although the relative rates of bone and muscle growth decline as mature size is approached, if energy intake exceeds requirements for maintenance and growth of muscle, bone and other tissues, adipose tissue growth is stimulated and both the relative and absolute rates of fat deposition are increased at a stage of growth where muscle and bone growth rates are declining. This leads to obesity and less efficient use of feed for growth of meat animals. Bone growth is more complex in that it involves several different cell types and synthesis of cartilage that is later replaced by hard or 'compact' bone. Several hormones control this process of continued formation of new cells and replacement of older cells throughout growth. Changes in cell number and size of these cell types cannot be used to characterize bone growth in the manner that they are used to describe muscle and adipose tissue growth.

Physiology of Skeletal Muscle Growth

Hormonal control of skeletal muscle growth after birth involves two primary targets of control: (1) rates of proliferation and incorporation of satellite cells into existing muscle fibers and (2) independent or concurrent changes in rates of muscle protein synthesis and degradation. Muscle fibers are long tubular-shaped cells that contain many nuclei, the total number of which depends primarily on muscle fiber length and radial area. Each nucleus in a muscle fiber provides information for the synthesis and maintenance of a specific amount of cytoplasm, primarily muscle proteins, in the cell. Growth of the multinucleated muscle fibers is characterized by a several-fold increase in their length, the number of myonuclei (total DNA) and total ribonucleic acid (RNA) and protein present. As the number of fibers in each muscle remains constant, increases in each of these components leads to a proportional increase in the total protein content and weight of the muscle. The number of fibers in each muscle does not increase by hyperplasia after birth in meat animals and humans, but the total number of nuclei, total amount of protein, and the total muscle weight do. Muscle fiber nuclei are the site of transcription of the DNA that defines the specific messenger RNAs that are translated into individual proteins. Skeletal muscle growth is regulated by activation of transcription in the nucleus. ST, testosterone, and estradiol increase the rate of muscle protein synthesis, and this is initiated through hormone-receptor binding and a cascade of intracellular signaling events that lead to increased protein synthesis. These signal-transduction elements will not be discussed here.

As myonuclei cannot divide, the source of the multitude of nuclei that accumulate in muscle fibers during growth after birth is attributed to the 'satellite cell' population. These cells reside between the sarcolemma (plasma membrane) of each muscle fiber and the basal lamina that surrounds each fiber. Muscle growth by hypertrophy should not be confused with growth by hyperplasia. The strict definition of hyperplasia is the increase in muscle fiber number that occurs with terminal differentiation and fusion of myoblasts to form myotubes (immature muscle fibers) during embryonic and fetal muscle growth. The regulation of myoblast proliferation, differentiation, and formation of muscle fibers is a complex process called myogenesis, and it will not be discussed here. Anabolic hormones, such as testosterone and ST, do not directly stimulate myoblast or satellite cell proliferation and fusion and the stimulatory effects probably result from the production of insulin-like growth factor-1 (IGF-1) and other growth factors. IGF-1 is produced in the liver and enters the general circulation of the blood, providing availability to skeletal muscle and other tissues, but it is also produced by skeletal muscle cells. Whether effects are mediated via an endocrine influence through the circulation or whether effects are mediated locally via paracrine influence is not certain and is under current investigation.

Farm animal muscle growth exhibits a positive association between increasing total DNA mass, total RNA mass, and total weight or protein content of individual skeletal muscles during growth, without any change in muscle fiber number. This is true for normal postnatal growth in pigs, sheep and cattle, and most other mammalian species as well. A strong association also exists between muscle weight, DNA mass, and RNA mass when nutritional restriction is imposed to slow muscle growth. Likewise, the lesser muscle size and weight observed when small birth weights are compared with heavy ones during early postnatal growth, and when surgical removal of an endocrine gland or the source of the hormone is removed (i.e., hypophysectomy or thyroidectomy), and when hormone

administration is used to stimulate skeletal muscle growth. Characterizing changes in endocrine status that occur with changes in rates of DNA, RNA, and protein accretion during growth and nutritional manipulation provide useful information for determining how changes in nutritional status mediate control of skeletal muscle growth.

Effects of Somatotropin on Muscle Growth

Insufficient amounts of ST synthesis and secretion from birth to maturity slow muscle and bone growth, and replacement therapy can restore normal growth rates. Circulating concentrations of ST vary according to the episodic pattern of secretion, a phenomenon that was not known to be present until the late 1960s and early 1970s. The effects of ST on the animal must be determined by frequent sampling and integration of concentration changes over time. Circulating ST concentrations exhibit positive correlations with carcass muscle and total RNA in the muscle of growing steers, but too many factors influence ST status to make this a reliable index for muscle growth potential.

Daily administration of ST allows a growing animal to achieve its genetic potential for muscle growth. Dose-dependent increases in absolute mass and percentage of muscle in carcasses of pigs, lambs, and cattle provide compelling evidence for the importance of the influence of ST on skeletal muscle growth. Relative increases in total muscle mass at the same live weight range from 28% with a low dose of 50 μg per kg live weight to 38% with a high dose of 200 μg porcine ST per kg live weight in animals fed an adequate diet. Smaller responses are observed if nutritional adequacy is not provided. Results from recent studies demonstrated a 27% increase in the composition of skeletal muscles in the half-carcass of 360 kg beef steers treated for 157 days with 160 mg bovine ST per week. Variation in growth response between muscles is quite small in pigs. Percentage increases of individual muscle weights were dose dependent but were similar across anatomical locations at the same dose. The chronic protein anabolic effect of ST on skeletal muscle growth appears to be achieved exclusively through enhancement of protein synthesis, with little or no difference in protein degradation rate.

The demonstrated effects of ST on protein accretion and metabolism in skeletal muscle appear to involve the IGF system. The degree to which increased circulating concentrations or locally produced IGF-1, IGF-2, and the IGF-binding proteins (IGFBPs) are involved is still in question. Administration of ST to growing pigs and ruminants beyond the first several weeks of age markedly increases plasma concentrations of IGF-1, IGF-2, and the major binding protein, IGFBP-3. IGF-1 stimulates protein synthesis and reduces protein degradation in muscle cell cultures, and circulating IGF-1 concentrations are positively correlated with protein gain in growing steers.

It is presumed that the major source of circulating IGFs and IGFBPs is the liver, as ST stimulates transcription of IGF-1, IGFBP-3, and the acid-labile subunit of the complex in the liver. Skeletal muscle and other tissues may also be a significant source, however. Increased levels of dietary protein intake in pigs and growing steers increase muscle IGF-1 messenger RNA (mRNA) abundance and whole-animal protein gain.

Relationships between growth rates, circulating concentrations of endogenous metabolic hormones, IGF-1, and IGF-1 mRNA concentrations in skeletal muscle and liver were compared in growing bulls, steers, and heifers. Bulls had higher mean ST and ST peak amplitude than heifers, whereas values for steers were intermediate. Bulls also had 1.6-fold and 3.0-fold greater liver IGF-1 mRNA concentrations than steers and heifers, respectively, whereas steers had 1.8-fold greater IGF-1 mRNA concentrations than heifers. Bulls had 1.3-fold higher plasma concentrations of IGF-1 than steers, and values for steers were 1.8-fold higher than those for heifers. Liver IGF-1 mRNA concentrations, liver slice IGF-1 production, and plasma IGF-1 concentrations were all significantly correlated with average daily gain and mean ST peak amplitude, but not with ST baseline concentrations, peak frequency, or plasma thyroid hormone (thyroxine (T_4) and triiodothyronine (T_3)) concentrations. These results suggest a relationship between sex steroid status and an endocrine influence of IGF-1 in growing cattle, but more definitive information is needed.

Effects of Estrogenic Steroids on Skeletal Muscle Growth

A recent study provides additional information regarding independent and combined estrogenic steroid and ST effects on composition of gain, skeletal muscle growth, and other growth responses. Daily administration of bovine ST (bST) alone and in combination with administration of estradiol-17β via the commercial estrogenic steroid implant Synovex (Syntex Animal Health, West Des Moines, IA, USA) increased average daily gain by 16% and 28%, respectively, without significant effect on feed intake, over a 56-day treatment period. Weights and rates of weight gain were determined for skeletal muscles taken from various parts of the carcass. The combination of Synovex plus bST resulted in additive effects on carcass protein gain, individual muscle weight gain, and muscle fiber size in the rectus femoris, triceps brachii and semitendinosus muscles. Although it is known that administration of estradiol increases ST secretion rate and plasma concentrations of ST, results from this experiment and others clearly show that estrogenic treatment and bST treatment exhibit additive effects on skeletal muscle growth.

Chronic estradiol alone and bST administration alone increase circulating insulin concentrations in growing cattle. The suggestion that increased insulin secretion is part of the pathway for the estrogenic or bST response is uncertain. Chronic insulin administration in young, rapidly growing pigs has no effect on growth rate, growth efficiency, and muscle or adipose tissue weights. Insulin serves an important role as a homeostatic (over acute time intervals) regulator of nutrient metabolism, and chronic alterations in nutrient partitioning are mediated by changing tissue sensitivity to insulin.

Effects of Androgenic Steroids on Skeletal Muscle Growth

Entire males in most species grow faster and contain greater absolute mass of skeletal muscle and less fat than castrated males. These differences are attributed to the presence of greater concentrations of testosterone in circulation. Intact males exhibit greater rates of protein gain at equal feed intakes.

Episodic testosterone administration (9 mg day^{-1}) in wether lambs increased nitrogen balance by 2.9 g day^{-1} (96%) during three successive 5-day treatment periods. Rates of muscle protein synthesis were not different between treatment and pre- and posttreatment periods.

Testosterone is known to invoke biphasic muscle growth patterns in neck muscles of intact male cattle and sheep, whereas most other muscles or muscle groups exhibit only a single-phase or linear growth pattern. It is during the latter part of the growth period to market weight (approximately 4–9 months of age in sheep and 12–18 months of age in cattle) that this divergence is noted. It is difficult to conclude just how testosterone influences muscle growth, but cautious speculation would include the possibility that the protein anabolic effect is expressed through decreased protein degradation. Similar conclusions were drawn from studies with a synthetic androgen, trenbolone acetate, administered in cattle.

Effects of Insulin on Skeletal Muscle Growth

Insulin is the metabolic hormone most responsible for facilitating nutrient uptake by tissues, organs, or cells, and thus plays a critical essential role in protein synthesis and muscle growth as well as lipid synthesis and adipose tissue growth. As mentioned earlier in the Section Growth Physiology Concepts, insulin is considered an acute-acting metabolic hormone and as such it exhibits concentration changes in the blood that follow feed ingestion patterns. After feed is consumed and digested, nutrients are absorbed into the bloodstream and insulin release from the pancreas is triggered. Synthesis of skeletal muscle proteins is sensitive to this cyclicity of nutrient intake and insulin concentration changes. Insulin stimulates glucose and amino acid uptake by muscle, and it has been shown to stimulate protein synthesis and decrease protein degradation in muscle in short-term *in-vitro* and *in-vivo* experiments. When compared with nonruminants, ruminants exhibit slower rates of insulin secretion, smaller postprandial changes in plasma concentrations, and less effectiveness in glucose removal from the blood because the volatile fatty acids (acetate, propionate, and butyrate) are the primary substrates of energy metabolism.

Physiology of Adipose Tissue Growth

Adipose Tissue Form and Function

Adipose tissue is sometimes referred to simply as fat. One of its major functions is to store energy for the body through the synthesis of lipid and its accumulation in adipocytes (fat cells). When feed intake is restricted, adipose tissue undergoes a process called lipolysis. Lipolysis is the separation of fatty acids from the stored form of fat (triglycerides), and release of the fatty acids into the blood so that they can be used by tissues and organs as a source of energy. These fatty acids are commonly called 'free fatty acids' or 'nonesterified fatty acids'. Adipose tissue is a specialized type of connective tissue found in most animals to accumulate around visceral organs, just under the skin, between skeletal muscles, and, to a small degree, within skeletal muscles. These locations where fat deposits appear are called adipose depots or fat depots. They begin as areas of loose connective tissue containing many very small blood vessels and fibroblast-like cells, where differentiation of adipocytes (fat cells) occurs and leads to the processes of synthesis of fatty acids and accumulation of lipid droplets in the cells. Fat cells are present before birth, but many new fat cells are formed during growth to puberty and beyond. Both the number of fat cells and the size of these spherical cells determine the size of a fat depot or the total amount of fat in the carcass of meat animals. Some animals, such as pigs, contain only 2–3% fat at birth and may have as much as 25–30% fat at market weight. As is the case with muscle growth, the rate of fat accumulation and ultimate fatness of meat animals is also influenced by age, gender, genotype, and nutrition.

Age effects

The composition of adipose tissue changes with age. The percentage of water and protein decline and the percentage of lipid increases with increasing age. Growth of adipose tissue with age includes hypertrophy of existing cells through lipogenesis (the metabolic processes involved in the accumulation of lipid) and hyperplasia, the formation of new cells. As long as the nutritional status provides excess energy intake, both processes will occur, leading to increasing fatness. A wide range of fat cell size exists during all phases of growth, but the number of largest cells is greater at market weight. The ability to continue fat accumulation explains why older animals that are 'overfed' become obese. There are species differences with regard to the biochemical pathways of fatty acid formation, and these differences explain the variation in fatty acid composition of adipose tissue between species.

Genetic effects

Different breeds or strains within a species of meat animal vary in their rate of fat deposition at typical market weights. Breed differences also exist in the proportion of fat stored in the different depots in the body. Dairy breeds of cattle contain far less subcutaneous fat (the depot under the skin) and more fat between muscles and in the visceral region than beef breeds. Genetic selection for decreased thickness of subcutaneous fat has been used very successfully for decades to reduce carcass fat in meat animals. This type of genetic selection results in fewer cells and smaller average cell volume at a given live weight.

Gender effects

Gender differences in fatness of meat animals are similar across species. In ruminants, intact males contain the least fat, females generally contain the most, and castrated males fall in between. Boars (intact males) contain less fat than barrows (castrates) or gilts (females) in swine, but barrows are usually fatter than gilts. These differences become most pronounced at puberty and beyond when testosterone in males and estrogens in females exert strong influences on growth and metabolism. Testosterone reduces fat synthesis and deposition, whereas estradiol enhances fat synthesis and deposition in female cattle and sheep. Gender differences result

in female ruminants being marketed at lighter weights than castrated males.

Nutritional effects

Following ingestion and digestion of a meal, insulin secretion into the blood is stimulated, leading to activation of receptors on cell membranes. Insulin stimulates or accommodates uptake of fatty acids and glucose by adipose cells. Glucose and fatty acids (predominantly acetate in ruminants) are the substrates for *de-novo* synthesis of fatty acids and triglycerides, respectively. Triglycerides are the stored form of fat in adipose cells. The greater the excess of nutrients absorbed following a meal, the greater the rate and extent of triglyceride synthesis and fat storage. To counter fattening of dairy heifers, which impairs milk production later, and breeding animals of other species, restricted feed intake is used to control fat content of these animals.

Hormonal effects

ST is known to have direct effects on lipogenesis and the rate of lipid accumulation in fat cells (adipocytes) in meat animals. This hormone blunts the sensitivity of fat cells, but not of skeletal muscle cells, to insulin. Hence, adipocytes are directed by ST to reduce uptake of glucose and fatty acids and reduce formation of triglyceride for storage. This is a potent and dynamic effect that depends closely on the amount of ST in circulation. The effects of ST are consistent in all genders and breeds of meat animals. The gender effects of testosterone and estradiol are still present, and are generally additive to the effects of ST.

Special conditions, such as onset of lactation and acute shortage of feed intake, will reverse the lipid accumulation processes through reduced synthesis and secretion of insulin and increased synthesis or action of adrenaline to stimulate lipolysis. This leads to release of fatty acids from the triglyceride molecules in fat cells and can lead to overall weight loss if sustained over a significant period.

These examples show how sophisticated monitoring of nutritional status and changes in nutrient demands are coordinated through actions of the endocrine system to accommodate the highest-priority physiological function present at any given stage or phase of growth.

Intervention strategies

Research into the complexity of growth processes led to the discovery of compounds that can be fed safely to growing meat animals to improve carcass composition and feed efficiency. The United States Food and Drug Administration, Center for Veterinary Medicine approved ractopamine, a synthetic beta-agonist compound, for use as a feed additive for growing-finishing swine (Paylean), cattle (Optaflexx), and turkeys (Topmax). More recently, zilpaterol (Zilmax) was also approved by the FDA as a feed additive to be fed the last 20 days before harvest of beef cattle. These orally active compounds fed during the latter part of the finishing period enhance skeletal muscle growth and reduce the relative amount of fat in the carcass, without altering quality characteristics of meat.

Physiology of Bone Growth

Bone Form and Function

Bone formation occurs through transformation of connective tissue during fetal development and growth following birth to the time when mature size is achieved. Five or six distinct cell types are involved in this intricate process. The complex series of events involving these cells determine not only growth rates but also the strength of the bones as well. Complexity is also added by the presence of three different types of bones throughout the body, and growth processes are slightly different for each type. The discussion presented here focuses on long bones, those responsible for locomotion and support of the animal, which determine the ultimate stature or height and mature size.

Long-bone growth centers on activities in the growth plate, or epiphysis, located in the enlarged end or ends of long bones. When activity here ceases, growth stops. This is sometimes referred to as epiphyseal 'plate closure,' the benchmark for cessation of long-bone growth and achievement of mature size. Once plate closure occurs, long bones grow only in diameter and thickness. Again, as with growth of muscle and adipose tissue, bone growth is influenced by age, gender, genetics, and nutrition. In contrast to the importance of nutrition for muscle and adipose tissue growth, nutrition has the least impact on bone growth, primarily because most normal diets for meat animals provide adequate nutrients to sustain bone growth.

Age and gender effects

Bone is the most mature tissue at birth in comparison with muscle and adipose tissue. A higher priority exists for viable bone form and function to assure survival at birth than for adipose tissue growth. Accordingly, bone represents a higher percentage of total weight at birth, and a declining percentage as the animal grows to maturity, whereas the pattern of adipose tissue growth is opposite. Significant age effects are associated with the onset of puberty. In meat animals, the estrogenic influence is responsible for earlier reduction of activity of the cells in the growth plate and ultimate conversion of this to compact bone, as compared with intact or castrated males. The females of most mammalian species are of smaller stature and lighter weight at mature size than intact males. Castrated males represent an interesting contrast to intact males or females. These animals exhibit delayed plate closure and longer times of bone growth, presumably related to lower testosterone production, than either females or intact males.

Endocrine and nutritional effects

ST exerts positive stimulatory effects on long-bone growth. Stimulation of cell division in the growth plate may involve local production of IGF-1 as a critical influence. ST stimulates bone growth in all genders and all common genotypes. Other hormones are associated with mineral balance and cartilage and bone matrix formation. A very high concentration of calcium salts is present in bone and endocrine influences on mineral absorption and incorporation into matrix material are essential for normal growth and maturation. Calcitonin, secreted by the thyroid gland, and parathyroid hormone,

secreted by the parathyroid gland, are key regulators of calcium metabolism in bone and other tissues.

Vitamin and trace mineral deficiencies can result in abnormal bone growth. Most meat animal diets contain supplements to ensure adequate availability of minerals for normal bone growth. Amounts of nutrients contributed from each individual feed source must be known for the determination of appropriate levels of supplementation for each species. Feed formulations must accommodate a balance of nutrient availability that meets the needs of bone, muscle, organs, and other metabolic tissues. Stage of growth, gender, and genotype contribute to the variation in nutrient requirements of meat animals.

Conclusion

The physiology of growth in meat animals is an orchestrated process characterized by the sophisticated coordination of cell proliferation (hyperplasia) and increase in cell size (hypertrophy) in organs, skeletal muscle, bone, and adipose tissue from birth to market weight or mature size. Total cell number is a primary determinant of skeletal muscle size and propensity to accumulate fat. Although the total number of skeletal muscle fibers present in each muscle is achieved by birth in meat animals, the number of cells in adipose tissue depots exhibits several-fold increase during postnatal growth. Organ, bone, and skeletal muscle growth exhibit a higher priority for use of absorbed nutrients than adipose tissue because they support survival functions and mobility. Adipose tissue growth results from excess energy produced from feed consumed in amounts greater than that required to support the genetic potential for organ, muscle, and bone growth. Metabolic hormones and sex hormones control metabolic pathways affecting the cellular processes that lead to cell growth and maturation (protein synthesis and degradation in skeletal muscle and lipogenesis and lipolysis in adipose tissue). Genetic blueprints exist in each animal for control of gene expression in each tissue or organ that culminate in phenotypic characteristics defining complex traits such as muscle fiber number and size, long-bone growth and mature size, and propensity to deposit fat at differential rates among the four major fat depots. These growth patterns determine the overall body growth rate, feed efficiency, and body composition. Comparisons of factors that influence growth must be made under carefully controlled conditions and at constant live weights (to remove confounding effects of allometric growth) if we are to understand the mechanisms that create differences and their impact on the relative amounts of muscle, fat, and bone that exist at any point during the various phases of growth. Animal well-being, product quality characteristics, environmental impacts, and economics ultimately determine which management strategies are used in meat animal production.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. Growth of Meat Animals: Adipose Tissue Development; Endocrinology; Growth Patterns; Metabolic Modifiers; Muscle. Nutrition of Meat Animals: Ruminants

Further Reading

Beermann, D.H., DeVol, D.L., 1991. Effects of somatotropin, somatotropin releasing hormone, and somatostatin on growth. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 373–426.

Dayton, W.R., Hathaway, M.R., 1991. Control of growth by glucocorticoids, thyroid hormones, autocrine and paracrine growth factors. In: Pearson, A.M., Dutson, T. R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 17–45.

Dodson, M.V., McFarland, D.C., Grant, A.L., Doumit, M.E., Velleman, S.G., 1996. Invited review: Extrinsic regulation of domestic animal-derived satellite cells. Domestic Animal Endocrinology 13, 107–126.

Etherton, T.D., Bauman, D.E., 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiological Reviews 78 (3), 745–761.

Gerrard, D.E., Grant, A.L. (Eds.), 2003. Animal Growth and Development. Dubuque, IA: Kendall/Hunt.

Hancock, D.L., Wagner, J.F., Anderson, D.B., 1991. Effects of estrogens and androgens on animal growth. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 255–297.

Mersmann, H.J., 1991. Regulation of adipose tissue metabolism and accretion in mammals raised for meat production. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 135–168.

Purchas, R.W., 1991. Effect of sex and castration on growth and composition. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 203–254.

Scanes, C.G., 2003. Hormones and growth. In: Scanes, C.G. (Ed.), Biology and Growth of Domestic Animals. Ames, IA: Iowa State Press, pp. 59–111.

H

HAM PRODUCTION

Contents

Cooked Ham

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Glossary

Delta-T Delta-T (ΔT) cooking means that the oven temperature rises to remain constant at a temperature above that of the food core (monitored by a core probe). The constant difference between the core temperature and cooking chamber is user-definable. The food temperature increases gradually until it reaches the end-of-cooking temperature set on the probe.

Tenderization Tenderization is a mechanical process used in the manufacture of cooked ham, which produces cuts in the muscle that break the structure of the connective tissue and puncture the brine collection areas between muscles, facilitating brine distribution and increasing the extraction surface. It therefore eliminates the necessity of thorough trimming, and reduces the effect of retraction of the connective tissues during cooking.

Tumbling Is a fall massage where meat pieces are lifted by baffles to the upper part of the machine and then fall onto the meat mass below, producing an intense mechanical action that softens the meat. This type of massage results in considerable cellullar breakage and a high extraction of proteins.

Introduction

Cooked ham is the complete ham, or part of the ham, cooked at a temperature high enough to coagulate the meat proteins, ensuring that, once packed, the product remains unchanged in normal storage conditions at refrigerated temperature.

The most important criteria to bear in mind for cooked ham production are: selection of raw material, trimming, brine composition and injection rate, tenderization, type and duration of mechanical treatment, stuffing or placing in molds, the cooking process, chilling, and preparation for marketing.

Selection and Preparation of the Raw Material

Raw material selection is of paramount importance for products of optimal quality. It is important to follow good manufacturing practices during slaughtering and cutting to avoid high bacterial counts, which decrease shelf-life and can produce color defects. It is also important to select hams with a good water-holding capacity (WHC). Hams with a pH less than 6.0 at 45 min postmortem will ultimately lead to high moisture loss, paler color, binding problems, and softer texture, and it is advisable to avoid this in the production of high-quality cooked hams. The optimal pH at 24 h postmortem for cooked ham

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production is between 5.8 and 6.2. Pale, soft, and exudative (PSE) meat has a lower WHC, whereas meat with pH > 6.2 is termed dark, firm, and dry (DFD). Even though DFD meat has a higher WHC, it is more prone to bacterial deterioration, and reduces nitrite to nitric oxide more slowly, which can produce discoloration problems, especially when nitrite content is reduced or ascorbate/erythorbate is not added. The selected pork legs should be chilled to an internal temperature between 2 and 4 °C, and classified according to weight.

Traditionally, hams were prepared with bones included, but nowadays most hams are boned. This operation is done manually due to the lack of appropriate machinery. Sometimes the original muscle structure of the ham is retained, but in order to trim out the intermuscular fat and connective tissue surrounding the muscles, hams are often separated into several pieces that can reproduce the entire ham if they are put together before cooking. Subcutaneous fat and rind are sometimes left on to give a more traditional appearance. A piece of cured rind with a standardized shape and subcutaneous fat layer is sometimes added during stuffing close to the m. biceps femoris, but fat and rind are usually trimmed off to obtain a leaner appearence. Trimming of fat and connective tissue facilitates the extraction of salt-soluble proteins, which increases the binding of muscles, improves the cohesiveness of the slices, and decreases the risk of formation of brine pockets during injection and of formation of gelatine between muscles during cooking.

Brine Preparation and Injection

The process of curing meat requires the addition of a number of ingredients and additives that are necessary to obtain the desired color, texture, and flavor. These substances are dissolved in water to form the brine. Traditionally, hams were submerged in brine and the curing salts diffused slowly into the meat. This process took several weeks and the hams were not homogeneous. Brine can be injected into the arteries, but the process is labor-intensive and the brine distribution is not uniform. Nowadays, brine is injected in such a way that the meat tissue is not torn and the brine is distributed evenly into all parts of the muscles, reducing the process time to one or two days.

To prepare the brine, it is necessary to decide the percentage to be injected. The water used must be potable. To ensure a complete solution, polyphosphates should be dissolved in the water first, followed by the nonmeat proteins, sugars, nitrite and nitrate, salt, carrageenan, and finally ascorbate. The final temperature of the brine should be adjusted to 2–5 °C. Some proteins foam when added to agitated brines; to prevent this, the use of edible antifoaming agents is advised, if allowed by local legislation; otherwise the brine should stand until the foam disappears. The amount of brine injected should be as close as possible to the target. The weight after injection is checked and adjusted by adding the required amount of loose brine to the tumbler in the process that follows the injection. An uneven distribution of brine will result in a deficiency or an excess of ingredients and additives in various areas, resulting in cooking loss and uneven coloring, binding, and flavor. The accuracy of the injection, a good brine distribution, and a low drip loss after injection are particularly important for products that are not subjected to a massaging process. Brine added during tumbling/massaging to adjust the injection weight gain should be at a minimum (< 2%) to prevent the hams swimming about in the brine, which hinders the desired tumbling effect. It is important to filter the brine to avoid blocking of the injector needles and to clean and check the filters and needles to guarantee a uniform injection.

The percentage of brine injected is determined by the desired quality of the finished product. The concentration of each ingredient in the brine depends on the amount required in the injected ham and the injection weight gain. Equation [1] relates the concentration of an ingredient x in the ham to the concentration in the brine.

$$\%B = \%P \cdot (100 + \%I)/\%I \qquad [1]$$

In eqn [1], %B = percentage of ingredient x in the brine; %P = percentage of ingredient x in the product; and %I = percentage of injection: (g of brine per 100 g of uncured meat).

The brine is injected into the muscles by means of a multineedle injector. Uniform distribution of brine in the muscles is important to reduce to a minimum the time required for the brine to migrate to the uninjected areas. This improves the color homogeneity and final product yield and prevents visible color variation along injection lines. A good brine distribution allows a better brine retention and consequently reduces the drip loss. This effect is improved when the brine penetrates deeply between the meat fibres and more muscle volume is covered with brine.

A precise injection rate ensures a minimum standard deviation of the brine content in different meat pieces and thus increases finished product quality. In some statutory requirements, the injection rate is regulated according to quality categories. The lower the standard deviation, the lower the number of under-injected pieces (which could cause sensory problems and a lower water-holding capacity) and the lower the number of overinjected pieces that would be above the statutory limit, thus producing a cooked ham with more attractive sensory characteristics.

It is important to be able to achieve the desired injection gain, ranging from a very low level such as 5%, which presents difficulties in distribution, up to a rate of 100%, which presents capacity problems.

Thorough cleaning of equipment is necessary at the end of each working day in order to avoid risk of contamination. Mechanical reliability and low maintenance requirements of the equipment used are also important in avoiding production stoppages.

Injection can be carried out with low-pressure or high-pressure injection machines. During the needle stroke, low-pressure injectors deposit the brine through the meat with needles that each have several holes more than 1 mm in diameter. This process forms brine deposits, which must then be distributed by mechanical action. High-pressure injectors (0.6–1.2 MPa) introduce a specific volume of brine when the needles have completely penetrated the meat and have stopped at the end of their downstroke. This system improves the uniformity of the injection rate from piece to piece compared to continuous pumps for brine injection, with which the pressure can change owing to variation in resistance offered by the meat.

The brine is distributed more homogeneously (particularly when pieces with bone are injected), because the needle holes are distributed along the needles and the diameter of each hole is approximately 0.6 mm. High-pressure injection, which is also called 'spray injection,' has a higher precision and gives a more uniform brine distribution with lower drip loss.

Tenderization

Tenderization is a mechanical process producing cuts or punctures in the muscles, increasing the extraction surface and eliminating the necessity of thorough trimming, as the structure of the connective tissue is broken down and consequently the effect of retraction of the connective tissue during cooking is reduced. The cuts also puncture the brine collection areas between muscles, facilitating brine distribution. Tenderization reduces cooking loss, binding defects, and holes. The degree of tenderization depends on the desired characteristics of the product. Products in which the fibrous structure of the meat is to be preserved do not undergo tenderization and the products go directly to the massaging process. For products where the primary objective is to improve tenderness, the tenderization will be light; in general, the higher the yield desired, the greater the required degree of tenderization.

There are three types of tenderizing machines available on the market:

- Tenderizer with needles: this consists of a head with needles that penetrate the meat, producing a series of small punctures in the muscles.
- Tenderizer with rollers: this consists of two sprung rollers between which the meat is passed. Some have knives that make superficial cuts; some have rollers with prongs. For rind-on products, one of the rollers is replaced with a plastic roller, which leaves the rind structure intact.
- Presses: These consist of two rollers that flatten the meat, breaking its intramuscular structure and brine pockets. This is especially useful for large pieces that do not need a great degree of binding between them.

Tumbling-Massaging

Extraction of salt-soluble proteins is important to bind the individual muscles together during cooking. The formation of exudate depends on the action of the brine ingredients and additives (NaCl, polyphosphates), the tenderization treatment, and the massaging process. The muscular structure is loosened as a result of the mechanical treatment, facilitating distribution and absorption of brine. There are two main systems: tumbling and massaging. The tumbling system provides an intense mechanical action on the meat that facilitates extraction of the salt-soluble proteins. The meat pieces are lifted by baffles to the upper part of the cylindrical machine and then fall onto the meat mass below, producing an intense mechanical action, suitable for high-yield products. This type of massage results in considerable cellullar damage and therefore an optimum extraction of proteins. The friction massaging system is smoother and acts through a stirring action that produces friction between the different muscles and with the walls and baffles of the massaging machine, producing a much gentler effect than the tumbling system. This type of massage is particularly suitable for products in which the pieces and the structure must be kept intact.

These two systems of mechanical treatment correspond to different types of cooked hams. Some of the parameters of the tumbling/massaging that influence the final result are:

- Meat characteristics: aging time before curing, age of animal, and degree of removal of fat and connective tissue.
- Brine composition: NaCl and polyphosphates contribute to the extraction of proteins, and their effect is increased by massaging.
- Massaging time: The longer the massaging time, the greater the extraction of myofibrillar proteins. However, an excessive massaging time could negatively influence the slicing quality.
- Rotation speed: A higher speed results in a greater extraction of proteins but also in greater damage to the muscle structure. For this reason, it is necessary to find an appropriate balance for each product.
- Vacuum: It is important to have a high level of vacuum to avoid foaming, which can cause air bubbles to appear between and inside the muscles. Vacuum enhances protein extraction and development and stabilization of color.
- Temperature: The mechanical action increases the temperature of the meat. Although the massaging efficiency is greater at a higher temperature, there is also a risk of bacterial growth. For this reason, it is advisable to have a cooling circuit in the equipment to control the temperature. If the tumbling equipment does not have a cooling circuit, vacuum is recommended only during tumbling, so as to avoid foaming. The temperature of the meat during tumbling should be 3–6 °C to minimize bacterial growth and allow enough protein extraction.
- Maturation period: A combination of massaging and maturation will result in the desired extraction of proteins. A minimum time of 24 h is recommended for good results. In the high-injection process it is important to reduce the processing time. In these cases the maturation is reduced to 4–6 h.
- Tumbler size: When using tumbling, the result will depend on the height from which the meat falls, and therefore the size of the batch in the machine is a relevant factor. The smaller the amount of meat, the shorter the tumbling time should be. However, it is advisable to prevent hams from falling directly onto the steel surface of the tumbler because the hams may be damaged excessively. Good results are achieved if tumblers are filled at approximately 40–50% of their capacity.

Stuffing and Molding

The matured muscles are stuffed and placed into molds to give the product the desired shape during cooking. Regardless of the material used for the mold, a polyethylene layer must be used to prevent the meat from sticking to the mold if the

product has to be repacked after cooking. If it is cooked in the bag, the shrinkable or thermoformed plastic provides protection. When thermoshrinkable multilayer plastic is used, the plastic can sometimes act as a mold through its ability to shrink. The product is clipped and placed, without a mold, between metal plates that exert pressure on the meat pieces during cooking. In this case the bags should have low oxygen permeability to prevent superficial green discoloration during cooking.

When thermoforming is used, the lower thick film is first thermoformed and then filled with the cured meat and lidded under vacuum. This system, which can also be used in combination with a rigid mold, gives the finished product a less regular shape than when using a rigid mold and shrinkable plastic, but is suitable for products for which a more traditional appearance is desired.

Molds of several shapes are used, sometimes resembling the original pear shape of the natural ham and sometimes rectangular or cylindrical, especially if the ham is to be sold sliced. The uniform cross-section of the rectangular or cylindrical shapes allows almost a complete use without waste.

The shape of the mold depends on whether or not one is attempting to reconstitute the original shape of the ham. In this case, it must be molded manually with care. Careless molding can result in products with a higher cooking loss and cavities inside the ham. A prevacuum is applied after molding to eliminate air locked between muscles. This increases the intramuscular binding and eliminates the spherical holes that are produced by expansion of the trapped air during cooking. When the air removal is completed, the bag must be vacuum-sealed either by a clipping system or by thermosealing. For thermosealing, the bags must be very clean in the seal area to avoid leaking seals.

Cans are sometimes used for cooked hams. The hams are then hermetically sealed under vacuum.

If it is not necessary to reconstruct the natural shape of the ham, the process of filling the molds can be done automatically with a stuffing machine, reducing the operations on the production line. Products that are stuffed automatically under vacuum do not require air removal and can go directly to the clipping stage. In this case, the muscles will be distributed at random in the finished product.

Cooking

One of the aims of the cooking process is to eliminate the vegetative forms of the microorganisms, but spores are not destroyed. The time required for thermal processing should be equivalent to heating the center for 40–60 min at a constant temperature of 70 °C. The heat coagulates the muscle proteins, gives the finished product consistency and firmness, and binds the muscles together. The red pigment in cured meat (nitrosylmyoglobin) is denatured into the pink pigment characteristic of cooked hams (nitrosylmyochromogen). Endogenous muscle enzymes such as proteolytic and lipolytic enzymes are inhibited and new compounds are formed that contribute to the flavor.

The packaged and/or molded hams are usually placed in cooking trolleys or baskets and cooked in steam cookers or water baths. When using steam cookers it is important to allow a good distribution of the steam; recirculation is advisable in water baths. Water baths are quicker than steam cookers. The forced and diffuse convection cooking/cooling system increases thermal efficiency and homogeneity in temperature distribution and reduces time-consuming processes by performing cooking and cooling in a single location.

Three types of cooking can be used:

- Cooking at a constant temperature: in this case the temperature is maintained constant from the beginning to the end of the thermal processing. The cooking is completed when the center of the ham (core temperature) reaches a defined temperature (between 65 and 72 °C). This is the most common method and gives acceptable results.
- Cooking at increasing temperature can be of two types: step-by-step cooking and Delta-T cooking. In step-by-step cooking, the applied temperature is increased in stages. This type of cooking produces good results, specially in cook-in systems, although the cooking time is longer than in the system using a constant temperature. In Delta-T cooking, the temperature is increased continuously in relation to the increase in temperature at the core of the product (e.g., Delta-T 25 °C, meaning that the cooking temperature in increased to maintain a 25 °C differential). When the cooker temperature reaches a certain value, it is maintained constant, as in the constant-temperature heating method. In this system, overcooking of the surface is minimized and the yield is improved, but the cooking time is longer.
- Cooking at decreasing temperatures begins with a high initial temperature (e.g., 80–90 °C) until the center reaches a predetermined temperature. The temperature is then reduced (to 70–75 °C) for the rest of the cooking process. This process is rapid, but it may result in overcooking of the surface, lower yield, and poor cohesion of the slices.

Cooling

The cooling process is important because it influences the level of pasteurization and can influence the yield and slice quality. At the end of the cooking process, the product is showered with cold water or immersed into water before being transferred to a refrigerated room in order to minimize growth of surviving microorganisms. The time taken to chill the product from 40 to 15 °C is considered critical, and should be restricted to a maximum of 4 h where possible.

Preparation for Marketing

The hams are removed from the molds and vacuum-packed. The product can be recontaminated as a result of the handling in this process. To decrease the surface flora after repacking, thermal processing of the surface (postpasteurization) is advisable. This is not necessary if the hams are cooked in their final packaging (cook-in-ham). The final products should be stored in refrigeration at 2–4 °C until they are supplied to the customer.

Nowadays, cooked hams are often marketed sliced and vacuum-packaged or packaged in a modified atmosphere. In vacuum-packaged ham, plastic film is sometimes placed between slices to ease their separation. The shelf-life depends on the bacteriological characteristics of the final product, the additives used in the brine (e.g., sodium or potassium lactate), and the packaging method. Recently, some companies have begun to treat sliced, vacuum-packed cooked ham at high hydrostatic pressures (>400 Mpa) to increase the shelf-life.

Modified-atmosphere packaging is used to improve the shelf-life of sliced cooked ham and to ensure ease of separation of slices. The gases used are mixtures of nitrogen and carbon dioxide. Oxygen concentration should be reduced less than 0.15% in the headspace to maintain the typical pink color; for this reason it is important to use packaging materials with a low oxygen permeability.

See also: Curing: Brine Curing of Meat. Packaging: Modified and Controlled Atmosphere. Processing Equipment: Brine Injectors; Tumblers and Massagers

Further Reading

Baracco, P., Berger, Y., Durand, P., *et al.*, 1982. In: Frentz, J.C. (Ed.), L'Encyclopédie de la charcuterie. Orly: Soussana. ISBN: 2-904007-00-8.

Freixenet, L.L., 1993. Spray injection of meat: Influence of the brine pressure in the quality of injected products. Fleischwirtschaft 73 (5), 547–550.

Gaze, J.E., Shaw, R., Archer, J., 1998. Identification and Prevention of Hazards Associated with Slow Cooling of Hams and other Large Cooked Meats and Meat Products. Chipping Campden: Campden and Chorleywood Food Research Association Review No. 8.

Hugas, M., Garriga, M., Monfort, J.M., 2002. New mild technologies in meat processing: High pressure as a model technology. Meat Science 62 (3), 359–371.

Hugenschmidt, G., Hadorn, R., Scheeder, M.R.L., *et al.*, 2010. The effects of early post-mortem pH and ultimate pH on level and amount of destructured zones in cooked cured hams. Meat Science 85 (4), 632–639.

Reichert, J.E., 1988. Tratamiento Térmico de Productos Cárnicos. Zaragoza: Acribia

Scheid, D., 1986. Manufacture of cook-in-ham. Fleischwirschaft 2, 31–34.

Valous, N.A., Mendoza, F., Sun, D.W., 2010. Emerging non-contact imaging, spectroscopic and colorimetric technologies for quality evaluation and control of hams: A review. Trends in Food Science and Technology 21 (1), 26–43.

Xargayó, M., Lagares, J., 1992. Computerized massaging of meat. Fleischwirschaft 73 (5), 654, 656–658, 660.

Xargayó, M., Lagares, J., Freixenet, L., Fernandez, E., 1995. Production of cooked ham and similar products. Use of an automatic vacuum stuffing-dosing machine for stuffing of whole muscle. Fleischwirschaft 75 (6), 770–775, 808.

Relevant Websites

http://www.hiperbaric.com
Hyperbaric.

http://www.metalquimia.com
Metalquimia.

Dry-Cured Ham

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Glossary

Bayonne hams Bayonne ham is a 'protected geographical indication' (PGI) dry-cured ham that takes its name from the ancient city of Bayonne in the South West of France.

Iberian ham Iberian ham is a type of dry-cured ham produced in Spain and Portugal. It is made from pure black Iberian pigs or cross-bred pigs, which must be at least 75% Iberian.

Parma ham Parma ham is a 'protected designation of origin' (PDO) dry-cured ham from the Italian region Emilia-Romagna (Parma Province) made from pigs that are born and bred only there. The production is regulated by a quality consortium that recognizes qualifying products with a distinctive label. Curing uses relatively little salt and no nitrate or nitrite is used in the process.

Quick-dry slice The QDS process is an accelerated drying process for sliced meat products that is applied to the sliced product directly after the fermentation step before the long drying phase. This process results in a dramatically reduced total processing time.

Serrano ham Jamón Serrano is a 'traditional speciality guaranteed' (TSG) type of dry-cured ham produced mainly in Spain. The majority of Serrano hams are made from white pigs and should not to be confused with Iberian ham, which is entirely different.

Water activity (a_w) Water activity is defined (for practical purposes) as the ratio of the vapor pressure of water in a material (p) to the vapor pressure of pure water (p_o) at the same temperature. When vapor and temperature equilibrium are obtained, the water activity of the sample is equal to the relative humidity of air surrounding the sample in a sealed measurement chamber. Multiplication of water activity by 100 gives the equilibrium relative humidity (ERH) in percentage $a_w = p/p_0 = \mathrm{ERH}(\%)/100$.

Introduction

The production of cured ham has been established since time immemorial as a process of preservation through salting and subsequent drying. Nowadays, efforts are made to obtain a product with added value that is safe, of high quality, and appreciated for its typical sensory characteristics.

The product quality at the end of the process is influenced by the raw materials and the technological processes. There are various production technologies for cured hams, but the basic aim of all of them is to furnish a product that can be kept at room temperature without jeopardizing health, without the risk of deterioration, and to facilitate the development of the desired sensory characteristics. (Spain, Italy, Germany, and France are the main producers of dry-cured hams. In 2010 the production in the European Union was over 600,000 Tm.)

This article reviews some aspects of the production process and compares different technologies.

Raw Material

Effect of the Quality of Raw Material

The age of the pigs providing the meat has to be taken into account as this can affect, among other things, the activity of the proteolytic enzymes, the color, the amount of fat, and the influence of the muscles on the drying process. It has been shown that meat with a high proteolytic potential is less suitable for the production of dry-cured hams, especially if a low salt content is desired. The selection of the raw material can be improved by evaluation of the activity of cathepsin B, which is one of the proteolytic enzymes thought to be responsible for the development of soft texture. The current methods of production are not adapted for online measurements of proteolytic activity. However, new technologies in the field of molecular genetics, such as genetic markers, show promise for the prediction of the proteolytic activity of the raw material.

The legs of mature pigs are considered to be the most suitable for the production of traditional dry-cured hams, because they have more pigmentation, greater fat content, and less proteolytic potential.

The fat content and the weight of the ham are the main criteria used in the selection of the raw material, and they also determine the processing time of the ham. The weight of green hams ranges from 9 to 14 kg. Whereas in northern European countries consumers prefer lean hams, in some countries in the Mediterranean region there are consumers who prefer a certain amount of intramuscular fat in this product, which prolongs the maturation time; this results in certain sensory characteristics, for which the product is highly valued. In Iberian hams and dry-cured hams that have undergone a long aging process, the presence of interior fat and a certain amount of superficial fat slows down the drying process and impregnates the musculature, so that mastication produces an oily sensation in the mouth and an aged flavor that is highly appreciated (a pleasant flavor associated with the oxidation of the fat). Hams that contain almost no subcutaneous fat must be avoided, as in these hams the salting and drying process in the rind area is greater than in the rest of the ham. This results in a product with a saltier taste, a higher weight

loss, and lower quality, and therefore it is rejected by the majority of consumers.

The pH of the meat is another important parameter affecting the maturation of the ham. For microbiological safety, the majority of authors recommend avoiding hams that have an ultimate pH (pH_u) > 6.2. A pH_u lower than 6.2 also leads to improved salting, a lower percentage of deteriorated hams, fewer appearance problems (such as a shiny appearance of the lean meat and phosphate precipitates), and an undesirable soft texture in the internal part of the ham (when water content is still high), as well as crust development at the lean surface (when water content is low) due to the lower proteolysis at high pH during the aging of ham. This type of problem is greater in larger hams. Furthermore, the pH values in a ham will vary considerably between muscles. Consequently, in order to obtain a pH measurement that is representative of the ham as a whole, it is advisable to carry out the measurement on an external muscle of considerable size, such as the m. semimembranosus. Hams with a $pH_u < 5.6$ show higher proteolysis especially at low salt content and consequently more incidence of pastiness occurs. PSE (pale, soft, exudative) hams absorb more salt than normal hams when dry-cured, which increases the variability in salt content among dry-cured hams.

Elimination of residual blood from the blood vessels of the hams, manually or by mechanical systems, before curing is advisable to reduce possible microbiological problems and to improve the appearance. The best time to carry out this operation is during the cutting process, as the blood is easier to remove. When there is no assurance that this operation has been carried out, it should be done when the raw material is received.

Shape of the Hams and Types of Cut

Cured hams are produced using whole joints or parts of the leg. The whole legs can be presented in different ways. In Spain, presentation with the hoof is common for Iberian and 'Serrano' hams. Parma and Bayonne hams, as well as the majority of the hams that end up boned, are produced without the hoof. The presence of the hoof can reduce the incidence of color fading of the shank, caused by the entrance of air, although the reason for producing hams with hooves seems to be mostly cultural.

In Spain, unlike other countries, it is usual to make a cut in the rind in the shape of a V. Possibly this facilitates the use of the rind and part of the fat for other purposes. In addition, greater standardization of the thickness of the subcutaneous fat is achieved, the retraction of the lean meat is facilitated during the drying process, and the slicing of the ham is also facilitated.

In Spanish hams, the aitch bone is left in, which means that the morphology of the muscles remains intact; in French (jambon de Bayonne) or Italian hams (Parma and San Danielle), this bone is eliminated, leaving a small part ('anchetta') in place in order to avoid cavities that hinder the drying process. The elimination of the bone allows a more rapid absorption of salt and faster water loss, especially in the biceps femoris muscle.

Pieces of ham muscles are used instead of whole hams for the production of jambon d'Ardennes, for Culatello and Fiocchetto in Italy, or bayona in Spain.

In all cases, it is important to avoid damaging the surface structure, as this could lead to the entrance of microorganisms, causing deterioration of the product.

Refrigerated versus Frozen Raw Material

The use of refrigerated raw material is usual in areas where there are abattoirs and cutting rooms in abundance. Frozen hams are often used when long transport is involved because it avoids changes in temperature that could spoil the hams. The use of frozen hams facilitates the absorption of salt on the surface, its migration toward the interior, and probably the diffusion of water toward the outside as well. The use of frozen meat makes the evaluation of the quality of the raw material difficult and facilitates the crystallization of tyrosine, which is formed during the maturation process, leading to a higher number of white crystals on the surface of the hams. If there are many cycles of temperature during the storage process, or if the frozen storage period is prolonged, superficial dehydration of the rind and the lean meat of the ham can occur. This causes surface `freezer burns.' Freezer burns can hinder the salting process and can damage the final appearance of the product. To prevent this, it is advisable to cover the ham with a close-fitting protective plastic film while freezing. To avoid problems with the growth of undesirable microorganisms, it should be ensured that no point of the surface of the ham reaches a temperature above 5 °C during the thawing stage. However, if several days are required to prepare a batch of hams for curing, which is frequent in small cutting rooms with low production capacity, it is preferable to freeze the hams rather than keep them refrigerated for excessively long periods.

Classification Methods

Classification of hams is common practice in most companies. However, if the hams are classified in batches, the individual information of each one is lost. At present, classification systems that identify each ham by means of a bar code or electronic identification are being commercialized. This individual identification signifies an extraordinary advance, as it means that the information on each ham can be stored (supplier, pH, impedance, weight, data, etc.) and it ensures traceability. Moreover, the suppliers can be classified, the return of the hams can be recognized, losses can be more easily standardized, and variability can be reduced.

Salting

Massaging

To facilitate the penetration of curing salts, to eliminate blood that is present in the veins and arteries, and to mold the ham, a massage is carried out with a mixture that contains salt, nitrate, nitrite, ascorbate, and sugars. Although the massaging can be done manually, it is normally carried out continuously by

machines or in drums. In this way, the absorption surface is increased and the penetration of the salt is enhanced. Similarly, moderate pressing done by pressing machines, to reduce the thickness of the hams and to facilitate the penetration of the salt into the deepest internal muscles, is recommended.

Covering in salt must be done as soon as a temperature between 1 and 3 °C is reached in order to inhibit the growth of undesirable bacteria to reduce the percentage of rejected hams at the end of the process due to off-flavors.

Salting Methodology

In Mediterranean countries, the dry-salting process is used; in northern Europe this process is also used, as well as salting in brine. In the dry-salting process, a higher level of osmotic dehydration is obtained, whereas curing in brine reduces the amount of salt, which would have a positive environmental impact.

The dry-salting process can be carried out by two different methods. The hams may be covered with salt. This can be done by individual salting of the hams (San Danielle) or by stacking them in stainless steel containers for a period of approximately 1 day kg^{-1} for the refrigerated hams and 1–3 days less for the frozen ones (typical of Serrano and Iberian hams). The salt has a moisture content of approximately 4–5% to allow correct salting, and the atmospheric humidity is high to avoid drying out and to facilitate the formation of saturated brine on the surface of the ham. The level of atmospheric humidity should not be below 75%, as this results in dehydration of the salt. The stacking of the hams creates pressure, which is especially high in the lower strata, facilitating water loss.

Salting can also be achieved using a quantity of salt that is calculated in proportion to the weight of the ham. The best-known example of this method is for Parma hams, where the relative humidity is maintained between 70% and 75% minimum and 85–90% maximum. With this method, salting of the areas covered in salt is achieved, at the same time allowing the drying of those areas that are not covered with salt. In this case, the hams remain in a horizontal position for a period of 3–4 weeks, which results in the thickness being less than it would be if the hams were left hanging. Penetration of the salt and evacuation of water from the inside is enhanced.

In other cases, the salting takes place in salting drums, with a subsequent resting period in containers, where the hams are in contact with the exuded brine. It is important to perform two applications of salt, so that the parts of the hams that have received little salt during the first treatment are treated again a second time. In this type of process, it is better not to excessively prolong the resting period in containers as the exuded water can be reabsorbed and encourage the growth of undesirable microorganisms in areas that are not covered in brine.

Ingredients and Additives

Salt is essential for the production of cured hams and is the only substance that is permitted for the production of Parma ham. Nitrate is present at very low levels in pork, and has been the preferred nitrifying agent in products for long aging, as it is transformed into nitrite by bacterial action. The cured color due to nitrosylmyoglobine is obtained faster with nitrite than with nitrate, which is preferable in rapid production processes. Ascorbate accelerates the transformation of nitrite into nitric oxide; it prevents the development of greenish discoloration resulting from the reaction of nitric oxide with oxygen, and inhibits the formation of nitrosamines in the lean meat. Zn-protoporphyrin IX complex is the pigment formed during aging that is responsible for the color of hams without the addition of nitrite and nitrate. Sugars, on the one hand, favor the establishment of the surface flora and lend a light, sweet flavor, but on the other they can bring about acidic flavors, when the lactic flora develops in the internal parts of the hams.

Parma hams usually have pepper added to the head of the femur or in the fat that is used to cover the ham, whereas spices are hardly ever added in Spanish Serrano and Iberian hams. For pieces of ham produced by a short-duration process, it is usual to add spices to improve their sensory quality.

Washing

In the past, hams were desalted by immersing them in running water to eliminate the excess salt picked up during the long salting process. However, nowadays, because the temperature can be maintained between 1 and 5 °C during the stabilization phase, it is possible to reduce the quantity of added salt; in the industrial production of Spanish hams, washing is done only to remove any salt crystals remaining from the salting process.

Italian hams are usually washed after the resting period, and at this stage it is possible to maintain a lower water activity at the head of the femur. The washing cleans the ham of slime that has formed on the piece, and phosphate crystals and salt are eliminated. In addition, this method ensures that a later drying process can be accomplished more quickly, because drying is carried out at a higher temperature than can be used after the washing of Serrano hams. In Parma hams the temperature varies from 25 to 28 °C during the first 8 h and is then reduced to 16–20 °C. The relative humidity is approximately 60%.

The use of tepid water allows a more effective washing than the use of cold water. The ideal time to carry out the washing is also determined by the cost of the handling involved and the dehydration capacity of the resting drying rooms.

At the end of the salting process, the blood vessels usually contain a certain amount of brine and it is advisable to perform a light pressing operation to eliminate this, along with any residual blood. By pressing and forming the ham, it is also possible to improve the evenness of the cut of the product. However, this should not damage the ham's structure, which could lead to the entrance of microorganisms that could cause spoilage. At whatever stage the washing is performed, it is always recommended to carry out a quick drying of the lean meat of the ham to prevent the growth of undesirable flora. However, it must be taken into consideration that fast drying reduces the water activity of the surface to values lower than 0.75, which will cause local crystallization of salt. This could lead to drying of the lean meat or cause white-colored stains on the rind and result in deterioration of the appearance.

Smoking

In countries with cold damp climates, hams have traditionally been smoked. The smoke provides the typical smoked color and flavor, increases antioxidant activity and impedes growth of surface bacteria and molds.

Resting Period

The aim of the resting period is to achieve an even distribution of the salt while at the same time achieving a slight dehydration. On the surface of the hams, the flora will be affected by the environmental humidity, and often a growth of molds is observed. In the interior of the ham, Gram-positive, catalase-positive cocci become the dominant flora. At this stage, it is advisable to carry out a more energetic drying during the first and second weeks, as it is important to reduce the surface water activity to impede the growth of undesirable microorganisms that can give the hams a slimy appearance. The accumulated weight loss at the end of this phase usually varies between 10 and 15%.

For a regular drying process, the quantity of water that evaporates must be compensated by the diffusion of water from the inner part to the outer part of the ham. The temperature at this stage must be less than 5 °C until a water activity lower than 0.96 has been reached in all parts of the ham. To avoid whitening of the rind, brought about by the crystallization of the salt, relative humidity above 75% is recommended. Once the majority of the salt from the rind has penetrated through the fat to the lean part of the ham, the relative humidity can be reduced below 75%. This facilitates a slight oil drip of the external subcutaneous fat, which in turn facilitates flavor development.

The duration of the resting period varies according to the size of the piece, the surface of the exterior lean part, the type of trimming, and the intermuscular and intramuscular fat, among other factors. In small hams, a minimum period of 1 month is recommended. For Parma hams it is necessary to prolong this period, sometimes for 3 months, due to the low salt content and absence of nitrite. For Serrano hams this period should be longer than 40 days (In Iberian hams the resting period lasts 2–3 months because of the high fat content, which hinders salt diffusion.).

Drying-Maturation and Cellar Phase

During the drying-maturation phase, the hams continue to dehydrate (e.g., for a minimum of 110 days in the case of Serrano hams), and the processes of proteolysis and lipolysis, which provide the aroma, continue. In Spanish Serrano hams, the temperature is slowly increased from 10 to 12 °C until it reaches a maximum of 28–34 °C, with a relative humidity between 60 and 80%. However, in recent years there has been a decrease in the length of time at high temperature in order to reduce the incidence of hams with a soft texture and a white film, which can be problematic for the selling of sliced products. When the temperature is increased, the fat melts and impregnates the muscle tissue, which provides one of the typical characteristics of Serrano hams.

During this phase, a fine layer of fat is frequently applied to prevent surface cracking, excessive drying, and mustiness, and to slow down the growth of mites. This application of fat can be preceded by washing with water and drying of the ham surface. For long processes, Serrano hams are subjected to a final phase termed the 'cellar phase' at temperatures from 12 to 20 °C and a relative humidity of 50–80% to dry the ham until the desired texture is obtained. This phase begins after the drying period and lasts up to a minimum processing time of 7 months. However, high-quality Serrano hams may have a processing time of up to 18 months.

For Parma hams, the drying phase takes place at a temperature of approximately 15 °C and a relative humidity of approximately 65–80%. After 6–8 months, the drying phase is slowed down as a result of several applications of a mixture of fat, flour (to make the paste more permeable), pepper, and salt. This paste is added several times as the ham becomes drier. Matured hams are stamped with the Parma ham brand after at least 10 months of processing for pieces weighing between 7 and 9 kg, and after at least 12 months for pieces more than 9 kg; pieces less than 7 kg cannot be branded (After the resting period, Iberian hams are kept for approximately 90 days in a chamber where the temperature is slowly increased until reaching 18–20 °C. Subsequently, the temperature is again slowly increased for a period of approximately 1–1.5 months until it reaches approximately 30 °C with a relative humidity of 60–80%. Finally, the hams are held for 12–18 months at temperatures ranging from 10 to 22 °C and with relative humidity of 65–80%.).

The Development of New Quick-Maturation Products

To obtain quicker maturation processes, it is necessary to accelerate the movement of the salt toward the inside and of the water toward the outside of the ham. This can be achieved by eliminating the fat (subcutaneous and intermuscular), injecting a brine, and reducing the thickness of the pieces by pressing. For sliced products, the slices can be dried by the Quick-Dry Slice System in a period of only 30–60 min when the salt content is uniform in the whole piece.

For whole pieces, if the original structure of the ham is not maintained, microorganisms can grow inside the hams. This causes acidification of the product or development of an odor that is typical in fermented products. To ensure a good final aroma of the product, it is necessary to select the raw material properly (reject dark, firm, dry (DFD) and boar-tainted hams and use slightly marbled hams). During production, the final aroma can be influenced by the addition of flavorings, spices, and/or smoke, the use of a selected microbial flora, and optimization of the relative humidity at the different stages of the aging process.

Commercial Presentation

The commercial presentation of dry-cured ham has traditionally been as whole pieces. However, in recent decades there has been an increase in the selling of boned and vacuum-packaged products. Vacuum-packaging facilitates the slicing process and even texture of the ham, and prevents problems with mites or excessive drying. Prolonged vacuum packaging causes

re-moistening of the surface, which can result in an unpleasant aroma. If the water activity is high, undesirable microorganisms can proliferate; in this case, it is necessary to keep the product refrigerated or to freeze the vacuum packages. Although the storage of hams by freezing permits the preservation of the flavor for long periods and slows down the formation of white film in sliced products, it also facilitates the formation of white crystals. Freezing of unpacked pieces causes a loss of volatile substances, which decreases the aroma of the product.

Before slicing, hams are partially frozen in order to obtain a uniform texture to facilitate the cutting process. To increase the ham microbial stability, high-pressure treatment at 400–600 MPa may be applied to hams packaged under vacuum or in modified atmosphere with flexible packaging.

Packaging in a modified atmosphere allows a more natural presentation of the slices as it reduces their adhesion as well as the waxed appearance of slices typical in vacuum-packaged hams. To prevent the deterioration of color and aroma, the gas mixture must not contain oxygen. The gas most frequently used is nitrogen, although carbon dioxide can be useful to improve the shelf-life of hams with high water activity. The plastic used must be high barrier type to prevent the gas mixture being altered. In this type of packaging, abrupt change of temperature must be avoided, as this can result in condensation on the inside of the packaging, causing a local increase in water activity and a reduction of the microbial safety of the product.

See also: Additives: Functional. Curing: Brine Curing of Meat; Dry; Production Procedures. Drying. Ethnic Meat Products: Mediterranean. Packaging: Modified and Controlled Atmosphere. Refrigeration and Freezing Technology: Freezing and Product Quality

Further Reading

Arnau, J., Guàrdia, M.D., Guerrero, L., Claret, A., 2011. Propuesta de guía metodológica para la evaluación sensorial de jamón curado de cerdo blanco. SENSOJAM PROJECT – RTA 2006-00060-00-00. Barcelona: IRTA, INIA. Available at: http://www.irta.cat/ca-ES/RIT/Noticies/Documents/Jornada_pernil_sensojam_guiametodologica_def.pdf (accessed 27.08.13).

Arnau, J., Hugas, M., Monfort, J.M., 1987. Jamón Curado: Aspectos Técnicos. Girona: Grafis-Sant, S.A. ISBN: 8440415753. Available at: http://www.scribd.com/doc/20577065/Jamon-curado-Aspectos-tecnicos (accessed 27.08.13).

Arnau, J., Monfort, J.M., 1998. El Jamón Curado: Tecnología y Análisis de Consumo. Simposio Especial-44th International Congress of Meat Science and Technology. Madrid, Spain: Estrategias Alimentarias SL-Eurocarne. ISBN: 84-930010-3-1.

Arnau, J., Serra, X., Comaposada, J., Gou, P., Garriga, M., 2007. Technologies to shorten the drying period of dry-cured meat products. Meat Science 77 (1), 81–89.

Baracco, P., Berger, Y., Durand, P., *et al*., 1982. In: Frentz, J.C. (Ed.), L'Encyclopédie de la charcuterie. Orly: Soussana. ISBN: 2-904007-00-8.

IV Congreso Mundial del Jamón, 2007. Ponencias, Comunicaciones y Pósteres. Salamanca: Comité Organizador IV Congreso Mundial del Jamón.

Consejería de Agricultura y Pesca, 2001. Primer Congreso Mundial del Jamón. Córdoba, Spain: Junta de Andalucía. ISBN: 84-8474-029-3.

Lopez-Bote, C.J., 1998. Sustained utilization of the Iberian pig breed. Meat Science 49 (Suppl. 1), S17–S27.

Monfort, J.M., 2000. II Symposium Internacional del Jamón Curado. Madrid, Spain: Estrategias Alimentarias SL-Eurocarne. ISBN: 84-930010-5-8.

Monfort, J.M., Guillén, F., 2005. Conference proceedings. In: Monfort, J.M., Guillén, F. (Eds.), III Dry Cured Ham World Congress on Science, Technology and Marketing. Teruel: Consejo Regulador de la Denominación de Origen "Jamón de Teruel." ISBN: 8493407801.

Olmos-Llorente, J.V., 2006. Atlas Monte Nevado de anatomía del jamón serrano. In: Jamones, S. (Ed.), Madrid: Gráficas Berlin, S.L., pp. 226. ISBN: 8460995755.

Poma, J.P., 1998. Le Jambon Sec et les Petites Salaisons. Paris, France: ERTI. ISBN: 2–903-524-80-7.

Smulders, F.J.M., Toldrà, F., Flores, J., Prieto, M., 1992. New Technologies for Meat and Meat Products: Fermentation and Starter Cultures, Muscle Enzymology and Meat Ageing, Quality Control Systems. Utrecht, The Netherlands: ECCEAMST. ISBN: 90-800360-2-1.

Ventanas, J., 2001. Tecnología del Jamón Ibérico: de los Sistemas Tradicionales a la Explotación del Sabor y el Aroma. Madrid, Spain: Mundi Prensa. ISBN: 84-7114-944-3.

Ventanas, J., 2003. Conjamón (II Congreso Mundial del Jamón). ISBN: 84-7723-629-1.

Virgili, R., Schivazappa, C., 2002. Muscle traits for long matured dried meats. In: Università di Parma (Ed.), 48th International Congress of Meat Science and Technology, pp. 45–55. Rome.

Relevant Websites

http://en.wikipedia.org/wiki/Dry-cured_ham
Wikipedia.

http://es.wikipedia.org/wiki/Jam%C3%B3n_serrano
Wikipedia.

HAZARD ANALYSIS CRITICAL CONTROL POINT AND SELF-REGULATION

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Glossary

Failure mode and effects analysis (FMEA) A procedure that involves reviewing as many components, assemblies, and subsystems of a process as possible, to identify failure modes and their causes and effects.

FAO/WHO Codex Alimentarius Commission The Codex Alimentarius Commission harmonizes international food standards, guidelines, and codes of practice, to protect the health of consumers and ensure fair practices in food trading.

Good manufacturing practices (GMPs) A system for ensuring that all aspects of production, from the starting materials, premises, and equipment to the training and personal hygiene of staff, meet with recognized standards.

Hazard analysis and critical control point (HACCP) system A systematic approach to identifying biological, chemical, and physical factors in production processes that can give rise to health hazards in finished products; and to implementing measures to reduce the risks from the identified hazards to acceptable levels.

Introduction

Terms, such as self-regulation, self-control, and quality assurance, are widely used to indicate ways in which food manufacturers take responsibility for the safety of their products. In recent years, food laws in many countries have been amended to require companies to take a systematic approach to self-regulation, to ensure the hygienic/toxicological status of food products is safeguarded. Many management systems for safeguarding the quality and safety of outputs from manufacturing facilities have been devised. Total quality management (TQM) is a well-established, wide-ranging management approach that is applied horizontally across an operation and extending backward and forward to include both suppliers and customers. TQM is only one of many systems that focus on quality. Other systems include continuous quality improvement, statistical quality control, quality function deployment, quality in daily work, and fault tree analysis (FTA). FTA, for example, is a top-down deductive failure analysis system which, like many top-down systems, might identify only major failure modes in a system because it does not systematically identify many lower level causes and failures or incorporate them within a preventive framework. Three types of systems commonly used by the food industry for the purpose of ensuring product safety are good manufacturing practices (GMPs), hazard analysis critical control point (HACCP) systems, and failure mode and effect analysis (FMEA). GMP systems have been used for many years and often provide a basis for more recent systems. HACCP systems are widely regarded as the preferred means of ensuring food safety; implementation of HACCP systems for food production processes is now required by many regulatory authorities. FMEA is a more recently developed system that is increasingly seen as having application for assurance of food safety. The aim of all of these systems is to prevent, and counteract when necessary, loss of control over product quality that could lead to consumer illness. Each of these systems will be described and their application illustrated by reference to a process for production of Vienna sausage (Figure 1).

Self-Regulation

Good Manufacturing Practices

GMPs are general rules for the safe production and handling of food products, including meats. The principles of GMP, developed over a number of years, provide a foundation on which the production of safe food is based. To be successful, any quality assurance system implemented in a meat processing plant must adhere to the fundamentals of GMP.

Although GMPs are not product- or process-specific, they can be adapted to provide detailed instructions for handling food items. GMPs appropriately modified for different kinds of food, and different steps in food production chains,

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Processing step		Material									
No.	Description	Amount (kg)	Type	Specification	Temperature (°C)	Time (min)	Activity	Transport	Retardation	Check	Storage
1	Preparing/weighing raw material	24.0 18.0 18.0 5.0	Beef Pork Pork fat Pig rind mix	R 2b S 3b S 9 S 14	+ 4 to + 10 + 4 to + 10 + 4 to + 10 0 to + 2		•				
2	Transport				+ 8			•			
3	Mincing				+ 8		•				
4	Slow cutting (3 cycles)	1.45	Curing salt		+ 10		•				
5	Fast cutting	20.0	Crushed ice		Down to +1 Up to + 12	Within 4	•				
6	Slow cutting (5 cycles)	0.92	Spice mixture	HSV 15	Up to + 14		•				
7	Transfer in container							•			
8	Interim storage					30			•		
9	Transfer in filler							•			
10	Filling (108 g/pc.)	280 m	Natural casings	∅ 18/20	+ 14 to +18		•				
11	Smoking			Prog.23	+ 60 to + 70	60	•				
12	Cooking				Core: + 72	Core: 15	•				
13	Washing/cooling				+ 10 to + 15	30	•				
14	Transfer in cold storage							•			
15	Cold storage				+ 6 to + 8						•

Figure 1 Concise representation of a Vienna sausage production process providing a detailed overview of the process parameters.

including those related to meats, are available as guidelines from various professional organizations and associations. In addition, numerous product-specific legal regulations specify GMPs, especially those with relevance to meat production and trade. Both the Codex alimentarius commission and the United States Food and Drug Administration have published collections of GMP-based regulations for food.

Basic information and documentation

In GMP systems, as for other self-regulatory systems, records of all manufacturing and controlling activities must be maintained, so that the methods of control can be assessed for appropriateness and, when possible, improved. These records also provide a basis for establishing external quality credentials and for quality audits.

Documentation should record at least the type and volume of production; staff, with identification of functions, responsibilities, and working relationships; a list of contractors providing services, such as cleaning; and a list of suppliers. Building plans of the working area, in which water supply and sewage pipes, temperature zones, clean and unclean processing areas, product flow, and personnel routes are identified, which are also required. A detailed process flow diagram of the type shown in Figure 1 must be available for every product. This basic documentation should be integrated with or used with other systems of quality control, such as

ISO 9001 quality management systems, HACCP systems, or FMEA.

Fundamental requirements

Fundamental requirements for GMP are related to control of structural, environmental, and personnel resources. The production of wholesome and safe food requires a sanitary processing environment. Therefore, companies must follow appropriate rules for design, utilization, and maintenance of facilities and equipment. Generally, buildings should be sufficiently large and constructed in ways that facilitate not only sanitary processing but also cleaning and sanitizing procedures. Personnel and material flows must be arranged so that manufacturing areas do not become thoroughfares. Cleaning and sanitizing procedures must be carried out as often as is necessary according to a documented cleaning and sanitation plan (Figure 2). The cleanliness of the facility must be checked daily, by visual inspection, before production starts (the so-called preoperation checks) as well as microbiologically at intervals appropriate to the facility's production capacity. Furthermore, pest control measures must be used and their efficacy must be regularly checked and recorded. Rules for the health, cleanliness, training, and supervision of personnel must be developed and maintained. Rules for waste storage, treatment and removal, water supply, and wastewater removal must be implemented.

Manufacturing conditions

Implementation of GMP requires procedures to ensure the appropriateness of raw materials and ingredients entering the facility. To achieve this, material specifications should be detailed and material checks should be performed at delivery to establish compliance with the specifications.

All manufacturing operations should be performed in ways that prevent product contamination as far as possible. Written standard operating procedures for each operation should be prepared. Flow diagrams for processes are very useful, because these are easy to understand. Instructions for processing operations must identify factors that might affect product safety as well as measures that should be taken when misprocessing occurs. All food-handling activities, from raw material delivery to packaging, labeling, and distribution, must be covered in a GMP system.

Adjuncts to good manufacturing practice

A facility's documented GMPs can be augmented by adjunct control systems. Some definition of an adjunct control system is needed. For example, *Listeria* that persist in chillers and

TMC The Meat Company	Cleaning and sanitation plan	Document number 6.5.d/10	Page 1/1
		Revision date : 30.09.2003	Version: 1

Cutting and deboning room

Surfaces	Frequency	Activity	Day: Monday	Tuesday	Wednesday	Thursday	Friday	Material	Visum staff member: Week ..... Monday	Tuesday	Wednesday	Thursday	Friday	Week ..... Monday	Tuesday	Wednesday	Thursday	Friday
Meat-contact	Daily	Precleaning	×	×	×	×	×	Brush, water 58 °C										
- Conveyor belt		Cleaning	×	×	×	×		Contrasoil MT 4, 2 %										
- Cutting tables							×	Antilime F5, 3 %										
- Saw blade		Sanitation	×		×		×	Mibideath 100, 1 %										
- Punching machine		Rinsing	×	×	×	×	×	Water, 20 °C										
		Drying	×	×	×	×	×	Air stream										
Nonmeat-contact	Daily	Precleaning	×	×	×	×	×	Broom										
- Floor		Cleaning	×	×	×	×		Contrasoil MT 4, 2 %										
- Walls							×	Antilime F5, 3 %										
- Doors		Sanitation					×	Mibideath 100, 1 %										
		Rinsing	×	×	×	×	×											
		Drying	None															
- Ceiling	Monthly	Precleaning	None															
		Cleaning					×	Contrasoil MT 4, 2 %										
		Sanitation	None															
		Rinsing					×											
		Drying					×											

Figure 2 Example of a cleaning and sanitation plan, including record preparation, for the cutting and deboning area in a meat processing establishment.

contaminate ready-to-eat meats can be controlled by use of a chiller heat treatment as an adjunct to an existing GMP program. For GMP adjuncts to be effective, a multidiscipline approach needs to be taken at the facility level. Engineering, operations, and quality assurance personnel need to be involved in the planning as well as the implementation of adjunct systems.

Hazard Analysis Critical Control Point Systems

HACCP systems are used to identify, evaluate, and control hazards that are significant with respect to food safety. HACCP is a concept of science-based control of hazards that might arise during processing. HACCP systems are both product and process specific. This specificity makes it almost impossible to transfer a HACCP system developed for one product or processing line to another without modification. The HACCP concept has been adopted by the FAO/WHO Codex Alimentarius Commission. It is considered to be the quality management tool applied in food production that is most effective for systematically safeguarding the hygienic and toxicological quality of food.

Approach to implementation

The principles of HACCP require examination of every single product in detail (i.e., they are product specific) and matching of the principles to the actual production process (i.e., they are process specific).

Seven principles must be applied when establishing and maintaining a HACCP system (Table 1). Application of the principles requires detailed and documented knowledge of the production processes in question. Each principle includes a number of subprinciples.

Table 1 Stages in the implementation of hazard analysis and critical control point (HACCP) concepts according to the principles given in Alinorm 97/13 A

Alinorm principle no.	*HACCP implementing procedure: Product and process description*
1	*Hazard analysis* ● Hazard identification ● Risk assessment
	Risk management 1. Prevention analysis
2	a. Determine critical control points (CCPs)
3	b. Establish critical limits
	2. Create process monitoring system
4	a. Establish monitoring procedures for CCPs
5	b. Establish corrective actions when monitoring indicates that a particular CCP is out of control
6	c. Establish verification procedures to confirm that the HACCP system is working effectively
7	d. Establish a documentation system concerning all procedures and records appropriate to these principles and their application

Source: Reproduced from Codex Alimentarius Commission, 1997. General Principles of Food Hygiene. Supplement to vol. 1B, Revision 3. Rome: Food and Agriculture Organization of the United Nations and World Health Organization.

Company requirements

Before implementation of HACCP, some organizational requirements have to be fulfilled. A clear commitment by the management to implement HACCP is required. The HACCP team should together have comprehensive knowledge of relevant fields, such as epidemiology, chemistry, microbiology, toxicology, processing technology, veterinary medicine, and quality management.

Product and process description

A detailed description of all products and technological processes to be included in the HACCP system is required. For a single product, the description must include the formula and ingredient specifications, as well as a detailed description of all process parameters. Companies developing a HACCP system will benefit from existing documentation for a GMP and quality management system. The product's intended use, its target market, and its distribution must be detailed.

Hazard analysis

A hazard is defined as 'a biological, chemical, or physical agent in; or conditions of food with the potential to cause an adverse health effect.' All possible occurring hazards should be listed, including those inherent in the ingredients as well as those derived from the processing environment. A hazard must be designated specifically. General terms like 'pathogenic microorganisms,' 'foreign bodies,' or 'heavy metals' are not sufficient.

After hazard analysis, a risk assessment must be performed. Not all hazards are equally important as some occur often, others seldom; some cause severe illness, others are associated with only mild symptoms; and some are distributed worldwide, others are regional. The probability of each hazard's occurrence (i.e., the risk) varies depending on a broad range of factors, such as its innate characteristics (e.g., growth requirements of microorganisms and reactivity of chemicals), consumers' eating habits, sensitivities and health, the rates of exposure, and the maximum damage to health that might be caused. Unfortunately, epidemiological studies are lacking for many hazards, and statistical data are often limited. Frequently, only a rough estimate of the real situation is feasible; but this is often sufficient for HACCP risk assessment purposes. Risk assessment should allow the hazards list to be reduced to only hazards relevant to the product in question. As an example, for the Vienna sausage production process, an outline characterization for HACCP purposes of the hazard *Salmonella* spp. and evaluation of its relevance to the process is shown in Figure 2.

Risk management

After risk assessment, the frequency and degree to which each relevant hazard can occur must be assessed at every step of the process. Points at which the process can be controlled to reduce the hazard's probability of occurrence and magnitude in the finished product (i.e., the hazard can be prevented, eliminated, or reduced to an acceptable level) are called critical control points (CCPs). Decision trees have been developed to assist with the CCP identification procedure.

A proper identification and exploitation of CCPs requires:

- detailed knowledge of the processing technology;
- detailed knowledge of the hazard characteristics;

- the possibility of establishing a critical limit for safe operation for at least one process parameter (i.e., principle 3);
- the possibility of continually monitoring the critical process parameter (i.e., principle 4); and
- the possibility of adjusting the process on-line (i.e., principle 5) to prevent production of unsafe product.

Table 2 Characterization and judgement of the relevance of the hazard '*Salmonella* spp.' in a hazard analysis and critical control point concept for Vienna sausage production

Hazard characterization		
Disease		
Occurring worldwide		
Very common (epidemic)		
Serious incapacitation with moderate duration Symptoms		
	• Nausea	
	• Stomach cramps	
	• Vomiting	
	• Diarrhea	
	• Fever	
Mostly not life threatening		
	• Fatality rate $<1\%$	
	• Fatality rate for young and elderly: *Salmonella enteritidis* in hospital/nursing homes outbreaks approximately 4%	
Sequelae infrequent		
	• Reactive arthritis (Reiter's syndrome) in $<2\%$ of the cases	
Infectious dose		
	• <100 cells, depending on strain virulence, health status, and age of host	
Potential control measures[a]		
	• Temperature >55 °C	
	• pH value <3.8	
	• Water activity <0.94	
	• Preservatives (e.g., *D*-value propionic acid 0.5 mol l^{-1}: 0.8 h)	
	• Irradiation (*D*-value: 0.5–1.1 kGy)	
Occurrence during Vienna sausage production (**Figure 1**)		
No.	*Processing step*	*Source*
1	Preparing/weighing raw material	Pork: approximately 10% positive Beef: approximately 3% positive
6	Slow cutting	Spices: approximately 5% positive
All	Generally during manipulation	Persons: intestinal carrier rate up to 5%
Conclusion: *Salmonella* spp. = relevant hazard		

[a]Deviations might occur owing to summation and differences between strains.

For example, when applying these requirements to the control of *Salmonella* spp. in Vienna sausage production, the cooking step (processing step 12) can be identified as a CCP because the cooking temperature of 72 °C exceeds the minimum temperature of 55 °C needed for *Salmonella* destruction (**Table 2**). Establishing critical limits for cooking temperature and time requires knowledge of the rates of inactivation of *Salmonella* spp. at temperatures attained during cooking. According to **Table 3**, the minimum thermal treatment needed for adequate destruction of salmonellae would be 45 s at 70 °C. Product heated for shorter times or to lower temperatures must be regarded as unsafe with respect to salmonellae.

The procedure described above is repeated for all hazards recognized as relevant to identify a number of CCPs. Not all identified hazards are necessarily controllable without changing the process. Sometimes additional measures are needed to control a specific hazard. An example is the insertion of metal detectors in a processing line to allow items containing metal objects or fragments to be identified and removed.

If more than one hazard can be controlled at the same CCP, the most rigorous of the critical limits for the hazards must be adopted. With respect to Vienna sausage production, elimination of *Listeria monocytogenes* requires a cooking time of 1 min at 70 °C provided that the contamination level is the same as for salmonellae. Therefore, for safety with respect to both *Salmonella* spp. and to *L. monocytogenes*, the products should be cooked to a minimum core temperature of 70 °C for at least 1 min.

Monitoring procedures that are related to the critical limits for CCPs should then be introduced. It is necessary to list the

Table 3 Practicable approach for establishing critical limits for the cooking process for control of *Salmonella* in a hazard analysis and critical control point system for Vienna sausage production

Establishing critical limits		
Estimated requirements		
• Estimated contamination level of sausage meat: <100 cells g^{-1}	$\geq$100 000-fold reduction ($=10^{-5}$) of *Salmonella* cells required	
• Security factor: 1000		
Destruction of Salmonella spp.		
Thermal sensitivity of Salmonella senftenberg (most heat-resistant strain)[a]:		
• 10-fold reduction in cell numbers within 0.15 min at 70 °C, D_{70}: 0.15 min		
Critical limit		
Required core time–temperature combination for 100 000-fold reduction:		
• Minimum 45 s (5×0.15 min $= 0.75$ min) at 70 °C		
Actual process parameters		
No.	*Processing step*	*Actual process parameter*
12	Cooking	Core temperature $+72$ °C for 15 min

[a]In glucose solution of water activity 0.98 and pH 5.5–6.2.

process parameters to be checked, the methods to be applied, the frequencies of monitoring each parameter and who is responsible for the monitoring. Monitoring of the Vienna sausage production process should include a continuous time–temperature surveillance system, ideally with automatic regulation of cooking (see principle 5). Monitoring is only useful when appropriate actions follow detection of deviation from the critical limit, as required by principle 5.

Principle 6 requires that effective working of the HACCP system is verified. This includes measures, such as periodic checks of monitoring equipment calibration, laboratory testing of product, and collection of data that confirm the absence of hazards. Onsite auditing procedures can be used for verification.

As with other self-regulation and quality management systems, HACCP systems require documentation (principle 7). This must include basic descriptions of the products and processes covered by the system, a list of hazards dealt with, a list of CCPs where hazards are controlled and a monitoring plan that specifies the critical limits, corrective measures, and verification procedures.

Failure Mode and Effect Analysis

FMEA is generally performed during product development to avoid costly failure during full-scale processing. FMEA is widely used in the nuclear, aviation, and automobile industries. Its use in the food industry is currently limited, but growing.

Practical approach

FMEA is used to assess the significance of potential product failures and to identify appropriate measures to prevent them. Issues identified by FMEA lead to immediate and specific actions. Two types of FMEA can be distinguished:

- A 'product FMEA' regards each raw material as a potential source of failure. The consequences of the failure with respect to end product safety and quality are identified and evaluated.
- A 'system FMEA' looks for weak points in the production process. A step-by-step approach is used to identify and evaluate potential causes of product failure.

As for other self-regulatory systems, FMEA requires a detailed description of the product and the processing technology.

Structured failure analysis

A failure analysis is usually present in the form of a table. By following the processing line, stepwise potential failures and their resulting effects as well as their potential causes are identified. Thus, for the Vienna sausage production process, the FMEA can be represented as in Figure 3.

The potential 'failure' of undercooking the product will cause softness, discoloration, and color instability as well as reduced storability due to microbial spoilage. In addition, pathogenic microorganisms might survive to pose health risks for consumers.

Production line: Vienna sausage

Processing step: 12 Cooking

Actual settings: + 72 °C, 15 min (sausage core)

FMEA team:	Leader:	*N.N. (P)*
	Members:	*X.Y. (QS)* *Y.Z. (P)* *K.E. (TS)*
Date:	start:	*01/01/03*
	close:	*30/05/03*
Documents:	flowchart (Fig 1)	
	Manual	

Potential failure	Potential effect	Potential cause	Current status: Control measures	O	I	D	RPN	Future status: Recommended measures	Responsibility Schedule	O	I	D	RPN
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Undercooking	▪ *Softness* ▪ *Discolouring* ▪ *Colour instability* ▪ *Spoilage* ▪ *Health risks*	*Lack of water*	*none*	*7*	*10*	*6*	*420*	*Water level alert system*	*TD 05/03*	*7*	*10*	*1*	*70*
		Faulty thermometer calibration	*monthly calibrations*	*4*	*10*	*6*	*240*	*Short term:* ▪ *daily calibration* ▪ *sensory product testing*	*P, QS 02/03*	*4*	*10*	*1*	*40*
								Long term: ▪ *automated temperature control and regulation system*	*TD, P, QS 06/04*	*1*	*10*	*1*	*10*

O: Probability of occurrence
I: Importance to clients
D: Probability of detection
RPN: Risk priority number
TS: Technical Support
QA: Quality Assurance
P: Production

Figure 3 Excerpt from a FMEA for Vienna sausage production.

Table 4 Classification system for assessing the probability of occurrence (O), the importance (I) to consumers, and the probability of detection (D) in failure mode and effect analyses

Risk assessment no.	*O*	*I*	*D*
1	Unlikely	None	High
2	Very low	Minor	Moderate
3	Very low	Minor	Moderate
4	Low	Medium	Moderate
5	Low	Medium	Moderate
6	Low	Medium	Low
7	Moderate	Serious	Low
8	Moderate	Serious	Low
9	High	Very serious	Very low
10	High	Very serious	Unlikely

Risk assessment

The significances of failures are evaluated based on their probability of occurrence (O), their importance (I) with respect to the client, and their probability of detection (D) before distribution of the product. These parameters are assessed on scales from 1 to 10 (Table 4).

In Vienna sausage production, the lack of water might result in the product being undercooked. This might occur with moderate frequency, for which a risk assessment number of seven would be appropriate. The consequences for consumers, however, might be very serious because of the survival of pathogenic microorganisms. The risk assessment number for client importance is then 10. Although marked undercooking will be detected through deviations in consistency, borderline cases might be difficult to detect so, the risk assessment number for the probability of detection is six.

The three risk assessment numbers (O, I, and D) are multiplied together resulting in a risk priority number (RPN), which indicates a failure ranking within the potential failure list. For Vienna sausage production, lack of water results in a RPH of 420. High RPNs indicate a need for urgent action to improve control in this area.

Optimization measures

After assessment of the potential risk of failure, measures are suggested that will optimize the processing conditions for control of potential failure. This might be achieved by reduction of one or more of the probability of occurrence or detection, or of the importance to clients. Ideally, all three parameters should be reduced. Suggested improvements are identified, and responsibilities and schedules for implementation are developed. To determine the effects of the measures, a new RPN is calculated using the reduced O, I, and D numbers.

For Vienna sausage production, there is a high probability that the lack of water will be detected by installation of a water level alert system. This should be done by technical support staff by a fixed date. Although neither the risk of occurrence of the failure nor its importance to consumers will be changed, the RPN is reduced to 70 because of the high probability of detection ($D=1$). Performing the same procedure for all processing steps will result in a process optimized with respect to safety and quality.

Future Prospects

As responsibility of food manufacturers for the safety of their products increases, self-regulatory systems that effectively control food safety risks will undoubtedly be applied to a greater extent. Cooperation between food processors and food inspection authorities will be needed for the systematic application and implementation of these systems.

The systems described here, namely GMP, HACCP, and FMEA are only a small selection from the large number of quality management tools potentially applicable for safeguarding food. Because some concepts are complex, food processors should initially only use a single, well-understood concept, and should seek expert advice. In addition, implementation should begin with a single product or process and take account of only a limited number of control parameters or hazards. All self-regulation systems must grow progressively.

See also: Environmental Contaminants. Foreign Bodies. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Yeasts and Molds; *Yersinia enterocolitica*. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Benlloch, A., 1992. Integrated systems analysis in meat industry practice; an example from Spain. In: Smulders, F.J.M., Toldrá, F., Flores, J., Prieto, M. (Eds.), New Technologies for Meat and Meat Products. Utrecht, NL: ECCEAMST, pp. 317–326.

Codex Alimentarius Commission, 1997. General Principles of Food Hygiene. Supplement to vol. 1B, Revision 3. Rome: Food and Agriculture Organization of the United Nations and World Health Organization.

Deutsches Institut fur Normung (DIN) 10503 Beiblatt 1, 2000. Food Hygiene – Flow Diagrams for the Utilization in the HACCP-Concept – Symbols, Kind of Presentation. Berlin: Beuth.

Eglezos, S., Thygesen, S., Huang, B.X., Dykes, G.A., 2010. A simple method to reduce *Listeria* in blast and holding chillers. Food Protection Trends 30, 472–476.

Godderz, A., Schmitz, T., Mack, A., Petersen, B., 2007. Failure mode and effect analysis (FMEA) as a decision support tool within a quality information system in pork production chains. In: Theuvsen, L., Spiller, A., Peupert, M., Jahn, G. (Eds.), Quality Management in Food Chains. The Netherlands: Wageningen Academic Publishers, p. 139.

Jarvis, B., 1999. Good manufacturing practice. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 2. London: Academic Press, pp. 961–972.

Kainz, R., Gombos, J., Bernert, G., 1996. Fleisch/fleischerzeugnisse: Modell eines eigenkontrollsystems. Ernährung – Nutrition 3–64.

Leaper, S., 1999. Critical control points. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 2. London: Academic Press, pp. 990–992.

Mahmutoğlu, T., Bozoğlu, F., 1999. Hazard appraisal (HACCP) – Establishment of performance criteria. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 2. London: Academic Press, pp. 992–1000.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 1998. Hazard analysis and critical control point principles and application guidelines. Journal of Food Protection 61, 762–775.

Untermann, F., 1999. Hazard appraisal (HACCP) – The overall concept. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology, vol. 2. London: Academic Press, pp. 982–990.

U.S. Food and Drug Administration, U.S. Department of Health and Human Services, 1999. Current good manufacturing practice in manufacturing, packing, or holding human food. Code of Federal Regulations Title 21, Part 110. Silver Spring, MD: U.S. Food and Drug Administration.

Varzakas, T.H., 2011. Application of ISO 22000, failure mode, and effect analysis (FMEA) cause and effect diagrams and pareto in conjunction with HACCP and risk assessment for processing of pastry products. Critical Reviews in Food Science and Nutrition 51 (8), 762–782.

HUMAN NUTRITION

Contents

Cancer Health Concerns

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Glossary

Carcinogenesis The collection of processes that allow cells to undergo uncontrolled cell division and prevents the normal removal of damaged cells, resulting in cancer formation.

Heterocyclic amines (HCAs) A class of chemicals that include compounds that are formed during high temperature or open-flame cooking of meat.

Mutagens The chemicals capable of causing deoxyribonucleic acid mutations.

***N*-Nitroso compounds** These compounds include nitrosamines produced from heme-iron and nitrates in meat in the presence of an acidic environment (e.g., processed meat or in the stomach).

Polycyclic aromatic hydrocarbons (PAHs) A family of chemicals which can be produced during high-temperature cooking of meat, for example, while grilling.

Introduction

The morbidity and mortality associated with cancer is a major health concern in most of the world, with the primary sites of cancer being dictated based, at least in part, on where individuals live and/or their socioeconomic status. In the USA, one in four deaths is attributed to cancer, even though the incidence rates over the last 5 years in which data are available (2004–08) has remained stable in women or has decreased slightly in men. Even though incidence rates are not increasing, approximately 1 660 290 new cases of cancer are expected to be diagnosed in 2013, which does not include noninvasive cancers (except bladder) or basal and squamous cell skin cancers. More importantly, long-term projections that include the impact of aging population suggest that by 2030 there will be 2.3 million cases of cancer in the USA, which is a 40% increase above current estimates.

The transition from normal tissue into a cancerous tissue involves multiple stages – from the initiating event that causes genetic mutations to promotion of cell transformation and the final stage of progression during which the potential for metastasis develops. Initiators of the transformation process include those factors which are capable of causing deoxyribonucleic acid (DNA) mutations, such as radiation, viruses, and chemicals. However, a single mutation is not sufficient to cause a cancer. Multiple insults must occur before sufficient modifications to the DNA exist for cells to attain the capacity for tumor development. These changes eventually allow cells to escape normal controls on growth and proliferation, which is characteristic of all known neoplasms. The eventual tumor mass that forms contains a variety of cell types, all of which contribute to an environment capable of supporting carcinogenesis and the eventual capacity for metastasis to other sites.

Several experimental approaches have been used to determine factors that might be involved in promoting or reducing cancer incidence. Among them are population studies that attempt to understand the disparities in cancer rates observed in various regions of the world. Others monitor cancer incidence rates among genetically identical groups that migrate from areas of low incidence to a part of the world with higher rates of specific cancers. All these approaches have

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demonstrated that environment is a major contributor to cancer development. Aspects of the environment that have been linked to carcinogenesis include chemical exposures, radiation, pollution, infectious agents, tobacco use, and diet.

Diet and Cancer

Approximately one-third of the cancer deaths predicted during 2012 were linked to overweight, obesity, physical inactivity, and poor nutrition, suggesting that modifications to these characteristics and environmental inputs would contribute to a reduction in cancer deaths. A systematic review of the literature performed by an expert panel convened by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) determined the relationships between meat and cancer overall and importantly, defined the relationships between meat intake and specific cancer types. This systematic review found that there was convincing evidence that red meat and processed meats contributed to increased risks of colorectal cancer. Limited evidence was found to support an increased risk of cancers in the esophagus, lung, pancreas, and endometrium with red meat consumption and for esophagus, lung, stomach, and prostate cancer with processed meat consumption. The group also found evidence to suggest a probable increase in risk for cancer of the nasopharynx with consumption of Cantonese-style salted fish. Data were only suggestive for the link between iron-containing foods and an increased risk of colorectal cancer and between smoked meats or meats that were grilled or barbecued and stomach cancer. Limited evidence exists to suggest a decrease in colorectal cancer with the consumption of fish or foods containing vitamin D. More recent analyses and reviews have also found evidence to suggest that diets with greatly elevated intakes of meat, extensively cooked red meat in particular, may be associated with an increased risk of some forms of cancer.

As the strongest evidence for a link between meat intake and cancer incidence is found for colorectal cancer, a greater extent of discussion will occur for this cancer. However, a discussion of data for other cancer sites will be presented as well. Processing of meats, such as salting, smoking, curing, and the addition of preservatives, as well as the methods used for cooking contribute to chemical exposures that may promote carcinogenesis. As a result, a presentation of the compounds developed during meat processing and/or cooking and their potential for contributing to carcinogenesis is provided.

Cancer Sites

Colorectal Cancer

When the data are combined for men and women, there will be an estimated 102 480 new cases and 50 830 deaths from colon cancer in the USA during 2013. The WCRF/AICR review found that red meat, processed meat, body fatness, abdominal fatness, and alcoholic drinks ($>30\ g\ day^{-1}$ in men) produce the most convincing link with colorectal cancer. The consumption of alcoholic drinks by women is probably linked with colorectal cancer, and there is limited, but suggestive, evidence of a relationship between consumption of foods containing iron, animal fats, or sugars and colorectal cancer. There are also limited data to suggest that consumption of fish may protect against colon cancer, whereas the data to support a link between consumption of poultry, total fat, and cholesterol and colon cancer are insufficient to support a conclusion. Inclusion of additional studies in the continuous update project by WCRF/AICR led to a relatively reduced risk of colon cancer with elevated red and processed meat consumption, but the relationship remained. One of the studies used in the continuous update report was from the UK Dietary Cohort Consortium, where mean intakes of meat were relatively low ($38.2\ g\ day^{-1}$ for men and $28.7\ g\ day^{-1}$ for women). This study found little evidence for an association between the consumption of red meat, processed meat, poultry, white fish, or fatty fish and colon cancer. A paper published after the continuous update project reports on the relationship between red meat intake and the risk of colon cancer in Japan, a population who also has a low intake of red meat (median $=46\ g\ day^{-1}$ for men and $43\ g\ day^{-1}$ for women). An increased risk of colon cancer was found with the highest level of red meat intake in women and the highest intake of total meat in men. This group found no association between colon cancer and the intake of processed meat. These observations suggest that red meat might induce a modest increase in the risk of colon cancer among populations consuming otherwise low levels of red meat, but as the level of intake approaches that observed in Westernized cultures, the risk of colon cancer is increased. Part of the relationship is more likely due to the confounding effects of increased body and abdominal fatness that are prevalent in those who are consuming diets with elevated energy densities and reduced level of foods thought to protect against colon cancer, for example, fruits, vegetables, and whole grains that contain high levels of dietary fiber and chemopreventive bioactive compounds.

As mentioned earlier, processing, both for preservation purposes and for cooking, has the potential to incorporate or develop mutagens and carcinogens in meat. The components of fresh meat that may promote carcinogenesis include fat, protein, heme iron, and the mutagens developed during high-temperature or open-flame cooking – heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs). The HCA found in meat include 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo [4,5-f]quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). The most prominent PAH in meat is benzo(a)pyrene (BaP). Processed meats also contain these compounds as well as added nitrite, nitrate, and salt. One class of potential carcinogens formed in the gastrointestinal tract is *N*-nitroso compounds, which can be formed from heme iron and nitrite. The relative risks of colon cancer are typically higher with consumption of processed meat than with fresh meat, although this observation is not true for all studies. The disparity between studies may be due to designs that combine fresh red meat and processed meats into a single red meat category, as opposed to determining their independent effects. One animal study found that nitrite-treated cured meat increased formation of early lesions of colon cancer and the effect occurred in parallel with an increase in fecal levels of total *N*-nitroso compounds.

Esophageal Cancer

Although the estimated incidence rate in 2013 for esophageal cancer was relatively low (17 990 new cases) in the USA, the estimated number of deaths from this cancer was high (15 210). The most convincing relationships between cancer of the esophagus and diet found in the WCRF/AICR review was with elevated body fatness (due to excess energy intake and/or reduced physical activity) and alcoholic drinks. At the time of that review, there were limited data to establish a link between red meat or processed meat and esophageal cancer incidence. However, subsequent publications have found positive relationships (odds ratios of 1.79–3.15 for the highest red meat intake) between red meat intake and esophageal cancer incidence. Analysis of data from the large NIH-AARP Diet and Health study detected a positive association between red meat intake and squamous cell carcinomas in the esophagus, but this study did not detect a relationship between BaP, nitrate, or nitrite consumption and cancer incidence. Another study conducted in Ireland found an increased risk of esophageal cancer in subjects with elevated intakes of total, saturated, and monounsaturated fats and fresh red meat intake. Together, these results suggest that a dietary pattern containing an excess of total and saturated fats and diets enriched in animal-based foods and low in plant foods may promote cancer in the esophagus.

Stomach Cancer

According to current estimates there will be 21 600 new cases and 10 990 deaths from stomach cancer in the USA in 2013. Although an infection with the bacterium *Helicobacter pylori* is prevalent in most cases of stomach cancer, it is thought that the incidence rate of stomach cancer can be reduced through dietary means. The WCRF/AICR review found the most convincing links between stomach cancer and diets that include elevated intakes of salt and salted or salty foods. There is only limited evidence suggesting an increase in risk with elevated intakes of chili, processed meats, smoked foods, and grilled or barbecued meats; moreover, there is limited evidence linking the consumption of unprocessed meats and stomach cancer. One recent study found no association between processed meat, nitrate, nitrite, or BaP intake and stomach cancer, yet the study did note an increase in stomach cancer with the highest intake of DiMeIQx.

Pancreatic Cancer

Overall, pancreatic cancer incidence rates are relatively stable, but there will be an estimated 45 220 new cases and 38 460 deaths from this disease in the USA in 2013. The most convincing link between pancreatic cancer and lifestyle is an increase in body fatness. However, in the WCRF/AICR review, there was only limited evidence to suggest a link between red meat consumption and pancreatic cancer. Recent data suggest that pancreatic cancer may be linked with the consumption of well-done meat that has been cooked at high temperatures, which is more likely due to the elevated levels of mutagens (MeIQx and DiMeIQx) produced with this form of cooking.

Kidney Cancer

In the USA, there will be an estimated 65 150 new cases of kidney cancer and 13 680 deaths in 2013. The most convincing evidence for an enhanced risk of renal cancer is associated with elevated body fat. The data linking meat consumption and kidney cancer were deemed insufficient to support a conclusion by the WCRF/AICR review team. However, data from a case-control study provide some evidence to suggest that the polycyclic hydrocarbon BaP formed during barbecuing is associated with an increase in kidney cancer. Interestingly, that study found a negative relationship between broiled meat intake and kidney cancer and found no association between total or individual meat types and kidney cancer incidence. These observations suggest that cooking style, and the formation of BaP, is more likely to be the base of the relationship rather than meat itself.

Bladder Cancer

Bladder cancer occurs more frequently in men than in women, and in 2013, there will be an estimated 54 610 and 17 960 new cases and 10 820 and 4390 deaths in men and women, respectively. The WCRF/AICR review concluded that there was insufficient evidence to support a conclusion concerning the association of any food and bladder cancer risk. Two newer studies have produced conflicting results, with the large European Prospective Investigation into Cancer and Nutrition study finding no association of meat, nitrosamines, or heme iron intake on bladder cancer. In contrast, data from the NIH-AARP Diet and Health Study found modest support for a positive relationship between the consumption of dietary nitrate or nitrite in processed meat and bladder cancer, as well as a borderline significance for the relationship between both red meat and PhIP intake and bladder cancer.

Lung Cancer

The most prevalent form of cancer in the USA is lung cancer, with 228 190 new cases and 159 480 deaths being estimated to occur in 2013. Fruits, as well as foods containing carotenoids, are probably capable of reducing the incidence of this disease. Although the evidence is limited, data suggest a positive relationship between lung cancer and the consumption of high levels of red meat, processed meats, and total fat. Outcomes from the European Prospective Investigation into Cancer and Nutrition suggest that no relationship exists between consumption of red meat, processed meat, white meat, or fish and lung cancer incidence. A study of Chinese women who had never smoked found that there was no association between lung cancer and the consumption of processed meats or heterocyclic amines and lung cancer. In this population, most of the meat consumed (72%) was white meat (chicken or fish) and they did note an inverse relationship between consumption of meat (total) or fish and lung cancer, suggesting white meat or fish consumption may be protective against lung cancer in those who have never smoked.

Endometrium Cancer

In the USA, the estimated number of new cases and number of deaths from endometrial cancer in 2013 are 49 560 and 8190, respectively. The most convincing and probable evidence for factors with an association to endometrial cancer risk are body fatness and abdominal fatness, respectively. The most recent publication describing the relationships between meat consumption and endometrial cancer was based on the Canadian Study of Diet, Lifestyle, and Health prospective cohort. This study found a nonsignificant increase in the risk associated with increased consumption of red meat, processed meat, and all meat combined. However, there were no clear associations found with poultry or fish consumption.

Breast Cancer

Breast cancer is the most frequently diagnosed cancer in women, with the exception of skin cancer. In 2013, there will be an estimated 232 340 new cases of invasive breast cancer diagnosed in women and 2240 in men, and 39 620 women and 410 men will die from the disease. The factors identified as having the greatest impact on breast cancer risk are consumption of alcohol, body fatness, adult attained height, with abdominal fatness, and adult weight gain having a probable role in an increased risk of breast cancer. Conclusions derived by the WCRF/AICR review, as well as the continuous update project by the same group, indicate that there are limited data to suggest a relationship between red meat or processed meat and breast cancer incidence. Studies since that review have found marginal increases in risk of breast cancer among postmenopausal women and total meat intake, whereas others have suggested no association. An evaluation of the relationship between meat, meat mutagens, and iron and the risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial failed to find a dose response relationship for red meat, processed meat, or heme iron. The most recent review of the literature concluded that red meat and processed meat intake were not associated with an increased risk of breast cancer, but the author cautions that effect modifiers, such as hormone receptor status in the breast, should be considered in order to determine whether these factors would influence the lack of association.

Prostate Cancer

Prostate cancer is the most frequently diagnosed cancer in men and is the second leading cause of death from cancer in men. In 2013, there will be an estimated 238 590 new cases and 29 720 deaths from prostate cancer in the USA. There is limited evidence to suggest that processed meats are associated with an increase in prostate cancer. It is more likely that the nitrates, nitrites, and salt found in processed meats may contribute to prostate cancer. Two studies published after the WCRF/AICR review have demonstrated an increase in aggressive/advanced prostate cancers with elevated intake of processed meat, meats cooked at high temperatures, or well-done red meat and the resulting mutagens (PhIP, MeIQx, and DiMeIQx), but one study found no association between white meat consumption and prostate cancer. Therefore, meat consumption may not be associated with localized and nonaggressive tumors, but consumption of highly processed meat or meat cooked at high temperatures might promote development of more aggressive forms of the disease.

Summary

Data suggest that diets with greatly elevated intakes of meat, extensively cooked red meat in particular, may be associated with an increased risk of some forms of cancer. The associations may be due to the fat contained in meat, the processing methods used to preserve meats, or the mutagenic/carcinogenic compounds developed during cooking where high temperatures or open flames are used. Although causation has not been established, individuals with the highest intake of cured meats and red meat have a modest (20–30%) increased risk of colon cancer. The relationship between meat intake and other cancers is not well defined, even though where data exist, they tend to suggest that processing may be in part responsible for the effects observed. Another putative indirect cause for the association between elevated intakes of meat and cancer may be the reduction of other dietary components including plant foods containing elevated levels of dietary fiber and other biologically active compounds that may be protective against cancer. These observations suggest that it might be possible to modify current processing or cooking conditions, as well as overall dietary pattern, in order to reduce the potential for meat to contribute to carcinogenesis in most tissues.

See also: Cooking of Meat: Cooking of Meat. Curing: Brine Curing of Meat; Dry; Natural and Organic Cured Meat Products in the United States; Production Procedures. Potential Chemical Hazards Associated with Meat. Preservation Methods of Animal Products. Smoking: Liquid Smoke (Smoke Condensate) Application; Traditional

Further Reading

ACS, 2013. Cancer Facts And Figures, 2013. Atlanta, GA: American Cancer Society. Available at: http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-figures-2012 (accessed 04.09.13).

Alexander, D.D., Morimot, L.M., Mink, P.J., Cushing, C.A., 2010. A review and meta-analysis of red and processed meat consumption and breast cancer. Nutrition Research Reviews 23, 349–365.

Anderson, K.E., Mongin, S.J., Sinha, R., *et al.*, 2012. Pancreatic cancer risk: Associations with meat-derived carcinogen intake in the prostate, lung, colorectal, and ovarian cancer screening trial (PLCO) cohort. Molecular Carcinogenesis 51, 128–137.

Corpet, D.E., 2011. Red meat and colon cancer: Should we become vegetarians, or can we make meat safer? Meat Science 89, 310–316.

Cross, A.J., Freedman, N.D., Ren, J., *et al.*, 2011. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. American Journal of Gastroenterology 106, 432–442.

Daniel, C.R., Schwartz, K.L., Colt, J.S., *et al.*, 2011. Meat-cooking mutagens and risk of renal cell carcinoma. British Journal of Cancer 105, 1096–1104.

Egeberg, R., Olsen, A., Autrup, H., *et al.*, 2008. Meat consumption, *N*-acetyl transferase 1 and 2 polymorphism, and risk of breast cancer in Danish postmenopausal women. European Journal of Cancer Prevention 17, 39–47.

Ferguson, L.R., 2010. Meat and cancer. Meat Science 84, 308–313.

Ferrucci, L.M., Cross, A.J., Graubard, B.I., *et al.*, 2009. Intake of meat, meat mutagens, and iron and the risk of breast cancer in the prostate, lung, colorectal, and ovarian cancer screening trial. British Journal of Cancer 101, 178–184.

Ferrucci, L.M., Sinha, R., Ward, M.H., *et al.*, 2010. Meat and components of meat and the risk of bladder cancer in the NIH-AARP diet and health study. Cancer 116, 4345–4353.

Jakszyn, P., Gonzalez, C.A., Lujan-Barroso, L., *et al.*, 2011. Red meat, dietary nitrosamines, and heme iron and risk of bladder cancer in the European prospective investigation into cancer and nutrition (EPIC). Cancer Epidemiology, Biomarkers, and Prevention 20, 555–559.

John, E.M., Stern, M.C., Sinha, R., Koo, J., 2011. Meat consumption, cooking practices, meat mutagens, and risk of prostate cancer. Nutrition and Cancer 63, 525–537.

Kabat, G.C., Cross, A.J., Park, Y., *et al.*, 2010. Intakes of dietary iron and heme-iron and risk of postmenopausal breast cancer in the National Institutes of Health-AARP diet and health study. American Journal of Clinical Nutrition 92, 1478–1483.

Lim, W.-Y., Chuah, K.L., Eng, P., *et al.*, 2011. Meat consumption and risk of lung cancer among never-smoking women. Nutrition and Cancer 63, 850–859.

Linseisen, J., Rohrmann, S., Bueno-de-Mesquita, B., *et al.*, 2011. Consumption of meat and fish and risk of lung cancer: Results from the European prospective investigation into cancer and nutrition (EPIC). Cancer Causes Control 22, 909–918.

van Lonkhuijzen, L., Kirsh, V.A., Kreiger, N., Rohan, T.E., 2011. Endometrial cancer and meat consumption: A case-cohort study. European Journal of Cancer Prevention 20, 334–339.

O'Doherty, M.G., Cantwell, M.M., Murray, L.J., Anderson, L.A., Abnet, C.C., 2011. Dietary fat and meat intakes and risk of reflux esophagitis, Barrett's esophagus and esophageal adenocarcinoma. International Journal of Cancer 129, 1493–1502.

Pan, A., Sun, Q., Bernstein, A.M., *et al.*, 2012. Red meat consumption and mortality. Archives of Internal Medicine 172, 555–563.

Punnen, S., Hardin, J., Cheng, I., Klein, E.A., Witte, J.S., 2011. Impact of meat consumption, preparation, and mutagens on aggressive prostate cancer. PLoS ONE 6 (11), e27711. doi:10.1371/journal.pone.0027711.

Santarelli, R.L., Vendeuvre, J.-L., Naud, N., *et al.*, 2010. Meat processing and colon carcinogenesis: Cooked, nitrite-treated, and oxidized high-heme cured meat promotes mucin-depleted foci in rats. Cancer Prevention Research 3, 852–864.

Seigel, R., Naishadham, D., Jemal, A., 2013. Cancer statistics, 2013. CA: A Cancer Journal for Clinicians 63, 11–30.

Smith, B.D., Smith, G.L., Hurria, A., Hortobagyi, G.N., Buckhholz, T.A., 2009. Future of cancer incidence in the United States: Burdens upon an aging, changing nation. Journal of Clinical Oncology 27, 2758–2765.

Spencer, E.W.A., Key, T.J., Appleby, P.M., *et al.*, 2010. Meat, poultry and fish and risk of colorectal cancer: Pooled analysis of data from the UK dietary cohort consortium. Cancer Causes Control 21, 1417–1425.

Takachi, R., Tsubono, Y., Baba, K., *et al.*, 2011. Red meat intake may increase the risk of colon cancer in Japanese, a population with relatively low red meat consumption. Asia Pacific Journal of Clinical Nutrition 20, 603–612.

Taylor, E.F., Burley, V.J., Greenwood, D.C., Cade, J.E., 2007. Meat consumption and risk of breast cancer in the UK Women's Cohort Study. British Journal of Cancer 96, 1139–1146.

WCRF/AICR, 2007. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: World Cancer Research Fund/American Institute for Cancer Research.

WCRF/AICR, 2008. The associations between food, nutrition and physical activity and the risk of breast cancer. WCRF/AICR Systematic Literature Review Continuous Update Report. Washington, DC: World Cancer Research Fund/ American Institute for Cancer Research.

WCRF/AICR, 2010. The associations between food, nutrition and physical activity and the risk of colorectal cancer. WCRF/AICR Systematic Literature Review Continuous Update Report. Washington, DC: World Cancer Research Fund/ American Institute for Cancer Research.

Relevant Websites

http://www.cancer.org
American Cancer Society.

http://www.aicr.org
American Institute for Cancer Research.

http://www.cancer.gov
National Cancer Institute.

Cardiovascular and Obesity Health Concerns

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Glossary

Beef in an Optimal Lean Diet (BOLD) Study A controlled clinical study that demonstrated cholesterol lowering effects of a heart healthy diet containing lean beef.

Cardiovascular disease (CVD) It includes diseases of the heart and blood vessels.

Dietary Approaches to Stop Hypertension (DASH) A recommended dietary pattern for reducing risk of CVD, that includes fruits, vegetables, low-fat/fat-free dairy products, and foods low in sodium and saturated fat.

Low-density lipoprotein (LDL) A lipoprotein fraction that transports cholesterol into blood; which is a major risk factor for CVD.

Therapeutic Lifestyle Changes (TLC) A recommended diet for decreasing LDL-C, that is low in saturated fat and cholesterol.

Introduction

According to the World Health Organization (WHO), the leading causes of mortality worldwide in 2008 were major chronic diseases, including heart disease, stroke, cancer, chronic respiratory diseases, and diabetes, accounting for 63% of all deaths. Of the 36 million deaths from chronic disease, 25% were premature deaths (less than 60 years of age).

Cardiovascular diseases (CVD) are the leading cause of death globally. In 2008, approximately 17.3 million people died from CVD, which accounted for 30% of all deaths. Approximately 7.3 million deaths were due to coronary heart disease, and stroke accounted for 6.2 million deaths. By 2030, the WHO's projections are that 23.6 million people will die from CVD, primarily from heart disease and stroke.

Overweight and obesity are a major public health problem globally (ranked as the fifth major risk factor for deaths). In 2008, 1.5 billion adults, 20 years and older, were overweight, and 10% of the world's adult population was obese. In addition, in 2010, approximately 43 million children less than 5 years of age were overweight. Excess body fat beginning in childhood markedly increases risk of overweight and obesity later in life and the accompanying comorbidities associated with it. There are at least 2.8 million deaths each year due to overweight or obesity. Overweight and obesity contribute to the development of chronic diseases, including CVD, diabetes, musculoskeletal disorders, and cancers. For example, 44% of the diabetes burden, 23% of the ischemic heart disease burden, and 7–41% of certain cancer burdens are due to overweight and obesity.

Good nutritional practices are the foundation for reducing risk of many major chronic diseases. Dietary guidance has been issued by many government agencies and health organizations worldwide to decrease the risk of developing chronic diseases and associated morbidity and mortality. Because red meat is an important part of the diet in many populations, it is vital to discuss how it can be incorporated in a healthy diet that reduces risk of major chronic diseases, with emphasis on CVD and overweight/obesity.

Cardiovascular Disease

CVD represents many disorders of the heart and blood vessels that include: coronary heart disease, cerebrovascular disease, peripheral arterial disease, heart failure, arrhythmias, heart valve problems, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism. Many CVDs develop over decades. CVD is a chronic inflammatory disease that frequently begins with endothelial damage that leads to inflammation of the vessel wall, with the consequent formation of atherosclerotic lesions that cause myocardial infarction and stroke. The underlying pathogenesis also involves dysregulated lipid metabolism and a pathological immune response.

An elevated cholesterol level and, in particular, elevated low-density lipoprotein cholesterol (LDL-C) are contributing factors to atherogenesis. Oxidative modification of LDL facilitates cellular uptake via the scavenger receptor and promotes atherogenesis via a process involving the production of macrophages and inflammatory cytokines. This sequel of events results in conversion of activated macrophages to foam cells, resulting in the formation of a fatty streak. In an abundance of atherogenic lipoproteins, the activated macrophages or foam cells endocytose until death by either apoptosis or necrosis. The death of the foam cells catalyzes the inflammatory process. In addition, there is smooth muscle cell proliferation and migration into the intima in response to inflammatory cytokines secreted by the damaged endothelium. This leads to the formation of a fibrous plaque that covers the fatty streak. Plaques that have a thin fibrous cap are susceptible to plaque rupture, resulting in a blood clot that is either released into the circulation or resides in place, thereby impeding blood flow to organs and tissues. In contrast, stable plaques tend to be asymptomatic, and over time they progressively occlude blood flow.

Cardiovascular Disease Risk Factors

The major modifiable and nonmodifiable CVD risk factors are presented in Table 1. There are other CVD risk factors that

Table 1 Modifiable and nonmodifiable risk factors for atherosclerosis

Modifiable	*Nonmodifiable*
Overweight/obesity	Age
Abnormal lipid/lipoproteins (elevated low-density lipoprotein cholesterol and triglyceride; low HDL-C)	Sex
Hypertension	Family history/genetics
Elevated blood glucose	Race/ethnicity
Endothelial dysfunction	
Chronic inflammation	
Cigarette smoking	
Physical inactivity	

have been identified recently (referred to as emerging CVD risk factors). Targeting these risk factors is central to reducing CVD.

Overweight and obesity are defined as a BMI of 25–29.9 kg m^{-2} and >30 kg m^{-2}, respectively. The optimum total cholesterol level is <200 mg dl^{-1} and the optimum LDL-C is<100 mg dl^{-1}, and less than 70 mg dl^{-1} for individuals with coronary disease. A high triglyceride (TG) level is ≥150 md dl^{-1}. Hypertension is defined as a blood pressure (BP) of greater than 140/90 mm Hg, and prehypertension is defined as a BP of 120–129/80–89 mm Hg. An elevated blood glucose level is >100 mg dl^{-1}. Having any one of these CVD risk factors increases the risk of coronary morbidity and mortality. The presence of two or more risk factors increases risk markedly. A condition known as metabolic syndrome in which individuals have three of the five following criteria (elevated waist circumference – >40 inch for men and >35 inch for women; elevated TG; low high-density lipoprotein cholesterol (HDL-C) – <40 mg dl^{-1} for men and <50 mg dl^{-1} for women; elevated BP – >130/≥ 85 mm Hg; and elevated blood glucose – >100 mg dl^{-1}) significantly increases CVD risk. Approximately one-quarter of the world's adult population has metabolic syndrome. These individuals are at two times greater risk of a fatal heart attack and three times as likely to have a stroke as healthy individuals. Healthy lifestyle behaviors are the first line of therapy for treating modifiable CVD risk factors and are central in preventing the onset of one or more of these risk factors.

Diet Effects on Cardiovascular Disease Risk Factors

Nutrition is the cornerstone for the prevention and treatment of CVD by reducing modifiable risk factors. The Therapeutic Lifestyle Changes (TLC) Diet is the gold standard dietary intervention for decreasing LDL-C, and the Dietary Approaches to Stop Hypertension (DASH) Diet is recommended for BP lowering. The TLC Diet recommends decreasing saturated fat (<7% of calories) and cholesterol intake (<200 mg day^{-1}), controlling calories to achieve a healthy body weight and increasing physical activity. The DASH Diet is low in saturated fat, dietary cholesterol, and sodium and high in fruits and vegetables (8–10 sv day^{-1}) and low-fat dairy products (2–3 sv day^{-1}). Both diets advocate a food-based approach that integrates all nutrient recommendations, including sodium reduction (<1500 mg day^{-1}). Because of the cardioprotective effects of fish consumption, two servings per week (preferably fatty fish) are recommended.

Red Meat and Cardiovascular Disease

Epidemiologic studies have linked red meat consumption to increased total CVD and cancer mortality, acute myocardial infarction, and metabolic syndrome. However, a number of epidemiologic studies have not reported an association between red meat consumption and CVD. It is important to note that epidemiologic studies typically do not adequately account for fat content, meat processing, and cooking methods. These factors can modify the health effects of lean red meat (i.e., specifically, beef) in the diet. In addition, red meat as a classification group may not just contain beef but also lamb, which is significantly higher in total and saturated fat. In one study in which the saturated fatty acid (SFA) content of the red meat was taken into consideration, the increased disease risk was no longer statistically significant. In another study in which increased red meat consumption was not associated with an increase in CVD mortality, individuals with higher red or processed meat intake also had lifestyle behaviors that increased their risk of CVD and mortality.

Clinical trials have shown that the cholesterol-lowering effects of a National Cholesterol Education Program (NCEP) Step-I diet or American Heart Association (AHA) diet containing lean red meat are equivalent to diets containing poultry and fish. In these studies, hypercholesterolemic subjects consumed 4–6 oz of lean beef or lean chicken/fish as part of the NCEP Step-I diet. Importantly, subjects in the lean red meat group were more compliant with the cholesterol-lowering diet.

There is little information about the effects of incorporating lean red meat into the DASH diet. Because the DASH diet is a recommended diet for reduction of CVD risk, and because beef is a popular food, it is important to determine whether inclusion of lean beef in the DASH diet elicits the same effects on CVD risk factor response.

The Beef in an Optimal Lean Diet Study

The Beef in an Optimal Lean Diet (BOLD) Study was conducted to examine the effects of a cholesterol-lowering diet containing lean beef (BOLD 4.0 oz day^{-1} and BOLD+5.4 oz day^{-1}) on LDL-C compared with a Healthy American Diet (HAD) and a DASH diet. Because beef often is avoided by individuals following a cholesterol-lowering diet as a means of controlling saturated fat, the authors sought to evaluate a healthy dietary pattern that contained lean beef and met food-based dietary recommendations for heart health and achieved the SFA goal of <7% calories on multiple CVD risk factors.

In the BOLD Study, 36 moderately hypercholesterolemic individuals (LDL-C >110 mg dl^{-1}) completed a randomized crossover-controlled feeding study. Participants were fed four intervention diets: HAD (33% total fat, 12% SFA, 49% carbohydrate (CHO), 19% protein (PRO), and 0.7 oz beef per

day); DASH (27% total fat, 6% SFA, 50% CHO, 19% PRO, and 1.0 oz beef per day); BOLD (28% total fat, 6% SFA, 54% CHO, 19% PRO, and 4.0 oz beef per day); and BOLD+ (28% total fat, 6% SFA, 46% CHO, 28% PRO, and 5.4 oz beef per day) for five weeks with a 1-week washout between diets. This design was employed to evaluate a 'dose-response' effect of lean beef on lipids, lipoproteins, apolipoproteins, and BP.

There were significant reductions ($p < .05$) in total cholesterol (TC) and LDL-C in response to the DASH (-19.0 ± 4.3 and -14.4 ± 3.7 mg dl^{-1}), BOLD (-18.6 ± 4.1 and -13.5 ± 3.6 mg dl^{-1}), and BOLD+ (-19.6 ± 4.1 and -13.5 ± 3.8 mg dl^{-1}) diets versus the HAD (-8.5 ± 4.1 and -5.5 ± 3.9 mg dl^{-1}). The BOLD and BOLD + diets reduced HDL-C by approximately 6%, and DASH also decreased HDL-C comparably. Systolic BP (SBP) was significantly reduced following the BOLD+ diet compared with HAD (-4.24 mm Hg). Compared with the HAD, the BOLD+ diet was the only treatment diet that significantly decreased apolipoprotein B. Apolipoprotein B is the predominant protein in non-HDL particles (i.e., the atherogenic lipoprotein particles). Significant reductions were also observed for apolipoprotein A1 (the major protein in HDL), apolipoprotein C-III, and apolipoprotein C-III bound to apolipoprotein A1 particles following the BOLD and BOLD+ diets ($p < .05$). Changes in total apolipoprotein C-III in lean beef diets reflected a decrease in a subfraction (apolipoprotein C-III HS), which represents the number of apolipoprotein C-III molecules bound to apolipoprotein A1-containing particles. It has been suggested that apolipoprotein C-III bound to HDL inhibits the antiinflammatory properties of HDL. Although total apolipoprotein A1 was decreased in the BOLD and BOLD+ diets, the decrease in apolipoprotein C-III bound to apolipoprotein A1-containing particles suggested that the anti-inflammatory capacity of the apolipoprotein A1-containing particles was improved. Collectively, the test diets with lean beef had beneficial effects on lipids and lipoproteins and BP as well as potential antiinflammatory responses.

The BOLD Study demonstrated that lean beef can be included in a cholesterol-lowering diet that is low in SFA and meets contemporary food-based recommendations. This dietary pattern elicited reductions in TC and LDL-C that were equivalent to heart-healthy plant protein-rich diets, such as the DASH diet. Of note was that increasing lean beef while controlling SFA (<7% calories) did not attenuate the decrease in LDL-C. The study also showed that increasing total dietary protein by including lean beef and other animal proteins was an effective strategy for reducing BP. Thus, the decrease in two major risk factors for CVD would be expected to correspond to a lower overall CVD risk in response to a heart-healthy diet that includes lean beef.

The Role of Lean Beef in a Healthy Diet

The 2010 Dietary Guidelines for Americans recommend three healthy, food-based dietary patterns that meet food-based and nutrient recommendations (Table 2). A variety of protein foods is recommended, including lean meat; poultry; eggs; fish/seafood; beans and peas; and nuts, seeds, and soy products. The US Department of Agriculture (USDA) Food Patterns recommends 1.8 oz lean meat daily. Including lean beef in a lipid-lowering, heart-healthy diet plan contributes to the intake of essential nutrients, such as iron, zinc, and B-vitamins. Specifically, beef is the number one food source of zinc in the diet, providing 39% of the daily value (DV) per 3-oz portion. It is also an excellent source of vitamin B_{12} (37% of the DV per 3-oz serving) and selenium (24% of the DV per 3-oz serving) and a good source of vitamin B6 (16% of the DV per 3-oz serving) and iron (14% of the DV per 3-oz serving). Compared with other protein sources, it would take 13.5 3-oz servings of salmon (2363 kcal), 7.5 3-oz servings of chicken breast (1050 kcal), 4.5 3-oz servings of tuna (491 kcal), or 6 tbsp peanut butter (570 kcal) to match the zinc, vitamin B_{12}, riboflavin, and protein in one 3-oz serving of lean beef, respectively.

Lean beef contains less than 10 g total fat, no more than 4.5 g of SFA, and less than 95 mg of cholesterol per 3-oz serving, a nutrition profile that readily fits into a heart-healthy diet. Based on the dietary fat recommendations of the AHA, individuals following a 2000-cal diet are advised to consume between 56 g and 78 g of total fat and 16 g or less of SFA. A 3-oz serving of lean cooked beef contains amounts of total fat and SFA well below these limits and cholesterol levels well below the AHA recommendation of <300 mg day^{-1}. There are currently 29 cuts of beef that meet the USDA's guidelines to be labeled lean. To select a lean cut, choose those with Loin or Round in the name labeled as Select or Choice. Some examples are listed in Table 3. Portion control and cooking technique are also important to control calories, total fat, and saturated fat.

Like most other foods containing fat, beef comprises a variety of fatty acids. Despite the common reference to animal fats as 'saturated,' less than half of all fatty acids in meat fat are saturated. For example, in lean beef, 54% of the fatty acids are monounsaturated (MUFA) or polyunsaturated (PUFA). In addition, one-third of the SFA in beef is stearic acid, which has a neutral effect on blood cholesterol levels unlike the other long-chain SFAs, which increase LDL-C.

The American Council on Science and Health concludes that 'lean beef, in moderate servings, fits well in a heart-healthy diet.' As a naturally nutrient-rich source of many nutrients, including high-quality protein, iron, zinc, and many B-vitamins, incorporating lean beef in the diet can help individuals meet their nutrient needs, as well as provide variety and flexibility in their diet, which may improve long-term adherence to a healthy dietary pattern.

Understanding Meat Labeling

According to a US Department of Agriculture Food Safety and Inspection Service (FSIS) ruling, nutrition labels are required on all single-ingredient raw meat and poultry products, including ground meats, sold in supermarkets. This information will assist individuals in meeting the 2010 Dietary Guidelines recommendations to include lean meat and poultry products as part of a healthy balanced diet. The meat and poultry industry has long awaited this ruling, which will now highlight the 29 cuts of beef, pork, and lamb that are considered lean.

Table 2 Eating pattern comparison: Usual US intake, Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and US Department of Agriculture (USDA) Food Patterns, average daily intake at or adjusted to a 2000 cal level

Pattern	*Usual US intake (adults)*[a]	*Mediterranean Patterns*[b] (Greece (G) Spain (S))	*DASH*[b]	*USDA Food Patterns*
Food groups				
Vegetables: *total* (*c*)	1.6	1.2 (S) – 4.1 (G)	2.1	2.5
Dark green (c)	0.1	nd[c]	nd	0.2
Beans and peas (c)	0.1	<0.1 (G) – 0.4 (S)	See protein foods	0.2
Red and orange (c)	0.4	nd	nd	0.8
Other (c)	0.5	nd	nd	0.6
Starchy (c)	0.5	nd – 0.6 (G)	nd	0.7
Fruit and juices (*c*)	1.0	1.4 (S) – 2.5 (G) (including nuts)	2.5	2.0
Grains: *total* (*oz*)	6.4	2.0 (S) – 5.4 (G)	7.3	6.0
Whole grains (oz)	0.6	nd	3.9	≥ 3.0
Milk and milk products (*dairy products*) (*c*)	1.5	1.0 (G) – 2.1 (S)	2.6	3.0
Protein Foods:				
Meat (oz)	2.5	3.5 (G) – 3.6 (S) (including poultry)	1.4	1.8
Poultry (oz)	1.2	nd	1.7	1.5
Eggs (oz)	0.4	nd – 1.9 (S)	nd	0.4
Fish/seafood (oz)	0.5	0.8 (G) – 2.4 (S)	1.4	1.2
Beans and peas (oz)	See vegetables	See vegetables	0.4 (0.1 c)	See vegetables
Nuts, seeds, and soy products (oz)	0.5	See fruits	0.9	0.6
Oils (*g*)	18	19 (S) – 40 (G)	25	27
Solid fats (*g*)	43	nd	nd	16[d]
Added sugars (*g*)	79	nd – 24 (G)	12	32[d]
Alcohol (*g*)	9.9	7.1 (S) – 7.9 (G)	nd	nd[e]

[a]US Department of Agriculture, Agricultural Research Service and US Department of Health and Human Services, Centers for Disease Control and Prevention. What we eat in America, NHANES 2001–2004, 1-day mean intakes for adult males and females, adjusted to 2000 cal and averaged.
[b]See the 2010 DGAC Report for additional information and references available at: http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm (accessed 28.10.13).
[c]nd, Not determined.
[d]Amounts of solid fats and added sugars are examples only of how calories from solid fats and added sugars in the USDA Food Patterns could be divided.
[e]In the USDA Food Patterns, some of the calories assigned to limits for solid fats and added sugars may be used for alcohol consumption instead.
Source: Reproduced from the US Department of Agriculture and US Department of Health and Human Services, 2010. Dietary Guidelines for Americans, 2010. seventh ed. Washington, DC: US Government Printing Office. Available at: http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm (accessed 28.10.13).

Table 3 Fat and cholesterol content per 3-ounce cooked serving of lean[a] beef

	Total fat (g)	*Saturated fat (g)*	*Cholesterol (g)*
Eye round roast and steak[b]	4.0	1.4	65
Top round roast and steak[b]	4.6	1.6	71
Bottom round roast and steak[b]	4.9	1.7	65
Top sirloin steak	5.0	1.9	67
93% lean ground beef	6.8	2.9	67
Chuck shoulder steak	5.0	2.1	70
Top loin (strip) steak	6.0	2.3	69
Flank steak	6.3	2.6	66
Tenderloin roast and steak[b]	6.7	2.5	69

[a]Lean: Less than 10 g of total fat, 4.5 g or less of saturated fat, and less than 95 mg of cholesterol per serving and per 100 g.
[b]Cuts combined.
Source: Reproduced from National Cattlemen's Beef Association Beef Facts. Available at: http://www.beefnutrition.org/CMDocs/BeefNutrition/Updated%20Materials/Lean%20Beef/ManyOfAmericasFavoriteCutsAreLeanWebFinal.pdf (accessed 28.10.13).

Commonly approved claims to be aware of when purchasing meat and poultry include: raised without added hormones, raised without antibiotics, not fed animal by-products, free range, free roaming, grass fed, corn fed, grain fed, and certified organic (by certifying entity).

According to the FSIS, hormones are only approved for use in beef cattle and lamb production. They are not approved for use in poultry, hogs, veal calves, or exotic, nonamenable species. Therefore, the phrase 'no hormones administered' on a chicken label cannot be approved unless it is followed (directly) with the statement 'Federal regulations prohibit the use of hormones in poultry.'

Antibiotics may be given to prevent or treat disease in cattle. A washout period is required from the time antibiotics are administered until it is legal to slaughter the animal so that any residue can exit the animal's system. The FSIS randomly samples cattle at slaughter and tests for residues. Animal-raising claims, such as these, may be approved for labeling if the animal production information submitted with the label application supports the claims being made and the claim is truthful and not misleading.

Thus far, the USDA has not adopted an official definition of grass-fed beef. There are two terms, often used interchangeably, that are found on labels: grass-fed beef and grass-finished beef. Grass-fed beef generally means beef from cattle that have eaten only grass or forage throughout their lives; however, some producers market their beef as grass fed but then finish the animals on grain for the last 90–160 days before slaughter. A more specific definition is grass-finished beef. Finishing is another term for the time that cattle grow to market weight during the last few months before processing. Typically, feed lots finish cattle for 90–160 days on a total mixed ration (TMR), whereas grass-finished cattle are fed grass only, until they are processed.

Most beef cattle raised in a contemporary production system, as well as most organic and natural beef, are finished with a TMR. The only distinction between organic or natural is that the grain is certified organic or natural. Although some grass-fed beef is organic, not all organic beef is grass fed or finished. In fact, many of the organic and natural products are TMR fed, in feedlots; it is just that the ration that is fed is certified organic or natural.

Can the Composition of Meat be Modified?

Feeding practices implemented can modify the total fat and fatty acid profile of meat from ruminant and nonruminant animals. For ruminants, the change is less than for non ruminants. Grass-fed beef has a lower total fat content compared with conventionally fed cattle. In addition, it is higher in conjugated linoleic acid (CLA), *trans* vaccenic acid (a precursor of CLA), and long-chain omega-3 fatty acids. The total SFA content is similar; however, grass-fed beef is higher in stearic acid (a cholesterol-neutral SFA) and lower in myristic and palmitic SFA (hypercholesterolemic fatty acids). Conventionally fed beef is higher in total fat, which reflects a higher content of monounsaturated fat. Because of the lower fat content, appropriate cooking techniques are necessary for grass-fed beef. For nonruminant animals (such as pigs and chickens), the fatty acid content and profile of the meat reflect diet composition more closely. Consequently, feeding techniques have been used to increase PUFA and MUFA and decrease SFA content specifically to help consumers to meet current dietary guidelines.

Obesity and Weight-Reducing Diets

Obesity is a complex physiological state and the underlying etiology is poorly understood. Obesity and overweight are related to the amount of body fat (i.e., adipose tissue). The two important adipose tissue depots are subcutaneous (located beneath the skin) and visceral (around internal organs). The distribution of adipose tissue is dependent on many factors, including sex, age, race, ethnicity, genotype, diet, physical activity, hormone levels, and medication. Although both subcutaneous and visceral fat are important, visceral adiposity has been the focus of attention because of its association with multiple comorbid conditions, such as impaired glucose and lipid metabolism, insulin resistance metabolic syndrome, CVD, and several malignancies, including prostate, breast, and colorectal cancers.

Given the ongoing obesity epidemic, there is interest among the general public and health professionals in effective treatment strategies. For the most part, weight loss programs tend not to be successful over the long term, although there are many 'success stories.' Nonetheless, there is great interest in identifying effective weight loss programs. There are countless diet programs available, many of which are fad diets or lack scientific support for efficacy. A key focus of different weight loss diets has been the macronutrient profile. A popular strategy over the years has been to decrease carbohydrates and increase protein (and also fat). In these diets, meat has been a focal point. Another popular strategy is a very low-fat diet. As noted in the Dietary Guidelines Advisory Committee 2010 Report, there are no differences in weight loss with differing macronutrient proportions, if diets are followed for longer than 6 months. In shorter term studies, high-protein diets tend to result in greater weight loss than low-fat diets; however, these differences are not sustained over time. Moreover, with diets that restrict many foods and food groups, adherence is a problem.

In summary, there is no optimal proportion of dietary fat, carbohydrate, and protein that is recommended to maintain a healthy body weight, to lose weight, or to avoid weight regain after weight loss. Thus, lean beef can be part of a healthy weight loss diet. Of importance are the total calories consumed and the nutrient quality of the diet. The three eating patterns (i.e., Mediterranean, DASH, and USDA Food Patterns) presented in **Table 2** all offer a healthy food-based strategy that can include lean beef to achieve weight loss and maintenance of reduced body weight.

Conclusion

CVD and obesity continue to be major public health problems. Both can be prevented and treated with healthy dietary practices. Healthy eating patterns include the Mediterranean

Diet, DASH Diet, and USDA Food Patterns. Research has shown that lean beef can be incorporated into each of these eating patterns and confer many health benefits, especially for CVD risk reduction and weight control. Lean beef is a nutrient-dense food that facilitates the achievement of nutrient adequacy. Advances in beef production and available consumer information have made it easier to procure lean beef and to enjoy it in a heart-healthy diet that is designed to achieve and maintain a healthy body weight.

See also: Human Nutrition: Macronutrients in Meat; Meat and Human Diet: Facts and Myths; Micronutrients in Meat

Further Reading

American Heart Association Nutrition Committee, Lichtenstein, A.H., Appel, L.J., Brands, M., *et al.*, 2006. Diet and lifestyle recommendations revision 2006: A scientific statement from the American Heart Association Nutrition Committee. Circulation 114, 82–96.

Appel, L.J., Brands, M.W., Daniels, S.R., *et al.*, 2006. American Heart Association. Dietary approaches to prevent and treat hypertension: A scientific statement from the American Heart Association. Hypertension 47 (2), 296–308.

Blumenthal, R., Foody, J., Wong, N.D., 2011. Preventive Cardiology: Companion to Braunwald's Heart Disease: Expert Consult. Philadelphia, Pennsylvania: Saunders, Elsevier Inc.

Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A., Larson, S., 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9, 10.

Gilmore, L.A., Walzem, R.L., Crouse, S.F., *et al.*, 2011. Consumption of high-oleic acid ground beef increases HDL-cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. Journal of Nutrition 141 (6), 1188–1194.

Lloyd-Jones, D.M., Hong, Y., Labarthe, D., *et al.*, 2010. American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: The American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation 121, 586–613.

Roussell, M.A., Hill, A.M., Gaugler, T.L., *et al.*, 2012. Beef in an Optimal Lean Diet study: Effects on lipids, lipoproteins, and apolipoproteins. American Journal of Clinical Nutrition 95 (1), 9–16.

Sacks, F.M., Bray, G.A., Carey, V.J., *et al.*, 2009. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. New England Journal of Medicine 360 (9), 859–873.

Sacks, F.M., Svetkey, L.P., Vollmer, W.M., *et al.*, 2001. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. New England Journal of Medicine 344 (1), 3–10.

US Department of Agriculture and US Department of Health and Human Services, 2010. Dietary Guidelines for Americans, 2010, seventh ed. Washington, DC: US Government Printing Office. Available at: http://www.cnpp.usda.gov/DGAs2010-DGACReport.htm (accessed 28.10.13).

Van Horn, L., McCoin, M., Kris-Etherton, P.M., *et al.*, 2008. The evidence for dietary prevention and treatment of cardiovascular disease. Journal of the American Dietetic Association 108, 287–331.

Relevant Websites

http://www.choosemyplate.gov/supertracker-tools.html
United State Department of Agriculture.

http://www.fsis.usda.gov/Fact_Sheets/Meat_&_Poultry_Labeling_Terms/index.asp
United State Department of Agriculture.

http://www.who.int/mediacentre/factsheets/fs311/en/index.html
WHO.

http://www.who.int/dietphysicalactivity/diet/en/index.html
WHO.

http://www.who.int/topics/chronic_diseases/en/
WHO.

Macronutrients in Meat

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Glossary

α-Linolenic acid Long-chain polyunsaturated fatty acid.
Monogastric Animals having a stomach with one compartment.
***n*-3 Polyunsaturates** The first double bond appears at the third carbon–carbon bond from *n*-terminal.
***n*-6 Polyunsaturates** The first double bond appears at the sixth carbon–carbon bond from *n*-terminal.
Oleic acid Long-chain monosaturated fatty acid.
Palmitic acid Long-chain saturated fatty acid.
Phospholipids Phosphorus-containing lipid.
Ruminant Animals having a stomach with four compartments (rumen, reticulum, omasum, and abomasum).
Saturated fatty acid Fatty acid with no unsaturated bonds.
Stearic acid Long-chain saturated fatty acid.
Trans fatty acids Trans-isomer (E-isomer) fatty acids.

Introduction

The macronutrients of foods, proteins, lipids, and carbohydrates are the major components that provide essential nutrients for growth, body maintenance, and other body functions. That is, they supply energy through metabolism and provide essential chemical subunits such as amino acids, fatty acids, phospholipids, and sugars.

Boneless meat is composed of skeletal muscle fibers, connective tissue, and adipose tissues and has a water content of approximately 70–75%. It is these main cellular and extracellular components that contribute to the macronutrients of meat.

Macronutrient Content of Meat

Unlike with most food types where, within a food, the composition is quite similar, for example, eggs, the macronutrient content of meat can be highly variable because of different fat contents of the individual meat cuts, different animal species, effects of animal age, or feeding regimen. Because lean muscle cells have moisture contents of approximately 70 g/100 g, compared with just 10 g/100 g for adipose tissues, as more adipose tissue is deposited in a lean muscle, the moisture content decreases markedly as the fat content increases. In fact, the relationship between fat and moisture content is so strongly negative that it is possible to estimate fat content of meat based on its measured water content. In addition to changes in moisture content, for a given weight of meat, an increase in fatness also leads to a small reduction in protein contents because muscle cells are the main providers of protein. In lean raw meat, the protein content may be 25 g/100 g but this may be reduced to less than 20 g/100 g in more fatty meat.

Although in nutritional terms the content of carbohydrates is very low in meat (~1 g/100 g), it does play an important role in the conversion of muscle to meat, with postmortem metabolism of glycogen enabling the meat to attain an ultimate pH of 5.5–5.7. However, carbohydrates in meat are usually not considered in terms of overall energy contribution, except perhaps where a cereal has been added to a processed meat product. In this article, only raw, whole muscle foods are considered. The important macronutrients of meat are, therefore, proteins and lipids.

Meat Consumption and Macronutrients in Meat from Various Countries

The per capita consumption of meat is largely dependent on the level of a country's socio-economic development, with developed countries usually being higher consumers. Meat consumption, particularly the type of meat, is also affected by religious and ethical beliefs. In certain countries, such as in Africa and Central and South America, pork consumption may be depressed due to fears of trichinosis infestation. Table 1 shows meat consumption data for the main meat species for some of the major meat-consuming countries. Countries such as Argentina, Australia, and Brazil tend to consume a greater percentage of beef compared with other meats whereas European and Asian countries consume more pork. In some countries, the consumption of chicken meats has continued to increase over the past 30 years at the expense of red meats.

In line with previous findings from the UK, the fat content in retail cuts of meat for most meat types has continued to fall. For example, in Australia, the fat content of pork loin chops decreased from 31% in 1984 to 9% in 2005, and in the same period, lean meat increased from 55% to 75%, largely as a result of consumer demand for leaner meat products in line with current health recommendations. This has been achieved through genetics, selective breeding, feed types, weight at slaughter, and level of trim.

The macronutrient composition of relatively lean meats from various species obtained from a number of developed countries is presented in Table 2. Lean raw meat cuts were chosen to avoid the huge variation in fat contents that can exist in some muscles/meat cuts, which could have been confusing and induced large variations in energy, protein, and moisture

Table 1 Meat consumption in selected countries (kg/capita)

Country	*Beef and veal*[a]	*Pork*[a]	*Broiler chickens*[a]	*Total meats*[b]
Argentina	55	7	37	91
Australia	36	22	36	123
Brazil	38	13	49	80
Canada	30	23	29	98
China	4	36	10	53
European Union 27	15	40	18	85
Hong Kong	20	69	38	
Japan	10	20	13	46
Russian Federation	18	20	22	61
South Africa	14	4	33	49
United Arab Emirates	21	–	71	85
USA	35	27	43	123

[a]*Source*: USDA, estimate for 2012.

[b]2007 data from Food and Agriculture Organization of the United Nations (FAO) 2010, Livestock and Fish Primary equivalent, 02 June 2010, Available at: http://faostat.fao.org/site/291/default.aspx (accessed 31.10.13).

contents. Instead, a selection of meats having higher fat contents is presented separately in Table 3. It is likely, however, that differences observed in fatness between the meat from various countries only reflects differences in the mean slaughter weights of the animals in those countries and the level of trim. However, for the majority of the lean cuts, where the fat content was less than approximately 6 g/100 g raw meat, the energy content ranged from approximately 450–600 kJ/100 g. In the UK, this equates to approximately 17–18% of the daily energy intake for adults between 19 and 64 years.

Of all the lean meats selected, beef steak/fillet from each country consistently had the highest, but modest, fat content (4.0–8.3 g/100 g). Beef round steak (from leg) was generally very lean. The lean meat originating from Australia, Canada, and the UK (1.7–2.7 g/100 g) was lowest in fat, possibly reflecting the predominant pasture grazing systems used in these countries. The content of fat in lamb steak/fillet was in a similar range to that found for comparable cuts of beef (3.7–6.0 g/100 g). Over the past 20–30 years, there has been a move toward lowering the fat content through breeding and production, and this has been particularly effective in pork; although perhaps at the expense of optimal eating quality. With some exceptions, it is apparent (Table 2) that pork loin/fillet is very lean (mainly approximately 1–2 g fat/100 g), somewhat lower than that observed for beef and lamb fillet.

The white breast meats from chicken and turkey were consistently very low in fat irrespective of country of origin. Skinless breast meat contained approximately 1–3 g fat/100 g whereas with skin-on breast meat, the fat content increased to 6–7 g/100 g meat.

The preceding discussion shows that lean meat is a high protein, low fat food; however, the fat and energy content of meat can be very high depending on the production process, meat cut, and extent of fat trimming. Table 3 shows the macronutrient contents in a selection of meat cuts with high fat content from a number of species.

Meat Proteins

Raw muscle contains approximately 20–25 g protein/100 g meat, which with loss of moisture and fat on cooking may attain a value of 28–36 g/100 g cooked meat. Protein from muscle tissue is an excellent source of essential amino acids and it has a high net protein utilization (NPU 0.75–0.8) (FOA/WHO 1985) and high digestibility. The high content of lysine in particular makes animal proteins attractive compared to cereal proteins. The nutritional quality of protein derived from connective tissue is somewhat lower than that from muscle tissue, although as it is present at only approximately 1–2 g/100 g meat, it is of little concern nutritionally. The main protein components of connective tissue, collagen, and elastin have lesser contents of the sulfur-containing amino acids and are, therefore, regarded as inferior. Further, because of their structure, they are less heat-susceptible to breakdown during heating and digestion.

Although it is the macronutrients of meat that contribute to energy intake, the physical/chemical forms of the meat as consumed affect the net energy intake. The energy required to digest raw or rare meat appears to be greater than that required when meat has been well done or over cooked. In controlled studies with pythons fed raw and cooked meat, it was determined that oxygen consumption was greater when the pythons had been fed raw meat, suggesting that there had been greater energy expenditure during the digestion process. Similarly there were differences in oxygen consumption dependent on whether the meat was intact or ground. Thus the degree of cooking and/or processing may be a factor to consider in terms of overall macronutrient content with respect to energy contribution.

The effect of cooking on the nutritional quality of proteins may also be important in some situations. Generally, this is not an issue at lower cooking temperatures but at higher temperatures ($>100\,^{\circ}C$) it is possible that some essential amino acids, such as lysine may interact with other chemical components (sugars) and become unavailable following digestion. Under similar cooking conditions it is also possible for the sulfur-containing amino acids to become modified and, therefore, nutritionally unavailable.

Humans require approximately 0.8 g protein kg^{-1} body weight day^{-1} to maintain skeletal muscle and organ tissue turnover. Thus a 70 kg person requires approximately 56 g of protein daily. Given the average consumption of meat for a

Table 2 Macronutrients, energy, and cholesterol contents of selected trimmed, raw lean meats sourced from various countries[a]

	Energy (kJ)	*Moisture (g per 100 g)*	*Protein (g per 100 g)*	*Fat (g per 100 g)*	*Cholesterol (mg per 100 g)*
Beef steak/fillet					
Australia	608	72.2	22.0	6.3	58
Canada	631	70.3	22.0	6.4	50
Denmark	594	72.7	20.0	6.4	58
Germany	508	73.4	21.2	4.0	100[b]
UK	586	72.3	21.2	6.1	61
USA	684	68.7	20.1	8.3	59
Beef round steak					
Australia	420	72.5	21.0	21.7	62
Canada	502	73.9	23.3	2.1	47
Denmark	606	71.0	21.5	6.5	63
Germany	519	73.4	20.9	4.4	50
UK	491	72.8	23.0	2.7	50
USA	642	72.0	20.5	7.0	62
Lamb steak/fillet					
Australia	582	73.0	21.7	5.8	62
Canada	544	73.3	21.2	4.4	80
Germany – muscles	491	74.3	20.8	3.7	63
USA	608	72.6	20.9	6.0	66
Pork loin/fillet					
Australia	478	74.3	23.3	2.2	46
Canada	598	72.2	21.4	5.7	59
Denmark	448	74.7	22.2	1.9	61
Germany	448	74.8	22.0	2.0	55
Japan	845	65.7	21.1	11.9	61
UK (steak)	507	73.9	22.4	3.4	62
USA	463	76.0	21.0	2.2	65
Chicken breast					
Australia	438	75.0	22.3	1.6	59
Canada	468	74.8	22.7	1.6	58
Denmark (skin)	621	70.0	21.5	6.9	64
Germany (skin)	607	70.3	22.2	6.2	62
Japan	506	72.8	24.4	1.9	73
UK (light meat)	449	74.2	24.0	1.1	70
USA	485	75.8	21.2	2.6	64
Turkey breast/lean					
Australia	490	73.0	21.6	3.3	45
Canada	464	75.1	23.4	1.2	57
Germany	446	73.7	24.1	0.99	44
Japan	444	74.6	23.5	0.7	62
UK (light meat)	444	74.9	24.4	0.8	57
USA	471	74.1	24.6	0.7	62

[a]Australia: FSANZ, NUTTAB (2010); Canada: Health Canada, Canadian Nutrient File (2009); Denmark: © Danish Food Composition Databank, version 7.0 (2008); Japan: Standard Tables of Food Composition in Japan. Food Composition Database, Sugiyama Jogakuen University (2004); UK: McCance and Widdowson's, The Composition of Foods Integrated Dataset (CoF IDS); USA: U.S. Department of Agriculture, Agricultural Research Service. 2011. USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page, Available at: http://www.ars.usda.gov/ba/bhnrc/ndl (accessed 31.10.13).
[b]High value possibly result of different methodology.

number of developed countries is in the range 140–300 g day^{-1}, this would equate to 28–65 g protein day^{-1}, thus accounting for the majority of the daily protein requirements. However, in individuals where there has been muscular inactivity as a consequence of an illness or trauma, a loss of muscle mass and muscle weakness usually results, and this is known as sarcopenia. This is particularly an issue with the aged population where the rate of muscle loss is believed to reach approximately 1% per year. A recent study has shown that, compared with an iso-nitrogenous amount of soy protein, beef protein was more effective in synthesizing myofibrillar proteins when fed to middle-aged men, either when at rest or after a resistance exercise. This is thought to result from relative differences in rates of protein digestion, as well as dietary meat proteins supplying the specific amino acids required for myofibrillar protein synthesis.

Table 3 Macronutrients, energy, and cholesterol contents of selected untrimmed, raw meats from various countries

	Energy (kJ)	*Moisture (g per 100 g)*	*Protein (g per 100 g)*	*Fat (g per 100 g)*	*Cholesterol (mg per 100 g)*
Beef rump[a]	815	65.7	18.9	13.3	62
Beef loin[b]	1385	56.4	16.8	27.5	78
Beef loin[c]	1046	61.7	19.8	17.9	85
Lamb leg[a]	786	66.5	19.4	12.3	69
Lamb loin chop[a]	1276	52.1	22.0	24.4	69
Pork loin[b]	904	64.6	20.6	13.6	62

[a]Australia: FSANZ, NUTTAB (2010).
[b]Japan: Standard Tables of Food Composition in Japan. Food Composition Database, Sugiyama Jogakuen University (2004).
[c]USA: USDA, National Nutritional Database for Standard Reference, Release 24.

Meat Lipids

Lipids in meat are located predominantly in fat cells (adipocytes) in a number of cellular sites, including subcutaneous (under skin), intermuscular (between muscles), and intramuscular (within muscles). Small lipid droplets are also located within muscle cells and are more prevalent in red muscles than white muscles.

Lipids in meat consist primarily of triacylglycerols (TAG) (mainly located in adipocytes) and phospholipids (in muscle and adipocyte cell membranes), with lesser amounts of cholesterol (in cell membranes and adipocytes). The lipid content of meat can vary widely depending on the fatness of the animal and the fat content of the actual meat cut and extent of trimming. Very lean meat may contain just 1 g fat/100 g meat, but for optimal eating satisfaction, a lipid content in the range of 4–7 g fat/100 g meat has been suggested. Extremely highly marbled and expensive meats from Wagyu beef may approach 40 g fat/100 g meat. Fat as a macronutrient is also important as it is a carrier for, and an aid in the absorption of, a variety of fat-soluble vitamins and various biologically active components such as carotenoids.

As the fat content of meat increases, the proportion of TAG (neutral lipids) increases and phospholipids decreases (**Figure 1(a)**). Because most lipids present in very lean meat are located in cell membranes, the total lipids consist primarily of phospholipids, and thus contain a relatively high percentage of polyunsaturated fatty acids (PUFA) (**Figure 1(b)**). As the fat content increases, the level of polyunsaturation decreases, being replaced with increased amounts of saturated and monounsaturated fatty acids.

Compared with other lipid fractions, cholesterol comprises only a very small part of the total fat content. Independent of meat species and country of origin (**Table 2**), the cholesterol content for lean meats was predominantly in the range of 50–60 mg/100 g raw meat. Thus the contribution of a lean meat meal portion to the total suggested maximum daily intake of cholesterol (200–300 mg) is approximately 20–30%.

The composition of fatty acids of meat is important in terms of its contribution to overall nutrition. Given current health recommendations that fat intake should be reduced to less than 30% of total energy intake, the PUFA:saturated ratio should be increased to above 0.4 and that the ratio of *n*-6:*n*-3 PUFA should be less than 4, it is possible that animal diets can be manipulated and meats can be selected to achieve these more favorable fatty acid compositions. However, apart from their role in nutrition, fatty acid composition is also important in terms of meat quality, with different fatty acid compositions affecting fat hardness/softness. This has implications for the following:

- carcase boning (hard carcase fat at chiller temperatures is difficult to bone and carcases are sometimes warmed to soften fat for ease of cutting – negative impact of meat quality),
- functionality in processed meats,
- visualization of marbling for chilled assessment (saturated fats are more visually evident),
- carcase fat color (yellow pigment is less evident with higher percentage of saturated fatty acids as fat tissue is more opaque at chiller temperatures), and
- susceptibility of the meat to oxidize, leading to meat color and flavor deterioration.

At a given level of meat fatness, the composition of TAG can affect its cooking and eating properties. This is very much dependent on the specific fatty acid composition of individual TAG and on the animal species from which the meat was obtained. Not only are the melting properties of the fat affected by the level of fatty acid unsaturation, but also by the individual fatty acids' positions in the TAG molecule (*sn*-1, *sn*-2, or *sn*-3). In beef fat, as with other ruminants, the *sn*-2 position is predominantly occupied by monounsaturated fatty acids whereas in the monogastric pig, the *sn*-2 position contains mainly saturated fatty acids. Such different configurations result in large differences in melting properties of TAG having the same fatty acid compositions. Placement of stearic acid, a saturated fatty acid, in the outer position of TAG can increase lipid melting points by as much as 10 °C relative to TAG with stearic acid solely in the *sn*-2 position.

The positional distribution of fatty acids in TAG also affects the absorption process in the small intestine and the resynthesis of absorbed lipids into TAG and phospholipids, and therefore can impact on their nutrient value. Before absorption, TAG is subjected to pancreatic lipase, which results in fatty acids being hydrolyzed from the *sn*-1 and *sn*-3 positions leaving the *sn*-2-monoacylglycerol intact. This *sn*-2-monoacylglycerol is readily absorbed by passive diffusion into the enterocyte where it is resynthesized into TAG or phospholipids and then transported to other sites as chylomicrons. Thus, the stereospecific structure of the ingested TAG is retained. However, for the long-chain fatty acids from the other two positions, a protein-mediated transport process is required which is somewhat slower than the passive diffusion process. Again, once absorbed by the

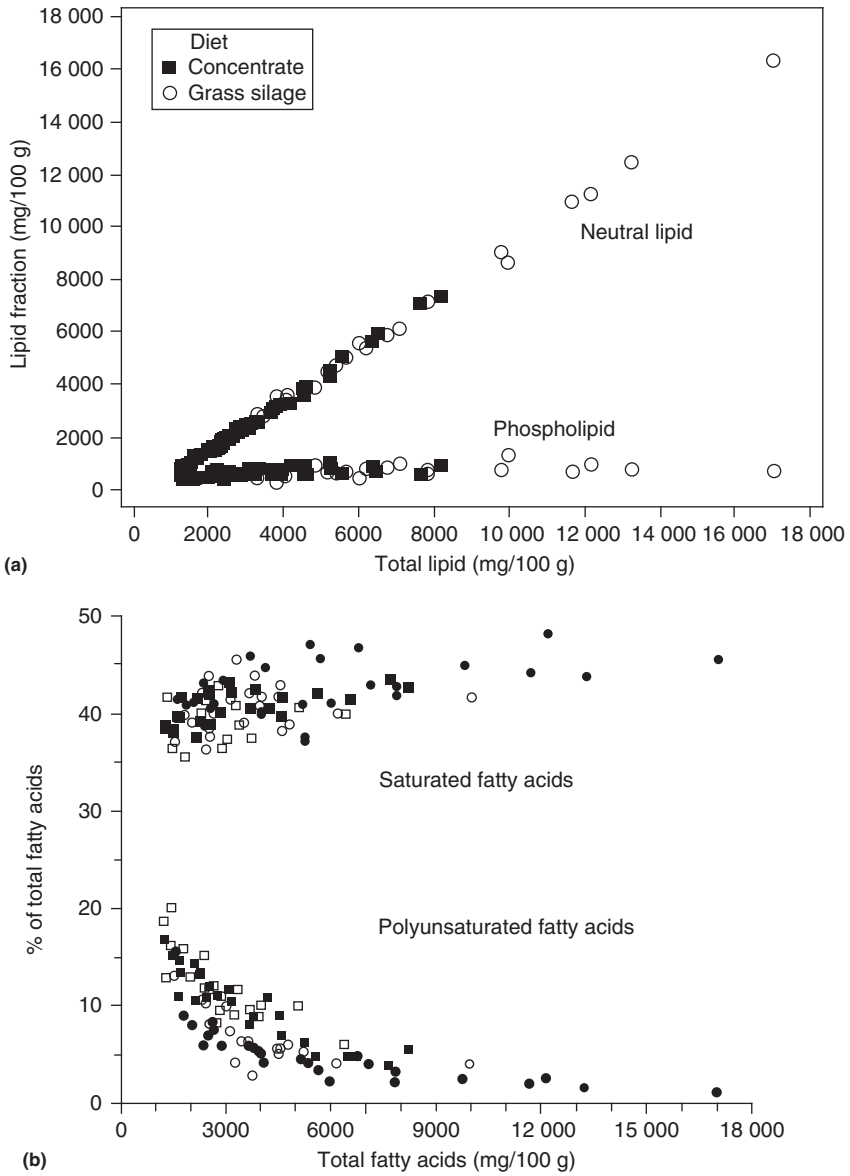

Figure 1 Lipid fractions (a) and fatty acid compositions (b) of beef *longissimus* muscles with increasing levels of fatness. Adapted from Wood, J.D., Enser, M., Fischer, A.V., *et al.*, 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Science 78, 343–358; and Warren, H.E., Scollan, N.D., Enser, M., *et al.*, 2008. Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. Meat Science 78, 245–269.

enterocyte, the fatty acids are reassembled into TAG but without retaining any regio- or stereo-specific structure.

Fatty Acid Composition of Meat

The fatty acid composition of meat adipose tissues from monogastric animals such as pigs and chickens can be highly variable as it is largely dependent on the feeds consumed. Inclusion of polyunsaturated oilseeds in feeds has been shown to result in high contents of polyunsaturated lipids in muscles and adipose tissues. Meat quality can then be affected as a result of a more rapid development of rancidity during chilled storage and other processing treatments. For ruminants, such as cattle and sheep, the composition is likely to be more constant among individuals because dietary unsaturated fatty acids are largely hydrogenated by microorganisms in the rumen before their absorption from the intestine. In ruminants, a significant amount of fatty acids is produced by *de novo* synthesis with palmitic acid being the end product of fatty acid synthase and stearic acid then produced by an elongase. This saturated fatty acid can then be desaturated by a Δ9-desaturase to produce the monounsaturated oleic acid, which is present in high amounts in adipose tissues. The extent to which this occurs depends on the genetic expression of stearoyl-CoA desaturase and it is this activity that largely accounts for the significant differences in unsaturation that exist among ruminant species and also among breeds. An outstanding

example of high desaturase activities and high levels of monounsaturated fatty acids in ruminant subcutaneous and intermuscular fat is that observed in Japanese Wagyu cattle where oleic and palmitoleic acids can account for more than 55–60% of the total fatty acids.

The major fatty acids present in meat (muscle and fat) from various species are indicated in Table 4. Irrespective of whether the meat is from monogastrics or from ruminants, palmitic and stearic are the main saturates and oleic the predominant monounsaturate. The percentage of the other fatty acids is generally low, except for some polyunsaturates, particularly linoleic, which vary markedly between ruminant and non-ruminant species, and this is most obvious in adipose tissue.

The ratio of *n*-6:*n*-3 polyunsaturates in meat is largely related to animal feed, with pasture-feeding resulting in a greater uptake and incorporation of *n*-3 fatty acids such as α-linolenic acid, and grain-feeding higher proportions of *n*-6 acids such as linoleic acid. These PUFA are also precursors for the *n*-3 long-chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the *n*-6 arachidonic acid found in meat. In beef, mean ratios of 1.5 and 7.7 have been reported for meat from grass and grain-fed cattle, respectively. Overall, lean meats from all species provide a valuable source of essential long-chain PUFA, even though the amounts present are usually quite low (< 200 mg/100 g muscle).

In addition to the fatty acids mentioned, two additional groups of fatty acids of nutritional interest are commonly present in ruminant fats; *trans* fatty acids and conjugated linoleic acids (CLA). The predominant *trans* fatty acid formed is vaccenic acid (18:1 *trans*-11), which is a product of incomplete biohydrogenation of linoleic acid (18:2*n*-6) in the rumen. Unlike the *trans* isomers of fatty acids formed synthetically by chemical hydrogenation of polyunsaturated oils, and which are of concern for human consumption, the naturally formed 18:1 *trans*-11 present in ruminant fat appears to be of little concern health-wise, particularly at its concentration of approximately 1–4 g/100 g fat. CLA is also produced in the rumen and the major isomer present is *cis*-9, *trans*-11 conjugated linoleic acid that may be present in amounts of 0.5–1 g/100 g fat. The content of CLA is generally higher in meat from pasture-fed animals. CLA isomers have received considerable interest for their anticancer, anti-inflammatory properties and potential to reduce cardiovascular disease. Although CLA is a product of rumen biohydrogenation, it appears that a more important source is via the desaturation of 18:1 *trans*-11 present in adipose tissue by stearoyl CoA desaturase.

Table 4 Fatty acid composition of muscle and fat from beef, lamb, and pork loin steaks containing high amounts of adipose tissue

	Beef	*Lamb*	*Pork*
Whole steak			
Fat content[a]	15.6	30.2	21.1
Muscle[b]			
16:0 palmitic	25.0	22.2	23.2
18:0 stearic	13.4	18.1	12.2
18:1 *n*-9 oleic	36.1	32.5	32.8
18:2 *n*-6 linoleic	2.4	2.7	14.2
18:3 *n*-3 α-linolenic	0.70	1.37	0.95
20:4 *n*-6 arachidonic	0.63	0.64	2.21
20:5 *n*-3 EPA	0.28	0.45	0.31
22:6 *n*-3 DHA	0.05	0.15	0.39
Total fatty acids[c]	3.8	4.9	2.2
Polyunsaturated:saturated	0.11	0.15	0.58
n-6:*n*-3	2.11	1.32	7.22
Fat[b]			
16:0 palmitic	26.1	21.9	23.9
18:0 stearic	12.2	22.6	12.8
18:1 *n*-9 oleic	35.3	28.7	35.8
18:2 *n*-6 linoleic	1.1	1.3	14.3
18:3 *n*-3 α-linolenic	0.48	0.97	1.43
C20–C22 *n*-3 PUFA	ND[d]	ND	0.56
Total fatty acids[e]	70.0	70.6	65.3
Polyunsaturated:saturated	0.05	0.09	0.61
n-6:*n*-3	2.30	1.37	7.46

[a]Percentage of steak.
[b]Percentage of total fatty acids.
[c]g/100 g muscle.
[d]Not detectable.
[e]g/100 g fat.
Source: Reproduced from Enser, M., Hallett, K., Fursey, G.A.J., Wood, J.D., 1996. Fatty acid content and composition of English beef, lamb and pork at retail. Meat Science 42, 443–456.

Macronutrients and Oxidation

The nutritive (and functional) value of meat may be compromised by oxidation of protein and lipid components. Generation of reactive oxygen species by processes such as storage, freezing/thawing, grinding, and cooking, etc., can result in structural covalent modifications to proteins such as aggregation, which can result in reduced susceptibility to proteolytic digestive enzymes and, therefore, lead to reduced uptake of amino acids. Further, this aggregation may be enhanced indirectly by the aldehydic products of lipid oxidation that are also generated by the various processing practices employed. In addition, the nonhydrolyzed peptides are not absorbed in the intestine and then pass on into the colon where they are fermented by flora, producing a range of potentially carcinogenic compounds.

Nutritive implications for ingestion of oxidized lipids mainly relate to their toxicity. Products of lipid oxidation such as hydroperoxides are known to damage DNA, and many secondary oxidation products are potential carcinogens (e.g., malondialdehyde), whereas carbonyl compounds may affect cellular signal transduction processes. Although lean meat has low lipid content, it does contain a higher proportion of the more susceptible PUFA, but their overall level of ingestion would likely be low compared with other fatty foods. Meats having high levels of the antioxidants α-tocopherol and ascorbic acid (provided by pasture-feeding or by supplementation) are better protected from oxidation than meats with lesser amounts of these compounds.

Summary

The macronutrient content of meat is highly variable, because of the large range of fatness that is possible in animals and

muscles. However, for lean meats across a wide range of species, breeds, and muscle cuts, there is little variation in the macronutrient content with protein and water making up more than 95% of its weight. Lean meat, therefore, provides an excellent source of high nutritional-quality protein supplying all the essential amino acids. Medical sources have recommended that wherever possible, fat should be trimmed from those cuts where fat is visible, preferably before cooking. This not only reduces the overall calorific value of the meat but removes a significant amount of the saturated fats, resulting in a higher proportion of PUFA in the total fat. Although the amount of *n*-3 PUFA contributed by meat is quite small when compared with oily fish, it still remains a significant and important source of these essential fatty acids.

See also: Chemical Analysis for Specific Components: Major Meat Components. Chemical and Physical Characteristics of Meat: Adipose Tissue. Human Nutrition: Cardiovascular and Obesity Health Concerns; Meat and Human Diet: Facts and Myths; Micronutrients in Meat. On-Line Measurement of Meat Composition

Further Reading

Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A., Larson, S., 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9, 10. Available at: http://www.nutritionj.com/content/9/1/10 (accessed 31.10.13).

Enser, M., Hallett, K., Fursey, G.A.J., Wood, J.D., 1996. Fatty acid content and composition of English beef, lamb and pork at retail. Meat Science 42, 443–456.

Müller, W.J., Bruce, H.L., Tume, R.K., *et al*., 2009. National sampling and gross composition of Australian retail pork cuts 2005/2006. Meat Science 81, 626–631.

Phillips, S.M., 2012. Nutrient-rich meat proteins in offsetting age-related muscle loss. Meat Science 92, 174–178.

Sante-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., Gatellier, P., 2008. Effect of meat cooking on physicochemical state and *in vitro* digestibility of myofibrillar proteins. Journal of Agriculture and Food Chemistry 56, 1488–1494.

Smith, S.B., Yang, A., Larsen, T.W., Tume, R.K., 1998. Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. Lipids 33, 197–207.

Tume, R.K., 2004. The effects of environmental factors on fatty acid composition and the assessment of marbling in beef cattle: A review. Australian Journal of Experimental Agriculture 44, 663–668.

Warren, H.E., Scollan, N.D., Enser, M., *et al*., 2008. Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. Meat Science 78, 245–269.

Wood, J.D., Enser, M., Fischer, A.V., *et al*., 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Science 78, 343–358.

Wyness, L., Weichselbaum, E., O'Connor, A., *et al*., 2011. British Nutrition Foundation. Nutrition Bulletin 36, 34–77.

Relevant Websites

http://www.ars.usda.gov/ba/bhnrc/ndl
Agricultural Research Service, United States Department of Agriculture.

http://faostat.fao.org/site/291/default.aspx
Food and Agriculture Organization of the United Nations.

Meat and Human Diet: Facts and Myths

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Glossary

Bioactive peptide A peptide able to display a physiological function including antihypertensive, antioxidative, opioid agonistic, immunomodulatory, antimicrobial, prebiotic, mineral binding, antithrombotic, and hypocholesterolemic effects.

Carnosine (beta-alanyl-L-histidine) It is a dipeptide of the amino acids beta-alanine and histidine. Carnosine is able to scavenge reactive oxygen species as well as alpha, beta unsaturated aldehydes formed from peroxidation of cell membrane fatty acids during oxidative stress. It is highly concentrated in muscle and brain tissues.

Caseinophosphopeptides These are bioactive peptides derived from the tryptic digestion of casein, which possess physicochemical properties that enable the chelation of various bi- and trivalent minerals, thereby enhancing mineral solubility in the lower small intestine.

Digestion rate of proteins It corresponds to the rate of peptides and amino acids released during digestion in the gastric or intestinal compartment.

Fructooligosaccharides These are oligosaccharide fructans, sometimes also called oligofructose or oligofructan, used as an alternative sweetener. They also have prebiotic characteristics.

Glutathion (GSH – L-γ-Glutamyl-L-cysteinyl-glycine) A tripeptide with a gamma peptide linkage between the amine group of cysteine and the carboxyl group of the glutamateside chain. It is an antioxidant preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.

Sarcopenia It can be defined as muscle wasting.

Thermolysin It is a thermostable neutral metalloproteinase enzyme. It specifically catalyzes the hydrolysis of peptide bonds containing hydrophobic amino acids.

Introduction

Meat has been an important part of the human diet for a very long time. Its consumption started approximately 1.9 million years ago by *Homo erectus*. The prehistoric discovery of cooking food is reported to result in a higher net energy value intake by human ancestors and probably also a higher food digestibility. As a consequence, it is believed that the increased ease of chewing cooked meat resulted in the molar teeth size reduction.

In the modern era, and especially in the developed Western societies, meat is often an object of a controversy if one refers to the number of epidemiological studies associating consumption of meat and meat products with the risk of developing diseases such as colorectal cancer. However, many controlled studies have not found such a close association when the various risks factors were correctly adjusted – admittedly difficult to do. This article focuses on balanced positive and negative aspects of meat from a human nutrition point of view, although the diverse aspects of the use of sodium or nitrites in processed meats associated with human health will not be addressed. In particular, it covers the positive nutritional aspects of meat in a diet that includes components like essential amino acids. Other articles in this encyclopedia cover aspects such as cardiovascular and obesity health concerns and cancer health concerns. Such studies should not be seen as being in conflict with this article. The way humans use meat, especially in excess, together with excesses of other food components appears to have a large bearing on health outcomes.

Proteins

The classic criteria for evaluating the quality of a protein source are based on amino acid composition and digestibility of the protein fraction. These basic criteria can only assess the ability of a food to provide available amino acids. It is now known that the definition of the quality of dietary proteins needs to integrate new concepts such as:

- The capacity of dietary proteins to release, during the digestion, peptides having a local or systemic biological impact;
- The rate of digestion which may, in some cases, have a direct influence on whole body assimilation of amino acids.

Classical Criteria for Evaluating the Quality of Protein Sources

Meat is a heterogeneous food with a composition that varies according to the origin of the muscle (ruminants, pigs, and poultry), the type of muscles, (which are themselves complex in terms of structural and biochemical properties), and the preparation used to process them into various dishes (curing, drying, sausage making, and fermentation). Yet, meat still presents some common nutritional features. Meat is protein rich compared with other foods. In addition, those proteins which are particularly rich in essential amino acids (i.e., amino acids that the human system cannot synthesize, such as lysine and histidine), are present in a balanced way meeting human requirements right through from childhood to adulthood. This means that proteins from meat products are efficiently used by the human body to remodel or create new proteins during growth. This arises because metabolically it is not necessary to supply surplus amounts of unnecessary proteins merely to meet the requirements for individual indispensable amino acids. Furthermore, the high concentrations of lysine in meat

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proteins are useful to improve the quality of other proteins from cereals in diverse human diets. Because meat proteins are easily digested in the small intestine, they do not induce any notable adverse reaction in the digestive tract, which could increase endogenous losses as might occur with high-fiber feedstuff.

Because of the favorable balance of indispensable amino acids, very high digestibility, and high bioavailability of amino acids, meat proteins have a high biological value. Thus, as part of a well-balanced diet, meat consumption does not need to exceed 120 g d^{-1} for healthy adults without any specific health requirements (i.e., 0.8 g kg^{-1} d^{-1} needed). With a 120 g d^{-1} protein supply situation, the part of the daily supply regarding other nutrients can exceed 60% for indispensable amino acids, vitamin B_{12}, and zinc; 40% for vitamin B_3 and cholesterol; and 20% for iron, selenium, riboflavin, vitamin B_6, and pantothenic acid as well a variable quantity of saturated fatty acids.

Bioactive Peptides

Currently, there is abundant available data that have described the physiological effects of certain food-derived peptides on the activity of the digestive tract and other physiological functions (i.e., antihypertensive, opioid, immunomodulatory, or antianxiolitic activities). Among the peptides found in meat, some are already abundant in the original feedstuff, and their synthesis does not follow the classical metabolic pathways of protein synthesis and degradation. These are, for instance, carnosine and glutathion present in meat products. Other peptides that are present in the proteins can only be released during the various processes of meat transformation or during digestion.

Carnosine is a dipeptide (β-alanine-L-histidine) present only in animal tissues. It is particularly abundant in brain and skeletal muscle from mammals. Its concentration is more elevated in glycolytic muscles, but it can also vary according to animal species, age, and/or food intake. The carnosine concentration in meat is not much affected by the type of meat aging or cooking processes.

The main biological activity of carnosine appears to be related to its buffering activity that allows, for instance, offsetting the decrease in intracellular pH linked to lactic acid production in muscles where anaerobic glycolysis is particularly active. Carnosine also has antioxidant properties by its ability to bind divalent metallic ions and its ability to trap free radicals. Moreover, carnosine seems able to reduce aldehydes formed from unsaturated fatty acids during oxidative stress and it has been suggested that carnosine also plays a prominent role in protection against glycation and crosslinking of proteins. Protein crosslinking interferes with tissue function and can, therefore, lead to aggregation of cellular material in the form of plates. Thus, carnosine may play an important role in preventing secondary complications in diabetes and in the protection against neurodegenerative disorders such as Alzheimer's disease. Carnosine-rich diets could, therefore, become increasingly important in geriatric human diets.

Glutathion is a tripeptide (GSH – L-γ-Glutamyl-L-cysteinyl-glycine) whose concentration is very high in the liver and is also present in the skeletal muscle. In contrast to carnosine, GSH is not specific to animal products and is also present in significant quantities in vegetables such as broccoli and spinach. It seems that a portion of the dietary glutathion can be absorbed intact, and it participates in the intracellular glutathion store in peripheral tissues. Because of the thiol function of cystein radical, glutathion exists as either a reduced (GSH) or an oxidized (GS-SG) form. GSH is the major hydrosoluble antioxidant in animal cells. It is an efficient free radicals scavenger, protecting cells from reactive oxygen species' (ROS) attacks. Any changes in GSH and GS-SG concentrations directly reflect alterations of their redox state. Glutathion also plays a role in xenobiotics detoxification, metabolism of various molecules (leucotriens, prostaglandins, formaldehyde, methylglyoxal, and nitric oxide), and the regulation of expression and/or activation of 'oxidation-sensitive' transcriptional factors that are necessary for the antioxidant response. Glutathion deficiencies contribute to the oxidant stress, which plays a key role in the aging process and the establishment of various pathologies (Alzheimer's, Parkinson's, and inflammation of the digestive tract). Studies in humans and animals show that an adequate protein intake is essential to maintain glutathion homeostasis. Moreover, GSH plays an important role in maintaining the integrity of the intestinal mucosa. Finally, dietary GSH, in addition to biliary GSH, participate in reduction of lipid peroxides present in the intestinal lumen.

Very few studies have described the ability of meat proteins to be a potential source of bioactive peptides. The most studied biological activity is the antihypertensive activity based on the inhibition of the angiotensin-converting enzyme (ACE). Several ACE-inhibiting peptides have been evidenced in controlled studies of muscle protein hydrolysates These studies have included a skeletal muscle hydrolyzed with thermolysin (i.e., myosin hydrolyzed with thermolysin), troponin C hydrolyzed with pepsin, and sarcoplasmic proteins hydrolyzed with a mixture of thermolysin, proteinase A, and protease type XIII. An *in vivo* study of an animal model shows that, following the ingestion of beef meat, numerous peptides are reproducibly released during the digestive process (Table 1) and many of them contain an amino acid sequence known to present an ACE-inhibiting potential. To be active at the peripheral circulation, these sequences will still need to be released intact by mucosal peptidases in order to enter the blood circulation and be resistant to the peptidases present in the blood plasma. The possibility of absorption of antihypertensive peptides has been demonstrated in humans after an oral administration of the dipeptide Val-Tyr, but the occurrence of such an absorption following the ingestion of dietary proteins containing bioactive sequences has so far not been reported. However, it was shown that a partial substitution of dietary carbohydrates by red meat can lower blood pressure in hypertensive patients.

Digestion Rate

It has been shown that the kinetics of the digestion of dietary protein determines the effectiveness of their assimilation, although optimal kinetics is not necessarily the same for all

Table 1 Peptides reproducibly released in duodenum and jejunum digestive contents during digestion of cooked beef meat in pigs

Parent protein	*Meat*	*Fragment position*	*Fragment sequences*	*m/z* ($M+H^+$)	*Bioactive sequences*	*Biological activity*
Duodenum						
Actin	TL, PP, and S	96–106	LRVAPEEHPTL	126.170	VAP	Antihypertensive
	PP and S	97–106	RVAPEEHPTL	1148.61	VAP	Antihypertensive
	PP and S	24–33	AGDDAPRAVF	1018.50	PR and VF	Antihypertensive
	PP	171–180	YALPHAIMRL	1184.66	YALPHA, ALPHA, and RL	Antihypertensive
	TL, PP, and S	171–178	YALPHAIM	915.48	YALPHA and ALPHA	Antihypertensive
	PP	181–191	DLAGRDLTDDYL	1251.62	YL	Antihypertensive and Opioid
	PP	31–41	AVFPSIVGRPR	1198.71	IVGRPR, GRP, VF, FP, PR, and RP	Antihypertensive
Myosin	PP	326–333	YFKIKPLL	1021.65	IKP	Antihypertensive
Fructose-1,6-bisphosphate aldolase	TL	19–31	IAHRIVAPGKGIL	1344.85	VAP PG	Antihypertensive Antithrombotic
Creatine kinase	PP	193–202	LFDKPVSPLL	1128.67	VSP and LF	Antihypertensive
	TL, PP, and S	193–201	LFDKPVSPL	902.5	VSP and LF	Antihypertensive
GA3PDH	TL, PP, and S	231–240	FRVPTPNVSV	1115.62	–	–
Myoglobin	S	111–124	AIIHVLHAKHPSDF	1584.87	LH	Antioxidant
		147–154	YKVLGFHG	920.50	–	–
Jejunum						
Actin	TL, PP, and S	31–41	AVFPSIVGRPR	1198.71	IVGRPR, GRP, VF, FP, PR, and RP	Antihypertensive
	PP and S	32–41	VFPSIVGRPR	1127.67	IVGRPR, GRP, VF, FP, PR, and RP	Antihypertensive
	S	33–41	FPSIVGRPR	1028.60	IVGRPR, GRP, FP, PR, and RP	Antihypertensive
	S	33–40	FPSIVGRP	872.50	GRP, FP, and RP	Antihypertensive
GA3PDH	TL, PP, and S	231–240	FRVPTPNVSV	1115.62	–	–
Creatine kinase	PP and S	193–201	LFDKPVSPL	1015.58	VSP and LF	Antihypertensive
	PP and S	194–201	FDKPVSPL	902.50	VSP	Antihypertensive

Note: Bioactive sequences within each peptide are given.

Abbreviations: S, shoulder; TL, top loin; PP, pectoralis profondus.

Source: Reproduced from Bauchart, C., Morzel, M., Chambon, C., *et al*., 2007. Peptides reproducibly released by *in vivo* digestion of beef meat and trout flesh in pigs. British Journal of Nutrition 98, 1187–1195 and Rémond, D., Savary–Auzeloux, I., Gatellier, P., Santé-Lhoutellier, V., 2008. Propriétés nutritionnelles des peptides et des protéines de la viande: Impact des procédés de transformation. Sciences des Aliments 28, 383–395.

subjects. For instance, for elderly people, it seems preferable to concentrate the daily protein supply on a single meal, or to ingest rapidly digested proteins, in order to accentuate the postprandial increase in plasma amino acids and stimulate protein synthesis. From these observations, nutritional strategies are now developed to counteract aging-related muscle wasting (sarcopenia). The meat in this context could be a very interesting food because of its high concentrations in highly digestible proteins allowing the concentration of the protein supply in one meal.

A recent study in old human subjects has shown that meat can be considered as a source of rapidly digested proteins. However, the rate of digestion depends on the chewing capacity of elderly people and more precisely on the level of boli disruption before swallowing. Being slower in subjects with reduced chewing efficiency (complete dentures) than among subjects of the same age normally toothed, it induces a lesser increase in postprandial protein anabolism. The interest of meat consumption to counteract muscle wasting in the elderly, especially when associated with physical activity, would require to take into account the decline in chewing efficiency associated with aging and to develop new and adapted meat preparation techniques, allowing one to benefit from meat's full nutritional potential.

Micronutrients

Meat is an essential source for key micronutrients because either it is the only source of them or it is characterized by their high bioavailability.

Iron

Iron plays an important role as an oxygen carrier in hemoglobin in blood, or myoglobin in muscle, and it is also required for many metabolic processes. Iron has a higher bioavailability when derived from meat as heme iron than plant-derived iron. Approximately 30% of heme iron is

absorbed in the intestines, against only 7% of nonheme. Concentration of heme iron in meat depends on the animal species. For example, 100 g of red meat supplies approximately 3 mg of iron, i.e., 30% of the daily recommended intake, whereas the same quantity of white meat provides only 0.4 mg of iron. In modern societies, meat and meat products provide approximately 20% of the iron actually used by the body. Iron is an essential micronutrient in human nutrition and its deficiency is a world nutritional problem. Owing to the high prevalence of anemia in developing and industrialized countries, almost two-thirds of the world's population, it is necessary to maintain a suitable iron intake through diet in order to achieve an appropriate status of iron in the body. For this reason, accurate knowledge of iron availability of foods is essential in order to plan intervention strategies that improve deficient situations of this nutrient. With regard to the two forms of iron present in foods, heme iron has greater bioavailability than nonheme iron. Beside this, nonheme iron bioavailability is conditioned by several dietary factors such as classical factors (meat, ascorbic acid, fiber, phytic acid, and polyphenols) and new factors (caseinophosphopeptides and fructooligosaccharides with prebiotic characteristics).

Furthermore, heme iron has been pointed as a major prooxidant agent in food or in the digestive tract. The Fenton reaction leads to HO formation and lipoperoxidation propagation as well protein oxidation in the product, which can affect the organoleptic properties. In the digestive tract, heme iron is suspected to promote adenoma in rat studies. However, this effect is reversed in the presence of calcium, suggesting calcium could act as a chelator of iron.

In addition, some epidemiological studies postulated a possible role of this food component in carcinogenesis. In numerous studies, the prevalence of cancer risk associated to meat intake often includes processed meat, i.e., meat cooked with nitrites forming nitrosomyoglobin. Therefore, it remains difficult to assess the difference between iron alone and iron added with nitrites.

Zinc

Zinc is associated with the reactivity of a wide variety of enzymes. By binding zinc, phytate decreases its bioavailability. Hence, meat is a better source of this compound than vegetables. Thus, 100 g of bovine meat supplies approximately 5 mg of zinc, i.e., 40% of the daily recommended intake. Meat contributes 17% of the total zinc intake in France and 31–34% in Denmark, the UK, and New Zealand. In the USA, the contribution of meat to total zinc intake is 56%. In some human populations, the serum levels of zinc are below the deficiency threshold, especially in children and in the elderly.

Selenium

Selenium plays a central role in human antioxidative defense systems and it has been suggested that high selenium intakes reduce the risk of cancer and cardiovascular diseases. Meat and meat products are a good source of selenium as 100 g of meat supplies 6–8 μg of selenium, i.e., approximately 10% of recommended intake. The liver is particularly rich in selenium (40–100 μg for 100 g).

Meat and meat products are also important sources of copper, cobalt, chromium, manganese, and nickel and greatly contribute to the recommended intakes of these compounds.

Vitamins

Two important micronutrients occur only in meat: vitamins A and B_{12}. Both cannot be compensated for plant-derived provitamins. Provitamin B_{12} does not exist and the provitamin A, β-carotene, has to be taken in high amounts due to a poor conversion rate (1:12).

Vitamin A

Vitamin A is involved in bone growth, eye pigment synthesis, and prevention of respiratory diseases. Meat is a better source of vitamin A than vegetables. Meat contains retinol or retinol esters, which are directly usable by the body, whereas vegetables contain β-carotene, a precursor of retinol, and the conversion rate of β-carotene into retinol is only 1:12. The concentration of vitamin A in meat ranges widely among species (2–100 μg per 100 g of meat). With 20 000 μg per 100 g, the liver is the best source of vitamin A in the human diet, and its contribution to the recommended intake is approximately 75%.

Vitamins B_9 and B_{12}

The B vitamins work as cofactors in different enzyme systems in the body and their deficiency is linked to many diseases. Meat and animal-derived foods are the only foods that naturally provide vitamin B_{12}, a water-soluble component. Meat contains 0.3–2 μg of vitamin B_{12}, oxidative muscle having the highest content, whereas the liver contains 65 μg. A daily consumption of 120 g of meat or a monthly consumption of 120 g of the liver supplies recommended intakes. Similarly, folic acid has a nearly 10-fold higher bioavailability from meat (especially liver) and eggs than from vegetables.

Vitamin D

Vitamin D is essential for the development and maintenance of bone. Although most vitamin D comes from the action of sunlight on 7-dehydrocholesterol in the skin, some human populations are particularly reliant on a dietary supply of vitamin D. With a level of 0.1 μg per 100 g, the contribution that meat makes to vitamin D dietary intake is important. In general, meat products contribute 20–30% of daily intake of vitamin D.

Vitamin E

There are small amounts of vitamin E in meat, but the recent trend to include seed oils or vitamin E in animal diet contributes to an increase of vitamin E content in meat.

Fat

Meat, like other animal products, is a source of lipids, mostly of phospholipids, triglycerides, and cholesterol. The proportion of

lipids in lean meat lies between 2% and 6%, according to species and muscle type. For example, in white meat such as deskinned poultry breast meat, the amount of lipid barely reaches 2%. The hydrolysis of triglycerides releases three fatty acids. These can be either saturated or unsaturated (monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs)). Because of its high saturated/unsaturated fatty acid ratio, beef consumption suffers unfairly from a poor image in terms of nutritional quality. To increase the amounts of PUFAs, and especially of *n*-3 PUFAs, in bovine meat, several strategies including diet have been implemented to reverse this ratio and achieve perceived positive health effects. Because PUFA and MUFA are highly reactive to oxidation in meat storage or during processing, addition of antioxidants in the diet of ruminants is recommended.

Meat Intake and Cancer

Several epidemiological cohort studies either have suggested a positive association between meat intake and colon/prostate cancer or have failed to detect such an association. However, the majority of the studies were not adjusted for total energy intake. It is more or less generally suggested that animal fat, rich in saturated fat, is closely associated with cancerogenesis and it is, therefore, implied that plant-derived mostly unsaturated fat (PUFA) is more protective. In animal models, the tumor-promoting effect of fat intake has been observed primarily for PUFA.

The suggestion that consumption of red meat as a source of dietary fat increases the risk of colon cancer is based on the premise that dietary fat promotes excretion of bile acids, which can be converted into carcinogens. However, this may not be true as fat derived from red meat might be less absorbed due to either its composition (stearic acid) or matrix (muscle) interactions. Also, it has been suggested that heterocyclic amines produced in barbecued meat might contribute to carcinogenesis.

The role of heme iron as a promoter is often suggested. Like many other transition elements, iron possesses unfilled atomic orbitals that allow it to coordinate electron donors and participate in redox processes. Iron can cause oxidative stress and DNA damage, and heme iron can catalyze endogenous formation of N-nitroso compounds (NOCs), which are potent carcinogens. Studies of chemically induced colon carcinogenesis in rats demonstrated a dose–response relationship between heme iron and promotion. The way in which the heme iron could promote cancer is described in Figure 1 via the fat peroxidation and the N-nitroso pathways. There is

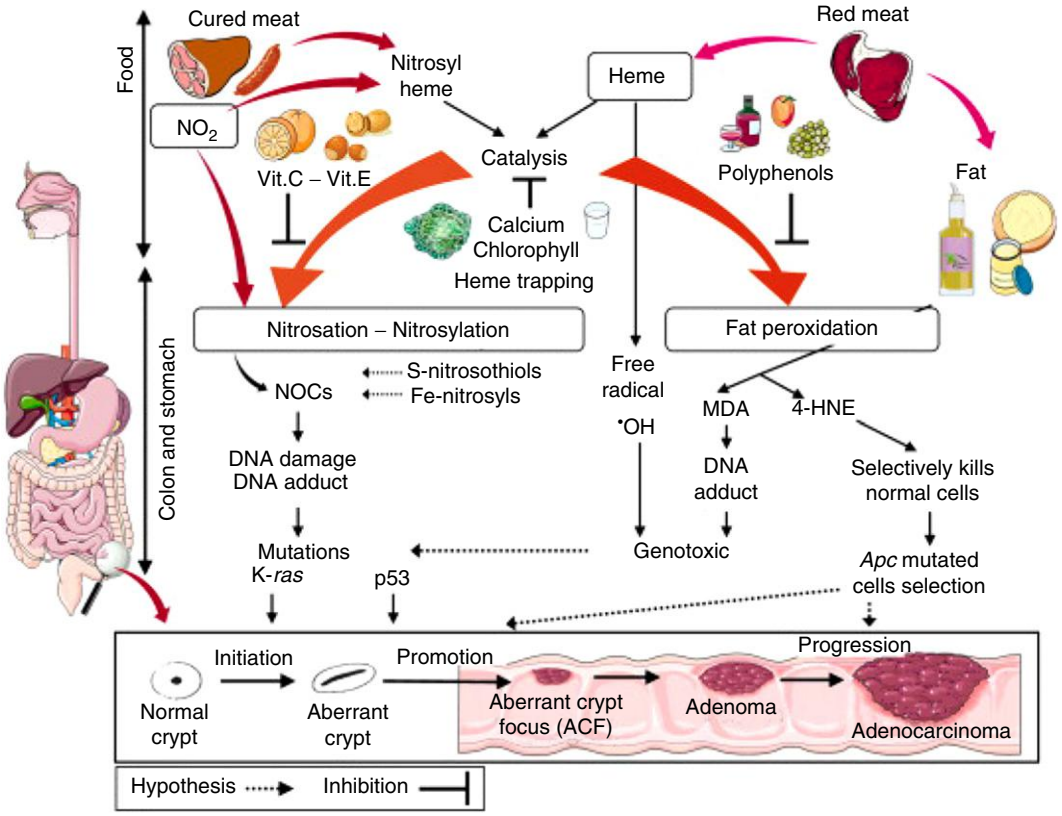

Figure 1 Catalytic effect of heme iron on fat peroxidation and N-nitrosation, and their inhibition by dietary means. Consequences for the development of colorectal cancer – Reprinted with modifications from Cancer Prevention Research – Bastide, N., Pierre, F., Corpet, D., 2011. Heme iron from meat and risk of colorectal cancer: A meta-analysis and a review of the mechanisms involved. Cancer Prevention Research 4, 177–184. Heme iron catalyzes nitrosation and fat peroxidation. End products are NOCs, malonaldehyde (MDA), and 4-hydroxynonenal (4-HNE). These pathways explain, at least in part, the promoting effect of red and cured meat on colorectal cancer. The catalytic effects of heme iron can be inhibited by trapping heme with calcium carbonate or chlorophyll. The endogenous formation of NOCs is inhibited by vitamin C and E. Ongoing studies suggest that specific polyphenols can inhibit fat peroxidation and/or nitrosation.

some evidence that carcinogenesis promotion by dietary heme iron is associated with the urinary excretion of a fat peroxidation biomarker, 1,4-dihydroxynonane mercapturic acid, the major 4-HNE – end products of lipoperoxidation of ω6 fatty acid – urinary metabolite. 4-HNE and MDA are toxic and bind DNA, forming mutagenic adducts. Those metabolic events could explain tumor promotion. Interestingly, in a study on rats, intake of beef meat with added calcium decreased the risk of tumor formation. The chelation of calcium by the heme iron complex would prevent the iron to act as a catalyst of lipoperoxidation. The N-nitroso pathway would mainly explain the hypothesis that nitrite-cured meat favors cancer. But this pathway is not limited only to cured meat, because a diet high in fresh red meat (600 g d^{-1} compared with 60 g d^{-1}) induces a 3-fold increase in fecal nitrosocompounds.

Conclusion

Although meat presents beneficial nutritional properties due to its essential amino acid composition, it is also very important to understand the function of biologically relevant peptides it contains, and its high digestibility. Therefore, there is a strong rationale for reassessing the processing that affects proteins in terms of their impact on the nutritional quality of meat. These postmortem changes (aging, processing treatments, etc.) affect the meat structure, which can decrease the bioavailability of essential amino acids, not only by changing the type of digestive end products (more or less resistant peptides) produced but also by affecting the digestion rate. New insights into the field of bioactive peptides make it possible to reexamine current concepts of the nutritional quality of dietary proteins, which to date have only been based on essential amino acid composition and digestibility. Thus, by integrating other properties having a known impact on health, the position of these foods within the framework of a balanced human diet can be revised.

Through its carnosine content and the potential of meat proteins to release bioactive peptides, especially antihypertensive peptides, meat could offer new nutritional benefits. Furthermore, the high protein content and fast rate of digestion of meat makes it the first-line choice in nutritional strategies designed to counteract aging-related sarcopenia. Digestion in the small bowel (rate and end products), which to date has been relatively unexplored, remains a key point to deeper insight into whether nutritional quality is positively or negatively impacted by the biochemical and structural changes of the major compounds that are caused by food processing procedures. There is clearly a need for more work in this area, given the uncertainties over the definition of meat and processed meat.

The increase in meat consumption has been associated with an increased risk of cancer (in societies where there is adequate food available), but there is also a concomitant increase of other foods almost to excess, a reduction of exercise and an increase in obesity, so meat is unlikely to be solely responsible. At the other end of the scale, addition of relatively small quantities of meat to a diet will have a positive effect, for the reasons indicated in this article, and certainly outweigh any disadvantages associated with its absence. It would be fair to say that the currently available epidemiologic evidence is not sufficient, on its own, to support an independent positive association between reasonable (i.e., not an excess) levels of red meat consumption and increased risk of colorectal cancer.

See also: By-Products: Edible, for Human Consumption. Chemical Analysis for Specific Components: Curing Agents. Chemical and Physical Characteristics of Meat: Protein Functionality. Cooking of Meat: Cooking of Meat. Curing: Brine Curing of Meat; Physiology of Nitric Oxide. Functional Foods. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns; Macronutrients in Meat; Micronutrients in Meat; Vegetarianism. Nutrient Claims on Packaging

Further Reading

Alexander, D., Cushing, C., 2010. Red meat and colorectal cancer: A critical summary of prospective epidemiologic studies. Obesity Reviews 12, 472–493.
Bastide, N., Pierre, F., Corpet, D., 2011. Heme iron from meat and risk of colorectal cancer: A Meta-analysis and a review of the mechanisms involved. Cancer Prevention Research 4, 177–184.
Bauchart, C., Morzel, M., Chambon, C., *et al.*, 2007. Peptides reproducibly released by *in vivo* digestion of beef meat and trout flesh in pigs. British Journal of Nutrition 98, 1187–1195.
Biesalski, H., 2005. Meat as a component of a healthy diet – are there any risks or benefits if meat is avoided in the diet. Meat Science 70, 509–524.
Carmody, R., Wrangham, R., 2011. The energetic significance of cooking. Journal of Human Evolution 57, 379–391.
Chao, A., Thun, M., Connel, C., *et al.*, 2005. Meat consumption and risk of colorectal cancer. Journal of the American Medical Association 293, 172–182.
Geissler, C., Singh, M., 2011. Iron, meat and health. Nutrients 3, 283–316.
Hagen, T., Wierzbicka, G., Sillau, A., Bowman, B., Jones, D., 1990. Bioavailability of dietary glutathione – effect on plasma-concentration. American Physiological Society 259 (4), G524–G529.
Mårtensson, J., Jain, A., Meister, A., 1990. Glutathione is required for intestinal function. Proceedings of the National Academy of Sciences of the United States of America 87, 1715–1719.
Rémond, D., Savary-Auzeloux, I., Gatellier, P., Santé-Lhoutellier, V., 2008. Propriétés nutritionnelles des peptides et des protéines de la viande: Impact des procédés de transformation. Sciences des Aliments 28, 383–395.
WCRF, 1997. World cancer research fund/ American institute for cancer research: Food, nutrition, physical activity and the prevention of cancer, a global perspective. Washington, DC: AIRC (American Institute for Cancer Research), 670 pp.

Relevant Websites

http://www.fao.org/docrep/
http://www.webmd.com/colorectal-cancer

Micronutrients in Meat

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Glossary

Bioavailability The amount of a nutrient which is absorbed and used/metabolized by the body. Techniques for assessing bioavailability include radioactive tracers, *in vivo* and *in vitro* studies.

Heme and nonheme iron Food iron is classified as heme and nonheme iron. Heme iron is derived from hemoglobin and myoglobin and its food sources are meat, fish, and poultry. Main food sources of nonheme iron include meat, cereals, fruits, vegetables, and nuts. Heme iron is more bioavailable than nonheme iron.

Irish Universities Nutrition Alliance (IUNA) IUNA is made up of four academic nutrition units from University College Cork, University of Ulster, Trinity College Dublin, and University College Dublin. Since it was established, IUNA has conducted a series of food consumption surveys among different age groups in Ireland. In addition to collecting information on habitual food and drink consumption, they also record, health and lifestyle characteristics and anthropometric measurements. The wealth of data is a valuable resource and is used to guide and develop nutrition policies and health promotion campaigns.

Meat factor Meat promotes the bioavailability of nonheme iron and the mechanism which is responsible for the enhanced absorption/bioavailability remains unknown. It is commonly referred to as the 'meat factor.'

Recommended dietary allowances The level of intake of nutrients that, on the basis of scientific knowledge, are judged to be adequate to meet the known nutrient needs of practically all healthy persons.

Introduction

This article examines the role that meat and meat products play in supplying iron, zinc, selenium, sodium, vitamin D, and the B vitamins to the diet. Data on the content of these nutrients in meat are taken from publications of McCance and Widdowson's 'The Composition of Foods.' These values should be used as a general guide; they are for raw meats and, therefore, may vary for processed and cooked meats.

Recommended dietary allowances (RDAs) are the level of intake of nutrients that are judged to be adequate to meet the known nutrient needs of practically all healthy persons. In this article, the RDA is cited for adult males and females. In the case of many nutrients, the RDA varies for age and special conditions (such as pregnant and lactating women). A full listing of the RDA values for each nutrient in Ireland is available from The Food Safety Authority of Ireland.

To put a figure on the contribution that meat makes to the micronutrient intakes, data will be taken from four food consumption surveys conducted in Ireland by the Irish Universities Nutrition Alliance (IUNA). The surveys were conducted among nationally representative samples of:

- Children: 5–12 years, 594 children, data collected between 2003 and 2004, used 7-days weighed food record.
- Teenagers: 13–17 years, 441 teenagers, data collected between 2005 and 2006, used 7-days semiweighed food record.
- Adults: 18–64 years, 958 adults, data collected between 1997 and 1999, used 7-days estimated food record. A second survey, 1274 adults, data collected between 2008 and 2010, used 4-days semiweighed food record. With the exception of selenium, adult micronutrient intake data will be taken from the most recent survey.
- The elderly: those aged 65 years and older, 226 persons, data collected between 2008 and 2010, used 4-days semiweighed food record.

A nutrient analysis software package, WISP©, analyzed the data, which uses the sixth edition of the UK compositional figures. It was added to using Irish food composition data for composite dishes, generic Irish foods and supplements. All foods were categorized into food groups, including a 'meat and meat products' food group.

Meat and meat products' food group contains fresh meat (beef, veal, pork, lamb, chicken, turkey, and game), meat dishes (offal and offal dishes; beef and veal dishes; and lamb, pork, and bacon dishes), and cured and processed meats (bacon, ham, burgers (beef and pork), sausages, meat products, meat pies, and pastries). Results from the surveys are summarized in Table 1.

Minerals

Iron

Iron deficiency is the most common nutritional disorder in the world (in both developed and developing countries) and it is estimated that nearly a quarter of the world's population is deficient in iron. Groups at greatest risk of iron deficiency include infants, children, adolescent girls, and pregnant women. For instance, iron deficiency is the most common nutritional disorder during early childhood and meat is recommended in

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Table 1 Contribution of meat and meat products to percentage micronutrient intake across different age groups in Ireland

	5–12 years old	*13–17 years old*	*18–64 years old*[a]	*> 65 years old*
Iron	13	17	18	18
Zinc	28	34	36	35
Selenium	N/A	N/A	30[b]	N/A
Vitamin D	31	35	30	22
Vitamin B_{12} (cobalamin)	20	26	27	27
Vitamin B_6	18	23	24	22
Niacin	30	39	39	39
Vitamin B_1 (thiamin)	16	19	20	21
Vitamin B_2 (riboflavin)	10	15	16	16
Pantothenic acid	17	23	25	23

[a]With the exception of selenium, data for the adults are taken from the most recent survey, where data were collected between 2008 and 2010.
[b]The selenium data are taken from the first adult survey, data were collected between 1997 and 1999.
Abbreviation: N/A, not available.
Source: Data taken with permission from Irish North–South Food Consumption Surveys – Irish Universities Nutrition Alliance (www.iuna.net).

the weaning diet and has been shown to make a significant contribution to the iron status of toddlers. Inadequate intake of iron can lead to varying degrees of deficiency; from low iron stores to iron-deficiency anemia. Conditions associated with iron deficiency include defective psychomotor development in infants, impaired education performance in school children, adverse perinatal outcome in pregnancy, and a diminished work capacity.

Bioavailability of iron refers to the proportion of ingested iron that is absorbed and utilized by the body. Low bioavailability of dietary iron is a major contributing factor to iron anemia. There are three elements in meat that make it so valuable in terms of iron nutrition.

1. Meat is a rich source of iron. A fillet steak contains 2.1 mg iron per 100 g. Fillet steak is an expensive cut of meat, but irrespective of the price, all meats are rich in iron. For instance, rib roast contains 1.7 mg iron per 100 g and liver contains 8–17 mg iron per 100 g. A 100-g portion of lamb and pork contains approximately 1.4 and 0.7 mg of iron, respectively.
2. There are two types of iron in meat – heme and nonheme iron. Dietary iron exists in two forms, heme and nonheme. Heme iron is found in foods that contain hemoglobin and myoglobin, that is, foods of animal origin and therefore meat and fish. Approximately 50–60% of the iron in meat is heme. In contrast, plant foods contain only the nonheme form. Heme iron is readily absorbed (20–30%) and utilized by the body and therefore is said to be highly bioavailable. The effect of cooking meat on heme iron is not fully understood; it is suggested that high temperatures convert heme iron into nonheme.
3. Meat promotes the absorption of nonheme iron. Meat also contains nonheme iron, and it is this iron that makes the largest contribution to the body's iron pool. Meat promotes the absorption of nonheme iron, not only from itself but also from other foods eaten with it in the same meal. For instance, it has been estimated that the amount of nonheme iron absorbed from cereals, fruits, and vegetables when eaten alongside meat can be as high as 15–25%. In contrast, when these foods are eaten on their own, nonheme iron absorption is between 1% and 7%. Nonheme iron absorption is inhibited by fibers (phytate and oxalate), phenolic (tannins) compounds, and other minerals (zinc, calcium, and copper) and enhanced by only two food components – vitamin C (ascorbic acid) and meat. The component in meat that enhances the absorption of nonheme iron is referred to as 'meat factor.' The 'meat factor' mechanism remains to be elucidated. Cooking meat does not impair the 'meat factor.' A functional food has a health benefit above and beyond the nutrients the food provides. Thus, presence of the 'meat factor' may, in the future, allow meat to be marketed as a functional food.

Meat makes an important contribution to iron intakes. Irish food intake survey data show that meat and meat products contribute 13%, 17%, and 18% of dietary iron in the diets of children (5–12 years), teenagers (13–17 years), and adults, respectively. The contribution that heme iron and the 'meat factor' makes to iron intake and status is not known. A thorough understanding of the bioavailability of both heme and nonheme iron from the diet is required to fully appreciate the role of meat in iron nutrition. Low intakes of meat are routinely associated with low iron stores and anemia. Therefore, the role of meat in iron nutrition cannot be overstated and including at least some meat in the diet guarantees an assured source of easily absorbable iron.

Zinc

Zinc is an essential nutrient required for numerous metabolic functions, including synthesis of proteins, nucleic acids, and insulin and sexual maturity in males. Deficiency of zinc results in growth retardation, high rates of infection, skin lesions, and impaired wound healing.

People readily associate meat with iron, but what they do not appreciate is that meat is an even better source of zinc and contributes twice as much zinc as iron. Data from the Irish nutrition surveys show that meat and meat products contribute 28%, 34%, 36%, and 35% of the zinc intakes in 5–12-year-old children, teenagers, adults, and the elderly, respectively. A general rule of thumb is that a third of humans' zinc intakes come from meat and meat products.

Similar to iron, meat also promotes the absorption of zinc and therefore the zinc in meat is classed as highly bioavailable. It is estimated that 20–40% of zinc is absorbed from meat. The mechanism by which meat promotes the absorption of zinc is not known. Zinc present in other foods such as plants (cereals, grains, legumes, and nuts) has a low bioavailability due to the presence of phytates, which are thought to bind the zinc and inhibit its absorption. The elderly, vegetarians, toddlers, and people with renal disorders are at risk of zinc deficiency.

Beef is the richest meat source of zinc; for example, a 100-g portion of trimmed lean beef contains approximately 4-mg zinc. The RDA for zinc is 9.5 mg day^{-1} for males and 7 mg day^{-1} for females. Therefore, a typical 100-g portion of beef provides approximately half its daily requirement. Lamb is also rich in zinc, with a typical 100-g portion containing approximately 3.3-mg zinc; lower levels are found in pork (2.1-mg zinc per 100 g). Red meats are generally better sources of zinc than white meats; for instance, chicken breast contains less than 1-mg zinc per 100 g. Zinc is stored in the liver and the zinc content of lamb and pork liver is 4–8 mg per 100 g and that of calf liver is 14–16 mg per 100 g.

Selenium

Selenium is a constituent of the enzyme glutathione peroxidase, which inactivates toxic hydrogen peroxide and hydroperoxides. Evidence suggests that selenium may protect against coronary heart disease and certain cancers, in particular prostate cancer. The RDA for selenium is 55 μg day^{-1} for adults. Meat and meat products contribute approximately 30% of selenium intakes.

Of the red meats, pork (13 μg per 100 g) contains the highest levels of selenium, followed by beef (7 μg per 100 g) and lamb (2 μg per 100 g). Organ meats such as kidney (150 μg per 100 g) and liver (42 μg per 100 g) are rich in selenium. The interest in selenium as an essential antioxidant nutrient is relatively new and research is required to find out more about selenium in meat, including its bioavailability.

Sodium

Fresh meat is low in sodium. Data from Irish nutritional surveys indicate that as a food group, meat and meat products supply approximately a quarter of sodium intakes and make the largest contribution of any food group (Table 2). Sodium is added to fresh meats as a curing agent to extend their shelf life by acting as a preservative; it also adds flavor and improves their color, taste, and texture. The Irish survey data separated the 'meat and meat products' food group into 'fresh meat and meat dishes' (beef, veal, pork, lamb, chicken, turkey, and game; offal and offal dishes; beef and veal dishes; and lamb, pork, and bacon dishes) and 'cured and processed meats' (bacon, ham, burgers (beef and pork), sausages, meat products, meat pies, and pastries). 'Cured and processed meats' contribute a fifth of total sodium intakes (Table 2). The contribution that meat and meat dishes make to sodium intake is 6% in children and teenagers, 9% in adults, and 7% among the elderly. Meat is indeed low in sodium, and it is the contribution of sodium by processed and cured meats that is having such a large impact.

Countries are looking at ways to reduce the amount of salt in processed foods and considerable inroads have been made. In Ireland, the Food Safety Authority of Ireland implemented the Salt Reduction Programme. Its aim was to reduce the intake of salt to 6 g day^{-1} by 2010 (the RDA is 4 g day^{-1}). The program worked with food processors to look at strategies to reduce salt, such as simply lowering salt in the formulations and using alternative ingredients such as low sodium salts, salt replacers, and other ingredients that may mask the effects of reducing salt. The program did achieve success but it did not reach its target of 6 g day^{-1}. Many processors have reduced the salt they use during processing but have indicated that they cannot reduce salt any further as it would negatively affect the taste, color, shelf life, texture, and safety of their products.

Vitamins

Vitamin D

Until recently, meat was not recognized as a valuable source of vitamin D – the sunshine vitamin. However, recent data indicate that meat and meat products are the richest natural dietary source of vitamin D. Children in Ireland aged between 5 and 12 years receive 31% of their dietary vitamin D from meat and meat products. Moreover, this food group contributes 35% dietary vitamin D in teenagers, 30% in adults, and slightly lower levels (22%) in the elderly. Vitamin D is fat soluble and higher quantities are found in meat fat than in lean meat.

The main source of vitamin D is sunlight. The endogenous synthesis of vitamin D begins when ultraviolet light converts dormant 7-dehydrocholesterol in the skin to cholecalciferol. Cholecalciferol is subsequently hydroxylated in the liver to 25-hydroxycholecalciferol ($25(OH)D_3$) and in the kidney to its most biologically active form, $1,25(OH)_2D_3$ (dihydroxycholecalciferol). $1,25(OH)_2D_3$ acts as a hormone and stimulates calcium absorption and maintains healthy bones.

Table 2 Contribution of meat and meat products to sodium (salt) intakes in Ireland among different age groups

	5–12 years old	*13–17 years old*	*18–64 years old*	*>65 years old*
Meat and meat products[a]	24	26	27	24
Meat and meat dishes[b]	6	6	9	7
Cured and processed meat[c]	18	20	18	17

[a]Meat and meat products – refers to all meats (beef, veal, pork, lamb, chicken, turkey, and game), meat dishes, and cured and processed meats.
[b]Meat and meat dishes – offal and offal dishes; beef and veal dishes; and lamb, pork, and bacon dishes.
[c]Cured and processed meats – bacon, ham, burgers (beef and pork), sausages, meat products, meat pies, and pastries.
Source: Data taken with permission from Irish North–South Food Consumption Surveys – Irish Universities Nutrition Alliance (www.iuna.net).

Historically, cholecalciferol was the only vitamin D metabolite in meat to be measured. Research indicates that $25(OH)D_3$ is five times more potent than cholecalciferol and this potency factor is included in Irish compositional data and the food intake surveys. Meat also contains $1,25(OH)_2D_3$ and it is suggested that it is 25 times more potent than cholecalciferol. This is not included in vitamin D analysis as further research is required to shed light on this issue.

In the 1920s, meat was shown to protect against rickets, a fact that has long been forgotten. Rickets (softening of the bones) occurs in children; its adult equivalent is osteomalacia. Rickets was endemic in industrial cities in Britain in the nineteenth century, whereas today it is practically nonexistent. The reason for this is thought to be the early introduction of meat into the diet of toddlers. It is hypothesized that there is a 'magic factor' in meat, which protects against rickets and it may be related to vitamin D. In Ireland, babies are recommended to take a vitamin D supplement up to their first birthday. In addition to maintaining bone health, vitamin D aids the immune system and it may protect against tuberculosis, muscle weakness, diabetes, certain cancers, and coronary heart disease.

B Vitamins

Meat is a significant source of many B vitamins. The B vitamins in meat are thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin, pantothenic acid, vitamin B_6, and vitamin B_{12} (cobalamin). As the B vitamins are water soluble, lean meat contains higher amounts of them than meat fat. They are not very stable and some losses of B vitamins occur during cooking. The amount of B vitamins lost during cooking depends on its duration and temperature. One way of replenishing the lost B vitamins is to use the juices of meat to make gravy or to make a casserole or a stew where the juices are eaten as part of the meal.

Vitamin B_{12} (Cobalamin)

Vitamin B_{12} was the last vitamin to be isolated; it has a complex structure and the largest molecular weight of all vitamins. Vitamin B_{12} is exclusively of animal origin as it is made through bacterial fermentation in the gut. Natural food sources of vitamin B_{12} are limited to meat, fish, milk, and eggs. Fortified foods such as breakfast cereals also contribute to intakes of this vitamin. The RDA for vitamin B_{12} is 1.4 μg day^{-1} for adults (males and females). Organ meats are the richest food source; examples include liver (23–110 μg per 100 g) and kidney (15–54 μg per 100 g). Beef, lamb, and pork contain 1–3-μg vitamin B_{12} per 100 g. Therefore, a 100-g portion of meat provides most, if not all, of the daily requirement of this vitamin. In Ireland, meat and meat products contribute approximately a quarter of vitamin B_{12} intakes in teenagers and adults and a fifth in children (5–12 years).

Vitamin B_{12} acts as a cofactor for many enzymes. It plays a role in red blood cell formation and a deficiency causes megaloblastic anemia. Vitamin B_{12} also plays a role in maintaining a normal central nervous system and another clinical syndrome of vitamin B_{12} deficiency is neuropathy (e.g., spinal cord disease).

As vitamin B_{12} is limited to foods of animal origin, vegans and strict vegetarians are at risk of deficiency and are advised to take vitamin B_{12} supplements. In addition, infants breast-fed by a vegan mother are also at risk of deficiency. Another vulnerable subgroup is the elderly, as they have a diminished capacity to absorb dietary vitamin B_{12}.

Vitamin B_6

The RDA of vitamin B_6 for adult males and females is 1.5 and 1.1 mg day^{-1}, respectively. Meat is a valuable source of vitamin B_6. Beef, lamb, and pork contain between 0.3- and 0.5-mg B_6 per 100 g. Calf (0.89 mg per 100 g), lamb (0.53 mg per 100 g), beef (0.52 mg per 100 g), and pig (0.64 mg per 100 g) liver contain higher amounts of vitamin B_6. In Ireland, meat and meat products contribute 18% of dietary vitamin B_6 in children (5–12 years) and 23%, 24%, and 22% in teenagers, adults, and the elderly, respectively. Vitamin B_6 is present in plants; however, it cannot be absorbed from some vegetables because of the presence of glycosides; this does not affect the vitamin B_6 in meat.

The term 'vitamin B_6' includes three pyridines: pyridoxine, pyridoxal, and pyridoxamine and their 5′-phosphorylated derivatives, which are interconvertible. In the liver, pyridoxine and pyridoxamine are converted into pyridoxal 5′-phosphate, which acts as a cofactor for many enzymes, including those related to protein metabolism and amino acid interconversion. Vitamin B_6 also plays a role in red blood cell formation and supplies energy by converting fat to energy.

The Role of Vitamins B_6 and B_{12} in Lowering Homocysteine

Homocysteine is an amino acid metabolite, and a raised plasma level is an independent risk factor for heart disease. Both vitamins B_6 and B_{12} act as cofactors for enzymes that break down potent homocysteine to innocuous amino acids.

Research shows that vegans and strict vegetarians who have habitually low intakes of vitamins B_6 and B_{12} have higher circulating levels of plasma homocysteine. This shows the important role that vitamins B_6 and B_{12} play in lowering plasma homocysteine and highlights the fact that meat and meat products are valuable sources of these vitamins.

Niacin

The RDA of niacin for adult males and females is 16 mg day^{-1}. Meat is the richest food source of niacin. A 100-g portion of meat contains approximately 5–7 mg of niacin. There are two sources of niacin in the diet – the vitamin nicotinic acid and the amino acid tryptophan. Half the niacin provided by meat is derived from tryptophan, which is more readily absorbed by the body than that from plant sources, which is bound to glucose. In Ireland, teenagers and adults get 39% of their niacin intakes from meat and meat products and this is 30% in children (5–12 years).

Niacin supplies energy to the body by converting carbohydrates and fat into fuel. It also promotes a normal appetite, healthy skin, and aids in digestion.

Vitamin B_1 (Thiamin)

The RDA of thiamin is 1 mg day^{-1} for adults. Thiamin is found in useful amounts in meats, particularly in pork. Pork and its products, including bacon and ham, are the richest meat sources of thiamin (1 mg thiamin per 100 g). Beef and lamb contain 0.1 mg thiamin per 100 g and the liver, kidney, and heart contain approximately 0.5 mg thiamin per 100 g.

Thiamin acts as a cofactor for enzymes, which convert fats and carbohydrates into fuel, thus supplying energy to the body. Thiamin also helps in promoting a normal appetite and normal nervous system function.

In Ireland, approximately a fifth of thiamin is provided by meat and meat products in teenagers and adults. Among children (5–12 years), slightly lower levels (16%) are supplied by meat and meat products.

Vitamin B_2 (Riboflavin)

Riboflavin acts as a cofactor for enzymes of the Krebs cycle, which supplies energy. It also promotes healthy skin, eyes, and vision and may prevent against cataracts. A deficiency of riboflavin reduces appetite, causes anemia, and impairs the conversion of tryptophan to niacin.

The RDA of riboflavin for adults is 1.6 mg day^{-1} for males and 1.3 mg day^{-1} for females. Offal meats are good sources of riboflavin; for example, kidney contains 2–3.3 mg per 100 g and liver contains 2–6 mg per 100 g. A 100-g portion of beef, lamb, and pork contains approximately 0.2-mg riboflavin. Meat and meat products provide 10% dietary riboflavin among children, this rises to 15% for teenagers and 16% for adults and the elderly.

Pantothenic Acid

The liver and kidney are rich sources of pantothenic acid. Although most of this vitamin is leached into the drip loss associated with frozen meat, this is unlikely to be of any nutritional consequence as pantothenic acid is universal in all living matter. There is no RDA for pantothenic acid, although it is suggested that an intake of 4–7 mg day^{-1} is required. All meats contain pantothenic acid, such as pork (1.46 mg per 100 g), beef (0.75 mg per 100 g), and lamb (0.92 mg per 100 g).

Pantothenic acid is involved in energy production and aids in the formation of steroid hormones. It is often referred to as the 'antigray' hair factor, as it is thought to prevent hair becoming gray. In Ireland, meat and meat products provide 17% dietary pantothenate among children, 23% to teenagers and the elderly, and a quarter of an adult's intake.

Summary

Iron is the main nutrient that people associate with meat, and it may come as a surprise to many people that meat is a valuable source of many other nutrients. In fact, meat and meat products contribute double or more than double the dietary intakes of zinc, selenium, vitamin D, and niacin than that of iron. The iron and zinc in meat are highly bioavailable. Meat enhances the absorption of iron from nonmeat sources and its contribution to iron nutrition has still to be quantified. Over the past 50 years, the contribution that meat makes to selenium intakes has grown. Recent advances in compositional analysis reveal that meat and meat products are the richest natural dietary source of vitamin D. In the past, little attention was given to the contribution that meat makes to the intake of the B vitamins. Recently vitamins B_{12} and B_6 have attracted much interest as they reduce plasma homocysteine and meat contributes between a fifth and a quarter of their dietary intakes. Meat is the richest food source of niacin. Vitamin B_{12} is exclusively of animal origin. The majority of sodium supplied by meat and meat products comes from its addition to processed meats.

Meat and meat products make a significant contribution to the dietary intakes of iron, zinc, selenium, vitamin D, vitamin B_{12}, vitamin B_6, niacin, vitamin B_1, thiamin, vitamin B_2, riboflavin, and pantothenic acid. In many cases, their offal meats contain even higher quantities of these micronutrients. However, nutritional science is relatively new and many mysteries have still to be unraveled about meat and its nutrients.

Acknowledgement

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See also: Cooking of Meat: Cooking of Meat. Functional Foods. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns; Macronutrients in Meat; Meat and Human Diet: Facts and Myths; Nutraceuticals; Vegetarianism. Nutrient Claims on Packaging

Further Reading

British Nutrition Foundation, 1999. Meat in the Diet. Briefing Paper. London: British Nutrition Foundation.

Chan, W., Brown, J., Church, S.M., Buss, D.H., 1996. Meat Products and Dishes, Supplement to McCance and Widdowson's The Composition of Foods. Cambridge and London: The Royal Society of Chemistry and Ministry of Agriculture Fisheries and Food.

Chan, W., Brown, J., Lee, S.M., Buss, D.H., 1995. Meat, Poultry and Game, Supplement to McCance and Widdowson's The Composition of Foods. Cambridge and London: The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Department of Health, 1991. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. London: HMSO, No. 41.

Dietitians Association of Australia, 2007. The role of red meat in healthy Australian diets. Nutrition and Dietetics 64 (Suppl. 4), S99–S195.

Dunnigan, M.G., Henderson, J.B., 1997. An epidemiological model of privational rickets and osteomalacia. Proceedings of the Nutrition Society 56, 939–956.

Gibson, S.A., Ashwell, M., 1997. New vitamin D values for meat and their implication for vitamin D intake in British adults. Proceedings of the Nutrition Society 56, 116A.

Higgs, J.D., 2000. An overview of the compositional changes in red meat over the last 20 years and how these have been achieved. Food Science and Technology Today 14 (1), 22–26.

Higgs, J.D., Mulvihill, B., 2002a. Enhancing the nutritional value of meat. In: Henry, C.J.K., Chapman, C. (Eds.), The Nutrition Handbook for Food Processors. Washington, DC: CRC Press, pp. 209–246.

Higgs, J.D., Mulvihill, B., 2002b. The nutritional quality of meat. In: Kerry, J., Kerry, J., Ledward, D. (Eds.), Meat Processing. Washington, DC: CRC Press, pp. 64–104.
Johnson, A., 2009. The role of red meat in a healthy New Zealand diet. Available at: www.beeflambnz.co.nz (accessed October 2011).
Mann, N.J., Li, D., Sinclair, A.J., *et al*., 1999. The effect of diet on plasma homocysteine concentrations in healthy male subjects. European Journal of Clinical Nutrition 53 (11), 895–899.
Mclean, E., Cogswell, M., Egli, I., *et al*., 2008. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Public health Nutrition 23, 1–11.
Mulvihill, B., 1996. The effect of meat systems on the bioavailability of non-haem iron. PhD Thesis, University College Cork, Republic of Ireland.
Wyness, L., Weichselbaum, E., O'Connor, A., *et al*., 2011. Red meat in the diet: An update. Nutrition Bulletin 36, 34–77.

Relevant Websites

www.beeflambnz.co.nz
Beef + Lamb New Zealand.
www.fsai.ie
Food Safety Authority of Ireland.
www.iuna.net
Irish Universities Nutrition Alliance.

Nutraceuticals

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Introduction

The importance of foods and food products in the prevention and treatment of diseases has moved beyond the simplicity of essential nutrients. Food and food products are now divided into many different categories based on the purposes of the food. Apart from conventional foods that are simply meant for sustenance, four categories of food or food products intended to improve or support health are medicinal foods, functional foods, supplements, and nutraceuticals. Although there is considerable overlap among these categories, there are distinct differences as well as nuances that determine which particular category a product belongs to. Medicinal foods are considered prescribed diets to treat medical conditions, such as diets designed to circumvent genetic abnormalities or severe allergies. Functional foods are foods thought to supply an additional benefit beyond meeting micronutrient or macronutrient needs, such as reducing oxidative stress, improving longevity, or lowering some disease risk factor.

Dietary supplements and nutraceuticals are distinguished from the other categories in that they are composed of extracts or components of foods meant to be consumed separate from food rather than as ingredients or whole foods. Dietary supplements are meant to supplement nutrient deficiencies in normal diets, such as vitamins and minerals. Some dietary supplements, such as herbal supplements, can also be used to introduce compounds not generally considered to be nutrients but that may still contain functional compounds that affect health.

The word nutraceutical itself is a portmanteau of the words 'nutrient' and 'pharmaceutical.' Thus, the concept of a nutraceutical is a component of food that has pharmaceutical properties. Nutraceuticals can therefore sometimes be considered a subset of dietary supplements in that eliminating a nutrient deficiency can cure or prevent some ailments (e.g., vitamin D and rickets). Often, however, nutraceuticals are considered components of foods that are given at pharmaceutical or superdietary doses to elicit responses beyond the effects known to be sufficient to prevent deficiencies (e.g., vitamin D and cancer prevention).

Definitions of these classifications of food are not clearly established, and vary among countries, researchers, and commercial operations. In particular, some consider nutraceuticals to be intact foods with health-promoting properties (defined as functional foods above). Thus, it is imperative that the chosen definition be made clear whenever discussing these categories of foods. The definition used herein corresponds to that of the Bureau of Nutritional Sciences of the Food Directorate of Health Canada:

> "A nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease."

The former part of the definition gives rise to the commercial reality that any compound or extract from food can be considered a nutraceutical. The latter part of the definition restricts nutraceuticals to products that can be demonstrated to improve health or impede disease. However, it can be difficult to prove that a purported nutraceutical improves health, which results in regulatory challenges discussed below.

Government Regulations

Regulation of categories of foods and food components varies from nation to nation, but generally there are no clear legal divisions or definitions for the use of such terms as 'functional foods,' 'nutraceutical,' or 'dietary supplement.' For instance, the Dietary Supplement Health and Education Act from the US essentially defines a dietary supplement as a product intended to supplement the diet that contains at least one dietary ingredient and is not to be consumed as conventional food, such as in capsule or liquid form. In addition, the product needs to be labeled as a dietary supplement. However, there is no regulatory definition of 'nutraceutical.' In Japan, food supplements are considered food, and need to comply with regulations for one of two categories: Foods with Nutrient Function Claims, such as the functions of vitamins and minerals, or Foods for Specified Health Uses, which are officially approved health claims. This is similar to 'structure/function' versus 'significant scientific agreement' health claims in the US.

Regulations divide medicines and foods. In the US, for example, compounds intended to treat or cure diseases that are not caused by nutrient deficiencies are considered drugs, with the exception of a few food components approved in the Code of Federal Regulations. For example, certain vitamin D- and calcium-containing foods or supplements can be advertised as lowering osteoporosis risk, which means these formulations are meant to prevent a disease. This differs from structure/function claims, in which compounds can be implicated in supporting health but not treating or preventing disease. Again using vitamin D as an example, a company marketing a vitamin D-containing supplement could suggest that vitamin D 'supports normal blood pressure' because there is some (but not conclusive) scientific evidence that vitamin D helps maintain normal blood pressure (a structure/function claim), but because strong scientific evidence does not exist they cannot claim that vitamin D lowers the risk of hypertension (a disease claim). Many individual countries, as well as the European Union, make analogous distinctions.

A common theme with nutraceuticals is that their efficacies are uncertain. Their functions go beyond the essentiality of nutrients where deficiencies result in clearly identifiable maladies. Moreover, the rigorous scientific investigation, fiscal backing, or dramatic phenotypic changes demonstrated by pharmaceuticals are often lacking. Therefore, the understanding of nutraceuticals' functions and effects are constantly being

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evolved. Because of the difficulty in establishing the strength of scientific evidence required to make legal disease claims, most dietary supplements and nutraceuticals are not approved to treat diseases or disease risk factors. Reaching a scientific consensus requires a significant financial investment. Often, however, a company cannot own the rights to a compound in food, but can merely own the rights to the methods of extracting or preparing the compound. Thus, the amount of time and money required to convincingly prove that a nutraceutical cures or prevents a disease is insurmountable for most companies.

Generalizability is often limited in nutraceutical research because of variability in the nutraceutical itself, such as differences in the exact chemical structure of the nutraceutical, the matrix in which the nutraceutical is supplemented, the dosage and frequency of administration, and the route of administration. For instance, glucosamine may have a sulfate or chloride counter ion; poorly preserved omega-3 fatty acids can oxidize; mineral absorption can be affected by the presence of other minerals; administration schedules affect absorption and functionality; and although nutraceuticals by definition should be orally administered, some studies administer them through alternative means, such as intramuscular and intraarticular injection of glucosamine. Understanding these details is imperative to elucidating the effects of nutraceuticals, as well as their potential contraindications. Therefore, even if a compound does amass the scientific consensus required to establish disease claims, companies that prepare the compound in a different way are not guaranteed to be able to use the approved disease claim because the preparation may alter the efficacy. That is, one proprietary formula may alter the efficacy of a nutraceutical compared to other preparations.

Examples of Animal-Derived Nutraceuticals

As described, any extract of food or food components may be considered a nutraceutical. The nutraceuticals discussed herein are restricted to a few of the more well-studied compounds that are found in animals or animal products, regardless of whether they are actually or commonly commercially isolated from animals.

Bile Acids

Hydrophilic and hydrophobic interactions drive important characteristics of the absorption of nutrients in the gastrointestinal tract. Lipids do not appreciably dissolve in aqueous environments such as the intestinal lumen. Bile secreted from the gall bladder contains chemicals such as bile acids that are important in solubilizing lipids and lipid-soluble nutrients for absorption. Thus, bile acids are integral to the efficient solubilizing and absorption of lipids in the intestines.

Chemically, bile acids are synthesized from cholesterol to be more hydrophilic by adding hydroxyl groups to a number of positions on the ring structure, as well as a carboxylic acid to the tail. The pattern of hydroxylation defines the chemical structure of the bile acid and gives rise to a variety of trivial names of bile acids, such as cholic, deoxycholic, lithocholic, chenodeoxycholic, hyodeoxycholic, ursocholic, and muricholic acids. Some of the trivial names are actually derived from animals, such as ursocholic acid for bears and muricholic acid for mice. Bile acids can also be conjugated to glycine or taurine, or remain in the nonconjugated state. For example, using cholic acid as an example, there are glycocholic, taurocholic, and nonconjugated cholic acids. Although bear bile has been used in traditional Chinese medicine for a long time, most bile acids commercially available today are highly purified from slaughterhouses.

Bile acids, particularly chenodeoxycholic acid and ursodeoxycholic acid, have been used as an alternative to surgery in gallstone patients, with an approximately 40% success rate in dissolution of gallstones in patients with symptomatic, small, radiolucent gallstones in otherwise functioning gall bladders. However, gallstone recurrence occurs in approximately half of those treated with bile acids within 5 years, though with a much lower recurrence thereafter. These recurring gallstones can usually be dissolved again following additional treatment with bile acids. The gallstones are typically crystalized cholesterol in the gall bladder, and therefore bile acids improve gallstones by resolubilizing the cholesterol in bile.

Bile acids have also been implicated in altering insulin sensitivity in diabetes and obesity. Most research up to this point has been conducted in animal and cell culture models, demonstrating beneficial metabolic effects, but very little work has been conducted in humans. One promising study in obese humans in which tauroursodeoxycholic acid was administered for 1 month demonstrated improved markers of systemic glucose tolerance driven by increases in muscle and liver insulin sensitivity, but not in adipose tissue. Other suggestions from animal, cell culture, and some human research indicate improvements in longevity, fatty liver diseases, and diet-induced atherosclerosis. However, more research needs to be conducted in humans to confirm these results.

It is important to note that bile acids play a key role in cholesterol homeostasis, and bile acid sequestrants that increase the excretion of bile acids have been used to decrease whole body cholesterol by requiring more bile acids to be synthesized from cholesterol. Rather than a benefit from supplementation, this is a benefit from a physiological decrease in bile acids.

Carnitine

Cells shuttle fatty acids into mitochondria through a carnitine-coupled transport system to utilize them for energy and metabolism. Fatty acids are coupled to carnitine through the carnitine-palmitoyl transferases, and can then enter the mitochondria for energy production through β-oxidation.

Humans are capable of synthesizing carnitine, and thus it is not an essential nutrient. In addition, most Western diets are sufficient in carnitine because of high contents in red meat, dairy, poultry, and fish, with the highest quantities found in red meat. In certain medical conditions, carnitine deficiency can occur, such as genetic primary carnitine deficiency, chronic renal failure, or specific disorders with intestinal absorption. In these cases, carnitine supplementation is used to fulfill a

nutrient deficiency rather than a nutraceutical function as defined above.

Tissue carnitine concentrations tend to decrease with age. In older adults, carnitine supplementation in the form of acetyl-L-carnitine reduced cognitive deterioration in individuals who had mild cognitive impairment or mild/early Alzheimer's disease. Benefits were detected as early as 3 months, with both clinical and psychometric measurements improving over time compared to a placebo. Carnitine has also been implicated in improving vitality in cancer patients low in carnitine. Carnitine supplementation in carnitine-deficient cancer patients appeared to improve signs of Cancer Related Fatigue, such as increasing vitality, decreasing fatigue, and improving quality of life scores. However, at least one randomized study failed to reproduce this effect.

Several studies have implicated carnitine in improving male fertility, such as by improving sperm quality, motility, fatty acid oxidation, and mortality in the testes.

One nutraceutical function attributed to carnitine is the boosting of athletic performance; however, the results of human studies have been inconclusive. In addition, using carnitine to alter body composition or as a weight-loss aid is not strongly supported by research in humans.

Conjugated Linoleic Acid

Conjugated linoleic acid, commonly called CLA, is a series of 18 carbon, doubly unsaturated fatty acids. The two most common isomers are *cis*9 *trans*11 and *trans*10 *cis*12, which are considered natural *trans*-fatty acids and are generally regarded as not having the same deleterious properties as artificial *trans*-fatty acids.

Interest in CLA blossomed when it was identified as an antitumorigenic compound found in beef. It is produced in ruminant animals through rumen biohydrogenation of unsaturated fatty acids. In addition, humans can synthesize some forms of CLA from specific precursors, such as *trans*-vaccenic acid (tVA), which is a naturally occurring monounsaturated *trans*-fatty acid. Through a delta-9 desaturase enzyme, humans can convert tVA into *cis*9 *trans*11 CLA.

Grass-fed cattle contain more CLA in their meat and milk than grain fed cattle, particularly in times of rapid grass growth. Feeding fish oil and flax seed to cattle has been shown to increase the CLA content of meat and milk as well. However, most studies looking for health effects in beef and dairy products enriched in CLA have shown no or modest health benefits. Most research showing benefits of CLA have demonstrated the effects in nutraceutical form.

Although it was originally investigated for potential antitumorigenic properties, human studies have not demonstrated clear results regarding the effects of CLA and cancer. One case-control study demonstrated a strong negative correlation between dietary and serum CLA and tVA and breast cancer in postmenopausal women; however, CLA content of breast adipose tissue appeared to be a modest risk factor. In addition, total CLA and tVA exposure was modestly associated with an increase in breast cancer in a cohort study in the Netherlands. Several other studies have demonstrated no association, and thus the effects of CLA on *in vivo* tumorigenesis are still inconclusive.

CLA has also been thought to affect attributes of the metabolic syndrome, such as body composition, body weight, and insulin sensitivity. These effects are purportedly from CLA acting on a transcription factor known as Peroxisome Proliferator Activated Receptor gamma (PPAR-γ), which is involved in regulating energy homeostasis. However, studies of overweight Caucasian males supplemented with approximately 3 g of CLA daily for several months have demonstrated decreases in insulin sensitivity. However, one meta-analysis concluded that CLA may induce a modest (0.05 kg per week) decrease in body fat mass. Although the effects of CLA on health appear to be isomer specific, too little data exist in humans to adequately characterize these effects.

Glucosamine and Chondroitin Sulfate

Glucosamine and chondroitin sulfate are two nitrogen-containing carbohydrates found as part of the extracellular matrix in joints. Two polymers of glucosamine are chitin and chitosan, which are components of crustacean exoskeletons; hydrolyzing these polymers is one source of glucosamine for dietary supplement preparations. The two common forms of glucosamine found in supplements are glucosamine sulfate and glucosamine hydrochloride. Chondroitin sulfate, however, is composed of a polymerized, variably sulfated disaccharide of glucuronic acid and acetylgalactosamine. The disaccharide unit can be sulfated at three different positions, which affects the overall structure and function of chondroitin sulfate. Although the sulfate in glucosamine preparations serves as a counter ion to the positive charge on the amine, the sulfate in chondroitin is covalently attached at the various positions. Two common sources of chondroitin sulfate are bovine and shark cartilage.

Glucosamine has been researched in terms of impeding the progression of osteoarthritis and decreasing associated pain. A number of studies have demonstrated a decrease in several measurements of osteoarthritis pain, including Lequesne's severity index, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and reports of daily pain. In addition, some studies have shown trends toward or significant slowing of joint space narrowing in patients with osteoarthritis. Although some studies demonstrated no improvements over placebo, glucosamine is well tolerated and resulted in fewer adverse events than ibuprofen administration for pain. One point of controversy is whether there is a difference in efficacy between the sulfate and chloride counter ions of glucosamine. Glucosamine sulfate has been more thoroughly studied than glucosamine hydrochloride, and although there is still some evidence that glucosamine hydrochloride may decrease pain outcomes, the evidence is less convincing than for the sulfate form.

Chondroitin sulfate has been evaluated for the same endpoints as glucosamine. Similarly, a number of studies have shown decreases in pain as measured by Lequesne's index, decreases in functional impairment, and retardation of joint narrowing. Again, although some studies demonstrated no improvements over placebo, chondroitin sulfate was as well tolerated as placebo.

The combination supplementation of chondroitin sulfate and glucosamine has also been investigated for osteoarthritis.

The Glucosamine/Chondroitin Arthritis Intervention Trial, a rigorous randomized clinical trial, did not demonstrate an improvement in joint pain; however, an exploratory analysis demonstrated that glucosamine plus chondroitin improved pain measurements in individuals who started with moderate-to-severe pain. Another study of middle-aged to elderly women showed improvements in WOMAC scores and subjective measurements of joint function. In addition, the combination of chondroitin sulfate and glucosamine resulted in a decreased need for nonsteroidal antiinflammatory drugs. Thus, the efficacy and effect size of glucosamine and chondroitin sulfate regarding osteoarthritis progression are still not certain.

Heme Iron

Iron is an essential mineral required for a number of functions in the body. When an iron atom is embedded in a porphyrin ring structure, it is called heme iron. Heme iron is the molecule responsible for the oxygen- and carbon dioxide-carrying capacity of hemoglobin in red blood cells; myoglobin serves a similar function in muscle. Heme iron is also an integral part of the cytochrome P450 system, which is responsible for a vast number of enzymatic oxidation reactions.

Dietary iron can be found either complexed to heme or in other forms. Heme iron is absorbed more efficiently than nonheme iron; furthermore, unlike nonheme iron, the absorption of heme iron is not negatively affected by dietary components known to chelate minerals, such as phytate, or in the presence of other minerals, such as calcium.

Individuals who have low intakes of iron, have iron deficient anemia, or in other ways have an insufficient iron status will benefit the most from taking iron. Extreme or intense athletes who otherwise have normal iron absorption can become iron deficient from increased iron requirements, blood loss, rhabdomyolysis, or hemolysis. Therefore, iron supplementation may support athletic performance in those at risk of becoming iron deficient through exercise. However, iron supplementation to individuals who are deficient does not necessarily impart nutraceutical properties beyond those of basic dietary supplementation.

As a nutraceutical, iron has not been shown to boost athletic performance beyond relieving a deficiency, and most other research on iron beyond basic requirements generally tends toward concerns regarding oxidative damage, coronary heart disease, and other maladies, although the results have generally been inconclusive. Because the human body does not have a mechanism to get rid of excess iron, supplementing the diet with excess iron could lead to toxicity.

Omega-3 Fatty Acids

Omega-3 fatty acids, also called n-3 fatty acids, are lipids that are characterized by an unsaturated bond three carbons from the end of the acyl tail. This contrasts with omega-6 (or n-6) fatty acids, which have the double bond six carbons from the end. Other positions of double bonds are possible, but omega-3 and omega-6 fatty acids are often discussed together, with some uncertainty as to whether it is the total quantity of omega-3 fatty acids in the diet that leads to their health-modulating effects, or the ratio of omega-3 to omega-6 fatty acids in the diet. Both omega-3 and omega-6 fatty acids are essential nutrients, meaning that the double bonds that define omega-3 and omega-6 fatty acids cannot be synthesized by humans. Very long chain, highly unsaturated omega-3 fatty acids are often considered more active in health promotion, including eicosapentaenoic acid ((EPA), a 20 carbon fatty acid with 5 double bonds) and docosahexaenoic acid ((DHA), a 22 carbon fatty acid with 6 double bonds). Humans can synthesize very long chain omega-3 fatty acids from long chain omega-3 precursors, such as α-linolenic acid (ALA), but cannot create the omega-3 bond itself.

One of the greatest sources of omega-3 fatty acids in human diets is fish and fish oil. Often, DHA and EPA are considered fish oil fatty acids, although the fish accumulate these fatty acids most often from consuming algae. Therefore, aquaculture in which fish are fed grains such as corn results in fish with lower omega-3 fatty acids. The greatest terrestrial source of omega-3 is flax seed, which has spurred the supplementation of animal diets with flax to increase the omega-3 fatty acid content of the meat. The omega-3 in flaxseed, as well as what little there often is in other terrestrial animal sources, is predominantly ALA, which is not considered as bioavailable or as health promoting as DHA and EPA. Therefore, livestock animals are not likely to be a good source of omega-3s for nutraceutical supplementation.

Omega-3 fatty acids decrease blood triglycerides in a dose-dependent manner. Elevated triglycerides are common in type II diabetes, and although omega-3 fatty acids decrease triglycerides, little convincing evidence exists regarding the effects of omega-3s on other hallmarks of diabetes, such as insulin resistance or blood glucose. Omega-3 fatty acids do, however, improve all-cause mortality related to cardiovascular diseases, particularly for EPA and DHA. To a lesser extent, evidence suggests that omega-3s modestly improve blood pressure.

A number of observational studies have linked increased EPA and DHA to decreases in prostate and breast cancers, with some of these studies focusing on an increased omega-3/omega-6 ratio. However, just as many studies have shown no effect, although there is little to no evidence of increased risk of these cancers with omega-3 consumption.

Omega-3s have also been implicated in improving joint tenderness in rheumatoid arthritis, but not necessarily joint pain. Other conditions studied include inflammatory bowel disease, renal disease, lupus, and bone fractures, but the evidence of improvement in these morbidities is much less certain.

Vitamin D

Vitamin D is a sterol-derived, lipid-soluble vitamin that can be synthesized endogenously or acquired through some foods, such as fish oil, egg yolk, beef liver, and fortified foods such as dairy products. There are multiple forms of vitamin D. Vitamin D_3, or cholecalciferol, is created from 7-dehydrocholesterol (a precursor in cholesterol synthesis) when skin is exposed to Ultraviolet (UV) light; it is also found in some foods. Vitamin D_3 is then processed in the liver to create 25-hydroxy vitamin D_3, or calcidiol. Calcidiol is further hydroxylated to

create 1,25-dihydroxy Vitamin D_3, or calcitriol, primarily by the kidneys. If the starting sterol is ergosterol instead of 7-dehydrocholesterol, the series of compounds are known as vitamin D_2; because these are primarily derived from non-animal sources, they will not be discussed further here. The vitamin D_3 used to fortify milk and other products can come from extraction of 7-dehydrocholesterol from animal hides, followed by exposure of the extract to UV light.

Calcitriol is generally considered the active form of vitamin D_3, functioning to facilitate calcium absorption from the intestines, as well as to act as a hormone in controlling calcium balance within the body, such as altering bone resorption and renal calcium filtration. These functions contribute to vitamin D's status as a vitamin, leading to the two well-known vitamin D deficiency diseases of rickets and osteomalacia.

Vitamin D has been linked to decreased risk of cardiovascular disease, stroke, hypertension, diabetes, metabolic syndrome, and cancer. Beyond the clear benefits to bone health, the US Institute of Medicine determined that all other health claims were inconclusive, inconsistent, and insufficiently characterized. However, a number of studies point to future potential for nutraceutical properties of vitamin D. For instance, observational studies have demonstrated an inverse relationship between diabetes risk and vitamin D status, although only in certain populations. Conversely, when individuals were supplemented with vitamin D in controlled studies, there appeared to be little if any improvement in diabetes risk. With regard to stroke, recent findings suggest that vitamin D supplementation may improve stroke risk, possibly by altering hypertension.

The relationship between vitamin D and cancer is more complex. Although some data exist to suggest that vitamin D may increase the risk of certain cancers, such as an increase in pancreatic cancer risk in a cohort of Finnish smokers, most evidence points to vitamin D having either beneficial or no associations with cancer risk, particularly regarding colon, breast, prostate, and overall cancer risk.

See also: Chemical Analysis for Specific Components: Micronutrients and Other Minor Meat Components. Functional Foods. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns; Micronutrients in Meat. Nutrient Claims on Packaging

Further Reading

Coates, P.M., 2010. Encyclopedia of dietary supplements, second ed. New York: Informa Healthcare.

Heinze, V.M., Actis, A.B., 2012. Dietary conjugated linoleic acid and long-chain n-3 fatty acids in mammary and prostate cancer protection: A review. International Journal of Food Sciences and Nutrition 63 (1), 66–78.

IOM (Institute of Medicine), 2011. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press.

MacLean, C.H., Mojica, W.A., Morton, S.C., *et al*., 2004. Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis. Evidence Report/Technology Assessment No. 89. AHRQ Publication No. 04-E012-2. Rockville, MD: Agency for Healthcare Research and Quality.

Vangsness, Jr., C.T., Spiker, W., Erickson, J., 2009. A review of evidence-based medicine for glucosamine and chondroitin sulfate use in knee osteoarthritis. Arthroscopy 25 (1), 86–94.

Relevant Websites

http://www.efsa.europa.eu/
European Food Safety Authority.

http:/www.fsc.go.jp/english
Food Safety Commission of Japan.

http://ods.od.nih.gov/
Office of Dietary Supplements, National Institutes of Health (United States of America).

http://www.fda.gov
United States Food and Drug Administration.

Vegetarianism

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Glossary

Ferritin An intracellular protein that stores and releases iron.
Hypercholesterolemic Having high levels of blood cholesterol.
Implicit association An automatic association between different concepts.
Socioeconomic status A measure of one's social standing or social class, typically based on education, income, and occupation.
Visceral Relating to or perceived as if in the viscera (internal organs).

Introduction

This article reviews the current literature on vegetarianism, exploring what motivates people to choose a vegetarian diet and how vegetarians and meat eaters often differ in their attitudes toward meat consumption, as well as how they tend to differ in their broader attitudes, values, and worldviews. Furthermore, this article provides an overview of the potential health benefits and health risks associated with vegetarian diets. Ethical and spiritual concerns have motivated vegetarianism since the time of Pythagoras, and these concerns were further expanded in the twentieth century to include specific concerns for animal rights, environmental welfare, and world hunger. In contrast, scientifically grounded arguments for the health benefits of a vegetarian diet are more recent, only emerging in the nineteenth century. Recent polls indicate that approximately 3% of the US Americans and 8% of Canadians identify themselves as vegetarian. Additional polls estimate rates of 3% in the UK, 1–2% in New Zealand, and 3% in Australia, with considerably higher rates of 6% in Ireland, 9% in Germany, 8.5% in Israel, and 40% in India. Although vegetarians are a minority in most cultures, they are not always small minorities, and the popularity of vegetarian diets is on the rise in many countries. As the popularity of vegetarianism has grown, so too has the number of scholars formally investigating the practice of vegetarianism.

Definitions of and Motivations for Vegetarianism

Although the most common definition of vegetarianism is the avoidance of red meat, poultry, and fish; considerable inconsistency among people's self-identifications arises within the literature, such that many people who claim to be vegetarian do not actually follow a strict vegetarian diet. In a 1997 Canadian survey, 78% of self-identified vegetarians reported that they sometimes consumed fish or seafood, 61% sometimes ate poultry, and 20% sometimes ate red meat. In 2001, 34% of self-identified Canadian vegetarians sometimes consumed red meat. Similarly, among a 2004 survey of self-identified vegetarians in the USA, only 30% said that they never ate fish, 36% said that they never ate poultry, and 64% said that they never ate red meat, and comparable discrepancies have emerged among Swiss and British populations. This inconsistency is not solely limited to lay people – among a 1990 survey of women physicians in the US, 38% of self-identified vegetarians had eaten fish, poultry, or red meat at least once in the past week.

A more precise way of assessing vegetarianism is along a continuum. At one end of this continuum are omnivores, who consume all manner of animal products. Further along the continuum are partial vegetarians, who avoid red meat but still consume poultry, fish, eggs, and dairy. Further along the continuum are pescatarians, who are similar to vegetarians but eat fish and shellfish in addition to an otherwise vegetarian diet. Further still along the spectrum are lacto–ovo vegetarians, who eat no meat, fish, or poultry but do eat eggs and dairy, followed by lacto-vegetarians, who also avoid eggs. At the end of the spectrum are vegans, who consume no animal products whatsoever. For the sake of parsimony, the term vegetarian will be used in this article to describe those who are following a lacto–ovo-, lacto-, or vegan diet, unless otherwise indicated.

Just as people's definitions of vegetarianism vary, so do their motivations for following a vegetarian diet. In Indian cultural contexts, family traditions and religious beliefs about the spiritually polluting effects of meat consumption emerge as the predominant motivations for vegetarianism, whereas in the majority of recent studies conducted in Western societies, the most common motivation for vegetarianism is concern that meat consumption is not consistent with animal welfare. Secondarily, people report concern for health and the environmental impact of meat consumption, followed by spiritual concerns and disgust at the taste and texture of meat (see Tables 1 and 2).

Research has uncovered a series of major differences between people whose vegetarianism is chiefly motivated by ethical concerns and those who are primarily motivated by concern for health. Compared with health vegetarians, ethical vegetarians consume a smaller range of animal products,

Table 1 Prevalence of common motivations for vegetarianism

Study	*Location*	*N*	*Percent giving reasons*				*Religion*	*Other*
			Animal welfare	*Health*	*Environment*	*Disgust toward meat*		
Beardsworth and Keil (1991)	UK	76	66	26	1	20		
Fox and Ward (2008)	USA, Canada, and UK	33	45	27	3			25
Hamilton (2006)	UK	47	49	34		11		19
Hussar and Harris (2009)	USA	16	72	6		9	9	16
Jabs *et al.* (1998)	USA	19	58	42				
Krizmanic (1992)	USA	301	19	46	4			30
Neale *et al.* (1993)	UK	174	91	39			6	37
Potts and White (2008)	New Zealand	155	54		7		14	
Preylo and Arikawa (2008)	USA	72	68					32
Rozin *et al.* (1997)	USA	104	64	77	61	53	23	55
Santos and Booth (1996)	UK	13	92	0		61		23
White *et al.* (1999)	USA	360	42	69	32	41	30	11

Source: Adapted from Ruby, M.B., Heine, S.J., 2012. Vegetarianism: A blossoming field of study. Appetite 58, 141–150.

Table 2 Summary of the most common motivations for vegetarianism

Reason	*Summary*
Animal welfare	Avoidance of meat due to the belief that modern meat production is unethical. Some avoid all meat products to alleviate this concern, whereas others consume meats only from sources whose animal welfare practices they find acceptable, such as local family farms
Environment	Avoidance of some or all types of meat out of the concern that meat production has negative effects on the environment and is unsustainable (e.g., carbon dioxide emissions, soil erosion, and resource use). This particular motivation is relatively new and its associations are largely unknown
Health	Avoidance of meat out of concern for their health. This motivation has three main components: (1) avoiding cholesterol and saturated fat, (2) avoiding substances in the meat that one considers harmful (e.g., hormones, antibiotics, and pathogens), and (3) attempting to reduce the risk of specific health problems that one believes to be associated with meat consumption (e.g., cancer, hypertension, and heart disease)
Disgust	Avoidance of meat due to disgust at the sight of it, especially visible blood or bodily juices. Some find the taste and/or smell of meat disgusting or dislike its texture and springy mouth feel
Religion	Avoidance of some or all meats for spiritual or religious reasons. Eating meat is sometimes believed to be spiritually polluting or to exert undesirable influences on one's character, such as increased levels of aggression. Religions that encourage vegetarianism include some branches of Hinduism, Jainism, and Buddhism

Source: Adapted from Gregory, N.G., 2004. Vegetarianism. In: Jensen, W.K. (Ed.), Encyclopedia of Meat Sciences, pp. 633–640.

transition more swiftly to a vegetarian diet, are more concerned with animal welfare, and respond to meat consumption with stronger feelings of disgust. At present, little is known about those whose vegetarianism is primarily motivated by other factors, such as concern for environmental sustainability.

After adopting a vegetarian diet, people often add and drop motivations over time. In general, people are more likely to add than to drop motivations, especially among those who are originally motivated by concern for animal welfare. Furthermore, there is evidence that people tend to move along a trajectory – in a 2002 study of women in Vancouver, Canada, most current vegetarians consumed a smaller range of animal products than when they first became vegetarians. Nonvegetarians hold relatively similar beliefs about why one might wish to follow a vegetarian diet, although they prioritize motivations in a different order than vegetarians do. In a 2003 South Australian study, nonvegetarian participants most commonly cited potential health benefits of a vegetarian diet, such as eating less saturated fat (65%) and controlling one's weight (40%), whereas concern for animal suffering (36%) and environmental welfare (22%) were cited less often.

Just as people identify common motivations for adopting a vegetarian diet, people also exhibit high levels of agreement on common deterrents to vegetarianism. In a 2003 study of Australian adults, the most commonly cited deterrent to vegetarianism was the enjoyment of eating meat (78%), followed by an unwillingness to change one's eating habits (56%), beliefs that humans are meant to eat meat (44%), and insufficient knowledge about vegetarian diets (42%). As with much of the literature on vegetarianism, pronounced gender differences emerged, such that more men than women believed that humans are meant to eat meat (49% vs. 39%) and that women were more likely than men to report the unwillingness of their family, spouse, or partner as a significant deterrent to adopting a vegetarian diet (39% vs. 18%). Although research on former vegetarians is rare, a 2002 study of formerly vegetarian women in Vancouver, Canada found that the most common reasons for resuming meat consumption were health concerns such as anemia and fatigue (29%), missing the taste of meat (23%), a change in living situation (such as moving in with a meat-eating family, 17%), and the perception that following a vegetarian diet was too time consuming (17%).

Attitudes toward Meat

Alongside fundamental differences in their eating practices, vegetarians and omnivores tend to hold very different attitudes toward meat. A 1998 study of teenage British girls revealed that vegetarians had strongly negative attitudes toward meat, linking it with the killing of animals, cruelty, the ingestion of blood, and visceral disgust. The nonvegetarian girls, however, had very positive attitudes toward meat, linking it with the concepts of luxury, social status, good taste, and special occasions such as holiday dinners. In recent years, several studies have found evidence for changing attitudes toward meat-based and vegetarian foods among omnivores in many Western societies. In a 2005 Canadian survey, 40% of respondents indicated that they sometimes seek out meatless meals; retail grocery sales of tofu and plant-based meat substitute products increased by 50% in Canada between 2000 and 2003; and in 2006, the US market for processed vegetarian foods (e.g., meat substitutes and nondairy milks) was estimated to be approximately US$1.17 billion. This trend also appears to hold outside the home – in a 2007 survey conducted by the National Restaurant Association, 71% of surveyed chefs in the USA considered vegetarian dishes to be 'hot' or 'a perennial favorite,' 63% described vegan dishes in the same terms. One reason cited for this shift is concern for animal welfare. A 2004 Canadian survey revealed that approximately 20% of respondents had boycotted food products out of concern for the treatment of the animals on the farm or during processing. Animal processing appears to be an area of particular concern – in a 1993 study of meat eaters in the UK, when presented with the hypothetical prospect of having to personally kill the animals that they wished to eat, the majority said that they would stop eating meat altogether. Obviously, an extremely high percentage of people would neither have access to a facility nor have the knowledge or skill to kill and process animals.

Values and Vegetarianism

A growing body of research indicates that, within Western populations, vegetarians and omnivores tend to endorse different sets of values, with endorsement of liberal values more common among vegetarians and endorsement of conservative values more common among omnivores. A 1990 study of women physicians in the USA found that those who self-identified themselves as 'very liberal' were two times more likely to be vegetarian than those who self-identified themselves as 'conservative.' Similar results emerged in a 1995 study conducted in the northeast USA, such that those endorsing 'traditional values' (e.g., social order, family security, and obedience) were more likely to be omnivores, whereas those who endorsed 'altruistic values' (e.g., equality, social justice, and environmental welfare) were more likely to be vegetarians. Concordantly, a 2004 Dutch study found that vegetarians were more concerned than omnivores with the impact of their food choices on the environment, and in a 2000 New Zealand study, those with a more pronounced omnivore identity more strongly endorsed right-wing authoritarianism and social hierarchies. A 2006 UK study found that, compared with omnivores, vegetarians reported greater opposition to capital punishment, and this antiviolence stance was especially strong among ethically motivated vegetarians. Similarly, in a 2008 study of the US Americans, vegetarians reported greater human-directed empathy than omnivores, and in a 2010 study conducted in Italy, ethically motivated vegetarians reported more concern for human suffering and showed increased recruitment of empathy-related areas of the brain when viewing scenes of human and animal suffering.

Perceptions of Vegetarians

Perceptions of vegetarians and vegetarianism have changed over time. Up through the early twentieth century, public and professional attitudes toward vegetarians remained very negative – indeed, in a 1946 paper on the psychology of vegetarianism, drawing on personal anecdotes, a prominent New York psychiatrist claimed that vegetarians were domineering and secretly sadistic, possessed little regard for their fellow human beings, and avoided eating meat in order to make difficulties for others.

In more recent times, people appear to hold common stereotypes about the personalities of vegetarians. In a 1986 study conducted in Arizona in the USA, meat eaters thought of vegetarians as typically pacifist, weight conscious, drug using, and liberal, and a similar pattern emerged among vegetarians who saw themselves as relatively intellectual, weight conscious, and sexy, with a tendency toward using recreational drugs. Further studies conducted in the US in the 1990s found that attitudes toward vegetarians were generally positive, especially among women, teenage girls, and self-identified liberals. A 2007 study of the US Americans obtained more nuanced results, in that meat eaters viewed vegetarians as more highly moral but relatively weak; parallel results emerged in a 2011 study of Canadian students, in which both meat eaters and vegetarians considered vegetarians to be more moral but less masculine than their omnivorous counterparts.

Vegetarianism and Health

Although research conducted in Western societies reveals that concern for animal welfare is often the most common motivation for vegetarianism, concerns for personal health are a notable and increasingly prevalent reason why people reduce or cease meat consumption. The most common concerns are about cardiovascular disease, cancer, obesity, high cholesterol, and the consumption of hormones and antibiotics. However, strict vegetarianism can introduce health risks, especially in the earlier stages of life. Although it was long maintained that meat was essential to human nutrition, recent research questions this stance. In their 2009 position paper, the American Dietetic Association holds that "appropriately planned vegetarian diets are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases. Well-planned vegetarian diets are appropriate for individuals at all stages of the lifecycle, including pregnancy, lactation, infancy, childhood, and adolescence, and for athletes." That said, proper planning is critical, as a poorly planned diet runs the risk of nutritional inadequacies and subsequent negative health outcomes.

Vegetarian diets are generally associated with lower body mass index (BMI) and lower rates of obesity. This pattern of results was found in a large cross-sectional study of 34 192 Seventh Day Adventists living in California in the USA, as well as in the Oxford Vegetarian study, a prospective study of 6000 vegetarians and 5000 omnivores from the UK. Similarly, in the European Prospective Investigation into Cancer-Oxford (EPIC-Oxford) study involving a cross-sectional study of 37 785 adults, meat eaters had the largest age-adjusted mean BMI, followed by vegetarians, and then by vegans. Furthermore, among a health-conscious subset of EPIC-Oxford, weight gain over a 5-year period was lowest among those who reduced their consumption of animal-based foods.

In both the EPIC-Oxford and the Adventist Health Study, as well as a 1999 meta-analysis of studies conducted in the USA, the UK, and Germany, vegetarians and vegans had a lower risk of death from ischemic heart disease than meat eaters. Notably, these differences held even after controlling for BMI, smoking habits, and socioeconomic status. Concordantly, studies indicate that vegetarians typically have lower levels of total blood cholesterol and low-density lipoprotein (LDL) cholesterol than meat eaters have. Among several cross-sectional and cohort studies conducted in the USA, the UK, and Barbados, vegetarians have lower rates of hypertension than meat eaters, and rates among vegans are even lower. Some research also suggests that vegetarians have lower rates of cancer. Within the Adventist Health Study, after controlling for age, gender, and smoking behavior, vegetarians had substantially lower risk of prostate and colorectal cancer but no significant difference in risk of lung, breast, stomach, or uterine cancer.

The exact mechanisms underlying differences in health outcomes between vegetarians and omnivores are yet unclear. These differences in outcomes are often confounded by differences in lifestyle and habits, and vegetarian diets tend to be lower in saturated fat and cholesterol, higher in dietary fiber, vitamins C and E, folate, magnesium, potassium, and various phytochemicals, and have a higher polyunsaturated to saturated fat ratio. Meta-analyses suggest that milk consumption reduces rates of colorectal cancer, and some vegetarians largely replace meat in their diets with milk products. These differences, rather than the presence or absence of meat per se, are often thought to underlie the various health benefits associated with vegetarian diets. Furthermore, most studies compare health outcomes of participants already following omnivorous or vegetarian diets, and experiments directly testing the health effects of meat consumption are rare. A series of studies conducted in Australia placed meat eaters on a lacto–ovo vegetarian diet for a period of time, then returned them to their normal diet. Among both healthy and hypertensive meat eaters, blood pressure dropped during the vegetarian diet phase and rose on return to the previous diet. The authors were unable to isolate the mechanisms underlying the change, but it was neither linked to changes in sodium, potassium, saturated fat ratio, fiber, or caloric intake, nor to body weight. A recent study comparing different types of omnivorous diets reveals that changes in the type and amount of meat one consumes can also lead to similar changes in health indicators. Among a sample of 36 hypercholesterolemic patients in the USA, participants following a diet with $<8\%$ of calories from saturated fat and 113 g of lean beef per day were able to significantly decrease their LDL cholesterol levels, compared with those following a standard Healthy American Diet. In a case control study of 8724 participants in Uruguay from 1988 to 2000 (6892 cancer cases and 1832 controls), researchers found a positive relationship between total meat intake and risk of several cancers including those of the stomach, esophagus, and colorectum, nervous system, and thyroid, but due to the correlational nature of the study, causality cannot be concluded.

In stark contrast to potential health benefits, some vegetarians and vegans will be at risk for several nutritional inadequacies, particularly calcium, iron, omega-3 fatty acids, vitamins B_{12} and B_6, and vitamin D. Although lacto–ovo vegetarians typically consume similar levels of calcium as nonvegetarians, vegans sometimes have lower intakes than both groups and could fail to meet recommended intakes. Indeed, in the EPIC-Oxford study, vegan participants' risk of bone fracture was approximately 30% higher than that of lacto–ovo vegetarians and meat eaters, more likely due to their lower calcium intake.

There are two forms of iron: that found in plant-based foods is nonheme iron and that in animal-based foods is heme iron. As nonheme iron is more difficult for the body to absorb than heme iron, vegetarians have an increased risk of iron deficiency anemia, especially for milk-fed infants and women who either have reduced iron intakes from dieting or a tendency to lose more iron than usual during menstruation. Indeed, research from Australia and New Zealand has found that vegetarian adults had lower average serum ferritin levels than meat eaters.

Although vitamin B_{12} deficiency is relatively uncommon among lacto–ovo vegetarians due to the consumption of eggs and dairy products, some vegans have markedly lower intakes of vitamin B_{12}, as no known unfortified plant-based food contains reliably high levels of vitamin B_{12}.Vitamin B_{12} is a critical nutrient, as it is required for the synthesis of myelin (the insulation sheath for nerves) and thereby helps maintain a healthy nervous system. Given this, the period following weaning can be especially risky if the parents do not provide adequate supplementation. Among some subgroups of vegans, resistance to supplementation is common, thus increasing the risk of vitamin B_{12} deficiency. As with vitamin B_{12}, a 2005 study of adults in California in the USA suggests that vegans might not consume sufficient levels of vitamin D, which plays an important role in bone health.

A commonly held belief is that vegetarian and vegan diets do not provide adequate levels of quality protein. However, a 1994 review indicates that by eating an assortment of plant-based foods throughout the day, vegetarians and vegans can obtain all essential amino acids, and a 2003 meta-analysis uncovered no significant difference in protein needs as a function of protein source. Vegetarians and vegans typically meet or exceed protein requirements, and a 2004 review indicates than plant-based diets can provide adequate levels of protein for athletes. That said, some plant proteins are more easily digested than others, for example, isolated soy protein is more easily digested than isolated wheat protein. Hence, a 2002 World Health Organization report recommends that those vegetarians whose protein comes mainly from sources that are more difficult to digest, such as legumes and cereals, should consume somewhat higher levels of protein.

Gender and Vegetarianism

One factor that routinely arises in the research and writings on vegetarianism is gender. There is broad evidence across a large array of cultural contexts that men and women approach meat on fundamentally different levels. The idea that meat is predominantly a man's food emerges within many geographical regions – from Europe and North America to Africa and Southeast Asia. A series of experiments conducted in the USA, the UK, and Western Europe in the past decade support claims for strong associations between meat and masculinity. Across a variety of studies and measures, participants both demonstrated implicit associations between meat and maleness and explicitly rated a variety of red meats as especially 'male' foods. These associations are echoed in broader relations to food – those activities which are associated with acquiring and preparing food (e.g., shopping, cooking, and serving) are often considered feminine tasks. Furthermore, a 1995 UK study revealed that men know considerably less about the nutritional properties of the foods that they eat, reporting a larger proportion of high-calorie foods and a lower proportion of fruits and vegetables, and are more likely to have suspicions about the benefits of healthy eating, insisting on large, 'masculine' meals that typically center around meat. In a 1993 study of the US Americans, women consider more nutritious meals to be more pleasurable, convenient, and healthy than men do and are more likely to report that they avoid eating red meat. Similar findings emerged among a 1999 study of Norwegians, such that men are more likely to believe that 'a healthy diet should always include meat.' Given this pattern of results, it is unsurprising that among Western societies, vegetarian women outnumber vegetarian men, and across most surveyed societies, women consume less meat than men do. Concordant with large gender differences in vegetarianism, research within Norwegian and British populations reveals that women are more likely than men to actively reduce their meat consumption.

Summary

In recent years, the prevalence of vegetarianism expanded in many cultural contexts, primarily due to concerns about animal welfare, personal health, and environmental sustainability. The current psychological literature suggests that omnivores and vegetarians tend not only to hold divergent attitudes toward meat consumption but also differ in their broader values, worldviews, and moral intuitions. Vegetarian diets have been associated with some potential health benefits and also with the risk of potential nutritional inadequacies.

See also: Environmental Impact of Meat Production: Primary Production/Meat and the Environment. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns; Macronutrients in Meat; Micronutrients in Meat

Further Reading

Amato, P.R., Partridge, S.A., 1989. The new vegetarians I Promoting health and protecting life. New York, NY: Plenum Press.
Appleby, P.N., Thorogood, M., Mann, J.I., Key, T.J., 1999. The Oxford Vegetarian Study I An overview. American Journal of Clinical Nutrition 70 (suppl. 3), 525S–531S.
Beardsworth, A., Keil, T., 1991. Health-related beliefs and dietary practices among vegetarians and vegans: A qualitative study. Health Education Journal 50, 38–42.
Beardsworth, A., Keil, T., 1992. The vegetarian option: Varieties, conversions, motives and careers. The Sociological Review 40 (2), 253–293.
Craig, W.J., Mangels, A.R., 2009. Position of the American Dietetic Association: Vegetarian diets. Journal of the American Dietetic Association 109, 1266–1282.
Fox, N., Ward, K., 2008. Health, ethics and environment: A qualitative study of vegetarian motivations. Appetite 50, 422–429.
Fraser, G., 1999. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. American Journal of Clinical Nutrition 70 (suppl.), 532S–538S.
Hamilton, M., 2006. Eating death: Vegetarians, meat, and violence. Food, Culture and Society 9, 155–177.
Hussar, K.M., Harris, P.L., 2009. Children who choose not to eat meat : A study of early moral decision-making. Social Development 19, 627–641.
Jabs, J., Devine, C.M., Sobal, J., 1998. Model of the process of adopting vegetarian diets: Health vegetarians and ethical vegetarians. Journal of Nutrition Education 30 (4), 196–203.
Krizmanic, J., 1992. Here's who we are. Vegetarian Times 182, 78–80.
Neale, R.J., Tilston, C.H., Gregson, K., Stagg, T., 1993. Women vegetarians: Lifestyle considerations and attitudes to vegetarianism. Nutrition and Food Science 1, 24–27.
Potts, A., White, M., 2008. New Zealand vegetarians. At odds with their nation. Society and Animals 16, 336–353.
Preece, R., 2008. Sins of the flesh: A history of ethical vegetarian thought. Vancouver: UBC Press.
Preylo, B.D., Arikawa, H., 2008. Comparison of vegetarians and non-vegetarians on pet attitude and empathy. Anthrozoös 21 (4), 387–395.
Roussell, M.A., Hill, A.M., Gaugler, T.L., *et al.*, 2012. Beef in an Optimal Lean Diet study: Effects on lipids, lipoproteins, and apolipoproteins. American Journal of Clinical Nutrition 95, 9–16.
Rozin, P., Hormes, J.M., Faith, M.S., Wansink, B., 2012. Is meat male? A multimethod framework to establish metaphoric or symbolic relationships. Journal of Consumer Research 39 (3), 629–643.
Rozin, P., Markwith, M., Stoess, C., 1997. Moralization and becoming a vegetarian: The transformation of preferences into values and the recruitment of disgust. Psychological Science 8 (2), 67–73.
Ruby, M.B., 2012. Vegetarianism: A blossoming field of study. Appetite 58 (1), 141–150.
Santos, M.L., Booth, D.A., 1996. Influences on meat avoidance among British students. Appetite 197–205.
Simoons, F.J., 1994. Eat not this flesh: Food avoidances from prehistory to the present, second ed. Madison: University of Wisconsin Press.
Sobal, J., 2005. Men, meat, and marriage: Models of masculinity. Food and Foodways 13 (1–2), 135–158.
Spencer, E.A., Appleby, P.N., Davey, G.K., Key, T.J., 2003. Diet and body mass index in 38 000 EPIC-Oxford meat-eaters, fish-eaters, vegetarians, and vegans. International Journal of Obesity 27, 728–734.
Twigg, J., 1979. Food for thought: Purity and vegetarianism. Religion 9, 13–35.
White, R.F., Seymour, J., Frank, E., 1999. Vegetarianism among women physicians. Journal of the American Dietetic Association 99 (5), 595–598.

Relevant Websites

http://www.eatright.org/
Academy of Nutrition and Dietetics.
http://www.llu.edu/public-health/health/index.page
Adventist Health Studies.
http://www.epic-oxford.org/
The EPIC-Oxford Study.

IRRADIATION

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Glossary

Food irradiation Process of exposing foodstuffs to a source of energy capable of stripping electrons from individual atoms in the targeted material.

Gray (Gy) 1 J kg^{-1} – a physical quantity. 1 Gy is the deposit of a joule of radiation energy in a kilogram of matter or tissue. Such energies are typically associated with ionizing radiation, such as X-rays or gamma particles or with other nuclear particles. It is defined as the absorption of one joule of such energy by one kilogram of matter.

Ionizing radiation Radiation composed of particles that individually carry enough kinetic energy to liberate an electron from an atom or molecule.

Irradiation The process by which an object is exposed to radiation originating from various sources other than background radiation and usually excludes the exposure to nonionizing radiation, such as infrared, visible light, microwaves, or other electromagnetic waves.

Nonionizing radiation Any type of electromagnetic radiation that does not carry enough energy to ionize atoms or molecules. It has sufficient energy only for excitation of atoms to a higher energy state and can produce effects in biological tissue that can lead to burns (e.g., UV radiation to cause sunburn), or accelerate some chemical reactions.

Pasteurization A process by which the microbial population present on food is reduced to improve the shelf life and safety of the food product. Pasteurization often consists of heating food to a specific temperature, for example, 72 °C and immediately cooling it, but can include any physical, chemical, or biological process that reduces the subsequent microbial growth.

Radiation Radiation is a process in which electromagnetic waves travel through a vacuum or matter. This spectrum of radiant energy can be divided into ionizing and nonionizing radiation.

Sievert (Sv) 1 J kg^{-1} – a biological effect. 1 Sv represents the equivalent biological effect of the deposit of a joule of radiation energy in a kilogram of tissue. A derived unit is used for radiation dose quantities such as equivalent dose, effective dose, and committed dose. It is a measure of the effect of low levels of radiation on the human body.

Introduction

Food irradiation is the process of exposing foodstuffs to a source of ionizing radiation emitted by a radioactive substance or generated by high-energy accelerators including X-rays. Radiation can also be used for nonfood applications, such as medical devices or even for examining tubes for gas pipelines, plastics, hoses for floor heating, and even automobile parts, wires and cables, and even gemstones.

Ionizing and Nonionizing Radiation

This spectrum of radiant energy can be divided into ionizing and nonionizing, according to whether it ionizes or does not ionize the atoms in ordinary chemical matter. Both ionizing and nonionizing radiation can be harmful to organisms. In general, however, ionizing radiation is far more harmful to living organisms per unit of energy deposited than nonionizing radiation, even at low radiation doses. Random effects in a

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cell can result in anything from harmless reactions to degradation of important structures in the cell; to killing it outright or triggering suicide (apoptosis); or modifying the deoxyribonucleic acid (DNA) in harmful, but yet temporary ways. By contrast, most nonionizing irradiation is harmful to organisms only in proportion to the thermal energy deposited (a prime example is microwaves generated in a microwave oven). Broiling and toasting use high radiant energies to cook food.

The radiation of interest in food preservation is ionizing radiation, also known as irradiation. These shorter wavelengths are capable of damaging microorganisms, such as those that contaminate food or cause food spoilage and deterioration. Due to the capability of microorganisms to contaminate foods and the fact that much of our food supply is lost due to spoilage and insects each year, scientists have been experimenting with irradiation as a method of food preservation since 1950. They have found irradiation to be a controlled and very predictable process.

Only certain radiation sources can be used in food irradiation. These are the radionuclides cobalt-60 or cesium-137 (used very rarely); X-ray machines having a maximum energy of 5 MeV (million electron volts); or electron machines having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in any material, including food. In addition, the radioactive source is never in contact with the foodstuffs and this also ensures the energy of radiation is limited below the threshold of induction of radioactivity (Figure 1).

Radiation dose is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. The units used are Gray (Gy), which is the unit of 'absorbed dose' (1 J kg^{-1} – a physical quantity) or in rad (1 Gy = 100 rads). This differs from the Sievert (Sv): which represents the 'equivalent dose' (1 J kg^{-1} – a biological effect). The Sievert is generally used as a measure of the effect of low levels of radiation on the human body. International health and safety authorities have endorsed the safety of irradiation for all foods up to a dose level of 10 000 Gy (10 kGy).

As the energetic particles or waves pass through the target material, chemical bonds are broken and, in particular, they can damage DNA, affect reproduction of microorganisms and other organisms such as insects, and thus preserve food. In this way, it can reduce the risk of foodborne illness, prevent the spread of invasive pests, and even eliminate sprouting or ripening.

Irradiation Compared to Pasteurization

As in the heat pasteurization of milk, the irradiation process greatly reduces but does not eliminate all bacteria. Irradiated poultry, for example, still requires refrigeration, but would be safe longer than untreated poultry. Strawberries that have been irradiated will last 2–3 weeks in the refrigerator compared to only a few days for untreated berries. Irradiation complements, but does not replace, the need for proper food handling practices by producers, processors, and consumers.

Two things are needed for the irradiation process:

- a source of radiant energy, and
- a way to confine that energy.

For food irradiation, the sources are radioisotopes (radioactive materials) and machines that produce high-energy beams. Specially constructed containers or compartments are used to confine the beams so that personnel will not be exposed.

Machines that produce high-energy beams offer greater flexibility. For example, they can be turned on and off unlike the constant emission of gamma rays from radioisotopes.

Regulation of Food Irradiation

Since 1986, all irradiated products must carry the international symbol called a radura, which resembles a stylized flower.

Treated with irradiation or Treated by irradiation

The United States Food and Drug Administration (FDA) requires that both the logo and statement appear on packaged foods, bulk containers of unpackaged foods, on placards at the point of purchase (for fresh produce), and on invoices for irradiated ingredients and products sold to food processors.

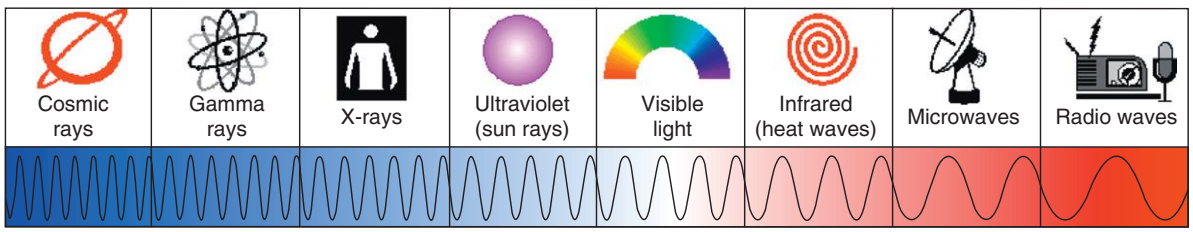

Figure 1 A diagrammatic representation of the energy spectrum. UW Food Irradiation Education Group (http://uw-food-irradiation.engr.wisc.edu/Process.html).

Processors may add information explaining why irradiation is used; for example, 'treated with irradiation to inhibit spoilage' or 'treated with irradiation instead of chemicals to control insect infestation.' Accurate plant records are essential to regulation because there is no way to verify or detect if a product has been irradiated, or how much radiation it has received.

The Food Irradiation Process

Irradiation is known as a cold process, and is sometimes called cold pasteurization. It does not significantly increase the temperature or change the physical or sensory characteristics of most foods. An irradiated apple, for example, will still be crisp and juicy. Fresh or frozen meat can be irradiated without cooking it. During irradiation, the energy affects unwanted organisms but is not retained in the food. Similarly, food cooked in a microwave oven, or teeth and bones that have been X-rayed do not retain those energy waves.

Irradiation of Muscle Foods

Irradiation Effects on Meat Flavor

Processing of meat, with exposure to ultraviolet (UV) light, heat, and oxygen can cause flavor changes and irradiation is no different. The changes due to irradiating fresh meat, even at low doses, therefore can result in off-odors and off-flavors (which have been described as rotten egg, bloody, fishy, barbecued corn, burnt, sulfur, metallic, alcohol, or acetic acid). The changes, however, are exacerbated by the other factors also known to have effects, such as oxygen exposure during and after the irradiation process. Methods to decrease any detrimental effects of irradiation include oxygen exclusion (vacuum packaging), replacement with inert gases (nitrogen), addition of protective agents (antioxidants), and postirradiation storage to allow flavor to return to near-normal levels (repackaging or double packaging in oxygen impermeable film). The changes through irradiation result from initiation or promotion of lipid oxidation or formation of free radicals from unsaturated fatty acids at double bond positions.

Quality

Irradiation might affect the quality of meat by processes other than those attributable to microorganisms. Radiation dose, dose rate, temperature and atmosphere during irradiation, and temperature and atmosphere during storage can all affect the outcome of specific foods. Radiolytic products can cause oxidation of myoglobin and fat, leading to discoloration and rancidity or other off-odor or off-flavor compounds. Ozone, a strong oxidizer, is produced from oxygen during food irradiation and may oxidize myoglobin, causing a bleaching discoloration.

Some scientists have observed that irradiated raw meat developed an off-odor compared with the nonirradiated control and the threshold dose for irradiation odor ranged from 1.5 kGy for turkey to 6.25 kGy for lamb. In some studies, an irradiation odor was detected but not objectionable in raw beef irradiated at low dose. Cooking appears to reduce or eliminate any irradiation-induced odor. Odor resulting from irradiation might thus be important only in raw meat. Further investigation would enable full characterization of irradiation-induced odor and better understanding of the conditions that affect its development.

Irradiation of frozen grass prawns at 10 kGy reduced levels of polyunsaturated fatty acids (C20:5 and C22:6) by 25–32%, possibly due to oxidation and decomposition of lipids into volatile compounds. The threshold dose for development of irradiation flavor in the frozen grass prawns was 4.5 kGy.

Color

Irradiation can also cause some color changes in meat that are greatly influenced by the packaging environment, just the same as in other processes. For example, irradiated vacuum-packaged meat can develop a fairly stable brighter red or pink color in pork, beef, and turkey breasts. In the presence of oxygen, however, irradiation can cause discoloration. The extent of chemical changes that occur in the frozen state is less than that in nonfrozen food due to decreased mobility of free radicals. With less mobility in the frozen state, free radicals tend to recombine to form the original substances rather than diffuse through the food and react with other food components. Irradiating foods at appropriate doses and under certain conditions, such as in a reduced oxygen or oxygen-free atmosphere, packaging, and the frozen state, can minimize or avoid the development of objectionable off-odors and off-flavors. The product quality (color and odor) issues can be controlled with proper processing conditions.

Four Areas in Which Irradiation Is Most Useful

- Preservation: Irradiation can be used to destroy or inactivate organisms that cause spoilage and decomposition, thereby extending the shelf life of foods. It is an energy-efficient food preservation method that has several advantages over traditional canning. The resulting products are closer to the fresh state in texture, flavor, and color, with the caveats of ensuring other processing factors are under control. Using irradiation to preserve foods requires no additional liquid, nor does it cause the loss of natural juices. Both large and small containers can be used and food can be irradiated after being packaged or frozen.
- Sterilization: Foods that are sterilized by irradiation can be stored for years without refrigeration just like canned (heat sterilized) foods. With irradiation, it will be possible to develop new shelf-stable products. Sterilized food is useful in hospitals for patients with severely impaired immune systems, such as some patients with cancer or acquired immunodeficiency syndrome. These foods can be used by the military and for space flights.
- Control sprouting, ripening, and insect damage: In this role, irradiation offers an alternative to chemicals for use with potatoes, tropical and citrus fruits, grains, spices, and seasonings. However, because no residue is left in the food, irradiation does not protect against reinfestation like insect sprays and fumigants do.

- Control foodborne illness: Irradiation can be used to effectively eliminate those pathogens that cause foodborne illness, such as *Salmonella*.

Nutritional Quality of Irradiated Foods

Scientists believe that irradiation produces no greater nutrient loss than what occurs in other processing methods, such as canning.

Effects of Irradiation on Biological Organisms

Irradiation exposes food to a source of ionizing radiation sufficient to create positive and negative charges. Depending on the dose of radiation energy applied, foods can be pasteurized to reduce or eliminate pathogens, or they can be sterilized to eliminate all microorganisms, except for some viruses. For example, low (up to 1 kGy) to medium doses (1–10 kGy) kill insects and larvae in wheat and wheat flour and destroy pathogenic bacteria and parasites. Low to medium doses also inhibit sprouting of potatoes and other foods and slow the ripening and spoilage of fruit. Higher doses (10–50 kGy) sterilize foods for a variety of uses, such as for astronauts during space flight and immune-compromised hospital patients who must have bacteria-free food.

When molecules absorb ionizing energy, they become reactive and form ions or free radicals that react to form stable radiolytic products. The Council for Agricultural Science and Technology (CAST, 1989) estimated that a dose of 1 kGy would break fewer than 10 chemical bonds for every 10 million bonds present, an extremely small percentage. Cooking, or applying infrared radiation to foods, produces similar changes in chemical bonds.

Microbiology Effects

As with cooking and thermal processing, higher radiation doses kill greater numbers of bacteria. The D values (decimal reduction, or dose required to destroy 90% of the microorganisms present) of several pathogenic bacteria that can be associated with raw meat and poultry. *Salmonella* is the most resistant non-spore forming pathogen, with a D value of approximately 0.6 kGy. The radiation doses approved for poultry (1.5–3.0 kGy) would destroy approximately 99.9% (3 logs) to 99.999% (5 logs) of *Salmonella*. Except for spores of *Clostridium botulinum*, all other pathogenic bacteria would be controlled within this dose range. A minimum dose of 1.5 kGy would destroy at least 6 logs of *Escherichia coli* O157:H7, which has a D value of approximately 0.24 kGy. Irradiation, therefore, would be extremely effective at eliminating this pathogen that was declared an adulterant in ground beef in 1994. The parasites *Toxoplasma gondii* and *Trichinella sprialis* are inactivated at doses of 0.25 kGy and 0.3 kGy, respectively.

Although the primary objective of irradiation of muscle foods is destruction of pathogenic bacteria, substantial reduction of spoilage microorganisms also occurs. Levels of aerobic and anaerobic bacteria were reduced by over 4 logs and almost 5 logs, respectively, in chilled ground beef irradiated at doses to 2.5 kGy. Shelf life of the ground beef stored at 4 °C was extended by 9 days before counts reached 7 logs. Studies showed that the refrigerated shelf life of vacuum-packaged beef sirloin cuts irradiated to 2 kGy more than doubled, from approximately 4 weeks for nonirradiated product stored at 0 °C to 10 weeks for irradiated product stored at 4 °C with a 3 log reduction in psychrotrophic aerobic bacteria in ground beef irradiated at 2.5 kGy. The irradiated ground beef had a shelf life of 10 days before counts reached 7 logs compared with the nonirradiated control which lasted only 1 day. Other studies on pork loin slices packaged under nitrogen and irradiated to 1 kGy had a 26-day shelf life (21 days more than the control) stored at 5 °C, and uninoculated ground pork, irradiated at 1.9 kGy, had no surviving bacteria when stored at 2 °C for up to 35 days.

The predominant food spoilage organisms are Gram-negative psychrotrophic microorganisms that are very susceptible to radiation. Several researchers have shown that irradiation of food at doses of at least 1 kGy virtually eliminate Gram-negative microorganisms, but has a much smaller effect on Gram-positive, lactic acid-producing microorganisms. *Pseudomonas* species and Enterobacteriaceae (common spoilage bacteria) are easily eliminated even with low doses of radiation. However, in all of these studies at doses in the range of 1–5 kGy, Gram-positive microorganisms survived and caused spoilage after prolonged refrigerated storage.

Approved Uses for Food Irradiation

Irradiation has been approved for many uses in approximately 40 countries, but only a few applications are presently used because of consumer concern and because the facilities are expensive to build.

In the United States, the FDA approved irradiation for eliminating insects from wheat, potatoes, flour, spices, tea, fruits, and vegetables. Irradiation also can be used to control sprouting and ripening. Approval was given in 1985 to use irradiation on pork to control trichinosis. Using irradiation to control *Salmonella* and other harmful bacteria in chicken, turkey, and other fresh and frozen uncooked poultry was approved in May 1990. In December l997, FDA approved the use of irradiation to control pathogens (disease causing microorganisms, such as *E. coli* and *Salmonella* species) in fresh and frozen red meats, such as beef, lamb, and pork.

Applications for Food Irradiation

Because the irradiation process works with both large and small quantities, it has a wide range of potential uses. For example, a single serving of poultry can be irradiated for use on a space flight. Or, a large quantity of potatoes can be treated to reduce sprouting during warehouse storage.

However, irradiation cannot be used with all foods. It causes undesirable flavor changes in dairy products, for example, and it causes tissue softening in some fruits, such as peaches and nectarines. Irradiated meat will be successful in

the market place only if consumers are satisfied with its sensory quality and most recent evidence suggests that this is a viable technology.

Industry Adoption of Meat Irradiation

There are several reasons to explain in the United States why irradiation is not widely used for meat:

- First, there is no strong consumer demand for the process because: (1) consumers are, in general, quite confident that food is safe and do not see a burning need for irradiation, (2) they are not familiar enough with irradiation to be comfortable about it, and (3) anti-irradiation activists have successfully raised consumer doubts about the process. Several studies have shown that once consumers are properly educated about irradiation, they will be willing to accept it and will purchase irradiated foods.
- Second, most companies in the industry are reluctant to assume the risk of being first with a controversial process, so they are holding back until there is strong demand or reduced controversy related to irradiation.
- Third, added cost for irradiated products is a barrier without distinct demand to justify the cost. Large-scale irradiation facilities require substantial financial investment and meat processors have not had strong enough economic signals to invest in such facilities. This assumes that not every processing plant would construct a facility and that means that products would need to be transported from some processing plants to an irradiation facility before transporting to retail or food service facilities and this adds extra cost.
- Fourth, there have also been some studies that show potential for some off-flavors or off-odors when products are held longer than ideal time periods.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment; Palatability. Cooking of Meat: Cooking of Meat; Flavor Development; Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor. Foodborne Zoonoses. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Safety of Meat: *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Hurdle Technology; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; *Yersinia enterocolitica*. Packaging: Technology and Films. Parasites Present in Meat and Viscera of Land Farmed Animals. Preservation Methods of Animal Products. Spoilage, Factors Affecting: Microbiological; Oxidative and Enzymatic

Further Reading

Brewer, M.S., 2004. Irradiation effects on meat color – A review. Meat Science 68, 1–17.
Brewer, M.S., 2009. Irradiation effects on meat flavor: A review. Meat Science 81, 1–14.
Fregonesi, R.P., Portes, R.G., Aguiar, A.M.M., *et al*., 2014. Irradiated vacuum-packed lamb meat stored under refrigeration: Microbiology, physicochemical stability and sensory acceptance. Meat Science 97 (2), 151–155.
Houser, T.A., Sebranek, J.G., Maisonet, W.N., *et al*., 2005. The effects of irradiation at 1.6 kGy on quality characteristics of commercially produced ham and pork frankfurters over extended storage. Journal of Food Science 70 (4), S262–S266.
Luchsinger, S.E., Kropf, D.H., Garcia-Zepeda, C.M., *et al*., 1996. Color and oxidative rancidity of gamma and electron beam irradiated boneless pork chops. Journal of Food Science 61 (5), 1000–1005.
Molins, R.A. (Ed.), 2001. Food Irradiation Principles and Applications. New York, NY: Wiley, Interscience.
Montgomery, J.L., Parrish Jr., F.C., Olson, D.G., 2000. Irradiation and storage effects on aroma and color of raw beef patties in anaerobic or aerobic packaging. Journal of Muscle Foods 11 (1), 19–33.
Murano, P.S., Murano, E.A., Olson, D.G., 1998. Irradiated ground beef: Sensory and quality changes during storage under various packaging conditions. Journal of Food Science 63 (3), 548–551.
Murray, D.R., 1990. Radiolytic products and selective destruction of nutrients. New York, NY: John Wiley and Sons, Inc.
Olson, D.G., 1998. Irradiation of food: Scientific status summary. Food Technology 52 (1), 56–62.
Pauli, G.H., Tarantino, L.M., 1995. FDA regulatory aspects of food irradiation. Journal of Food Protection 58, 209–212.
Thakur, B.R., Singh, R.K., 1994. Food irradiation – Chemistry and applications. Food Reviews International 10 (4), 437–473.
Yong, J., Hun, K., Sung, Y.P., *et al*., 2000a. Identification of irradiation-induced volatile flavor compounds in chicken. Journal of Korean Society of Food Science and Nutrition 29 (6), 1050–1056.
Yong, J., Hun, K., Sung, Y.P., So, J.K., Young, J.Y., 2000b. Identification of irradiation-induced volatile flavor compounds in beef. Journal of Korean Society of Food Science and Nutrition 29 (6), 1042–1049.

Relevant Websites

http://www.meatupdate.csiro.au/Irradiation.pdf
Australian Commonwealth Scientific and Research Organization.
http://www.foodstandards.govt.nz/consumer/foodtech/irradiation/Pages/default.aspx
Food Standards Australia New Zealand.
http://ftsi.us/irradiation
Food Technology Service, Inc.
http://www.extension.iastate.edu/foodsafety/irradiation/
Iowa State University Extension Food Safety.
http://www.sterigenics.com/services/food_safety/food_irradiation__questions_answers.pdf
Sterigenics International, Inc.
http://www.epa.gov/rpdweb00/sources/food_irrad.html
United States Environmental Protection Agency.
http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm261680.htm
United States Food and Drug Administration.
http://uw-food-irradiation.engr.wisc.edu/Facts.html
UW Food Irradiation Education Group.
http://uw-food-irradiation.engr.wisc.edu/Facts.html
University of Wisconsin Food Irradiation Education Group.
http://uw-food-irradiation.engr.wisc.edu/Process.html
University of Wisconsin Food Irradiation Education Group.
http://uw-food-irradiation.engr.wisc.edu/Facts.html
University of Wisconsin Food Irradiation Group.
http://en.wikipedia.org/wiki/Food_irradiation
http://en.wikipedia.org/wiki/Gray_(unit)
http://en.wikipedia.org/wiki/Ionizing_radiation
http://en.wikipedia.org/wiki/Non-ionizing_radiation
http://en.wikipedia.org/wiki/Radiation

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LABORATORY ACCREDITATION

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Glossary

Accreditation A process in which competency, authority, or credibility is certified.
Accreditation body Organization auditing compliance with the requirements to fulfill.
Codex Alimentarius Collection of internationally recognized standards, codes of practice, guidelines, and other recommendations relating to foods, food production, and food safety.
Equivalent By procedures of mutual evaluation and acceptance between accreditation bodies accreditation systems may be recognized as equivalent.
ISO 17025 standard Standard on general requirements for the competence of testing and calibration laboratories. Fulfilling the Requirements assures the quality of analytical test results.
Notification Process, in which laboratories are empowered to conduct official analyses of public responsibility.

Introduction

Food samples are generally analyzed in order to decide whether they are acceptable with respect to safety, quality, and regulatory requirements. For this decision to be made, the analytical results have to reflect the real condition of the sample. However, every analytical procedure is influenced by numerous external and internal factors, and confidence in analytical results is only justified if the laboratory performing the work (1) uses an appropriate analytical method and (2) controls all factors potentially influencing the accurate run of the analytical procedure. In other words, a quality assurance program for laboratory testing is essential.

Having introduced a system to assure the quality of test results, laboratories may subsequently strive for an official approval of their competence to perform specific tests or types of tests. This official approval of competence is called 'accreditation.' The term is derived from the Latin word *accredo*, which means 'to yield one's belief to another,' i.e., to believe unconditionally, to trust, and to accept wholeheartedly. Hence, this term in connection with laboratory work indicates that the client may trust the analytical results delivered.

Laboratories entrusted with tasks in the public food control sector are obliged to go through the trouble of a 'notification' procedure. Derived from the Latin expression *notum facere*, meaning 'to make well known,' this essentially means an administrative procedure resulting in the public listing of laboratories that have been state inspected and found to be competent to take over legally prescribed state control tasks.

Accreditation and Notification System

Development

One of the earliest approaches to promoting laboratory quality systems dates from 1947 in Australia, when the Australian National Association of Testing Authorities (NATA) was founded. The intention was to organize a national testing service by identifying what was important for the reliability of test results and by developing standards to be met. In the 1960s, many countries developed their own laboratory standards, the application of which became increasingly prescribed by legislation during the 1970s. However, it was recognized that the prescription of methods alone – without the exclusion of interfering factors during testing – did not result in a uniformly high level of laboratory performance. As a result, from the 1980s there was a general move toward the

prescription of a general quality system within which the laboratory must operate. This is best illustrated by Article 12 of regulation (EU) 882/2004: laboratories involved in official controls must "... operate and are assessed and accredited in accordance with the following European standards: (a) EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'; …". Although these requirements apply only to laboratories involved in public food control, this also affects food laboratories in the private service sector. For the results to be accepted as equivalent, the latter are advised to voluntarily adopt the same standards.

Standards to be Met

To guarantee the quality of test data, a system of quality standards has to be implemented in the laboratory. Such a system must comply with generally accepted and standardized norms.

As early as 1978, the International Laboratory Accreditation Cooperation (ILAC) provided a statement of technical criteria for accreditation of laboratories to the International Organization for Standardization (ISO). Published as ISO Guide 25:1984, this standard formed the basis for laboratory accreditation worldwide, and also for the European Standard EN 45001, which was adopted by the joint European Standards Institution (CEN, Comité Européen de Normalisation/European Committee for Standardization; CENELEC, Comité Européen de Normalisation Electrotechnique/European Committee for Electrotechnical Standardization) in 1989. The further sophistication of laboratory quality system requirements necessitated several revisions of ISO Guide 25. The last extensive revision finally resulted in the ISO 17025 standard, which brought the terms and requirements of laboratory quality systems in line with the quality management and quality assurance standards of the ISO 9000 series. This standard also replaces EN 45001.

Figure 1 shows the hierarchical structure of the private-law accreditation system as well as the relevant standards to consider. Since the term 'accreditation' is not limited to testing laboratories, calibration laboratories and inspection and certification services are also included in the figure. Some relevant norms for laboratory accreditation are further detailed in Table 1.

Organizations auditing compliance with the accreditation demands are called 'accreditation bodies.' Most accreditation bodies have adopted ISO/IEC 17025 as the basis for the accreditation procedure, which is crucial for safeguarding a uniform approach for determining laboratory competence. By procedures of mutual evaluation and acceptance between accreditation bodies according to ISO/IEC Guide 68:2002 accreditation systems may be recognized as equivalent and a mutual recognition agreement (MRA) may be signed. This helps test data that accompany exported goods on overseas markets to be more readily accepted, although it does not guarantee it.

Notification is a similar process, in which laboratories are empowered to conduct official analyses of public responsibility. Herein, competence assessment will be performed by public authorities, mostly requiring accreditation according to ISO/IEC 17025 as a prerequisit and demanding several additional requirements. Table 2 compares briefly accreditation

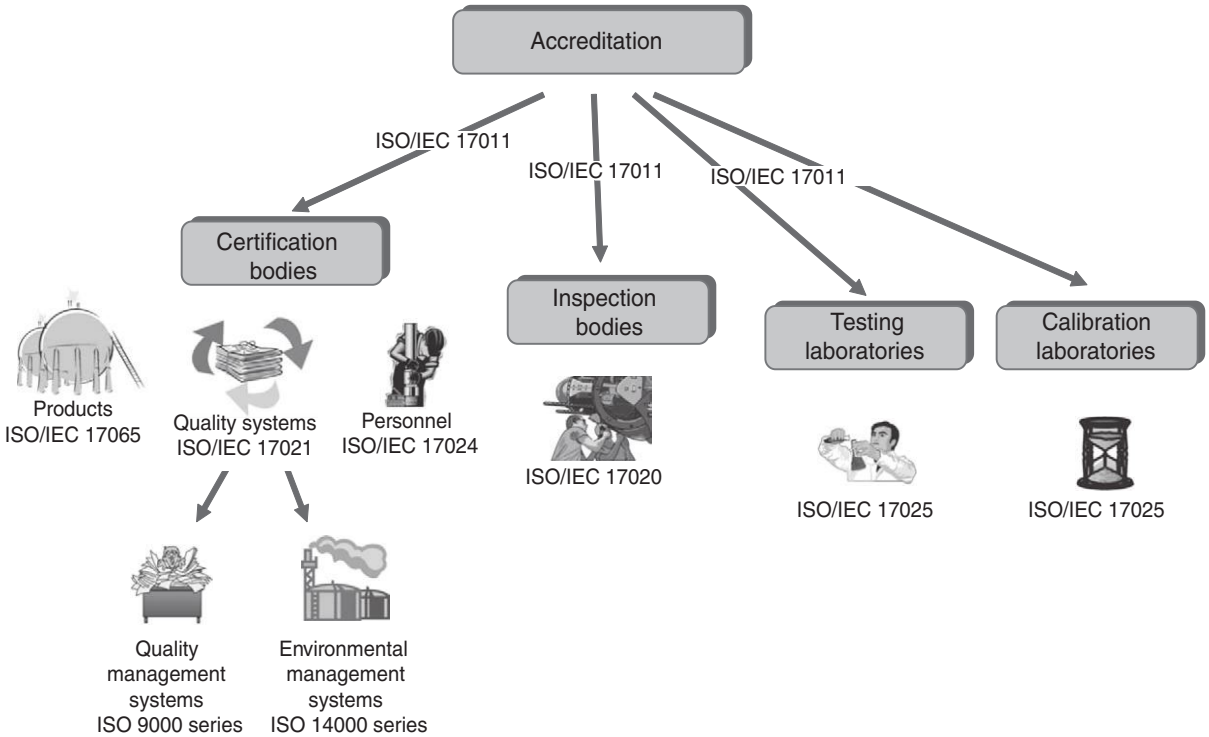

Figure 1 Accreditation system and relevant European and International standards in the privately organized sector. Adapted from Wessel, H., 1998. ...ierungen – Begriffe der Qualitätssicherungen. DACH-Zeitung 63 (10), 57, with permission from Reichenberger & Co. GmbH.

Table 1 Standards referring to accreditation and certification of analytical laboratories

Standard	*Description*
Terms	
● EN 45020:2006	Standardization and related activities – general vocabulary
● ISO/IEC Guide 2:2004	Standardization and related activities – general vocabulary
● ISO/IEC 17000:2004	Conformity assessment – general vocabulary
Laboratory requirements	
● ISO/IEC 17025:2005	General requirements for the competence of testing and calibration laboratories
● ISO/IEC 17043:2010	Conformity assessment – general requirements for proficiency testing
● ISO 15189:2012	Medical laboratories – requirements for quality and competence
● ISO 10012:2003	Measurement management systems – requirements for measurement processes and measuring equipment
● ISO 9000:2005	Quality management systems – fundamentals and vocabulary
● ISO 9001:2008	Quality management systems – requirements
● ISO/TR 10013:2001	Guidelines for quality management system documentation
Accreditation body requirements	
● ISO/IEC 17011:2004	Conformity assessment – general requirements for accreditation bodies accrediting conformity assessment bodies
● ISO/IEC 17040:2005	Conformity assessment – general requirements for peer assessment of conformity assessment bodies and accreditation bodies
● ISO 19011:2011	Guidelines for auditing management systems
● ISO/IEC Guide 68:2002	Arrangements for the recognition and acceptance of conformity assessment results

Table 2 Comparison between accreditation and notification systems

	Accreditation	*Notification*
Aim	To document the competence to perform specific tests for private clients	To carry out state control tasks
Basis	Private contract between laboratory and accreditation body	Specific legal regulation
Extent	Free choice of the applicant laboratory (a single method would be possible)	All methods fixed in the legal regulation concerned
Requirements	Testing environment and accommodation	Testing environment and accommodation
	Personnel	Personnel
	Technical equipment	Technical equipment
	Quality system and manual according to ISO 17025	Quality system and manual
	Participation in proficiency testing schemes desirable but not imperative	Successful participation in proficiency testing schemes imperative
	Subcontracting possible at any time	Subcontracting only exceptionally

and notification requirements. The Codex Alimentarius Commission Guideline CAC/GL 27–1997 concerning the 'assessment of the competence of testing laboratories involved in the import and export control of food' additionally laid down the use of internal quality control procedures, such as duplicate analysis or inclusion of particular reference materials into the analytical procedure. Owing to the formal acceptance of Codex standards in the World Trade Organization's SPS (sanitary and phytosanitary measures) and technical barriers to trade agreements, the significance of these Codex standards has dramatically increased over the past few years.

Procedure

In the private laboratory sector, accreditation is a voluntary procedure that a laboratory may choose to undergo. Nevertheless, since the beginning of the 1990s a strong movement toward conformity assessment has developed, resulting in a huge number of conformity assessment bodies for multiple purposes in nearly all economic areas (Figure 2). Some websites that are useful for finding information on laboratory accreditation are given in section Relevant websites.

Testing laboratory accreditation is usually restricted to defined testing procedures, for example, determination of dioxin in meat. By contrast, a notification demands the application of all analytical methods regulated in the legal norm applied, for example, Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

Generally, the assessment of competence includes the following five steps:

1. preparatory steps
2. application for approval of competence
3. auditing procedure
4. accreditation/notification
5. surveillance

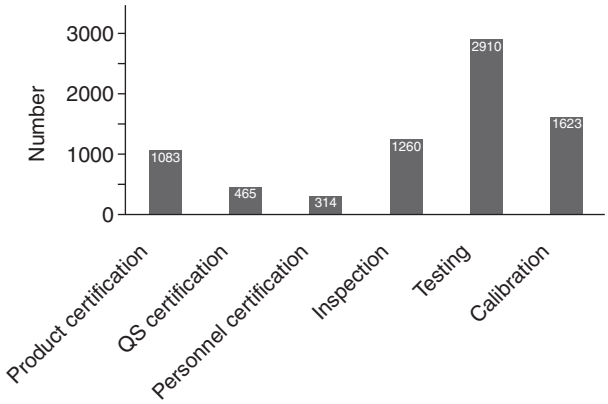

Figure 2 Fields of activity of 3535 conformity assessment bodies in 29 European countries organized in the European Organisation for Conformity Assessment (EOTC) in April 2002.

Preparatory steps

As a first step, it is necessary to become thoroughly informed about the requirements, so that it is clear what the accreditation's or notification's subject matter (analytical methods, testing procedures, and legal norms) is, and what the demands of the accreditation body are. Furthermore, the current laboratory's status quo must be established.

Since a quality system according to an appropriate norm (in general ISO/IEC 17025) is demanded, a concept for its implementation or revision must be prepared. It is necessary to determine which organizational and technical structures must be introduced, modified, or eliminated, and which documents must be produced (responsibility, schedule). This is the main area where resources are required. Depending on the existence and state of any current quality system, as well as the level of support provided by the laboratory's management, this preparatory phase may last from 6 months for well-organized laboratories to 3 years for institutions that have more changes to implement.

Application for approval of competence

For private laboratories, the application for accreditation must be directed toward the accreditation body chosen by the laboratory and a civil law contract is made. In the case of notification, the state authority responsible for the legal norm in question should be contacted.

Auditing procedure

The accreditation body or state authority assigns appropriate assessors and reviews in a first step the application and quality documentation. An on-site assessment is performed including review of the quality documentation, records, and sample handling. In addition, interviews with technicians may be held, demonstrations of tests or calibrations may be requested, and equipment and calibration records may be examined.

The assessor's findings will be summarized in a written report. Any deficiencies must be remedied before the next step can be taken.

Accreditation/notification

After elimination of deficiencies, the accreditation body or state authority decides – usually by a council vote – on the accreditation/notification. Where the accreditation/notification is granted, competence to perform the specific tests is testified by the issue of an official accreditation certificate and publication in the register of accredited organizations.

Surveillance

Subsequently, the laboratories are supervised according to the rules of the accreditation body or the state authority. Document reviews and periodical re-audits are a standing part of the surveillance activities.

Costs

The costs for accreditation and notification are considerable. The establishment and implementation of the quality system is responsible for about 90% of the total expenses, which is the focal point of each approval of competence. These costs result mainly from release of personnel in order to establish the documented quality system and to train the staff involved. Technical upgrading or reconstruction may be necessary. However, the latter is mostly the removal of shortcomings that already existed.

The fees charged by the accreditation body for the document review and auditing procedure, as well as the accreditation certificate, vary widely. They are different from country to country and depend on the size of the laboratory and the number of tests accredited. Laboratories in Germany, for example, must expect costs of €10 000–25 000. Costs in other countries may be lower. Owing to mutual recognition, private laboratories may choose foreign accreditation bodies. However, it should be recognized that accreditations that involve border crossing may incur considerable travel, translation, and shipment costs.

Benefits

The primary benefit of accreditation is obvious: the laboratory is formally recognized as being competent to carry out specific tests or specific types of tests and receives a written proof of this. This may be an effective marketing tool when laboratories are invited to tender for analyses. From the point of view of a client who wants a product to be checked, the availability of a list of accredited laboratories makes it easier to select a service, which is able to deliver accurate and reliable results.

Additionally, notification as well as accreditation in combination with the concept of mutual recognition will reduce costs in international trade. Once the principle 'once tested, accepted everywhere' is recognized all over the world, expensive resampling and retesting, as well as multiple second-party audits, will be avoided for the most part.

Furthermore, the laboratory itself will enjoy some internal advantages. The unbiased evaluation of the laboratory's work by a science-based and experienced third party counteracts the organization's natural myopia. The required quality system will result in clearly stated procedures and responsibilities.

This will make it easier for new personnel to become familiarized with their work, facilitate mutual substitution between staff members, and help to increase confidence when carrying out rarely applied analytical methods.

Laboratory Requirements

The main objective of a laboratory quality system is to ensure the consistency of laboratory results day to day and their conformity with defined criteria. Therefore, sample handling and all methodical procedure steps have to be documented, and all data relevant for the test as well as the test results have to be recorded in order to guarantee traceability.

There is no generally applicable plan for establishing a laboratory quality management system. Each organization will have its own idiosyncrasies and problems that require special consideration and treatment. However, the general principles and requirements are laid down in the International Standard ISO 17025. The requirements are grouped into two main categories, i.e., management and technical aspects.

Management Requirements

To guarantee objective analysis and data reporting, the laboratory has to ensure its integrity and independence from any undue internal and external commercial, financial, and other pressures. Also, the staff's duties and responsibilities must be specified and documented in order to avoid faults due to unclear organizational structures. In this respect, the role of the laboratory's senior management is very important: it maintains the general accountability, establishes the marketing strategy and the quality policy, and commits the required resources. Therefore, a quality system cannot be established without the senior management's support. Additionally, review and assessment of the quality activities and assurance of a continuous improvement are aspects of the management's duties.

Further management requirements are the establishment of a system for approval, issue, change, and access for documents and records, as well as rules for the review of requests, tenders, and contracts and for the service to clients including handling of complaints.

Standards for subcontracting work and for purchasing services and supplies must be set, in order to guarantee the fulfillment of the laboratory's own quality standards. Furthermore, rules for dealing with nonconforming work including corrective and preventive actions are required.

Technical Requirements

Technical requirements concern personnel, environment, equipment, reagents, culture media and reference materials, sampling and sample handling, test methods, and quality of performance. For laboratories carrying out microbiological testing of materials, products, and substances, the joint EA/EURACHEM working group document EA-04/10 Accreditation for Microbiological Laboratories provides detailed and specific guidance on the interpretation of ISO 17025. The guidance is applicable to the performance of all objective measurements, whether routine, nonroutine, or as part of research and development.

Personnel

Testing has to be either performed or supervised by an experienced person, qualified to degree level. Furthermore, staff should have relevant practical work experience before being allowed to perform work covered by the scope of accreditation. If the laboratory includes interpretation of test results in the report, this has to be done by authorized personnel with suitable experience and relevant knowledge of the specific application, including, for example, legislative and technological requirements. The laboratory management has to ensure that all personnel have received adequate training for the competent performance of tests and operation of equipment.

Environment

A typical laboratory is comprised of the testing facilities and ancillary facilities. In general, there are specific environmental requirements for the testing facilities, for example, to construct the premises according to the 'no way back' layout principle, to designate areas for sample receipt and storage, to separate the areas of sample preparation, examination of samples, media and equipment preparation, and sterility assessment and decontamination. Reduction of contamination may be achieved by having smooth surfaces on walls and minimal opening of windows and doors while tests are being carried out. Laboratory clothing appropriate to the type of testing being performed should be worn and removed before leaving the area. This is particularly important in the molecular biology laboratory, where movement from an area of high deoxyribonucleic acid (DNA) load to one of low DNA load may unwittingly introduce cross-contamination. Moreover, there should be a documented cleaning program for laboratory fixtures, equipment and surfaces. In addition, an appropriate environmental monitoring program should be devised, including all factors potentially influencing the test results. In microbiological laboratories, for example, the microbial counts of the air and the working surfaces should be monitored.

Equipment

As part of the quality system, a laboratory is required to operate a documented program for maintenance, calibration, and performance verification of its equipment. The maintenance has to be carried out at specified intervals determined by factors such as the rate of use. A calibration and performance verification program of equipment directly influencing the test results (e.g., scales and pH meters) has to be established. Even for rather simple equipment items this may be a sophisticated affair. For microbiological incubators, for example, the time required to achieve temperature equilibrium conditions, temperature stability, and uniformity of temperature distribution have to be established and documented, in particular with respect to its typical use. Subsequent to the initial validation of the equipment, the constancy of the characteristics should be checked and recorded after each significant modification. Moreover, the laboratory has to monitor the operating parameters during each use and retain records of the results.

Reagents, culture media, and reference material

The suitability of each batch of reagents, culture media, and diluents, critical for the test has to be verified initially as well as during its shelf life. Furthermore, all reagents, media, and diluents have to be labeled adequately to indicate identity, concentration, storage conditions, preparation date, validated expiry date, and recommended storage period. Reference materials, certified reference materials, and reference cultures have to be used, for example, to demonstrate the accuracy of results, to calibrate equipment, to monitor laboratory performance, or to validate methods.

Sampling and sample handling

While testing laboratories are responsible for primary sampling to obtain test items, for example, sampling of drinking water in food establishments, the sampling procedure also has to be covered by the quality assurance program. Transport and storage conditions have to maintain the integrity of the sample. The conditions should be monitored and records should be maintained.

The laboratory has to establish sample delivery and sample identification procedures. All relevant information such as date and time of receipt, and sample condition on receipt, have to be recorded. Samples have to be stored under suitable conditions in order to minimize changes, and storage conditions have to be defined and recorded.

Testing methods and quality of performance

All methods used in a laboratory have to be validated. The validation process should reflect the matrices and test conditions used in the laboratory. The specificity, relative trueness, positive and negative deviation, detection limits, matrix effects, repeatability and reproducibility of the method should be determined. Additionally, the uncertainty of measurement has to be estimated and the laboratories should be aware of the incidence of false positive and false negative results associated with qualitative tests used.

A program of periodic checks is necessary to demonstrate that variability (e.g., between analysts and between equipment or materials) is under control. The program may involve internal quality controls such as the use of reference materials, spiked samples, and replicate testing. The example in Figure 3 compares the variability of two microbiological methods during replicate testing. Furthermore, it is recommended (if not prescribed; see above) regularly to take part in external proficiency testing schemes, which are relevant to their scope of accreditation and matrices used.

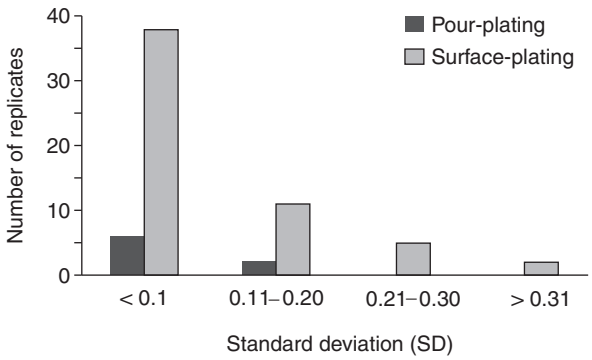

Figure 3 Variability during replicate microbiological testing for purposes of internal quality control. Standard deviations (SD) are shown for replicate testing using the pour-plating and surface-plating methods.

Prospects

As described above, laboratory accreditation and notification provide important benefits. Nevertheless, a further evolution of the whole system will support its effectiveness.

Generally, the establishment, implementation, and maintenance of a quality system in a laboratory bind relevant personnel and monetary resources, and the necessities of surveillance and documentation will usually result in an increased workload. However, the laboratory should be careful not to bureaucratize the processes. Over-organization and pure formalism only results in personnel demotivation and superfluous workload. The quality system should be a helpful tool to control factors influencing the analytical results, and not an obstacle to work!

Owing to the universal character of the standards, there may be some lack of clarity when realizing the requirements. Several organizations provide documents in order to interpret the normative text. However, this should not result in 'flooding' of the laboratory with documents of limited importance. Accreditation bodies and other organizations should restrict information to helpful and practical advice.

In the future, it is likely that the incorporation of specific analytical methods into legislation will be replaced by the specification of method performance characteristics, for example, proof of applicability, in-house method validation, specification of detection limits, determination of measurement uncertainty, etc. This will have the advantage that the analyst's expertise is emphasized, thus allowing a certain degree of freedom with respect to the choice of the method. Furthermore, laboratory automation will be furthered and administrative difficulties involved with changing a method delivering unsatisfactory or inferior results in comparison with another will be eliminated.

However, this increased degree of freedom must go hand in hand with a strengthening of the expertise of the accreditation bodies in order to ensure a uniform quality during competence assessments of laboratories. Furthermore, to overcome barriers to international trade, an international harmonization of the accreditation/notification requirements as well as the further development of mutual recognition of accreditations is mandatory. This should be a worldwide effort, otherwise accreditation itself will serve as a new barrier. Laboratories in many countries are still not in a suitable state to take part in this development since infrastructural problems such as unstable energy supply or a lack of local accreditation bodies render the establishment of an accredited quality system difficult.

See also: Chemical Analysis: Standard Methods. Microbiological Analysis: Standard Methods. Microbiological Safety of Meat: *Listeria monocytogenes*. Preslaughter Handling: Preslaughter Handling

Further Reading

AOAC Analytical Laboratory Accreditation Criteria Committee, 2001. Accreditation Criteria for Laboratories Performing Food Microbiological and Chemical Analyses in Foods, Feeds, and Pharmaceutical Testing. Gaithersburg, MD: AOAC International.

Codex Alimentarius Commission, 1997. Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and Export Control of Food. CAC/GL 27–1997. Available at: http://www.codexalimentarius.org/standards/list-of-standards/ (accessed 15.01.14).

Council Directive 93/99/EEC, 1993 on the subject of additional measures concerning the official control of foodstuffs. Official Journal L290, 14–17.

Garfield, F.M., Klesta, E., Hirsch, J., 2000. Quality Assurance Principles for Analytical Laboratories. Gaithersburg, MD: AOAC International.

Kohl, H., 1996. Qualitätsmanagement im Labor. Berlin: Springer-Verlag.

Prichard, F.E., 2002. Quality in the Analytical Chemistry Laboratory. Hoboken, NJ: John Wiley & Sons.

Ratliff, T.A., 2003. The Laboratory Quality Assurance System: A Manual of Quality Procedures and Forms. Hoboken, NJ: John Wiley & Sons.

Seiler, J.P., 2000. Good Laboratory Practice: The Why and the How. Berlin: Springer-Verlag.

Wessel, H., 1998. ...ierungen – Begriffe der Qualitätssicherungen. DACH-Zeitung 63 (10), 57.

Wood, R., 2001. Laboratories and analytical methods. In: Dillon, M., Griffith, C. (Eds.), Auditing in the Food Industry. Cambridge: Woodhead Publishing Limited, pp. 111–144.

Relevant Websites

http://www.a2la.org
American Association for Laboratory Accreditation.

http://www.aplac.org
Asia Pacific Laboratory Accreditation Cooperation.

http://www.aoac.org
Association of Analytical Communities.

http://www.citac.cc
Cooperation on International Traceability in Analytical Chemistry.

http://www.eurachem.org
Eurachem.

http://www.cenorm.be
European Committee for Standardization.

http://www.european-accreditation.org
European Cooperation for Accreditation.

http://www.eurolab.org
European Federation of National Associations of Measurement, Testing and Analytical Laboratories.

http://www.eoq.org
European Organisation for Quality.

http://iaac-accreditation.org
Interamerican Accreditation Cooperation.

http://www.iaf.nu
International Accreditation Forum.

http://www.ianz.govt.nz
International Accreditation New Zealand.

http://www.iasonline.org
International Accreditation Service.

http://www.ilac.org
International Laboratory Accreditation Cooperation.

http://www.iso.ch
International Organization for Standardization.

http://www.nacla.net
National Cooperation for Laboratory Accreditation.

http://www.apec-pac.org
Pacific Accreditation Cooperation.

http://www.sadca.org
Southern African Development Community Cooperation in Accreditation.

M

MANURE/WASTE MANAGEMENT

Contents

Manure Management

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Glossary

Aerobic An environment with air and oxygen.
Anaerobic An environment without air or oxygen.
Manure Combination of feces and urine excreted from animals that results in a mixture of organic matter and nutrients such as nitrogen, phosphorus, and potassium among others.
Monogastric An animal that has a simple single-chambered stomach, such as pigs and poultry, compared with a ruminant, such as cows or sheep, that have a four-chambered complex stomach.
Ruminant An animal that has a four-chambered complex stomach, such as cows or sheep, compared with a monogastric, such as pigs or poultry, which have a simple single-chambered stomach.

Introduction

Raising livestock responsibly and economically involves numerous management aspects, including genetic selection, reproductive success, adequate nutrition, and providing an overall environment that promotes proper husbandry. Although all are essential to the sustainability and viability of raising livestock, one area that continues to receive more attention is the management of the manure produced by livestock. Manure production is a natural process of excreting unused or unavailable consumed nutrients from the body. In fact, livestock generally utilize only 10–30% of the total nutrients (feed) they consume. When defining animal manure, feces and urine are the main components. Other materials or liquids can also get mixed with feces and urine and become part of the manure stream. These may include washwater of equipment used for flushing/cleaning pen floors, wastewater from drinkers, spoiled feed, bedding (straw, sand, savings, etc.), rain, runoff from pen surfaces, and soil scraped from dirt pen surfaces. Proper manure management can help to improve many aspects of livestock production, including animal growth, feed efficiency, and health. In addition to these, properly handled manure can be used to replace commercial fertilizer through the nutrients it contains and at the same time improve soil quality. However, when in storage or applied to the land, it must be done in a manner not to harm the environment, particularly surface water and groundwater. Some nutrients are volatile in storage and during land application, especially nitrogen compounds; thus, odor issues can arise and be undesirable for humans in surrounding areas. However, these challenges can be overcome through various management practices.

Manure Nutrients

The nutrients found in manure are directly related to the nutrients fed to the animal. Because livestock use feed nutrients with less than 100% efficiency, the nutrients not digested and absorbed by the body are excreted as manure through urine

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and feces. Therefore, just as feed rations are formulated to certain levels of nutrients, manure nutrient concentrations can be predicted based on them. When valuing the economics of manure nutrients, producers consider the nutrients that they generally purchase and apply through commercial fertilizer for crop growth. Thus, the main manure nutrients evaluated for concentrations are nitrogen, phosphorus, and potassium and, to a lesser degree, micronutrients of zinc, copper, and sulfur that might be required on the basis of soil type and location. Every manure analysis should include a moisture test to help to determine the level of nutrients in either a given manure volume (liquid manure) or a given manure weight (solid/stackable manure).

Nitrogen

Nitrogen in manure can be found in multiple forms. Manure is typically characterized by three reporting values based on an analysis: (1) total (Kjeldahl) nitrogen, (2) ammonia nitrogen (NH_4^+), and (3) organic nitrogen, which is calculated from the difference between total nitrogen and ammonia nitrogen concentrations. Although total nitrogen is valuable to account for, ammonia nitrogen is the form that is the most important to understand. This is the form that is immediately available to plants as a fertilizer source. However, ammonia nitrogen is subject to volatilization loss to air at any point during storage or land application. Thus, covered storage structures and incorporation of manure directly into the soil are ideal means to capture a larger portion of the ammonia nitrogen for plant utilization in order to replace a larger portion of commercial nitrogen fertilizer. Organic nitrogen is not immediately available to plants for growth; however, through mineralization (breakdown of organic material through decomposition) while in the soil, the organic nitrogen becomes available for plant use in future years. The degree of mineralization is heavily influenced by the activity of soil microflora, especially bacteria and fungi, as well as soil temperature, moisture level, and soil type. Thus, knowing that both ammonia and organic nitrogen are critical to accurately predict the amount of nitrogen immediately available to plants, the amount that will become available in subsequent years as the organic nitrogen is mineralized to ammonia nitrogen.

Phosphorus

Phosphorus in manure can be in multiple forms; however, all are available to plants. Thus, total phosphorus should be used in an analysis of manure. However, for agronomic purposes, P_2O_5 is used for the recommended amount for land application. The conversion is: total phosphorus $\times$ 2.27 = P_2O_5.

Potassium

Potassium is a manure nutrient that may or may not be needed for plant growth. This depends on the crop to be grown and soil type. Like phosphorus, the level of potassium in manure should be analyzed on a total basis. However, for agronomic purposes, K_2O is used for the recommended amount for land application. The conversion is: total potassium $\times$ 1.2 = K_2O.

Farm Nutrient Balance

Environmental stewardship of livestock and poultry operations can be defined in many ways. One practice to achieve this classification involves properly balancing nutrient inputs for operations (fertilizer, animals, and feed) and outputs (milk, meat, crops, and manure). This concept applies to all operations, both large and small. To comply with regulatory nutrient management plan requirements, regulated operations must demonstrate that manure will be handled and applied on an agronomic basis. For most small operations, the regulatory requirements are not as strict; however, efficiently minimizing excess inputs while utilizing manure nutrients is essential for sustainability. More often than not, as farms get larger in livestock numbers, their ability to achieve a nutrient balance can be more difficult. Consequences of an unbalanced farm is overapplying manure nutrients over time, which leads directly to increased soil nutrient levels above that needed for plant uptake and growth. This not only is an uneconomical method of managing and utilizing nutrients, but it also becomes an environmental issue due to potentially increased runoff and leaching of various nutrients. Nitrogen can be transported by water, both by means of runoff from excess rainfall and by leaching downward through the soil. Phosphorus is more likely to runoff saturated soil, and once in open surface water, it can cause algae growth, which reduces water oxygen levels. The lowering of water oxygen has negative effects on aquatic animals and can lead to death in extreme cases of algae blooms.

Feed Influences Manure Nutrients

To help to minimize the level of nutrients present in manure, providing diets to livestock and poultry that meet but do not provide excess nutrients is ideal. Nutrition research to determine nutrient requirements is an ongoing process and substantial progress has been made to determine daily requirements for all species of livestock and poultry. However, although daily requirements are established, historically, dietary margins of safety above the daily needs of animals, especially for phosphorus, have been used by nutritionists to not limit performance of both market and breeding animals. However, these margins have been reduced substantially as the price of ingredients, such as supplemental phosphorus, has dramatically increased. As feeding nutrients above the animal's requirement simply increases excretion, and not absorption and utilization to any great measure, the manure level of these nutrients simply increases.

Monogastrics

Swine and poultry have digestive systems that are generally similar in overall function and absorption capabilities. Although differences in digestive anatomy exist between them, most concepts of how nutrition influences nutrient excretion are similar. Swine and poultry diets are formulated on a digestible basis for many nutrients, particularly phosphorus and amino acids (nitrogen). Amino acids are the structural

components of crude protein, which are the nitrogen containing compounds of the diet. One of the challenges to reduce excretion of nitrogen and phosphorus is to improve the availability of those nutrients for digestion and absorption.

As an example, the phosphorus in cereal grains has a very low availability for digestion, approximately 14% for corn but as high as 50% for wheat. Thus, when diets are formulated, the majority of the dietary phosphorus passes through the animal into the manure because it is not digested and absorbed. This is due to the majority of phosphorus being in 'phytic' form, in which pigs and poultry cannot break the bonds of this phosphorus complex. The main reason for this is that they do not produce 'phytase,' which is the enzyme responsible for breaking the bonds of phytic acid. However, technology has allowed the synthetic production of phytase, and it is currently used worlwide as an additive in swine and poultry diets. A diet with added phytase allows a decrease in supplemental phosphorus and an increase in overall phosphorus availability, so the total level of phosphorus excreted is reduced. The use of dietary phytase has led to reductions in phosphorus manure concentrations, resulting in less phosphorus being applied to the land. Also, crop genetic advancements have led to the production of low-phytate corn. When these varieties are fed, for example, in swine diets, excretion can be lowered 13–50% compared with pigs fed regular corn.

For diet nitrogen management, methods to reduce the overall crude protein (crude protein = nitrogen content × 6.25) are also in use in swine and poultry diets. Because monogastric diets are balanced on an individual amino acid basis instead of on a crude protein basis, synthetic amino acids can be added directly to diets to replace ingredients used specifically for their amino acid content. These are typically plant proteins, such as soybean meal. However, as more protein sources are added to the diet, the crude protein level of the diet increases as well. It is well documented that reducing dietary protein content by using dietary crystalline amino acids reduces the amount of nitrogen excreted, which lowers the nitrogen content of manure directly. A general guideline in swine and poultry is that for every 1% reduction in dietary crude protein by using crystalline amino acids, nitrogen excretion is reduced 10%. Similar results have been found with poultry, where a 1.3% reduction in dietary protein resulted in a 21% reduction in manure nitrogen content. Because nitrogen excretion is reduced when crystalline amino acids are used in the diet, more swine and poultry manure can be applied per acre, leading to a reduction in the amount of land required for manure application.

Ruminants

Ruminant animals, such as beef and dairy cattle, have a different digestive system than that of monogastric animals in that they have four compartments (rumen, reticulum, omasum, and abomasums) of their stomach. The main compartment that makes a ruminant unique is the rumen, where fermentation of feedstuffs occurs. Also, in the rumen, wide arrays of microorganisms are present to help to break down the feedstuffs for absorption in the lower digestive tract. With this, ruminant animals produce phytase naturally in their digestive system, so they can digest and absorb phosphorus in feedstuffs to a much higher extent than by monogastrics. Thus, phytase is not fed to ruminant animals.

When balancing a ruminant diet for crude protein concentration, little, if any, attention is paid to the amino acid concentration, which is in contrast to monogastric species. This is due once again to the rumen, where the microorganisms actually break down the dietary protein and form microbial protein that contains a different level of amino acids than was fed in the diet. Feeding synthetic amino acids provide no measureable benefit as they are broken down before they have the opportunity to be absorbed in the small intestine. Therefore, practices to reduce the diet crude protein level of cattle are limited, as diets are formulated to crude protein requirements to meet daily needs.

Manure Treatment

Treatment of raw manure is generally practiced when a producer wants to change the characteristics of it to make it easier to handle or to bring more value to their operation. However, many producers do not utilize treatment methods as the excreted manure form is ideal for their individual situation (i.e., static swine pit underneath the flooring). However, manure characteristics can be altered by storage method (aerobic or anaerobic), anaerobic digestion, adding additives to influence its characteristics, attempting to separate solids from the liquid stream, or composting. Although many methods are used, only the most common types will be discussed.

Anaerobic Treatment

Anaerobic treatment involves several steps in which microorganisms degrade or break down organic waste products in the absence of oxygen. Different types of manure treatment are involved with this process, with the main ones being an earthen lagoon or digesters.

Anaerobic lagoons

Anaerobic lagoons serve as liquid manure storage structures. Most often they are dug into the soil and contain an outer clay liner to mitigate leaching. Lagoons can be plastic lined to prevent absorption of moisture into the ground soil. This type of manure treatment is popular in areas of low rainfall and high evaporation. Also, where irrigation of liquid manure is desirable, this type of structure and manure treatment is desirable. Anaerobic lagoons, however, can be negatively perceived as odor from a large open surface of wastewater because they are noticeable with the correct weather conditions. The undigested material, generally the minerals, must be cleaned or removed from the bottom, which can be costly.

Anaerobic digesters

Anaerobic digesters are simply an enclosed structure where anaerobic break down of manure organic matter takes place. The anaerobic microorganisms convert the organic matter into biogas, which then can be captured and utilized for energy as a flammable gas. Methane is the most commonly captured

by-product from the digestion of organic matter, but carbon dioxide, hydrogen, hydrogen sulfide, nitrogen, and water vapor are also present. Advantages of this type of manure treatment include a reduction in odor and greenhouse gas emissions and as renewable energy source. However, the initial cost, level of labor, and continual management of the microorganism balance have prevented the widespread use of digesters.

Aerobic Treatment

Aerobic treatment refers to biological treatment in the presence of oxygen in liquid-type manure storage structures. Aerobic microorganisms break down organic and nitrogenous compounds that help to reduce odor and ammonia emissions, which are the main advantages of this type of manure treatment. However, aerobic treatment is not widespread due to operational costs related to mechanical equipment, such as motors, compressors, turbines, or fans, required to infuse enough oxygen into liquid manure to support aerobic bacteria viability.

Composting

Composting is the natural decomposition of solid manure or other organic materials by aerobic (oxygen dependent) bacteria and fungi. However, the microorganisms require certain conditions to effectively break down materials. The main conditions essential for proper composting are: (1) carbon to nitrogen (C/N) ratio, (2) moisture, and (3) temperature. Microorganisms use C and N for energy, growth, and reproduction and ideally require a C/N ratio of 25–30:1. However, manure from feedlots or pens results in a ratio of 10–20:1. To increase C, fibrous materials (straw, corn stalks, wood shavings, newsprint, rotted silage, hay bales, etc.) can be added, as they contain high C/N ratios (60–850:1). To achieve desired C/N ratios, the manure is combined with a C source.

Moisture levels should optimally be between 50% and 60%, as active composting slows when it falls below 40% or can totally cease ($<15\%$). If the level is $>65\%$, pores for oxygen transfer may become blocked and odor emissions can increase. A liquid source can be added to the mixture if moisture levels are below the preferred range. Wells, ponds, lagoons, or other water sources can be used, but if the liquid is from waste storage containments, the nutrient content will increase, which can be an advantage or disadvantage depending on the use of the end compost. As a rule of thumb, the compost is too wet if water can be squeezed out of a handful and too dry if the handful does not feel moist to the touch.

Microorganisms responsible for effective composting require an optimum range of 104–150 °F for maximum efficiency. Pathogens (135 °F or greater for 3 days) and weed seeds (145 °F) can be killed from the heat generated during composting. However, temperatures more than 150 °F can kill microorganisms and the pile should be turned. Conversely, a pile with temperature less than 104 °F may indicate an inadequate oxygen level and should be turned. If the temperature fails to rise, the pile should be allowed to finish composting for at least 1 month.

The most common method of composting is storing the composting material in windrows. When using a bucket loader to turn the piles, the initial windrows can be 6–10 ft high and 10–15 ft wide. During composting, many nutrients are lost through microorganism degradation and atmospheric loss, including N (40%) and C (60%). This loss can be seen by a decrease in pile mass, which can be one-half from start to finish. Composting time may range from 2 to 5 months, depending on the management and composting conditions.

When selecting a site, regardless of size, several considerations need to be evaluated. Some of these factors include proximity to your home and neighbors, visibility of site, drainage, runoff control, soil type, and separation distance between the composting site and water sources and streams.

Land Application

Livestock manure is used as an economical means of providing nutrients for crop growth and can provide for increased soil organic matter and fertility. When preparing to land apply manure, keys items that should be known include the nutrient content of the manure, application method to assure a uniform application based on crop needs, soil type, and what type and level of commercial fertilizer may be needed to compliment the manure application process.

As application of manure has the potential to increase odor from the volatilization of various compounds, best management practices to reduce odor and capture more nutrients in the soil for crop uptake include: (1) injecting liquid manure streams directly into the soil and (2) incorporating surface-applied solid or liquid manure into the soil within 24 h. Injecting manure directly into the soil is the most effective application method to reduce odor and retain nutrients for crop use. Research evaluating the ammonia concentrations of manure has shown that up to 90% is retained in the soil if injected compared with surface applying. Incorporating surface-applied manure within 24 h retains the majority of nutrients. However, surface-applied manure will lose almost all the ammonia nitrogen after 5–7 days. Manure should be applied at agronomic rates to match the upcoming year's plant needs, so that runoff or leaching is minimized to prevent environmental degradation to groundwater or surface water.

Air Quality

As manure contains many volatile compounds, unwanted or unpleasant odor is a potential in nearby areas of livestock production. In some situations, odors are very objectionable to nearby residents or passersby. Odors and gases are emitted into the atmosphere from all livestock and poultry operations, but the amount of emissions depend on a variety of factors. Some of the factors include weather conditions (high temperatures, moisture, and humidity all can increase odor), wind, manure storage type (open structure produces more odors than that by covered), amount of manure accumulated in open pens or in stockpiled areas, and the amount of dust

coming off the production area. Overall, manure can produce between 80 and 200 odorous compounds.

As previously discussed in the Section Feed Influences Manure Nutrients, reducing the level of nitrogen in the feed directly lowers the nitrogen level in manure. Another benefit is that the reduced nitrogen content of manure leads to less ammonia loss due to a lower overall level in manure. Ammonia is a colorless gas and has an undesirable smell. Ammonia from livestock operations mainly originates from excreted urinary nitrogen, so any decrease in the concentration of nitrogen in urine and feces will reduce the amount of ammonia that is released into the air from manure storage areas. Thus, it is not surprising that numerous research trials have shown that including crystalline amino acids in swine and poultry diets to replace protein ingredient sources, such as soybean meal, reduces ammonia emissions from manure.

Greenhouse Gases

Greenhouse gas compounds of environmental concern that contribute to global warming are carbon dioxide, methane, and nitrous oxide. Generally, carbon dioxide receives the most attention as a contributor to climate change; however, methane and nitrous oxide are significant greenhouse gases because of greater global warming potential relative to carbon dioxide. Although greenhouse gases are being contributed by numerous sources into the atmosphere, these are also emitted from animal agriculture. The gases emitted by agriculture are predominantly methane from enteric fermentation in ruminants, carbon dioxide from nutrient metabolism and respiration, and all three compounds from manure decomposition. Thus, livestock and poultry operations can make improvements in reducing greenhouse gas losses by avoiding excess diet protein levels, improving overall feed efficiency, reducing time of manure storage, and injecting or incorporating manure into soil at the time of land application.

See also: Environmental Impact of Meat Production: Primary Production/Meat and the Environment. Quality Management: Farm Level: Pork Quality; Farm Level: Safety and Quality of Beef

Further Reading

American Society of Agricultural and Biological Engineers, 2005. ASABE Standards 2006: ASAE D384.2 MAR2005 Manure Production and Characteristics. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

Environmental Protection Agency, 1999. Livestock Manure Management. Available at: http://www.epa.gov/outreach/reports/05-manure.pdf (accessed 15.12.11).

Fulhage, C.D., 1993. Laboratory Analysis of Manure. EQ215. Columbia, MO: University of Missouri.

Godwin, D., Moore, J.A., 1996. Manure Management in Small Farm Operations. EM 8649. Corvallis, OR: Oregon State University.

van Heugten, E., van Kempen, T., 2010. Nutritional effects on swine nutrient excretion and air quality. In: Meisinger, D.J. (Ed.), National Swine Nutrition Guide, Chapter 31. Ames, IA: US Pork Center of Excellence, pp. 278–287.

James, R., Eastridge, M.L., Brown, L.C., *et al*., 1992. Ohio Livestock Manure Management Guide. Bulletin 604-06. Columbus, OH: Ohio State University.

Leikam, D.F., Lamond, R.E., 2003. Estimating Manure Nutrient Availability, MF-2562. Manhattan, KS: Kansas State University.

Relevant Websites

http://cfpub.epa.gov/npdes/home.cfm?program_id=7
Environmental Protection Agency.

http://www.extension.org/animal_manure_management
eXtension.

Waste Management in Europe

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Legal Background

The directive 2008/98/EC on wastes in the European Union (EU) is aimed to improve protection of the environment and climate as well as natural resources by boosting the waste prevention and the recycling of waste. For this, a five-step waste hierarchy is defined, i.e., waste prevention, reuse, recycling, energy recovering, and disposal. In this context, reuse means that the waste is used without further treatment; recycling means any recovery operation by which waste materials are reprocessed into products, materials, or substances for the original or other purposes.

The preference is always for the best option with regard to the protection of the environment. For the consideration of the options, technical, economic, and social consequences are estimated. The recycling management is, therefore, forcefully directed to waste prevention and recycling without endangering well-established ecologically high-class disposal techniques.

In the framework of enforced recycling strategies, the waste management of municipal waste shall achieve a rate of recycling of 65% until 2020. The rate of material utilization of building waste will be 70%.

Municipal Solid Waste

Municipal waste consists of disposals derived from private households and comparable institutions, as well as of domestic waste-like material from industry, i.e., waste of surgeries, schools, kindergarten, etc. In addition, bulky refuse, waste from markets, litter, biodegradable waste, glass, paper, feces, and sludge belong to municipal waste. Until June 2005, use of untreated biodegradable municipal waste for landfilling in Germany was allowed. Now, reusable waste material has to be treated either thermally or biomechanically before it is used in a repository.

Biowaste

Biodegradable waste can be commonly found in municipal solid waste as green waste, food waste, paper waste, and biodegradable plastics. Other biodegradable wastes include human waste, manure, sewage, and slaughterhouse waste. The reutilization of biowaste is one of the most important parts of waste management today. Figure 1 shows the percentages of separately collected biowaste and green waste.

Separation of biowaste from other waste and the removal of the remaining biodegradable elements in the residual waste through pretreatment was the turning point with regards to the waste management sector's climatic impact: the annual emissions of greenhouse gases, expressed in CO_2 equivalents, have been reduced by approximately 56 million metric tons compared with 1990 levels. That represented almost 25% of the total reduction in emissions of greenhouse gases achieved in Germany until 2006.

In the context of further efforts to produce renewable energy, for example, from energy plants, whose cultivation is sometimes in competition with food and fodder production, combined material and energy recovery from biowaste and green waste is now of particular interest.

In 2007, the German Advisory Council on the Environment (SRU, 2007) established that every year approximately 100 million metric tons of 'biomass residues,' i.e., biowaste and similar materials are generated in Germany from areas such as forestry, agriculture, or sewage and waste management. Of this, approximately 65% could be technically and ecologically useful. This has a potential of 4–5% of the country's primary energy requirement. High priority should be given to exploiting this potential, a major proportion of which falls within the sphere of responsibility of local authorities.

The EU Waste Framework Directive of December 2008 also underscores the need to make better use of biowaste. Article 22 of the Directive states that member states shall take appropriate measures to encourage the separate collection of

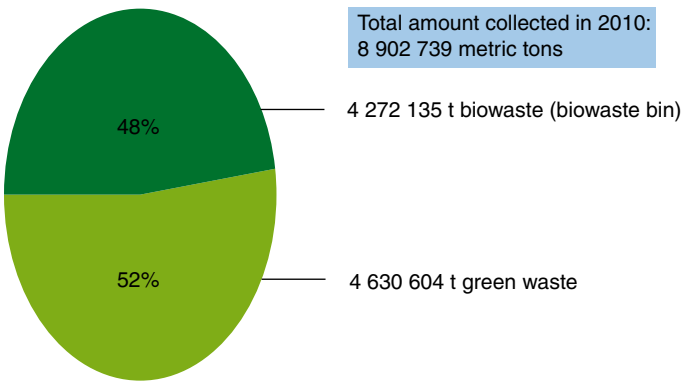

Figure 1 Percentage of separately collected biowaste and green waste of the total amount of biowaste. Reproduced from Federal German states waste inventories 2010 (NRW and Thuringia, 2009).

Utility of bio-waste in the different recovery paths

Recovery paths	Composting	Anaerobic digestion	
Product	Material -solid-	Energy/material -solid-[1]	Energy/material -liquid-
Humus reproduction	+ + +	+ + +	0
Peat substitution	+ +	+ +	0
Plant nutrients[2]:			
• Nitrogen	+	+	+ +
• Phosphorous	+ +	+ +	+ +
• Other nutrients	+	+ +	+ +
Energy, heat	(+)[3]	+ +	+ +

1. Composted digestates
2. Short- and medium-term availability
3. In energy recovery from sifting residue

Figure 2 Utility of biowaste in the different recovery paths. Modified from Bundesgutegemeinschaft Kompost (2008).

biowaste with a view to utilize it for composting and anaerobic digestion. Figure 2 summarizes the possible reutilization pathways of biowaste.

Sewage Sludge

Sewage sludge originates from municipal sewage plants that purify the wastewater derived from private households and from industry. It contains separated ingredients of the wastewater, i.e., sediments from the mechanical purification step, excess biomass from the biological purification step, and sludge from the third purification step containing precipitated phosphates. Owing to the rather high nitrogen and phosphate content, sewage sludge can be used as fertilizer on agricultural areas where the pollution burden is rather low. This usage of the sewage sludge is desirable with regard to political economy and protection of resources. Currently, approximately 50% of sewage sludge is used for fertilizing in Germany. The other part is used as refuse-derived fuels or for landfilling after biomechanical pretreatment or a treatment in a waste incinerating plant.

The application of sewage sludge as fertilizers in agriculture is administered in the EU according to the directive 86/278/EU.

Animal By-Products

All residual materials derived from animals that are not suited for human consumption are called animal by-products. They consist of entire animal bodies, parts of killed animals or animals that died, or products originating from animals that are not or no longer determined for human consumption including oocytes, embryos, and semen that are not used for breeding.

Animal by-products are considerable sources for infections and play an important role in the transmission of infectious agents, i.e., foot-and-mouth disease virus, swine fever viruses, or prions. Therefore, these materials should be utilized or depolluted without endangering the health of human beings and animals as well as the environment. Beyond this, it is to be ensured that no animal by-products end up in the food chain.

To achieve these goals, the differentiation and utilization of animal by-products in Europe is regulated by the regulations 1069/2009 and 142/2011 of the EU. These regulations deal with animal by-products and their follow-up products that are excluded from consumption. In addition, products and crude materials of animal origin that are excluded from the food chain due to the decision of producers and are, therefore, not used for human consumption are also considered in the regulation. The hygienic conditions and epidemic precautions for the collection, transport, storage, treatment, handling, usage, trading, and elimination of animal by-products are subjects of this regulation.

In accordance with the regulation 1069/2009, animal by-products are categorized into three groups with regard to origin and epidemiological risk for humans as well as animals. Based on this, the materials have to be variably treated and can be utilized in different ways.

Category 1 Material

Animal by-products belonging to Category 1 are considered to have the highest epidemiological risk potential. Category 1 material shall comprise the following animal by-products:

- Entire bodies and all body parts, including hides and skins, of the following animals:
  - animals suspected of being infected by a transmissible spongiform encephalopathy (TSE) in accordance with Regulation (EC) No 999/2001 or in which the presence of a TSE has been officially confirmed;
  - animals killed in the context of TSE eradication measures;
  - animals other than farmed and wild animals, including in particular pet animals, zoo animals, and circus animals;

  - animals used for experiments as defined by Article 2(d) of Directive 86/609/EC without prejudice to Article 3(2) of Regulation (EC) No 1831/2003; and
  - wild animals, when suspected of being infected with diseases communicable to humans or animals.
- Specified risk material, entire bodies or parts of dead animals containing specified risk material at the time of disposal.
- Animal by-products derived from animals that have been subjected to illegal treatment is defined as the use of unauthorized substances or products authorized under Community legislation for purposes or under conditions other than those laid down in Community legislation or, where appropriate, in the various national legislation (Article 1(2)(d) of Directive 96/22/EC or Article 2(b) of Directive 96/23/EC).
- Animal by-products containing residues of other substances and environmental contaminants listed in Group B (3) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation.
- Animal by-products collected during the treatment of wastewater required by implementing rules adopted under point (c) of the first paragraph of Article 27 from establishments or plants processing Category 1 material; or from other establishments or plants where specified risk material is being removed.
- Catering waste from the means of transport operating internationally (international catering waste, e.g., aircraft catering). Mixtures of Category 1 material with either Category 2 material or Category 3 material or both.

Category 2 Material

Animal by-products belonging to Category 2 are considered to have the medium epidemiological risk potential. Category 2 material shall comprise the following animal by-products:

- Manure, nonmineralized guano, and digestive tract content.
- Animal by-products collected during the treatment of wastewater required by implementing rules adopted from establishments or plants processing Category 2 material; or from slaughterhouses other than those covered by Article 8(e).
- Animal by-products containing residues of authorized substances or contaminants exceeding the permitted levels as referred to in Article 15(3) of Directive 96/23/EC.
- Products of animal origin that have been declared unfit for human consumption due to the presence of foreign bodies in those products.
- Products of animal origin, other than Category 1 material, that are: imported or introduced from a third country and fail to comply with Community veterinary legislation for their import or introduction into the Community except where Community legislation allows their import or introduction subject to specific restrictions or their return to the third country; or dispatched to another Member State and fail to comply with requirements laid down or authorized by Community legislation except where they are returned with the authorization of the competent authority of the Member State of origin.
- Animals and parts of animals, other than those referred to in Article 8 or Article 10, that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes; fetuses; oocytes, embryos, and semen that are not destined for breeding purposes; and dead-in-shell poultry.
- Mixtures of Category 2 material with Category 3 material.
- Animal by-products other than Category 1 material or Category 3 material.

Category 3 Material

Animal by-products belonging to Category 3 are considered to have the lowest epidemiological risk potential. Category 3 material shall comprise the following animal by-products:

- Carcasses and parts of animals slaughtered or, in the case of game, bodies or parts of animals killed, and which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons.
- Carcasses and the following parts originating either from animals that have been slaughtered in a slaughterhouse and were considered fit for slaughter for human consumption following an antemortem inspection or bodies and the following parts of animals from game killed for human consumption in accordance with Community legislation:
  - carcasses or bodies and parts of animals that are rejected as unfit for human consumption in accordance with Community legislation, but which did not show any signs of disease communicable to humans or animals;
  - heads of poultry;
  - hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus and metacarpus bones, tarsus and metatarsus bones, of animals, other than ruminants requiring TSE testing, and ruminants that have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001;
  - pig bristles, feathers.
- Animal by-products from poultry and lagomorphs (animals belonging to the order Lagomorpha, which includes the rabbits, hares, and pikas) slaughtered on the farm as referred to in Article 1(3)(d) of Regulation (EC) No 853/2004 that did not show any signs of disease communicable to humans or animals.
- Blood of animals that did not show any signs of disease communicable through blood to humans or animals obtained from the following animals that have been slaughtered in a slaughterhouse after having been considered fit for slaughter for human consumption following an antemortem inspection in accordance with Community legislation: animals other than ruminants requiring TSE testing; and ruminants that have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001.
- Animal by-products arising from the production of products intended for human consumption, including degreased bones, greaves (protein containing residues of

rendering, after partial separation of fat and water), and centrifuge or separator sludge from milk processing.
- Products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arises.
- Pet food and feeding stuffs of animal origin, or feeding stuffs containing animal by-products or derived products that are no longer intended for feeding for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arises.
- Blood, placenta, wool, feathers, hair, horns, hoof cuts, and raw milk originating from live animals that did not show any signs of disease communicable through that product to humans or animals.
- Aquatic animals, and parts of such animals, except sea mammals that did not show any signs of disease communicable to humans or animals.
- Animal by-products from aquatic animals originating from establishments or plants manufacturing products for human consumption.
- The following material originating from animals that did not show any signs of disease communicable through that material to humans or animals: shells from shellfish with soft tissue or flesh; the following originating from terrestrial animals: hatchery by-products, eggs, egg by-products (including egg shells), and one-day-old chicks killed for commercial reasons.
- Aquatic and terrestrial invertebrates other than species pathogenic to humans or animals.
- Animals and parts thereof of the zoological orders of Rodentia and Lagomorpha, except Category 1 material as referred to in Article 8(a)(iii), (iv), and (v) and Category 2 material as referred to in Article 9(a)–(g).
- Hides, skins, hooves, feathers, wool, horns, hair, and fur originating from dead animals that did not show any signs of disease communicable through that product to humans or animals, other than those referred to in point (b) of this.
- Adipose tissue from animals that did not show any signs of disease communicable through that material to humans or animals, which were slaughtered in a slaughterhouse and which were considered fit for slaughter for human consumption following an antemortem inspection in accordance with Community legislation.
- Catering waste other than as referred to in Article 8(f).

A strict separation of the three different categorized materials from the production up to the utilization or disposal is the basis for the biosafety of the whole process. In case of mixing materials belonging to different categories, the mixture is always allocated to the category showing the higher risk potential.

Waste Treatment

Composting Processes

Composting is a biological decomposition process for organic waste, in which the material is broken down by microbes and microorganisms under aerobic conditions. During the composting process, the temperature of the material increases up to 70–80 °C. This will sanitize the input material.

Input materials for composting plants can be, for example, biowaste, green waste, digestates from biogas plants, sewage sludge, and animal by-products of Categories 2 and 3 after sanitation. Composting has been used as a method of biowaste treatment on a large industrial scale in Germany since the middle of the 1980s.

As composts contain essential plant nutrients, they are good organic fertilizers and excellent soil improvers. Compost and composted solid digestates are especially suitable for humus reproduction.

The mechanical composting processes applied can be divided into various categories:

- composting in heaps (triangular, trapezium-shaped, or flat stacks),
- composting in bunkers/containers
- row/tunnel composting
- composting as briquettes
- other systems.

The processes differ in the way the heaps are constructed (open, covered, and in containers), the type of ventilation, and also the duration of the intensive rotting stage and the maturity of compost desired. If the intensive rotting system is set up for mature compost, the main and subsequent rotting phases are integrated. If the intensive rotting results in sanitized fresh compost, a second rotting stage can follow to achieve a higher degree of decomposition. In composting facilities, the second stage is carried out predominantly in flat or triangular heaps.

The following diagram provides an overview of the possible composting processes (Figure 3).

Marketing channels for compost can be found in many areas, i.e., private gardening, landscape recultivation, soil suppliers, agriculture, and horticulture.

The economic importance of using composts in agriculture is often underestimated. However, a constantly rising demand shows that compost is rapidly becoming an attractive substitute in the wake of higher mineral fertilizer prices. Whereas until a few years ago farmers were paid to accept compost and composted digestates, nowadays it is usually a source of revenue.

If one considers just the fertilizer value of compost based on mineral fertilizer prices, a metric ton of compost was worth up to 12 Euros in early 2012.

Anaerobic Digestion Processes

Unlike composting, the biological decomposition processes occur in the absence of oxygen. The most important end product of anaerobic digestion is biogas. Biogas can be used mainly in electricity and heat production. Besides this, a nutrient-rich digestate is produced during the fermentation process that can be used in liquid or solid form as a fertilizer in agriculture and related areas.

Separately collected biowaste as well as food scraps and the herbaceous parts of green waste, on the other hand, are

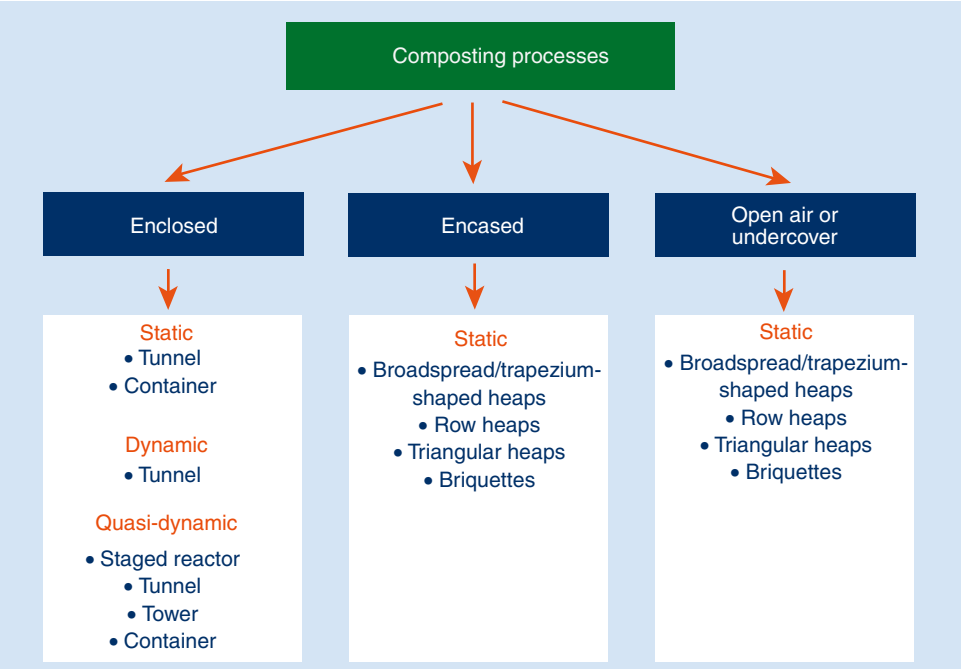

Figure 3 Overview of the composting plants.

generally well suited for anaerobic digestion. However, animal by-products can be used as input material of biogas plants, for example, manure, carcasses of slaughtered animals, etc. In most cases, biogas plants are operated using a mixture of manure and energy crops.

The digestates can be used directly in agriculture in liquid or solid form or marketed as a solid digestate product after composting. Anaerobic fermentation achieves a positive energy and climate balance due to biogas production, which can be used as a substitute for fossil fuels.

An important feature for distinguishing anaerobic digestion processes is the method of operation (Figure 4). There are continuous and discontinuous processes, which are operated at mesophilic temperature (35–37 °C) or thermophilic temperature (50–55 °C).

In the continuous process, input material is automatically fed at regular intervals into the anaerobic digestion reactor (fermenter). This process promotes continuous biogas production of consistent quality. In the discontinuous process, the digesters are filled manually, then after several weeks they are emptied and refilled (batch operation). Biogas production is not continuous, but parallel connection of several digesters working on a staggered system can largely compensate this. In addition, with regard to the dry matter of the input material, wet and dry fermentation is distinguished.

Anaerobic digestion achieves a positive energy and climate balance due to the emerging biogas and the resulting substitution of fossil fuels. With anaerobic digestion the climate credit amounts to approximately 99 kg (German average) or 194 kg (state-of-the-art facilities). For 1 metric ton of biowaste, depending on the input quality and process, between 80 and 140 m^3 of biogas with a methane content of 50–65% is produced. The energy from this equates to 50–80 m^3 of natural gas. As an example, an input of 20 000 metric tons of biowaste per year suffices to operate a cogeneration plant with a rated electrical capacity of 600 kW, producing enough electricity for 1000–1500 households. It is also possible to refine biogas to natural gas quality and feed it into the natural gas grid.

Disposal and Recycling of Animal By-Products

Animal by-products can contain infectious agents, which can infect human beings and animals. Therefore, the utilization possibilities for these very heterogeneous materials are dependent on the category to which the by-products belong. In the case of Category 1 materials harboring the highest epidemiological potential, the materials have to be sterilized (133 °C, 3 bar, and 20 min) and afterwards burned.

Materials of the Categories 2 and 3 can be recycled after sterilization and disinfection, respectively. In these cases, the materials can be composted or used as input materials in biogas plants. The production of animal feed derived from Category 3 materials is principally possible but more or less restricted to pet feed.

Waste Management and Hygiene

When using organic fertilizers in agriculture, the transmission of microorganisms infecting human beings and animals has to be considered. This is especially the case if sewage sludge, untreated slurry, and digestates are used. Therefore, the epidemiological risk of fertilizers produced during the recycling of wastes of different organic origin has to be estimated. For this, the risk assessment should include the raw material, the processing techniques, the end product, and the kind of application.

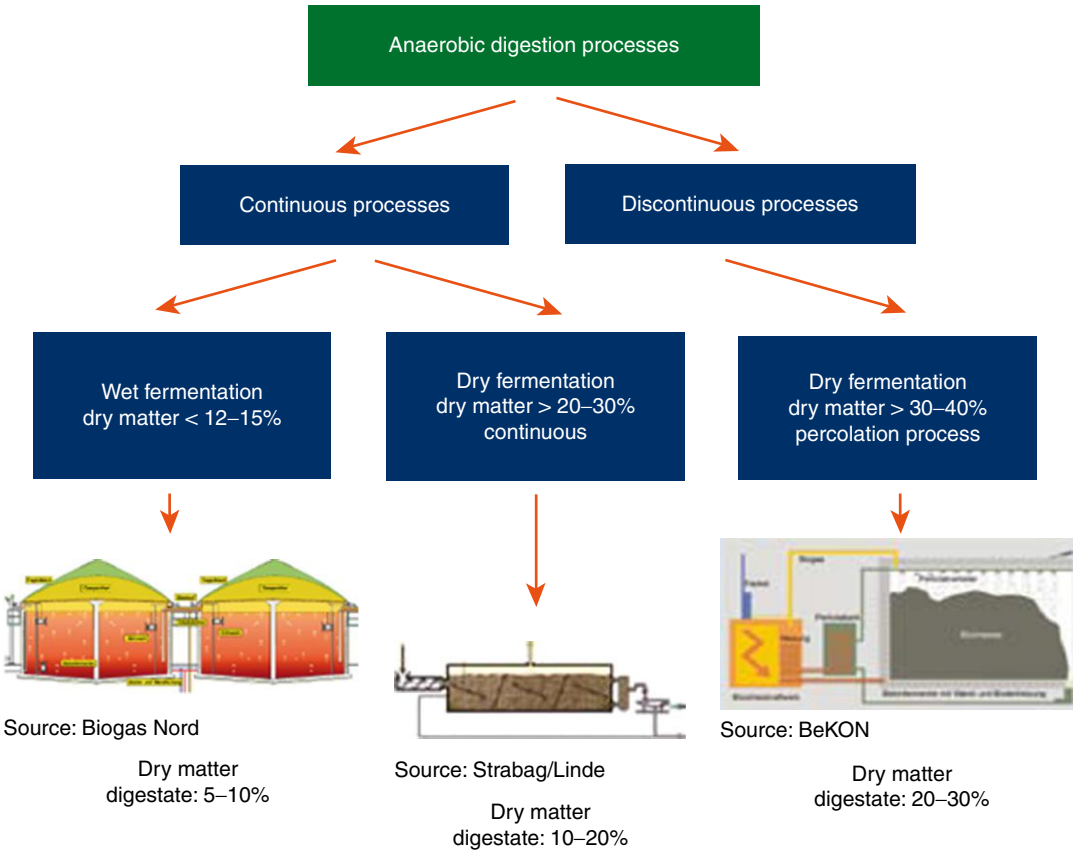

Figure 4 Overview of the anaerobic digestion plants.

In some cases the minimization of the epidemiological risk is only possible by treatment of the raw material (disinfection and sterilization), for example, animal by-products. Sometimes the treatment process is able to inactivate infectious agents leading to an epidemiologically harmless product, for example, composting. One additional possibility to minimize the epidemiologic risk is to perform microbiological investigations of the end product before application. This leads in some instances to restrictions for the scope of application.

In summary, blood, placenta, wool, feather, hair, horn, and raw milk from animals which are not suspicious for carrying agents that can be transmitted to humans and animals are allowed to be used in biogas plants after pasteurization (70 °C, 1 h according to the regulation 1069/2009). The same procedure is applied in the case of inedible fat (e.g., used fat from fryers). Fat from fat separators originating from the food industry and slaughterhouses is processed as Category 1 material.

According to Article 9(f) of the regulation 1069/2009, fetuses, oocytes, embryos, and semen which are not destined for breeding purposes as well as dead-in-shell poultry have to be disposed as Category 2 material using pressure sterilization (at least 133 °C, for at least 20 min with an absolute pressure of at least 3 bar). Male and female reproduction tracts as well as brain material that were not categorized as specified risk material are preceded accordingly.

Furthermore, high-quality soil improvers and fertilizers can be produced sustainably from biowaste and green waste by treating it in composting plants or combined anaerobic digestion and composting facilities. The material recovery of digestates is an important renewable source of plant nutrients and humus, and therefore is essential for the greenhouse gas balance.

Summary

Every year, some 2 billion tons of waste are produced in the EU, and this rate is rising steadily. Therefore, strict measures of waste management are necessary for protecting the environment and climate as well as human health and natural resources. The Directive 2008/98/EC on wastes comprises the legal background of waste management in the EU. This legislation introduces a five-step waste hierarchy where waste prevention is the best option, followed by reuse, recycling, and other forms of recovery (e.g., energy recovering), with disposal such as landfill as the last resort. The main waste categories are municipal solid waste, biowaste, sewage sludge, and animal by-products.

Animal by-products are an exception since they can be utilized for recycling only after disinfection. They must be dealt in accordance with strict regulations (regulations 1069/2009 and 142/2011 of the EU) designed to prevent harm to people, animals, and the environment. Animal by-products are categorized (Category 1–3 materials) by the risks they pose and the methods used to deal with them.

The two most important procedures for recycling of organic raw materials are composting and biogas fermentation. Composting is a biological decomposition process for organic waste, in which the material is broken down by microbes and microorganisms under aerobic conditions. Biogas can be used mainly for the production of electricity and heat. Besides this, a nutrient-rich digestate is produced during the fermentation processes that can be used in liquid or solid form as a fertilizer in agriculture and related areas.

Further Reading

Bischofsberger, W., Dichtl, N., Rosenwinkel, K., Seyfried, C.F., Böhnke, B., 2005. Anaerobtechnik, second ed. Berlin, Heidelberg: Springer Verlag.

BMU, 2012. Brochure: Ecologically Sustainable Recovery of Bio-Waste-Suggestions for Policy-Makers at Local Authorities Berlin, Germany: Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) Public Relations Division 11055. E-mail: service@bmu.bund.de. Available at: www.bmu.de/english (accessed 15.01.14).

EC, 2009. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation).

European Union, 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008. Official Journal of the European Union L 312/3.

European Union, 2011. REGULATION (EU) No 142/2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. Official Journal of the European Union L 54/1.

SRU, 2007. Sachverständigenrat für Umweltfragen Klimaschutz durch Biomasse-Kurzfassung des Sondergutachtens. Available at: http://www.umweltrat.de/SharedDocs/Downloads /DE/02_Sondergutachten/2007_SG_Biomasse_KF (accessed 15.01.14).

Statistisches Bundesamt (Federal Statistical Office), 2009, 2013. Wiesbaden. Available at: www.destatis.de (accessed 15.01.14).

Wiechmann, B., Dienemann, C., Kabbe, C., *et al*., 2012. Klärschlammentsorgung in der Bundesrepublik Deutschland. Dessau-Roßlau: Umweltbundesamt.

MEASUREMENT OF MEAT QUALITY

Measurements of Water-holding Capacity and Color: Objective and Subjective

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Glossary

Color space A color space maps a range of physically produced colors to an objective description of color sensations registered in the eye, typically in terms of tristimulus values. It is the terminology used to describe the mathematically derived languages for describing color, such as International Commission on Illumination (CIE) XYZ, CIE$L^*a^*b^*$, and RGB.

Diffuse reflection The reflection of light from a surface such that an incident ray is reflected at many angles rather than at just one angle.

Gloss The shine or lustre on a surface.

Isobetic A specific wavelength at which the extinction coefficients for two or more forms of myoglobin are the same.

***Lab* color space** A color-opponent space with dimension L for lightness and a and b for the color-opponent dimensions of redness–greeness and blueness–yellowness, respectively, based on nonlinearly compressed CIE XYZ color space coordinates.

RGB color space Any additive color space based on the RGB color model and defined by the three chromaticities of the red, green, and blue, the additive primary colors. It can produce any chromaticity defined by those primary colors.

Specular reflection The mirror-like reflection of light from a surface, in which light from a single incoming direction is reflected into a single outgoing direction.

Tristimulus Relates to the three levels of stimulus that color gives to the three types of cone cells in the eye. The cone cells (photoreceptors) in the eye have sensitivity peaks in three wavelengths: short, medium, and long, hence tristimulus refers to stimulation of all three types of cone cells.

Water-binding capacity The amount of water that can be added during cutting, heating, grinding, and pressing, including the use of phosphates and salts.

Water-holding capacity The amount of water that meat can hold during cutting, heating, grinding, and pressing and during transport, storage, and cooking. The water released can be variously described as drip, purge, cook loss, weep, exudate, or cook loss.

Introduction

The two most important quality traits of meat are the visual acceptability, which determines the initial impression of quality, and sensory acceptability when the meat is consumed, possibly justifying the visual impact. The surface color of meat and the weep in the tray are important for cues on visual acceptability of meat on retail shelves when it is purchased. Water-holding capacity (WHC) is related to sensory juiciness as well as the occurrence of weep in the tray. Meat color and WHC can be assessed subjectively or objectively and these methods are discussed below. In addition, the background to WHC and color measurement are briefly described along with the specifications necessary for selecting a colorimeter for meat color measurement.

This article focuses on methods that can be used in the laboratory or in the field.

Water-holding Capacity Measurement

WHC determines not only the visual acceptability but also the loss of water during transport, storage, and cooking. Muscle comprises approximately 75% water at rigor and the addition of water to meat, and the hydration of the meat after cooking, is closely related to taste, tenderness, color, and juiciness. The consequence of poor WHC is low cook yields and often 'dry (lack of juiciness)' meat, so these can also be used to indirectly measure WHC. This section describes the methods used to measure WHC.

WHC is the amount of water the meat can hold during cutting, heating, grinding, and pressing. If there has been added water, including phosphates and salts, the correct term would be water-binding capacity. The terms drip, purge, cook loss, weep, and exudate refer to the loss of water from muscle and are inversely related to WHC. Each of these traits is related to each other but do not always have a strong correlation. In particular, cooking loss can have quite different influencing factors and thus should not be inferred from measurements of the other traits.

Factors that Influence Water-holding Capacity

Water in the muscle cells (approximately 75% by weight) is bound primarily by capillary forces within the myofibrillar structure, as well as in the sarcoplasm. In the living animal, water is kept in cells by the sarcolemma (cellular membrane)

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and maintained by various membrane pumps. Postslaughter, water is moved to the sarcoplasm by shrinkage of the myofibrils and kept in the cell until the pH falls, adenosine triphosphate becomes unavailable, and water and ions can move and pass through the sarcolemma into the extracellular space between the cells. In muscle, approximately at pH 7, of a live animal, the myofibrils take up most of the space in the muscle cell. Postmortem, the myofibrils shrink both in transverse and longitudinal directions, the intracellular sarcoplasmic space increases, and over time, water moves to the extracellular space. In a muscle at approximately pH 7, more than 95% of the water is within cells; some days postmortem, approximately 15% is in the extracellular space and the water appears as drip at the surface of the meat. From this point, proteolysis of the cytoskeletal filaments occurs during aging and further water is produced over time as drip. Dark, firm, and dry (DFD) meat with a high ultimate pH (>6.2) loses less drip. This is because the myofibrils have had minimal transverse shrinkage, as at the high pH of DFD meat, there is a net negative charge on the proteins in the myofibrils, which causes the filaments to be repulsed from each other. During rigor, as the pH of the muscle approaches 5.4 or lower, the net charge on proteins in the myofibrils diminishes and filaments can approach each other, causing transverse shrinkage in the myofibril. Thus, there is always some loss of WHC when the pH of muscle drops from 7 to 5.5. When the sarcomere shortens, due to rigor, cold, or heat shortenings, the myofibril shortens longitudinally, with water being expelled to the intracellular space. Thus, meat from stretched muscles, with long sarcomeres, has higher WHC as measured by drip loss, purge, and cooking loss. In muscle prone to the pale, soft, and exudative (PSE) condition, the high temperatures shortly after slaughter (35–42 °C) and the already low pH lead to myosin denaturation, which causes additional transverse shrinkage in the myofibril, along with early membrane destruction. In PSE muscles, the three conditions of low pH, denatured myosin, and damaged membranes cause drip loss to occur virtually without any time lag. The strongest increase occurs in the first 2 days. This seems to be less of an issue in beef and lamb, although increased drip has been observed in beef muscle going through high temperature rigor. The WHC, therefore, can be influenced by the prerigor myosin denaturation, sarcomere shortening, decline in muscle pH from 7 to 5.5, and the release of water from the muscle cell as a result of cytoskeletal protein degradation postrigor during tenderization.

Weight loss during storage, cooking, freezing, and thawing are related to how much water is available and how easily it can leave the muscle structure network.

Methods to Measure Water-holding Capacity

The measurement of WHC usually involves the application of force to measure the water released. The force can either be natural, through gravimetric means, or be applied externally as pressure, through centrifugation, compression, or taking advantage of capillary action.

Methods applying no external force

1. Gravimetric (drip loss): This method involves the measurement of weight loss in free drip, bag drip, or cube drip, where the meat is left to itself under different environmental conditions. The most commonly used method involves using a standard size and weight block of meat (approximately 30–100 g) and suspending it in a bag, for 1–2 days at 1–4 °C, ensuring that the meat does not touch the sides of the bag (Figure 1). Variations of this method include chopping the meat and placing it in a tray, so that all the drip can be collected in the bottom of a tray, which is not in physical contact with the meat. Each method involves weighing the meat at the start and end, and the drip loss is expressed as a loss in weight of the sample over the defined period, expressed as a percentage of the initial weight. The surface area of the meat can influence the results. This method is widely used because of its simplicity and is often called the Honikel bag method.

   The EZ-DripLoss method has been developed as a simplified, convenient, and standardized variation of the procedure. A 25-mm slice is removed at a right angle to the muscle fiber direction. The sample is immediately cut using a 25-mm cork borer in the fiber direction. It is then placed in a special preweighed container equipped with a lid to avoid evaporation and loss of meat juice. This container contains lamellas with a minimum area touching the sample's surface. The container is stored for 24 h at 4–6 °C, and then the meat is removed and weighed from the container and carefully dried with absorbent paper and weighed.
2. Weep or purge: Purge, or weep, is defined as water lost from meat or muscle during storage postrigor, including during storage in trays (overwrap or modified atmosphere packs) on retail shelves. Measuring purge, or weep, involves measuring the loss in weight of meat over a defined period. Thus, the meat is weighed before being placed in the bag or tray, then weighed again once the bag is opened or the packaging is removed, at the end of the storage or display period. The fluid is mopped up and the meat reweighed at the completion of the storage period. This is a method that can be used when the meat is stored in a vacuum bag or retail-ready tray. The appearance of weep or purge is unsightly and implies an inferior product.

Figure 1 Illustration of the gravimetric method for measuring drip loss, involving suspension of a meat sample in an inflated bag and storage at 2–4 °C for 1–2 days. Credit: Kaufman, R., University of Wisconsin–Madison.

3. Subjective scoring of exudate: Visual scores for exudate have been used to assess the surface muscle of beef and pork carcasses. The score can be a simple 'yes' or 'no,' or a score from 0% to 100%, similar to that used for the rapid filter paper method discussed in the Section Methods Applying External Force under point 4.

Methods applying external force

1. The press method or compression: This was the first method developed to measure WHC. A predefined circular piece of meat (approximately 30 g) is placed on filter paper, between two plastic sheets, and a defined pressure is applied to the meat. The water squeezed out is absorbed by the filter paper and is related to the amount of 'loose' water in the sample. The amount of water released is either measured (1) indirectly as the area of the ring of expressed juice or (2) directly by weighing the filter paper. This method is no longer widely used as the results are variable and are dependent on the texture of the meat.
2. High-speed centrifugation: This involves subjecting samples of 1–20 g to centrifugal forces of 6000–40 000*g*. Water release is determined by weighing the water or the sample before and after centrifugation. This method removes more water than most of the other methods, due to the high centrifugal forces involved. But due to the elastic nature of meat, some of the water is reabsorbed once the centrifugal force is removed. Similar to the press method, the results obtained are influenced by the texture of the meat. This method has had very limited use.
3. Low-speed centrifugation: This method involves subjecting 3–15 g samples at 200–5000*g* for 15–30 min (or even longer) and measuring the weight loss of the sample or weight of the exuded fluid. The advantage of this method over high-speed centrifugation is that it uses specially designed centrifuge tubes with a perforated disc in the middle (e.g., Mobicols from MoBiTec). This disc allows moisture to spin down to the bottom while leaving the tissue on the top, thus preventing reabsorption of the fluid. One of the problems with this method, which has introduced inaccuracies in the results, has been the blocking of the pores in the membrane. This can be overcome by using superglue to glue the piece of meat to the bottom of the lid of the tube. Figure 2 shows the effects on water loss during centrifugation by gluing, or not gluing, the meat sample to the lid of the tube before centrifugation. This method has become very popular as it correlates well with drip loss measurements, is relatively simple to conduct, and samples can be centrifuged regularly (e.g., every 15 min) to follow changes in WHC.
4. Rapid filter paper method: This method relies on capillary suction forces of the filter paper applied to the meat surface. It is extremely rapid and involves placing a filter paper on a freshly cut surface at a defined time postcutting (e.g., 10 min), smoothing it out, then either scoring the filter paper for wetness (0–100% wet) or weighing the filter paper (Figure 3). Figure 4 shows the relationship between the filter paper score and temperature at rigor.
5. Other capillary suction methods: Other methods that utilize capillary suction rely on suction potential generated via different analytical filter papers and previous calibration of the filter papers or gypsum blocks combined with

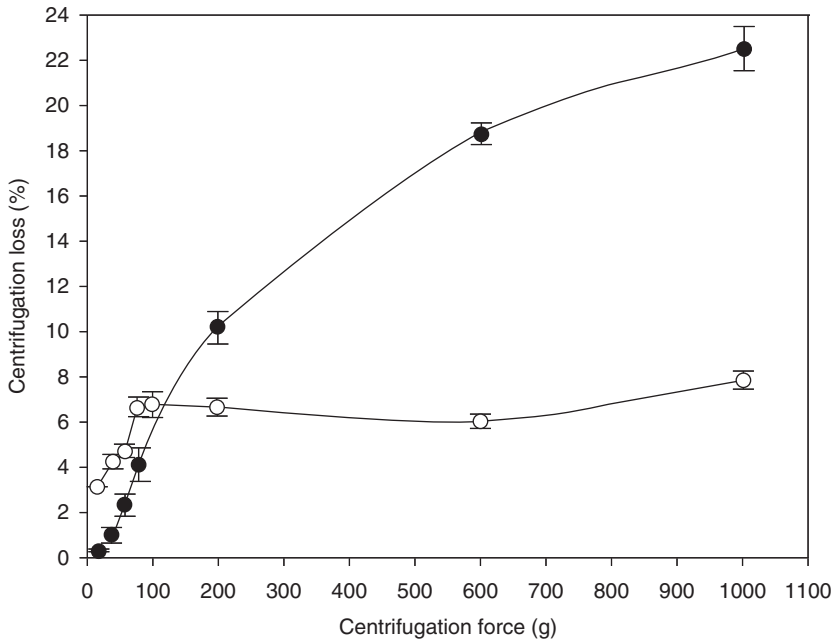

Figure 2 Water loss from meat samples (centrifugal loss %), as a result of 30-min centrifugation as a function of centrifugal force. Filled circles: samples glued to the bottom of the lid. Open circles: nonglued samples. Bars indicate the standard error of the mean. Ten replicates at each time point. Reproduced from Kristensen, L., Purslow, P.P., 2001. The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. Meat Science 58, 17–23.

compression. These two methods are time consuming and have not gained widespread use.

6. Application of heat (cooking loss): Cooking loss is defined as the loss in weight as a result of cooking, being expressed as a percent of the precook weight. A common method is to combine a measure of weight loss during cooking with the objective measurement of tenderness. Thus, samples destined for objective measurement of cooked meat are weighed before cooking, and then after cooking, the samples are cooled before removal from the bag, are blotted, and reweighed in order to determine cooking loss. Cooking loss is strongly related to degree of aging, cooking temperature, and cooking conditions and is quite an ill-defined property of meat. Of all the WHC measurements, cook loss has the highest correlation with the sensory trait juiciness, which is a complex and ill-defined trait in itself.

Figure 3 Illustration of the rapid filter method for measuring weep or exudate on a beef loin surface. The filter paper can either be weighed, for amount of fluid absorbed, or be scored for percentage of wetness. Credit: Athula, N., Department of Primary Industries, VIC, Australia.

Indirect methods

1. Protein solubility: Protein solubility is a good indirect indicator of WHC, which has been used to quantitate the low WHC of PSE meat muscle for a number of years. Measurement of sarcoplasmic and myofibrillar protein solubility of muscle involves homogenizing 1–2 g of meat in a low ionic strength and high ionic strength buffer, for sarcoplasmic and total protein solubility, respectively, then measuring the protein concentration in the supernatant after centrifugation at 1500*g* and 4 °C for 20 min. Myofibrillar protein solubility is determined by the difference between the protein concentration in the low and high ionic strength buffers. Alternatively, myofibrillar protein solubility can be determined by subjecting the pellet left, after centrifugation of the low ionic strength solution, to a high ionic strength buffer in order to solubilize the myofibrillar proteins.
2. Cook yield: This method is used to predict the yield of cooked meat products. Samples are weighed, homogenized in a food processor, placed into containers, cooked to a defined internal temperature, cooled, and then weighed for cook yield determination.

Color Measurement

Factors Contributing to Meat Color

The concentration and chemical state of the pigment myoglobin in the meat surface, as well as the texture of the meat and light scattering within the meat structure, determine the perceived color. The concentration of pigment in the meat surface, which determines the lightness and redness, usually varies with muscle and also with species (pork and chicken having low muscle myoglobin content relative to beef and sheep meat) and animal age. Young animals, such as veal, have less muscle myoglobin and thus the meat appears paler and less red. The main chemical states of myoglobin in a fresh meat surface are the reduced, purple deoxymyoglobin, red

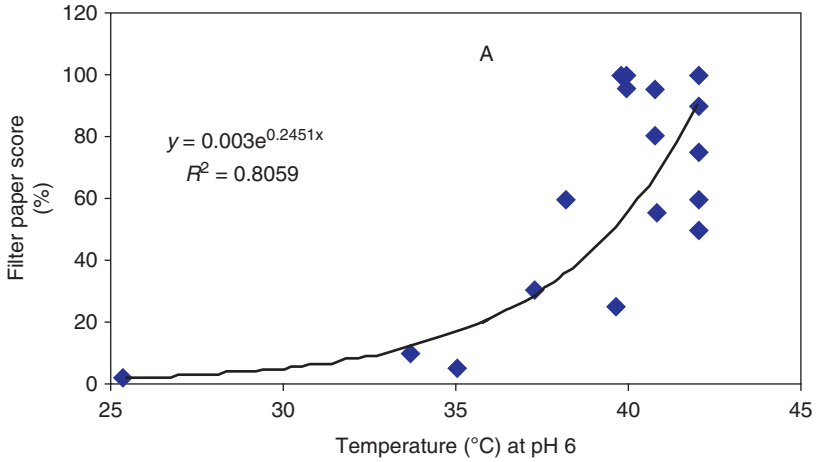

Figure 4 The relationship between filter paper score (rapid filter paper method) and temperature at pH 6 (rigor temperature) in the beef loin muscle. Reproduced with permission from Robyn Warner, Department of Primary Industries, Victoria, Australia.

oxymyoglobin with a bound oxygen molecule, and brown, reduced metmyoglobin. The consumer acceptability of the meat deteriorates rapidly when metmyoglobin starts accumulating in the meat surface. If deoxymyoglobin is the predominant form in the meat surface, the meat appears dark, purple red, and also has less consumer acceptability. Light scattering within the muscle structure occurs when the meat has undergone rapid pH fall prerigor at a high muscle temperature, muscle proteins have denatured, and there are only small gaps between myofibrils. These conditions are thought to cause increased scattering of incident light within the muscle structure and the muscle surface appears 'pale' or 'less light' to detectors (human or machine). This is particularly evident in the condition known as PSE pork.

These variations in pigment and structure will cause variations in the color of the meat surface, which can be visually seen and also measured by a machine.

Theory of Color Measurement

In the study of color perception, one of the first mathematically defined color spaces is the International Commission on Illumination (CIE) 1931 XYZ color space, created by the CIE in 1931. Owing to the distribution of three different types of cone cells, with different sensitivity peaks, in the eye (sensitivity to short, medium, or long wavelength light), the tristimulus values depend on the observer's field of view. To eliminate this variable, the CIE defined the standard (colorimetric) observer. Originally, this was taken to be the chromatic response of the average human viewing through a 2° angle, due to the belief that the color-sensitive cones resided within a 2° arc of the fovea in the retina. Thus, the CIE 1931 Standard Observer is also known as the CIE 1931 2° Standard Observer. A more modern and valid alternative is the CIE 1964 10° Standard Observer. Color spaces and color models of Yxy (derived from XYZ) and RGB (primary colors) have been described. A *Lab* color space is a color-opponent space with dimension L for lightness and a and b for the color-opponent dimensions of redness–greenness and blueness–yellowness, respectively, based on nonlinearly compressed CIE XYZ color space coordinates. The coordinates of the Hunter 1948 L, a, b color space are L, a, and b. However, *Lab* is now more often used as an informal abbreviation for the CIE 1976 (L^*, a^*, b^*) color space (or CIELAB). The difference between Hunter and CIE color coordinates is that the CIE coordinates are based on a cube root transformation of the color data, whereas the Hunter coordinates are based on a square root transformation. Thus, the CIE $L^*a^*b^*$ and Hunter Lab values are not directly comparable and the CIE $L^*a^*b^*$ is the preferred color space for meat measurement as it puts more emphasis on the red part of the spectrum.

In comparison to XYZ, Yxy, and RGB color spaces, the *Lab* color space is designed to approximate human vision. It aspires to perceptual uniformity, and its L component closely matches human perception of lightness, although it has its limitations.

Edge loss occurs when light that scatters through a translucent material, such as meat, which originally would be seen by the eye, is simply not measured by the instrument due to the configuration of the illuminant, sensor, and aperture. This occurs during conventional reflectance measurements of translucent materials when both the illumination and observation light paths travel through an aperture. Generally, the L^* value decreases when the window decreases in size from infinity to 3 mm. Thus, more accurate and valid measurements are obtained if a large aperture is used.

When incident light strikes a surface, the reflected light is described as $<4\%$ specular and $>96\%$ diffuse. Specular reflection is reflected from the surface at right angles to the incident light, whereas diffuse reflection is scattered. Color is seen in the diffuse reflection and gloss is seen in the specular reflection. If samples have the same color, but different surfaces, they will be perceived as a different color. Instrument geometry refers to the angle the light source and the detector make with the sample. Thus, geometries can be diffuse (sphere) or directional (45°/0° or 0°/45°). Directional geometry machines are recommended for meat as they exclude specular reflection, whereas sphere geometry includes specular reflection.

Instrumental Measurements of Meat Color

Surface measurements of color are difficult to make in a well-defined manner unless working under strict laboratory conditions. This is due to the blooming process that begins as soon as a fresh cut has been made in a piece of meat as well as due to the anisotropic characteristics of meat. However, standardized blooming conditions where the temperature and time are specified assist in obtaining accurate and representative measurements. Anisotropy means that the results obtained are different for measurements in different directions on the same material. Thus, the orientation of the fibers in a piece of meat will determine the color measured, showing the necessity for standardizing the fiber orientation in a meat surface being used for color measurement. A standardized measurement procedure is of utmost importance for reliable results. The recommendations for standardizing meat color measurement are for a minimum blooming time of 30 min, for the blooming to occur at 1–4 °C, duplicate measurements and preparation of the surface of the meat so that the fibers are always oriented the same way. The recommendations for the color machine are to calibrate it using standard color tiles and to use a constant (and recorded) degree of observer, size of aperture, and illuminant/viewer angle (Table 1).

Meat mainly reflects light in a diffuse way from the surface, thus the texture of the surface can influence the measurement. The texture or grain in the meat can influence the color measurements, emphasizing the need for standard procedures to be used. In addition, weep, exudate, or water on the meat surface, such as with PSE, can cause variable spectral reflectance to occur, again influencing the color measurements made. Because meat is partially translucent, a portion of the incident light is transmitted below the surface and reflected internally. Thus, samples must be sufficiently thick to ensure no light is reflected from the background. Ideally, the sample should be at least 2.5 cm thick. For high pH dark cutting (DFD) meat, the dark color is thought to be caused by internal reflection of light, thus preventing light from being reflected back to a detector (human or machine).

Table 1 Recommended specifications for preparation of meat for color measurement and for colorimeters, including alternatives and comments

Specification	*Recommended*	*Alternatives*	*Comments*
Time of blooming	30–60 min		Too short can result in inaccurate measurements
Thickness of sample	>2.5 cm		To ensure no effect of background color
Temperature of blooming	1–4 °C		Cold temperature allows better bloom due to higher solubility of O_2 at low temperature
Illumination/viewing angle	45/0 or 0/45	d/8 (diffuse/8)	45/0 allows exclusion of specular reflection
Degree of observer	10°	2° or 0°	Revolves around understanding of cones in eye
Aperture – Size of measuring port	As large a possible >8 mm		Small size can result in 'edge loss'
Light source (illuminant)	D65 (daylight at noon)	C (average daylight) A (Incandescent) F2 (Cool white fluorescent) U30 (Ultralume)	
Calibration tiles	Black and white	Color similar to sample	

Important specifications for any color machine are the angle of the viewer, size of the measuring head, presence of a glass or plastic plate in the measuring head, and type of incident light, as summarized in Table 1. As the color measurements obtained are not absolute, and are reliant on the viewing conditions and specifications of the machine used, the values that are obtained with different machines cannot be compared. An example of this is when color measurements of *L*, *a*, *b* are made with a Hunter Lab Mini Scan 4500 XE Plus; the size of the measuring head (large, 25 mm or small, 5 mm) has a substantial influence on the values obtained.

Lab or $L^*a^*b^*$, along with the calculation of hue and chroma/saturation, is one of the two main systems generally used to describe the color of fresh meat. The other system is percentage reflectance, which is described below along with CIE $L^*a^*b^*$.

1. CIE L*a*b* or Hunter Lab: The CIE $L^*a^*b^*$ or Hunter Lab systems report values for lightness (L^* and L), redness/greenness (a^* and a), and yellow/blueness (b^* and b) and are represented by a three-dimensional color space (see Figure 5). The hue and saturation (also called chroma) can be calculated from the a^* and b^* values. Hue angle = arctangent (b^*/a^*), defines the color, and also indicates the changes in the myoglobin pigment from red to brown. Saturation or chroma = $\sqrt{(a^{*2}+b^{*2})}$ indicates the intensity of the color. An increase in a^* (redness–greenness) value is predominantly related to an increase in the concentration of myoglobin in the meat surface, especially oxymyoglobin. An increase in L^* value (lightness) occurs when there is not only less myoglobin in the surface but also increased light scattering, due to protein denaturation in the muscle structure.
2. Reflectance ratios and percentage reflectance: Reflectance measurement closely relates to what the human eye and brain can see. It is a good method for examining the amount and chemical state of myoglobin in meat *in situ* and is rapid. Usually, the difference in color over time, between treatments or samples, is required and for this the calculation of the reflectance ratios is recommended. For a discussion of the analysis of the individual pigments present in the meat, using reflectance spectrometry or pigment extraction, see Further Reading. The proportions of the three major pigments in fresh meat, namely deoxymyoglobin, metmyoglobin, and oxymyoglobin, can be calculated from the spectra from a reflectance spectrophotometer. The calculations depend on wavelengths known as isobetic points, where the extinction coefficients for two or three of the pigments are the same. Isobetic points are 525 nm for all three pigments and 572 nm for the reduced pigments. The ratio of percentage reflectance at 630 nm to that at 580 nm (R630/R580) has commonly been used to measure the changes in fresh meat color,

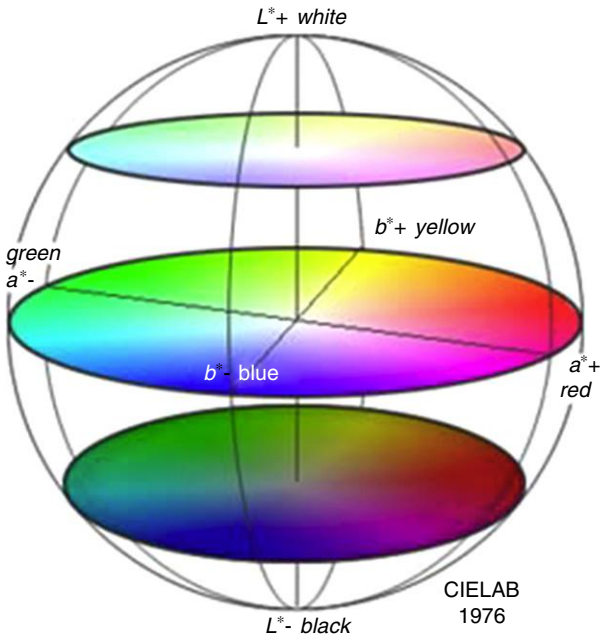

Figure 5 A representation of the CIE $L^*a^*b^*$ color space showing lightness (L^*) values, redness/greenness (a^*) values, and yellowness/blueness (b^*) values (http://www.zevendesign.com/category/glossary/browse-C/).

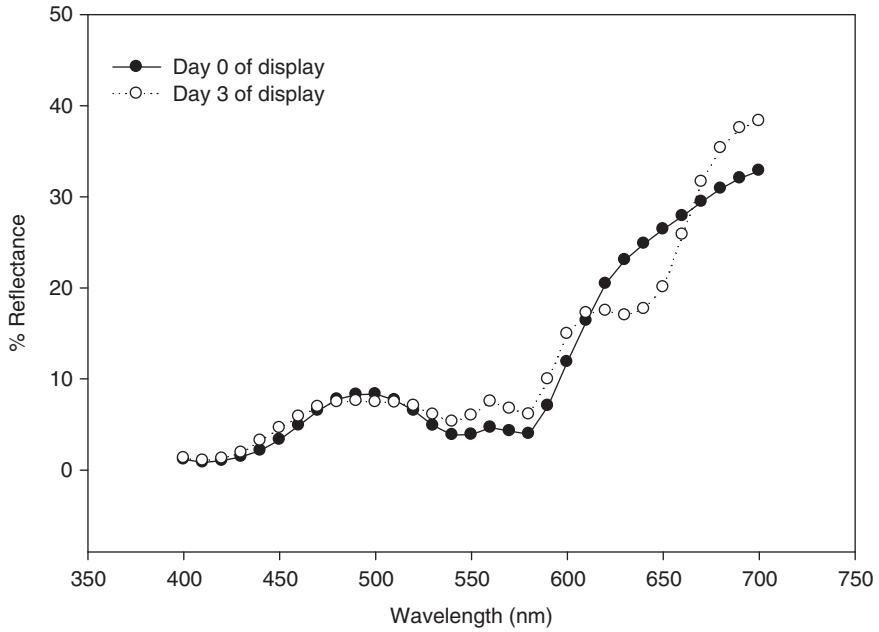

Figure 6 The percentage reflectance over 400–700 nm of lamb loin muscle overwrapped and under simulated retail display for 0 or 3 days. The shift to the brown pigment metmyoglobin is shown particularly around 550–700 nm (reproduced with permission from Robyn Warner, Department of Primary Industries, Victoria, Australia). The ratio of percentage reflectance at 630 nm to the percentage reflectance at 580 nm is recommended to monitor the change in the pigments in the meat surface during retail display.

especially during simulated retail display and shelf life studies. A decrease in ratio of R630/R580 measures a change in the predominant pigment in the meat surface from oxymyoglobin to metmyoglobin and is a measure of the 'browning' occurring in the meat surface. Figure 6 shows the change in the percentage reflectance across the visible spectrum from 400 to 700 nm, for lamb loin muscle which has been displayed for 0 or 3 days.

3. Laboratory and portable machines: The first portable color machines were produced by Minolta, which enabled easy measurement of meat surfaces in boning rooms, chillers, and in the field. They have gained considerable popularity because of their small size, cost, and ease of operation. The portable Hunter Lab color machines are also now popular and are more likely to be used for reflectance measurements in order to monitor changes from oxymyoglobin to metmyoglobin during shelf life studies (see above and Figure 7). Laboratory machines have been used for a number of years to measure spectrophotometry and color space values. The main considerations for choice of a machine are the need for portability, aperture size, illumination/viewing angle, and ease of use. A summary of the recommended specifications for colorimeters to measure the color of meat are presented in Table 1.

Figure 7 A portable Hunter Mini Scan instrument (Model 45/0-L) being used in the field to measure percentage reflectance on an exposed beef loin muscle. The muscle has been exposed to the air for 30 min and is done after rigor is completed. This machine is not used for grading purposes but certainly has potential to be used in this manner. Credit: Athula, N., Department of Primary Industries, VIC, Australia.

Subjective Scoring Methods of Color Measurement

Visual assessment of color is related to the consumer and ultimately any instrumental measurement must be verified against consumer scores of acceptability and unacceptability. To conduct visual assessments using consumers rating as acceptable or unacceptable, at least 10, and ideally more than 10, consumers should be used. Pictorial standards can substantially improve a panel's consistency and validity. Color space parameters have been correlated with many subjective scales and the scales used to grade meat usually refer to the paleness or darkness of meat. Semiobjective pictorial standards have been developed by industry for grading purposes and these are widely used around the world. It is very important to standardize the conditions at the slaughter facility or

processing plant during grading for meat color. Thus, a standard lighting system as well as a standard cut must be used. In addition, where possible, a defined blooming time and minimum time postslaughter should also be specified and standardized. In the case of beef, if carcasses are graded too early, before final rigor is attained (final rigor usually attained >24 h postslaughter), the perceived color can be darker than the actual color, costing the company significant money. This is because carcasses graded as 'too dark' incur a significant economic penalty. Examples of meat color chips are the Australian meat and fat color chips for beef and the Japanese meat color chips for pork. When using these color chips, it is important that measurements are made under exactly the same lighting conditions every time, because the color blocks have spectra in the visible region that look nothing like meat spectra. In addition, the lighting used to view the meat should be in the range of 800–1600 lux at the meat surface to be graded.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment; Water-Holding Capacity. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Rigor Mortis, Cold, and Rigor Shortening. On-Line Measurement of Meat Quality. Tenderness Measurement

Further Reading

Hamm, R., 1960. Biochemistry of meat hydration. Advances in Food Research 10, 355–463.

Honikel, K.O., 2009. Moisture and water-holding capacity. In: Nollet, L.M.L., Toldra, F. (Eds.), Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, pp. 315–334.

Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science 49, 447–457.

Hunt, M.C., Acton, J., Benedict, R.C., *et al*., 1991. Guidelines for meat colour evaluation. Reciprocal Meat Conference Proceedings 44, 1–17.

Judd, D.B., Wyszecki, G., 1975. Colour in Business, Science and Industry. New York: John Wiley & Sons.

Kauffman, R.G., Eikelenboom, G., Van der Wal, P.G., 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Science 18, 307–322.

Kauffman, R.G., Eikelenboom, G., Van der Wal, P.G., Merkus, G., Zaar, M., 1986. The use of filter paper to estimate drip loss of porcine musculature. Meat Science 18, 191–200.

Khliji, S., Van De Ven, R., Lamb, T.A., Lanza, M., Hopkins, D.L., 2010. Relationship between consumer ranking of lamb colour and objective measures of colour. Meat Science 85, 224–229.

Kristensen, L., Purslow, P.P., 2001. The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. Meat Science 58, 17–23.

McGlone, V.A., Devine, C.E., Wells, R.W., 2005. Detection of tenderness, post-rigor age and water status changes in sheep meat using near infrared spectroscopy. Journal of Near Infrared Spectroscopy 13, 277–285.

Nakai, H.F., Saito, T., Ikeda, S.A., Komatsu, A., 1975. Standards models of pork color. Bulletin National Institute of Animal Industry 29, 69–74.

NPPC, 1991. Procedures to Evaluate Market Hogs, third ed. Des Moines, IA: National Pork Producers Council.

Offer, G., 1991. Modelling of the formation of pale, soft, and exudative meat: Effects of chilling regime and rate and extent of glycolysis. Meat Science 30, 157–184.

Perez-Alvarez, J.A., Fernandez-Lopez, J., 2009. Colour measurements on muscle based foods. In: Nollet, L.M.L., Toldra, F. (Eds.), Handbook of Muscle Foods Analysis. Boca Raton, FL: CRC Press, pp. 467–477.

Trout, G., 1988. Techniques for measuring water-binding capacity in muscle foods – A review of methodology. Meat Science 23, 235–252.

Warner, R.D., Kauffman, R.G., Greaser, M.L., 1997. Muscle protein changes post mortem in relation to pork quality traits. Meat Science 45, 339–352.

Warner, R.D., Ponnampalam, E.N., Kearney, G.A., Hopkins, D.L., Jacob, R.H., 2007. Genotype and age at slaughter influence the retail shelf-life of the loin and knuckle from sheep carcasses. Australian Journal of Experimental Agriculture 47, 1190–1200.

Relevant Website

http://www.ausmeat.com.au/custom-content/preview/ham/pdf/chiller.pdf
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MEAT, ANIMAL, POULTRY AND FISH PRODUCTION AND MANAGEMENT

Contents

Antibiotic Growth Promotants

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Glossary

Antibiotic growth promotants Sub-therapeutic levels resulting in improved growth and reproductive performance.
Antibiotic resistance Continual use of antibiotics can involve a resistance and this resistance can be transferred to humans and other animals.
Feed efficiency A measure of an animal's efficiency in converting feed mass into increases of the desired output (meat in the case of meat animals and milk in the case of dairy cows).
Ionophore Ionophore antibiotics exert their antibiotic action by disrupting the transport of ions in the cell membranes; ionophores are absorbed in small amounts and are deposited in various tissues including liver, muscle, fat, and skin.
Sub-therapeutic Levels of antibiotic used that are below the dosage levels used to treat diseases.

Introduction

Antibiotics have now been used in livestock production for more than 60 years. Antibiotic growth promotants (AGPs) have provided a convenient dietary means of efficiently producing pork, beef and poultry meat and other animal products. AGPs are orally active at sub-therapeutic levels, resulting in improved growth and reproductive performance. Antibiotics are also used at moderate levels to prevent disease (prophylactics) and at therapeutic levels to treat a variety of diseases.

The swine and poultry industries have been the principal users of AGP, but they are also used for beef cattle, dairy calves, sheep, and companion animals. The major impediment to the use of AGP is the real and perceived risk of creating antibiotic-resistant microorganisms that can be transferred to humans and other animals. During the last 20 years, the growing consumer demand for food products with credence values such as safety, welfare friendly, healthy, and reduced environmental impact has placed even further pressure on the use of AGPs and the mandatory or voluntary removal of AGPs from a number of countries and market segments. The desire to reduce the use of AGPs in animal industries has resulted in much interest in the development of alternatives, many of these alternatives based on plant extracts, probiotics, blood and yeast based extracts, although none of these can yet provide the full efficacy of AGPs.

The Efficacy of Antibiotic Growth Promotants

The inclusion of growth-promoting antibiotics in pig feed can improve live weight gain by 5–6% and feed conversion efficiency by 3–4% (Figure 1). The most pronounced effects are

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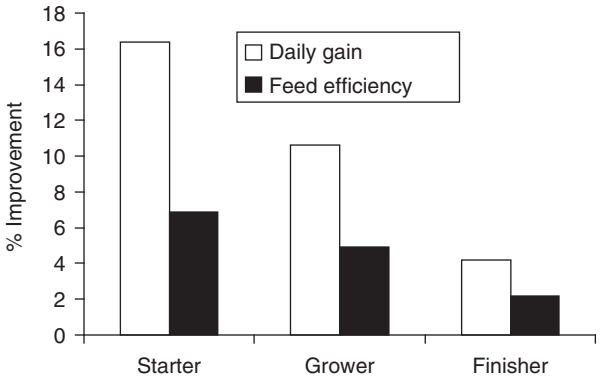

Figure 1 Effect of antimicrobial growth promotants on daily gain and feed efficiency in different classes of pigs. Data are modified from Gaskins, H.R., Collier, C.T., Anderson, D.B., 2002. Antibiotics as growth promotants: Mode of action. Animal Biotechnology 13, 29–42.

found in young pigs, with the response declining in finisher pigs. The efficacy of AGPs in broilers have also been well documented, particularly under adverse conditions such as high stocking rate, insufficient hygiene, and high pressure of infectious diseases.

The Mechanism of Action of Antibiotic Growth Promotants

The exact mechanisms by which AGPs improve growth performance have not been fully elucidated, although several have been proposed. The proposed mechanisms include (1) an inhibition of subclinical infections, (2) a reduction of growth-depressing microbial metabolites, (3) a reduction in competing microbial use of nutrients, (4) an enhanced uptake and use of nutrients through a thinner intestinal wall associated with AGP-fed animals, and (5) reduced protein turnover associated with immune stimulation and intestinal tissue synthesis. The common hypothesis is that intestinal bacteria depress animal growth and this is supported through a lack of effect of AGPs in germfree animals.

Although statistically significant differences in growth rate have never been well correlated with differences in bacterial counts, either in specific intestinal segments or in the gastrointestinal tract as a whole, several investigations have shown that AGPs do produce significant changes in the composition and activity of the gastrointestinal microbiota. The results strongly indicate that AGPs accomplish their effect in the small intestine, whereas they have little or no effect in the large intestine. It is generally accepted that the small intestinal microorganisms compete with the host animal for easily digestible nutrients and at the same time produce toxic compounds. Experiments with slaughter pigs have shown that as much as 6% of the net energy in the pig diet could be lost owing to microbial fermentation in the small intestine. However, the microorganisms in the hindgut are believed to have a beneficial effect on the host animal because they produce energy by fermentation of feed material that has escaped digestion in the small intestine. It has been calculated that on a normal Danish pig diet, 16.4% of the total energy supply for slaughter pigs is achieved by microbial fermentation in the hindgut. Zinc bacitracin, virginiamycin, and salinomycin have all been shown to reduce microbial activity in the small intestine in pigs, whereas virginiamycin, spiramycin, and salinomycin have been shown to reduce microbial fermentation of carbohydrates in the small intestine. Quantification of the results from these experiments indicates that the amount of energy saved for the animal by the reduced microbial fermentation in the small intestine almost equals the improved feed conversion. It is interesting to note that although different types of bacteria may generate one or more of the growth depression effects mentioned above, the Gram-positive bacteria such as streptococci, lactobacilli, and clostridia that predominate in the small intestine often contribute to these effects. As most of the AGPs target the Gram-positive bacteria, these observations are consistent with the involvement of small-intestine Gram-positive bacteria in the growth depression. AGPs exert no benefits on the performance of germfree animals, which also clearly points to their effect being on the microflora rather than being a direct interaction with the physiology of the animal.

Antibiotic Growth Promotants and Their Role in Resistance Development

Since the introduction of antibiotics as growth promoters in animal production in the 1950s, there has been great concern about the development of resistant bacteria. Several studies have shown that the occurrence of resistance is closely related to the use of a drug. This association has also been demonstrated for antibiotics used as growth promoters and it has been shown that food animals may serve as reservoirs of resistant bacteria or resistance genes that may spread to the human population. For these reasons, international public health organizations have recommended termination or a rapid phasing out of the use of AGPs that are also used for human therapy or that may give rise to multiple-resistant bacteria causing infections in humans. Examples include avoparcin and virginiamycin.

Antibiotics Allowed as Antibiotic Growth Promotants

Until 1969 there was almost no restriction in the types of antibiotics used as AGPs. In 1969, the Swann committee in the UK recommended tighter governmental control of the types of antibiotics used as AGPs, and stated that antibiotics used to treat human diseases should not be used in animal feed. This led in 1971 to a ban of tetracyclines and other therapeutic antibiotics as AGPs in many countries. In 1986, Sweden was the first country to ban the use of all AGPs.

Since 1995 major changes in the use of antibiotics as growth promoters have occurred in Denmark. Avoparcin was banned in May 1995, and virginiamycin was banned in January 1998. Furthermore, the food animal industries decided in 1998 to voluntarily stop all use of antibiotics as growth promoters by the end of 1999. The termination of the use of antibiotics as growth promoters in 2003 has led to a

Table 1 Timeline of the European Union (EU) withdrawal of nontherapeutic antibiotics (NTAs) in food animal production timeline

1963–1965	Epidemic of resistant *Salmonella typhimurium* in the UK
1969	Swann Committee in the UK recommends that antimicrobials for animals be divided into two groups: feed additives used without a prescription and therapeutic agents used with a prescription; recommends restricting use of antimicrobial growth promoters
1972–1974	The EU bans the use of tetracycline, penicillin, and streptomycin for growth promotion
1986	Sweden bans the use of antibiotics for growth promotion in agriculture, as requested by Federation of Swedish Farmers Sweden stops the use of all general prophylactic medications
1988	Vancomycin-resistant enterococci (VRE) is reported in food animals in the UK
1993	Denmark restricts direct sale of therapeutic antimicrobials from veterinarians and limits veterinary profits from antimicrobial sales. Denmark bans routine prophylactic use of antimicrobials
1994	Denmark bans the use of avoparcin for all purposes in agriculture. Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) is initiated. Sweden and Finland join the EU and lobby for the EU-wide ban on agricultural growth promoters
1995	Germany bans the use of avoparcin
1996	The EU bans the use of avoparcin. Netherlands bans the use of olaquindox and carbadox
1997	WHO Berlin meeting, 'The medical impact of the use of antibiotics in food animals,' concludes that use of medically important antimicrobials as growth promoters should be stopped
1998	The Copenhagen recommendations: recognition of antimicrobial resistance as a global threat; call for development of new antimicrobials and establishment of a European Surveillance System. Denmark bans use of virginiamycin.
1999	Scientific Steering Committee of the European Commission recommends phasing out antimicrobial growth promoters that are medically important and implementing disease-preventive methods. The EU bans olaquindox and carbadox; suspends authorization of bacitracin, tylosin, spiramycin, and virginiamycin. European Antimicrobial Resistance Surveillance System (EARSS) is established. Sweden bans the use of remaining AGPs: flavophospholipol and avilamycin. The UK's Advisory Committee on the Microbiological Safety of Food issues a report recommending improved veterinary training and surveillance of resistance
2001	European Surveillance of Antimicrobial Consumption (ESAC) launched to collect data on antimicrobial use in ambulatory and hospital care
2006	The EU ban on all AGPs
2008	European Surveillance of Veterinary Antimicrobial Consumption Project (ESVAC): European Commission asks the European Medicines Agency to harmonize surveillance programs collecting data on antimicrobial sales and usage

Source: Reproduced from Cogliani, C., Goossens, H., Greko, C., 2011. Restricting antimicrobial use in food animals: Lessons from Europe. Microbe 6, 274–279.

total reduction of the use of antibiotics in food animals in Denmark by 54%.

Before 1 July 1999, nine antibiotics were permitted as AGPs in the European Union (EU). In 2004, only four antibiotics remain authorized as AGPs in the EU: flavophospholipol, monensin sodium, salinomycin, and avilamycin. The distinction between the AGPs banned, and those still allowed, is based on whether the active component is used in human medicine or has similarities to a medical product used to treat human disease. It is an ongoing discussion whether AGPs really present a threat to human health. However, there is still – in spite of the fact that AGPs have been used for nearly 50 years – no convincing evidence about unfavorable health effects that can be directly linked to the use of sub-therapeutic levels of antibiotics. AGPs are nevertheless now considered politically unacceptable in the EU, and it is the plan that all AGPs were to be phased out in the EU by January 2006. In North America and many other parts of the world, the use of AGPs is still permitted, and they are used frequently. Even antibiotics used to treat infectious diseases in humans are allowed as AGPs in the United States.

Global Anitibiotic Growth Promotant Use Status

Growing concerns about antimicrobial resistance have caused a number of countries including the EU, New Zealand, and South Korea to implement restrictions or bans on the use of AGPs. These bans apply to both domestic production as well as imported products. The first ban on farm use of AGPs was enacted in 1986 in Sweden. Since then Denmark, the UK, and finally the EU banned the use of AGPs in food animal production. It is worth noting that initially, some of these bans were closely monitored from the outset, whereas others were not fully enforced at first (Tables 1 and 2).

Ionophore Antibiotics (Coccidiostats)

Ionophore antibiotics are fermentation products of different *Streptomyces* species and other fungi. They are polyether antibiotics, which exert their antibiotic action by disrupting the transport of ions in the cell membranes. In contrast to AGPs, ionophores are absorbed in small amounts following ingestion and are deposited in various tissues including liver, muscle, fat, and skin. To prevent antibiotic residues in the carcass, ionophore antibiotics are withdrawn from the diets before slaughter.

Ionophore antibiotics are used as coccidiostats to prevent coccidiosis in poultry, a disease caused by protozoa of the genus *Eimera* that may pass from one bird to another through contamination of the litter. The protozoa cause serious damage to the intestines of the animals, thus inhibiting the absorption of nutrients and growth. Infections are often fatal and can spread rapidly. Ionophore anticoccidials are mostly used in broilers, but also to some extent in turkeys and laying hens. To prevent residues in eggs, laying hens may only be fed with ionophore antibiotics during the rearing period.

Table 2 Country policies on AGP use in animal production. A summary of the country policies on AGP use in animal production

Country	*Overview of policies*
Australia	Allows use in feed of some drug classes that are important in human medicine, but is reviewing its policies for approved uses. Establishing a comprehensive surveillance system. Limited information is available on its data collection system
Brazil	Limited information suggests that Brazil does not currently restrict the use of these drugs in feed. Information is not available to determine if Brazil has surveillance and data collection systems in place
Canada	Allows use in feed of some drug classes that are important in human medicine, but is reviewing its policies for approved uses. Establishing a comprehensive surveillance and data collection system
China	Limited information on current activities, as well as information on existing surveillance and data collection systems
European Union (EU)	Prohibits use of antibiotics in feed for growth promotion. Most of the EU members have established surveillance and data collection systems. In 2011, the EU passed a resolution calling on its member states to 'ensure a better control over the implementation of the ban (2006) on antimicrobials being used as growth promoters,' and to 'work toward an international ban on antimicrobials as growth promoters in animal feed,' and to bring this matter up in its bilateral negotiations with other countries such as the United States
Hong Kong	Limited information on current activities, as well as information on existing surveillance and data collection systems
Japan	Some unconfirmed media reports indicate that Japan has increased or is considering increasing restrictions on antimicrobial use in food animal production, whereas other reports indicate it is continuing its review. Has established surveillance and data collection systems
Mexico	Limited information suggests that Mexico does not currently restrict the use of these drugs in feed. Limited information also suggests that Mexico is developing a surveillance and data collection system
New Zealand	Prohibits use of antibiotics in feed for growth promotion. Has established surveillance and data collection systems
South Korea	Effective in 2011, prohibits 'eight antibiotics (enramycin, tyrosine, virginiamycin, bacitracin methylene disalicylate, bambermycin, tiamulin, apramycin, and avilamycin) and one antimicrobial agent (sulfathiazole) in animal feed as feed additives,' effective in 2011. Limited information is available on its surveillance and data collection systems
Thailand	Some unconfirmed reports indicate that Thailand has increased or is considering increasing restrictions on antimicrobial use in food animal production. Information is not available on its surveillance and data collection systems
United States	Allows use in feed of some drug classes that are important in human medicine, but is reviewing its policies for approved uses. Has established surveillance and data collection systems

Source: Reproduced from Johnson, R., 2011. Potential trade implications of restrictions on antimicrobial use in animal production. Congressional Research Service Report 7–5700.

Compared to most chemical coccidiostats, the situation of coccidial resistance against ionophores does not seem to be a cause for alarm. However, following long-term application, a clear reduced efficiency of ionophore anticoccidial drugs has been observed.

Besides their anticoccidial effect, ionophore antibiotics inhibit the growth of Gram-positive bacteria and have been shown to effectively reduce the numbers of *Clostridium perfringens*, thus preventing necrotic enteritis. In this respect, ionophores seem to be able to substitute AGPs. The continued use of ionophores in broiler feed, after the AGP ban in Denmark, is very likely the reason why the removal of AGPs was not being followed by increased problems with necrotic enteritis, for example, increased mortality and liver condemnation at slaughter.

The antibacterial property of ionophores is the reason why some of these substances are also used as AGPs in animal feed. The ionophores salinomycin and monensin are applied as feed additives in the production of pigs and cattle, respectively. However, when used as AGPs, the concentration allowed for the use of salinomycin, for example, in pigs is lower (40 mg kg^{-1} feed), compared to the concentration allowed for use as an anticoccidial in broiler production (60 mg kg^{-1} feed).

Managing without ionophores will not be easy. Apart from their efficiency, the low price of these compounds is an advantage in favor of their continued use. A number of efforts have been made to prevent infection or to moderate intestinal lesions related to coccidioses by dietary means. However, at the present time the only real alternative to anticoccidial drugs in broiler feed seems to be the application of vaccines, which are now commercially available. However, use of vaccines will not solve the problems with necrotic enteritis caused by *Clostridium perfringens*. Despite these problems, the use of ionophores as anticoccidials is being discussed in the EU, and they will probably be phased out as feed additives by January 2012.

Impact on Resistance of Termination of the Use of Antibiotic Growth Promotants

A continuous national monitoring program for antibiotic resistance has been carried out in Denmark since the ban of AGPs in 1999. The data from this monitoring program show that it is possible to reduce the occurrence of antibiotic-resistant bacteria in a national population of food animals when the selective pressure is removed. This observation is supported by investigations carried out in Germany, Holland, and Belgium.

The termination of AGP usage has resulted in a marked reduction in the presence of resistant bacteria in food animals, but resistant bacteria have not completely disappeared. Several investigations have shown that, although the probability of randomly picking up an AGP-resistant enterococcus from an animal or a food product has been reduced, the resistant strains are still present in the farm environment, in the food animals, and even in the animal products. Removal of the selective pressure has thus reduced the population density of resistant bacteria and accordingly the possibility for transfer of resistant bacteria from animals to food, but the seeds of resistance remain in the farm environment for many years after

the termination. A readmittance of antibiotics for growth promotion would mean a quick increase in resistant strains to pretermination levels.

Managing without Antibiotic Growth Promotants

So far, only a few studies have investigated the effect of terminating the use of AGPs on animal health and productivity. To investigate whether and how removal of AGPs affected production results and mortality in Danish broiler flocks, data covering the period before and after the termination of AGPs were extracted from a database administered by the Danish Poultry Council. This database provides information about each broiler flock including parental flock, stocking rate, mortality, and feed consumption. The results of this study showed no evidence of decreased productivity (kg broiler per m^2) or increased mortality following the removal of AGPs. However, a minor increase in feed consumption was reported corresponding to 16 g feed per kg broiler. This decreased feed efficiency is, however, offset completely by saving the cost of AGPs. Necrotic enteritis was at most a minor broiler health problem following the termination of AGPs in Denmark, probably because producers continued to use ionophores for prophylaxis against coccidiosis.

In Danish pig production, there was a significant increase in therapeutic antibiotic treatments for diarrhea in the post-weaning period following the complete elimination of AGPs. The termination of AGPs also resulted in some loss of productivity in weaners. In finishers, however, no effect of the termination of AGPs was observed on productivity or feed efficiency. Similar results for pig production were observed earlier after elimination of the use of AGPs in other countries.

See also: Chemical Analysis: Sampling and Statistical Requirements; Standard Methods. Foodborne Zoonoses. Growth of Meat Animals: Metabolic Modifiers. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Aarestrup, F.M., Bager, F., Andersen, J.S., 2000. The association between the use of avilamycin for growth promotion and the occurrence of resistance among *Enterococcus faecium*. Microbial Drug Resistance 6, 71–76.

Aarestrup, F.M., Seyfarth, A.M., Emborg, A.-D., *et al*., 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicobial Agents and Chemotherapeutics 45, 2054–2059.

Armstrong, D.G., 1986. Gut-active growth promoters. In: Buttery, P.J., Lindsay, D.B., Haynes, N.B. (Eds.), Control and Manipulation of Animal Growth. London: Butterworths, pp. 21–37.

Atlef, M., Ramadan, A., Youseef, S.A.H., Abo El-Sooud, K., 1993. Kinetic disposition, systemic bioavailability and tissue distribution of salinomycin in chickens. Research in Veterinary Science 54, 254–268.

Borgen, K., Sorum, M., Wasteson, Y., Kruse, H., 2001. Van A-type vancomycin-resistant enterococci (VRE) remain prevalent in poultry carcasses 3 years after avoparcin was banned. International Journal of Food Microbiology 64, 89–94.

Callesen, J., 2002. Effects of termination of AGP use on pig welfare and productivity. In: Abstracts of the International Invitational Symposium: Beyond Antibiotic Growth Promoters in Food Animal Production, Foulum, Denmark, pp. 42–45. Copenhagen: Danish Veterinary Institute and Tjele: Danish Institute of Agricultural Science.

Cromwell, G.L., 2002. Why and how antibiotics are used in swine production. Animal Biotechnology 13, 7–27.

Dierick, N.A., Vervaeke, I.J., Decuypere, J.A., Hendericks, H.K., 1986. Influence of the gut flora and some growth promoting feed additives on nitrogen metabolism in pigs. II. Studies *in vivo*. Livestock Production Science 14, 177–193.

Emborg, H., Ersboll, A.K., Heuer, O.E., Wegner, H.C., 2001. The effect of discountinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Preventive Veterinary Medicine 50, 53–70.

Engberg, R.M., Petersen, J.S., 2005. Poultry production with and without questionable feed additives and ingredients. Zootecnica International 5, 38–45.

Jensen, B.B., 1998a. The impact of feed additives on the microbial ecology of the gut in young pigs. Journal of Animal Feed Science 7, 45–64.

Jensen, B.B., 1998b. Effect of diet composition and virginiamycin on microbial activity in the digestive tract of pigs. In: Proceedings of the 4th Symposium on Digestive Physiology in the Pig, pp. 392–400. Jublana, Poland: Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Poland.

Jensen, B.B., Mikkelsen, L.L., Christensen, D.N., 1998. Integration of ileum cannulated pigs and *in vitro* fermentation to quantify the effect of diet composition on microbial fermentation in the large intestine. In: Proceedings of NJF Seminar No. 274 on Energy and Protein Evaluation for Pigs in the Nordic Countries, pp. 106–110. Foulum, Denmark: Nordisk Jordbrugsforskning Nr.

Thonke, S., Elwinger, K., 1998. Growth promotants in feeding pigs and poultry. I. Growth and feed efficiency responses to antibiotic growth promotants. Annales de Zootechnie 47, 85–92.

Wegner, H.C., 2003. Antibiotics in animal feed and their role in resistance development. Current Opinion in Microbiology 6, 439–445.

Wierup, M., 2001. The experience of reducing antibiotics used in animal production in the Nordic countries. International Journal of Antimicrobial Agents 18, 287–290.

Relevant Website

http://www.fas.org/sgp/crs/misc/R41047.pdf
Congressional Research Service Report.

Beta-Agonists

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Introduction

Meat animals are managed to produce safe, nutritious palatable food in various forms ranging from retail cuts of beef, pork, lamb, veal, and poultry to a variety of fresh ground items and further processed products. The latter are produced using one or more of various processing technologies such as curing, smoking, fermenting, blending or chopping, and cooking. The fresh meat provided by each species varies in eating characteristics and composition according to the influences of management factors such as age, gender, genetics (breed or strain), and portion of the animal from which it is derived. Nutrition and other production strategies or practices also influence these characteristics.

Management strategies used in meat animal production are selected to maximize the efficiency of production, yield, and quality of meat with minimal impact on the environment and with accommodation of good health and welfare of the animals. One recently developed management strategy that increases efficiency of growth, improves the amount of meat, and improves carcass composition is the use of a class of feed additives called beta-agonists. These compounds have been intensively studied during the past two decades, and a few specific beta-agonists, but not all, have been approved for use in meat-producing animals in several countries. They are not used in dairy animals because they do not alter milk production, and they are also not to be used in breeding animals. At present, only one product is approved for use in turkeys, but not in chickens.

These compounds act to repartition the use of nutrients consumed by animals toward increased muscle growth and reduced amount of fat gain. These growth-regulating compounds are administered as a feed ingredient because they are orally active. They are usually fed at concentrations of 5 to 30 ppm (ppm) (g per tonne) near the end of the finishing period for 20 (zilpaterol hydrochloride) or up to 42 (ractopamine hydrochloride) days just before the harvest. Their use, therefore, requires careful planning to determine their start.

Currently, only four products are approved by the US Food and Drug Administration (FDA) for their commercial use in the US. Three products contain the beta-agonist ractopamine hydrochloride, which is produced by Elanco Animal Health, Indianapolis, IN, USA. These three products are marketed as Paylean® (approved in December 1999), Optaflexx® (approved in June 2003), and Topmax™ (approved in April 2009) for their use in swine, beef cattle, and turkeys, respectively. Paylean® is also approved for its use in 14 other countries around the world. Another compound, zilpaterol hydrochloride, is manufactured by Merck Animal Health, Whitehouse Station, New Jersey, USA. It is sold commercially as Zilmax® for its use in beef cattle and is fed during the last 20 days, with a 3-day withdrawal required before harvest. It is approved for its use in Canada, Mexico, South Africa, South Korea, and the US. The FDA approved its use in the US in 2006. Approval is being considered by other countries also. Specific guidelines for feeding animals with these commercial beta-agonist products are provided by the manufacturer. None of the beta-agonists are approved for their use in production of meat animals within the European Union.

Nature of Beta-Agonists

Beta-agonists are naturally occurring and synthetic organic compounds that share a common base chemical structure distinctive to a class of compounds called phenethanolamines. The general structure of a phenethanolamine is shown in Figure 1. It consists of a phenyl group ring structure attached to the ethanolamine group. Different substituents attached at the A, B, C, and R positions denote differences among the family of phenylethanolamine compounds called beta-agonists. These substituents influence how long the beta-agonist stays in circulation in the blood, which tissues are influenced, and what specific actions or effects they promote. Two naturally occurring beta-agonists are adrenaline (epinephrine) and noradrenaline (norepinephrine), but neither compound has beneficial effects on animal growth. Adrenaline is synthesized and secreted by the medulla of the adrenal gland, and noradrenaline is released by specific types of nerve endings in the body. These two phenethanolamines, called catecholamines, play important roles in the regulation of heart rate, blood flow, and other physiological and metabolic functions in humans and animals.

Synthetic beta-agonists such as clenbuterol (Figure 2) have been used in human and animal medicine for many decades to relieve respiratory distress symptoms associated with asthma, to treat pregnant women to control premature parturition (uterine smooth muscle relaxant), and as a cardiostimulant to treat cardiac irregularities. These compounds have no antibiotic activity.

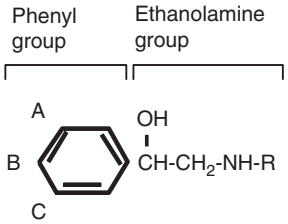

Figure 1 The base chemical structure of phenethanolamines. All contain the phenyl group coupled to the ethanolamine group, but they differ in substituents attached at positions A, B, C, and R.

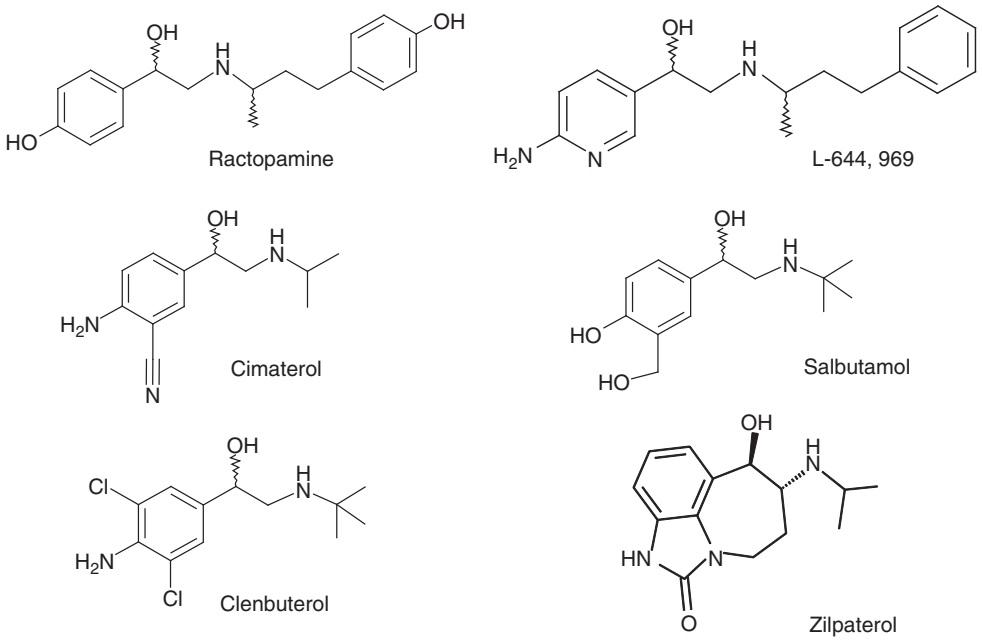

Figure 2 The chemical structures of beta-agonists, also called phenethanolamines, that have been studied in meat animal species. These compounds exert their influence by binding with receptors on the plasma membrane of cells in skeletal muscle, adipose tissue, and other tissues and organs.

During the past three decades, several beta-agonists including ractopamine, cimaterol, clenbuterol, L-644 969, salbutamol, and zilpaterol (Figure 2) were evaluated for their potential muscle growth enhancement and antiobesity effects. All were found to increase muscle growth rate and reduce the amount of fat in laboratory animals, meat animals, broiler chickens, and turkeys. However, these compounds, other than ractopamine and zilpaterol, are not approved for their use in meat animals or poultry and it is illegal to administer them to food-producing animals unless approved for therapeutic use by a veterinarian. For several reasons, the naturally occurring beta-agonists adrenaline and noradrenaline do not have significant effects on efficiency or composition of growth of meat animals or poultry. They tend to be cleared from the blood very rapidly, and certain tissues exhibit diminished responses or lack of response to continuous presence of their elevated concentrations in the blood or to continuous stimulation of cells by them.

Beta-Agonists as Feed Additives

Beta-agonists or phenethanolamines are often referred to as repartitioning agents because they redirect nutrients used for growth toward increased rates of muscle protein synthesis, leading to larger muscles, and away from deposition in adipose tissue (fat). It is more efficient to grow muscle than fat, so feeding meat animals with beta-agonists results, on average, in reduced feed intake at equal or greater rates of body weight gain (improved efficiency). The increased leanness of animals resulting from greater weights of individual muscles and the reduction in the amount of adipose tissue or fat also improves the meat yield of the animals. Experimental research has demonstrated that lambs and cattle fed with cimaterol or clenbuterol contained muscles in the hind leg that were up to 25–30% larger, whereas the amount of fat in the carcass was reduced by 15–25%. These two compounds would more likely reduce the 'value' of the carcasses because of decreased quality grade and greater incidence of 'dark cutters.'

Finishing pigs can be fed Paylean® at 4.9–9 g t^{-1} of complete feed from 68 kg to market weight, and it is required that the feed contains 16% protein. The muscle growth response depends on adequate nutrient availability (primarily amino acids) to produce larger muscles. Inadequate protein nutrition will preclude a positive muscle growth response. There is no withdrawal period required when feeding animals with Paylean®.

Paylean® fed to finishing swine for 30 days at 20 g t^{-1} (20 ppm) has been shown to increase average daily gain by up to 10%, decrease feed intake by 3–5%, increase carcass yield by 1 kg, and improve percentage of muscle in the carcass from 52% to 58%. This equates to an increase of 4.3 kg in the absolute amount of lean or muscle yield. Separable fat was reduced from 27% to 23% of carcass weight, equivalent to 3 kg less fat. These changes enhance the economic value of pork carcasses in addition to the positive impact of improving the efficiency of live animal production. Research results demonstrate that meat quality characteristics (muscle color, pH, degree of marbling, and moisture retention) and sensory traits of cooked pork (flavor, tenderness, and juiciness) were not different from pork derived from pigs not fed Paylean®. Caution should be exercised to strictly adhere to manufacturer's feeding directions to avoid the risk of reduced tenderness that has been observed with higher doses or longer feeding times. Nutrient composition of pork is also unaffected when it is fed Paylean®. Responses to Paylean® were smaller at the approved lower

Figure 3 Fresh pork loins (a) and hams (b) from market hogs fed Paylean® (left) and not fed Paylean® (right). Photographs provided by and reproduced with permission of Elanco Animal Health, Greenfield, IN, USA.

doses of 5 g t^{-1} and 10 g t^{-1} (5 and 10 ppm, respectively). The size of the growth responses varies among the individual beta-agonists studied, the species to which beta-agonists are fed, and the dose at which the beta-agonist is fed. The enhanced lean growth response to feeding with Paylean® appears to be proportional to the genetic potential for lean growth. One study observed a reduction in marbling in Berkshire pigs, which are known to contain a higher propensity for marbling than for most other breeds. Figure 3 illustrates the effects of feeding with Paylean®.

Finishing beef cattle can be fed Optaflexx® at dietary levels of 10–30 ppm for the final 28–42 days of feeding, but the recommended level of feeding is 200 mg per head per day. Studies involving more than 4000 cattle demonstrate 15–25% improvement in average daily gain and no change in feed intake, resulting in significant improvement in feed efficiency. Carcass yield is increased by 2–8 kg, and carcasses contain less fat and more muscle and protein on a relative basis. Optaflexx®-fed cattle contain approximately 10 kg more lean muscle with equal amounts of feed, compared with control animals. Meat quality factors and sensory panel characteristics (taste, tenderness, texture, and juiciness) of beef from cattle fed Optaflexx® are not consistently different from those of control cattle. Some data suggest statistical reduction in tenderness when the highest level of 300 mg per head per day is fed. Nutrient composition of the meat is not different. No withdrawal period is required.

Zilmax® improves growth performance, carcass yield, and the yield of muscle from carcasses when fed to finishing cattle. Studies involving more than 40 000 finishing cattle fed Zilmax® at 7.56 g t^{-1} dry matter (60–90 mg per head per day) for 20 days with a 3-day withdrawal period demonstrated that feed efficiency improved by 3%, carcass weights increased by 11–15 kg, and gross return to the feeder was increased. Zilmax® had no negative effects on meat quality when fed for 20 days, and meat was aged at least 21 days in studies conducted in the US. Because length of feeding may vary and some genotype by beta-agonist interactions may exist, caution must be exercised to avoid increasing the risk of reduced tenderness, particularly when a beta-2 agonist like Zilmax® is fed.

Mode of Action of Beta-Agonists

Beta-agonists that exhibit repartitioning effects act through receptors (beta-adrenergic receptors) on skeletal muscle and adipose cell membranes and generate signals that control metabolic activities in the cells. When ractopamine or other beta-agonists bind to the beta-adrenergic receptors on fat cells, biochemical signals are created throughout a pathway involving activation of several enzymes that lead to reduced rates of fat synthesis and storage (lipogenesis) in the cells, and at the same time stimulate fat mobilization from the cell (lipolysis). These changes result in a slower rate of fat accumulation in animals, and the magnitudes of the changes are influenced by the dose or amount and the length of time for which the beta-agonist is consumed.

Skeletal muscle cells, called muscle fibers, also contain beta-adrenergic receptors on their surface. The interaction of the beta-agonist with the receptor affects similar pathways of signaling events as in fat cells. In muscle, however, these signaling events increase the rate of synthesis of ribonucleic acid (RNA), which leads to increased rates of synthesis of muscle proteins in the cells. The effects on muscle result in an increase in cell size (hypertrophy) without an increase in cell number. The total number of muscle fibers in a muscle is generally set by birth in most domestic animal species. Growth occurs by increase in the length and radial dimensions of the cells. These are the same changes that occur with vigorous weight-lifting or isometric exercise, which lead to muscle enlargement (growth) in humans. When beta-agonists are fed, a shift in metabolic properties does occur in some muscle fibers to increase percentage of fibers with glycolytic metabolism, thereby reducing the percentage exhibiting aerobic metabolism. Ultimately, feeding with beta-agonists results in approximately 20–30% greater rates of muscle cell growth in pigs fed Paylean® for 28–42 days. The changes that occur in muscles of animals fed beta-agonists are not progressive, nor do they continue to occur over longer periods of time. Hence, the recommended time of feeding is near the end of the finishing period. Longer feeding has little or no benefit on growth performance or output and would result in markedly reduced economic benefit.

Because less energy is required to grow muscle than to grow adipose tissue or fat, use of feed for growth in animals fed beta-agonists for short periods (20–42 days) is more efficient overall. The beta-agonists stimulate muscle growth and reduce the rate of nutrient use for adipose tissue growth, resulting in less feed required to produce an animal of the same weight. Ultimately, there is less animal waste produced and less environmental impact when beta-agonists are fed to meat-producing animals.

Safety of Feeding Beta-Agonists to Meat Animals

Stringent safety criteria and extensive testing to address the safety of feed additives and animal health products are used to assure that approval of a product is warranted. The human food safety of beta-agonists approved for use in the US has been determined through a series of evaluations to satisfy the safety criteria. Ractopamine hydrochloride, the active ingredient in Paylean®, Optaflexx®, and Topmax™, has been

extensively tested in laboratory animals and meat animals to establish the safety of the approved use levels and conditions under which they are produced and used. The same testing was conducted in association with approval of Zilmax®. Assessments of chronic toxicity, mutagenicity, lifetime carcinogenicity, and effects on reproduction over two generations of laboratory animals are required by the FDA in the US. Approval of these compounds in other countries is often based on approval by this agency.

Ractopamine and zilpaterol were similarly evaluated for possible effects on animal welfare, and no adverse effects were found. Animal health, behavior, and well-being were extensively evaluated at usage levels at least 10 times higher than approved for use in meat animals. Studies revealed that clenbuterol and cimaterol have much longer half-lives in meat animals. These differences may contribute to problems of significant residues in edible tissues and associated possibilities of toxicity in humans when clenbuterol or cimaterol are fed to meat animals.

The European Union had banned all beta-agonists for use in meat animals in 1989; however, full scientific evaluation of beta-agonists has been conducted and it does not support the ban, of those approved for use elsewhere. Illegal and unsafe use of beta-agonists was a problem in many parts of the world, and this may have contributed to the ban by the European Union. Thorough review and approval of ractopamine use by FDA followed the ban, and safe use of approved commercial products such as Paylean®, Optaflexx®, Topmax™, and Zilmax® is assured when adherence to use guidelines is followed. Beta-agonists are not all alike. Differences in chemical structure and biological activity are significant; they determine where the use of beta-agonists is appropriate and allowed. Some beta-agonists, such as clenbuterol, are approved for use in human medicine but have not been approved for use in meat animals. The unique structure of specific beta-agonists and the way they are metabolized in the body influence safety considerations. Ractopamine and zilpaterol are approved for their use in meat animals. It is safe to consume food from them.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. Growth of Meat Animals: Endocrinology; Growth Patterns. Nutrition of Meat Animals: Pigs; Poultry; Ruminants

Further Reading

Beermann, D.H., 2002. Beta-adrenergic receptor agonist modulation of skeletal muscle growth. Journal of Animal Science 80 (E. Supplement 1), E18–E23.

Gerrard, D.E., Grant, A.L., 2003. Animal Growth and Development. Dubuque, IA: Kendall/Hunt, pp. 117, 196–197, 239.

Hill, R.A., Pell, J.M., Flint, D.J., 2003. Immunological manipulation of growth. In: Scares, C.G. (Ed.), Biology and Growth of Domestic Animals. Ames, IA: Iowa State University Press, pp. 316–341.

Mersmann, H.J., 1991. Regulation of adipose tissue metabolism and accretion in mammals raised for meat production. In: Pearson, A.M., Dutson, T.R. (Eds.), Growth Regulation in Farm Animals. London: Elsevier Applied Science, pp. 135–168.

Scanes, C.G., 2003. Growth performance enhancement. Biology and Growth of Domestic Animals. Ames, IA: Iowa State University Press.

Bovine and Porcine Somatotropin

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Glossary

β-agonist An adrenalin-like compound that binds to and stimulates β-receptors to have similar effects to adrenalin.
Average daily gain (ADG) The amount of live weight that an animal gains each day.
Growth hormone releasing factor (GRF or GHRH) A hormone produced in the hypothalamus that increases the release of somatotropin from the pituitary.
Intramuscular (IM) fat The fat, that is found within muscle, that is often referred to as marbling.
Somatostatin (SS or SRIF) A hormone produced in the hypothalamus that decreases the release of somatotropin from the pituitary.
Somatotropin (ST) A growth hormone (GH) that is slightly different for cattle (e.g., bovine ST) and pigs (e.g., porcine ST). Regulators are GH-releasing factor and Somatostatin or ST release-inhibiting factor.

Introduction

In the 1920s, it was discovered that a crude pituitary extract stimulated growth in rats, and this extract was referred to as growth hormone (GH) or somatotropin (ST) after the Greek derivation meaning tissue growth. Results were extended to farm animals when ST was shown to enhance growth rates in pigs and stimulate milk production in lactating ruminants. For many years, it was not possible to apply this knowledge to practice because of the limited supply of ST. However, this has changed, with the development of recombinant DNA techniques, and porcine ST (pST, pGH) has been approved since 1995 for commercial use as a daily injectable in growing pigs, first in Australia and then in other countries. Recombinant bovine ST (bST, bGH) is, since its commercial introduction in 1990, approved in many countries (including the United States) for stimulating milk production in dairy cows. However, bST is not approved for use as a growth promoter, possibly because of relative differences in the value of the different bovine products (i.e., meat vs. milk) and the potential for crossover of product applications. Use of bST and pST is not allowed in the countries of the European Union.

The main objective of this article is to review the effects of ST, or GH, on performance and meat quality in farm animals, with special emphasis on the use of bST and pST to growing cattle and pigs, respectively.

Somatotropin

ST is a single polypeptide chain consisting of 191 or 190 amino acids, although the amino acid composition varies somewhat between species. The bovine, porcine, and human growth hormones are rather different, whereas the bovine and ovine forms are almost identical. ST is a naturally occurring protein hormone produced by the anterior pituitary gland and secreted into the circulation. ST has several important roles in the regulation of development and growth of skeletal muscle, bone, adipose tissue, and the liver in growing animals and plays an integral role in the coordination of lipid, protein, and mineral metabolism in mammalian species. Elevation of plasma ST redirects nutrients toward increased muscle and bone growth and decreased adipose tissue growth in meat animals. It appears that the ST-induced shift in nutrient partitioning is mediated, in part, through inducing insulin resistance in adipose tissue. Other hormones and peptides regulate the secretion, but the main physiological regulators of ST release are growth hormone-releasing factor (GRF or GHRH) and somatostatin (SS or SRIF (somatotropin release-inhibiting factor)), both produced in the hypothalamus and with quite distinct upstream and downstream points of regulation.

Because ST is a protein, it cannot be administered orally but has to be administered by injection. In most early experiments, ST was administered by daily injection. However, sustained-release vehicles have also been developed for commercial application in dairy cattle as either biweekly or monthly implants (e.g., subcutaneously as oleaginous suspensions). In commercial pig production, pST dissolved in sterile water, is injected daily using a propane-powered applicator. However, daily injection with pST is problematic and so alternative injection regimes with less frequent injections have been investigated and demonstrated to have similar efficacy to daily injections.

Performance, Carcass Quality, and Health

Cattle and Sheep

In the late 1950s, the first published paper documented that the administration of exogenous pituitary-derived bST to growing heifers (female calves) resulted in a 10% increase in average daily gain (ADG). Similarly in the late 1970s, the first report with a positive effect of ovine ST (oST) treatment on growth rate in sheep was published. Following this, responses of the same magnitude have been observed using both pituitary-derived and recombinant ST. The increase in ADG of heifers has been as high as 25%. In males, the growth responses have varied: for example, an 18% increase in bull calves, a zero effect in young bulls. In general, the effects on

ADG are rather small in finishing feedlot steers, which may relate to their higher degree of fatness and the opposing effects of ST on lean and fat deposition. In wether lambs, improvements of ADG of approximately 10–20% are typical, but increases in ADG as high as 36% have been observed.

Feed intake may increase slightly in young cattle (e.g., calves), whereas a slight reduction may be seen in finishing animals (e.g., steers). This difference suggests that the changes in feed intake depend on the balance between the increase in protein accretion and the reduction in fat accretion. Thus, with a minor effect on feed intake and often an increased growth rate, ST treatment generally results in improved feed efficiency, ranging from 2% to 25%.

The effect of ST on absolute carcass weight is dependent on the growth response obtained, the length of the treatment period, and the developmental stage of the animals, because these factors influence the relative changes in deposition of protein, fat, and bone in animals. In general, there is a small positive effect on carcass weight, but increases as large as 8–9% have been observed in heifers. However, reduced carcass weights have been observed in some studies, perhaps where the increase in protein deposition is offset by a decrease in fat deposition.

The increase in protein deposition in ST-treated animals is generally considered to be due to an increase in protein synthesis rather than to a reduction in protein degradation, although the responses may differ between species, physiological age in the same species or even between different tissues within the same animal. Given the high rate of protein turnover in skeletal muscle, only subtle changes in either protein synthesis or degradation are sufficient to account for the differences in protein deposition. Although ST increases protein deposition in most muscles in the body there appears to be some variability in how different muscles respond. Also, the increased protein deposition is not restricted to skeletal muscle as ST increases protein deposition in all tissues including skin and visceral organs. Indeed, the proportional increases in protein deposition in skin and viscera may be greater than that in skeletal muscle contributing to a reduction in dressing rate ($\sim$1% in pigs). Also, ST can increase bone deposition impacting on boneless meat yield percentage. Therefore, the increases in growth rate observed with ST treatment need to be offset against the decreases in dressing and boneless meat yield percentage.

It is a consistent finding that ST administration increases the lean-to-fat ratio of carcasses. This is due to the previously mentioned increased protein deposition and a reduced fat deposition comprising subcutaneous fat, intermuscular, and intramuscular (IM) (i.e., marbling) fat. The longissimus dorsi area is increased in some but not all studies. In spite of the reduced carcass fat content and backfat thickness, carcass conformation is usually not changed by ST treatment of cattle. In sheep, the effects of exogenous ST on carcass weight and carcass composition are similar to those observed in cattle and the maximum observed changes are a 30% reduction in carcass fat accretion and a 36% increase in protein accretion.

The magnitude of growth response and changes in carcass composition following ST treatment is influenced by nutritional factors, such as feeding level and protein and amino acid levels and supply. Interactions between feeding level and ST treatment have been demonstrated and suggest that the response to ST in young growing cattle in many situations may be impaired by limitations in amino acid supply from microbial and ruminal escape protein. Thus, the effect of ST on performance can be enhanced by increasing the level of escape protein (e.g., a fish meal–rich diet); also, the effects of ST and abomasal-infused casein seem to be additive. There are still no definite and complete data available for estimation of energy and protein requirements of ST-treated cattle. However, amino acid availability and amino acid composition may in fact have limited the protein-anabolic response in some of the earlier ST experiments. It is also likely that nutrient density (i.e., protein, energy, and some minerals) of the diet should be increased in order to compensate in feeding situations where ST administration reduces feed intake and to ensure there are sufficient nutrients to support the increased protein and bone deposition.

Other possible sources of variation in the growth responses to ST include dose and pattern of administration. Using the maximum increase in ADG and/or decrease in plasma urea as criteria for optimizing the response, the optimum dose for cattle has been estimated at 41–64 μg per kg bodyweight per day. In sheep, the optimal dose seems to be higher (100–200 μg per kg bodyweight per day). The effect of pattern of administration is less clear, but injections four times a day seem to be more potent than injection once a day or continuous infusion. A possible explanation might be that a regimen of several injections per day resembles the normal episodic secretory pattern more closely than injection once a day or continuous infusion. Furthermore, the most effective dose is lower for daily than for weekly administration. The biological effect of ST does not seem to be attenuated with the length of the treatment period, and the marginal effect of ST does not diminish with time. In fact, the maximum reduction in carcass fat is obtained after a long treatment period (months).

ST seems to act additively with other growth promoters, such as β-agonists and sex steroids. This has been observed in experiments in which ST has been given together with zeranol (a steroid-like compound with female sex hormone activity) or implants of estradiol (female sex steroid) or estradiol, progesterone, and trenbolone (with male sex hormone activity) combined. Similarly, the effects of ST and a β-agonist (clenbuterol) seem to be additive.

Pigs

Exogenous pST treatment consistently improves ADG, feed conversion efficiency and protein deposition, and reduces fat deposition in pigs. Dose-dependent increases in lean deposition and reductions in feed intake, fat deposition, and carcass fat have been observed (Figure 1). Occasionally, no growth responses are observed because the reduction in fat deposition may offset the increase in protein deposition.

Despite the accelerated protein deposition, feed intake usually always decreases in ST-treated pigs under *ad libitum* conditions, with a 5–15% reduction being common. The increased ADG and the simultaneous reduction in feed intake are virtually always associated with a substantial improved feed efficiency, which can amount to 40%.

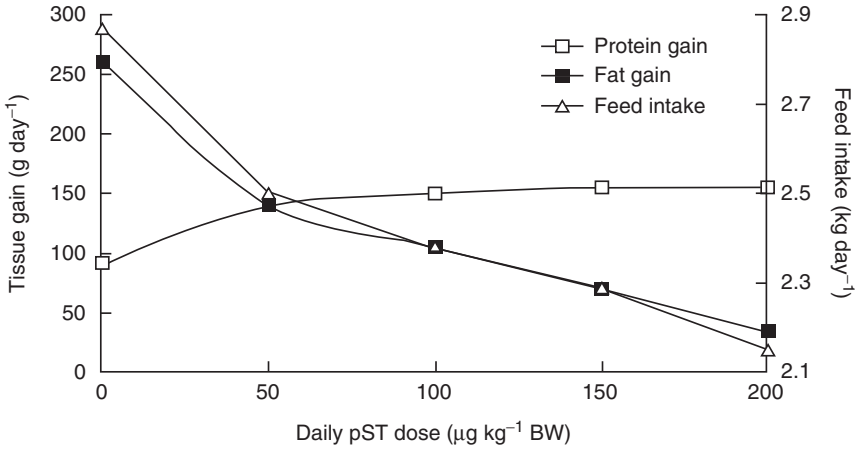

Figure 1 Relationship between porcine somatotropin (pST) dose and parameters of growth performance. Reproduced from Krick, B.J., Roneker, K.R., Boyd, R.D., *et al.*, 1992. Influence of genotype and sex on the response of growing pigs to recombinant porcine somatotropin. Journal of Animal Science 70, 3024–3034.

Table 1 Effects of growth hormone on performance in cattle and pigs. All means expressed relative to the control mean as 100

	Cattle	*Pigs*
Feed intake	90–110	70–96
Daily gain	93–127	95–130
Gain/feed	103–125	115–150
Dressing percentage	95–103	96–99
Carcass lean	100–102	110–150
Carcass fat	80–90	26–85

The reason for the reduction in feed intake is related to the mechanism of action of ST on adipose tissue metabolism. The ST-induced shift in nutrient partitioning is mediated, in part, through inducing insulin resistance in adipose tissue. This insulin resistance results in a reduced uptake of glucose in adipose tissue and a reduction in fat synthesis as well as accumulation of glucose in the circulation. Consequently, the ST-treated pig will reduce feed intake and fat deposition. All animals treated with ST contain less carcass fat with reductions in subcutaneous fat of up to 70% having been observed. Reductions in IM, belly, and leaf fat are also generally observed during pST treatment.

In contrast to adipose tissue, the amount of muscle tissue is generally increased in carcasses from ST-treated pigs. The magnitude of this increase is typically 5–20%. Also, animals that do not show an overall increase in growth show increased amounts of carcass lean. As in ruminants, protein-rich tissues other than muscles, such as visceral tissue, also increase after pST treatment. Thus, ST treatment generally causes a slight decrease in dressing percentage (1–2 units) (Table 1).

The large variation in response to ST treatment can, as in ruminants, be explained by (1) ST dose and mode of administration, (2) diet composition, (3) the animal's potential for tissue accretion, (4) the developmental stage, and (5) the breed or genotype of pigs. Concerning dose, carcass protein accretion increases with up to 150 μg pST per kg bodyweight per day, whereas lipid accretion continues to decrease up to a dose of 200 μg pST per kg bodyweight per day. It has been calculated that 94% and 84% of the maximum theoretical responses in ADG and feed efficiency, respectively, are met at 100 μg pST per kg bodyweight per day.

Owing to the increase in protein accretion, dietary levels of essential amino acids have to be elevated in order to maximize the ST response in pigs. For this reason, the full potential of ST was not revealed in some of the earlier studies in which relatively low levels of lysine (0.7–0.8%) were applied. Most likely the lysine requirement with ST treatment is 1.0–1.2%, which is 20–30% above the requirement for contemporary pigs.

Variation in growth response to ST treatment can also be attributed to the pig's potential for growth. For example, it has been shown that differences in protein accretion rate between gilts, barrows, and boars largely are eliminated by ST treatment. Thus, the magnitude of response was highest for animals with the lowest growth potential (i.e., gilts and barrows), and lowest for animals with the highest growth potential (i.e., boars). In line with this, the effect of ST on lean tissue gain seems to be inversely related to the animal's inherent potential for lean tissue growth. Furthermore, it has been reported that pigs with relatively low capacity for lean growth require a higher dose of ST than pigs with relatively high capacity. However, factors other than lean growth potential are probably important for the response to ST. One such factor might be muscle fiber size and, consequently, the number of fibers, because the relationship between muscle fiber size and response to ST suggests that pigs with relatively large muscle fibers will show smaller improvements in lean deposition rates than pigs with relatively small muscle fibers.

Health Aspects

In the large majority of studies concerning the effects of ST on performance, there have been no reports of adverse health or welfare aspects. However, only a few studies have specifically examined health aspects. Thus, it has been reported that ST treatment may increase the incidence and severity of osteochondrosis and cartilage unsoundness in pigs although this may be because insufficient dietary calcium and phosphorous were provided to support the increase in bone deposition.

Stomach ulcers may also be a problem in ST-treated pigs but this only appears to occur when very high doses of ST are used resulting in a large reduction in feed intake or where finely milled maize-based diets are used

In one dose–response study in steers, a higher incidence of abomasal ulcers and liver abscesses was observed in the groups receiving very high ST doses compared with the control group or a group receiving a low dose. In a second study, where five low-to-moderate doses were applied, there were no systematic differences in incidences of abomasal ulcers and liver abscesses in steers. These observations suggest that very high doses of STH probably may cause health problems in cattle.

Muscle Characteristics and Meat Quality

Because of the general improvement of growth performance after ST treatment, it is most relevant to examine the response in muscle and meat quality characteristics. The following discussion focuses on muscle fiber types, muscle metabolic potential, and physicochemical and sensory aspects of meat from ST-treated animals. The effects on chemical composition (e.g., fatty acid composition) of edible tissue will also be discussed.

Muscle Fiber Type Properties and Metabolic Potentials

Treatment with ST does not seem to affect the number of muscle fibers in a given muscle. Consequently, the increase in carcass meat content after ST treatment of pigs and cattle is reflected in increased muscle fiber hypertrophy. Studies in pigs have shown that the response to ST is dependent on the anatomical location, with muscles located in the hindquarter region and the loin responding most to ST.

The majority of studies have also reported no effect of ST on fiber type distribution. The major effect of ST administration is on increasing muscle fiber size which can result in increased shear force, an objective measure of tenderness. Also, the use of pST has also been reported to reduce calcium-activated proteolysis in the Longissimus muscle, thereby preventing improvements in tenderness during the aging process. In both heifers and pigs, an increased number of capillaries per fiber has been observed after ST treatment. These changes are probably not a direct consequence of ST treatment per se, but are probably secondary to the muscle fiber hypertrophy. Only small changes in the activities of citrate synthetase, hydroxyacyl-CoA dehydrogenase, and lactate dehydrogenase were found. These findings indicate that muscle function is not compromised by ST treatment and the hypertrophy only indicates a higher degree of muscle maturity.

Physicochemical Characteristics

The postmortem rate (pH measured in 45 min postmortem, pH_{45}) and extent (ultimate pH measured either 24 or 48 h postmortem, pH_u) of muscle pH changes affect several meat quality aspects, for example, water-holding capacity, color, tenderness, and juiciness. However, results for pH_u and pH_{45} are not consistent. Thus, the pH_u of the longissimus muscle was unaltered after ST treatment in some studies in pigs and cattle but not in others. A meta-analysis has shown that while there was a significant difference in pH_u in pST-treated pigs, the magnitude of the difference was negligible (5.63 vs. 5.64). The pH_{45} has been reported either to decrease or to be unaltered.

Meat color is also largely unchanged after ST treatment of pigs, heifers, and lambs of different breeds. A meta-analysis has shown that there was no difference in the L*(reflectance) value, but there were significant, but small, differences in a* ($+2.1\%$) and b*(-5.7%), indicating slightly redder and less yellow pork from pigs treated with pST. In agreement with relatively unchanged color, the heme pigment content of the longissimus muscle was unchanged after ST treatment in heifers and pigs.

Drip loss and cooking loss influence meat yield and the juiciness of meat, respectively. A meta-analysis has shown that there was a small, but significant, decrease in drip loss (4.8 vs. 4.5%) in pork from pigs treated with pST. Cooking loss seems to be unaffected by ST treatment in both pigs and cattle. Therefore, treatment with ST has little effect on these important quality characteristics.

Subjective fat marbling scores and chemically extractable IM fat in the loin are consistently reduced by ST. Although a reduction in IM fat would likely reduce the intake of saturated fats in the consumed portion of meat, low levels of IM fat can impact negatively on sensory perceptions of meat quality. The poly- and monounsaturated fatty acid composition of both subcutaneous and IM fat are significantly increased by pST treatment. The reduction in IM fat coupled with the favorable fatty acid profile in pork from pigs treated with pST should result in a more healthy product, although this may be offset against reduced eating quality. Furthermore, the dramatic reduction in *de novo* fatty acid synthesis means that proportionately more of the fatty acids incorporated into newly formed lipids would be of dietary origin and this offers the opportunity to further manipulate fatty acid composition through dietary intervention.

In general, it appears that pST causes a small increase in shear force ($\sim +9\%$) and sensory perceptions of toughness in pork but it is unsure whether this would be consistently detected by consumers as there is no difference in perceptions of flavor or juiciness. There are far fewer studies in ruminants but those that have been conducted suggest similar findings as for pigs. Most of the observed increase in shear force of pork chops with high doses of pST seems to be accounted for by the first yield of the force–distance curve, which indicates a change in tenderization of the myofibrillar proteins. In agreement with this, the ratio between noncollagen protein and collagen deposition rates was 49% greater in the carcasses of ST-treated pigs. In lambs, the ratio of collagen to noncollagen synthesis seems to be unchanged, and in young cattle, collagen content and solubility were found to be unchanged after treatment with ST.

Sensory Aspects of Meat from ST-Treated Animals

The limited data on consumer preferences would suggest that there is a decrease in tenderness ($\sim -9\%$), although there is no effect on juiciness or flavor. Importantly, there may be some interactions between muscle types, processing, gender, and genotype. For example, chops from pST-treated pigs

received significantly lower scores for initial tenderness, initial juiciness, sustained juiciness, and flavor than chops from control counterparts. However, there was no effect of pST on the sensory characteristics of the cured and processed semimembranosus obtained from these same pigs.

Final Remarks

GH clearly has potential as a growth promoter in pigs and ruminants. The general effects of ST are increased or unchanged growth rate, improved feed efficiency, and increased protein deposition concurrent with reduced rate of fat deposition. The net result is increased lean-to-fat ratio of the carcass. The response is known to depend on many factors including the dose of ST, the pattern and mode of administration, the developmental state of the animal, and nutritional factors, such as feeding level and amino acid supply. There are still many questions that remain unanswered concerning optimal doses, diet composition, and animal nutrient requirements.

Existing evidence indicates that muscle function is not compromised by administration of ST. The effects on meat quality are negative but generally marginal. There may be a slight decrease in meat tenderness, but most sensory panels do not detect this effect. Furthermore, it is possible that postmortem handling of the meat has been suboptimal in these experiments owing to the lean carcasses of ST-treated pigs, which may require less severe chilling.

See also: Chemical Analysis for Specific Components: Veterinary Drug Residue Analysis. Chemical and Physical Characteristics of Meat: pH Measurement. Growth of Meat Animals: Adipose Tissue Development; Endocrinology. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists. Nutrition of Meat Animals: Pigs; Ruminants. Residues in Meat and Meat Products: Residues Associated with Meat Production

Further Reading

Allen, P., Enright, W.J., 1989. Effects of administration of somatotropin on meat quality in ruminants: A review. In: Sejrsen, K., Vestergaard, M., Niemann-Sørensen, A. (Eds.), Use of Somatotropin in Livestock Production. London: Elsevier Applied Science, pp. 201–209.

Bonneau, M., 1991. The effects of exogenous growth hormone-releasing factor or somatotropin administration on growth performance, carcass characteristics, meat quality and reproductive function in poultry and pigs. In: van der Wal, P., Weber, G.M., van der Wilt, F.J. (Eds.), Biotechnology for Control of Growth and Product Quality in Meat Production: Implications and Acceptability. Den Haag: Pudoc Wageningen, pp. 67–89.

Burton, J.L., McBride, B.W., Block, E., Glimm, D.R., Kennely, J.J., 1994. A review of bovine growth hormone. Canadian Journal of Animal Science 74, 167–201.

Collier, R.J., Vicini, J.L., Knight, C.D., McLaughlin, C.L., Baile, C.A., 1992. Impact of somatotropins on nutrient requirements in domestic animals. Journal of Nutrition 122, 855–860.

Dunshea, F.R., D'Souza, D.N., Pethick, D.W., Harper, G.S., Warner, R.D., 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Science 71, 8–38.

Etherton, T.D., Bauman, D.E., 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiological Reviews 78, 745–761.

Foster, T.P., 1999. Somatotropin delivery to farmed animals. Advanced Drug Delivery Reviews 38, 151–165.

Krick, B.J., Roneker, K.R., Boyd, R.D., *et al*., 1992. Influence of genotype and sex on the response of growing pigs to recombinant porcine somatotropin. Journal of Animal Science 70, 3024–3034.

National Research Council, 1994. Metabolic Modifiers: Effects on the Nutrient Requirements of Food-Producing Animals. Washington, DC: National Academy Press.

Prusa, K.J., 1989. Nutritional and sensory characteristics of pork from pigs administered somatotropin. In: van der Wal, P., Nieuwhof, G.J., Politiek, R.D. (Eds.), Biotechnology for Control of Growth and Product Quality in Swine. Implications and Acceptability. Den Haag: Pudoc Wageningen, pp. 183–189.

Disease Control and Specific Pathogen Free Pig Production

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Infectious and Noninfectious Diseases

Diseases of pigs may have infectious or noninfectious etiologies. Infectious diseases are caused by pathogens, such as viruses, bacteria, or parasites. Noninfectious diseases may be hereditary, or of metabolic, nutritional or injury origin.

In intensive production systems, where large groups of relatively young animals are kept in confinement with limited airspace and in close contact, infectious diseases tend to predominate. Under such conditions infections may spread easily through direct contact, short-distance airborne transmission, or fecal uptake. This means that diseases may from time to time become a problem for animal welfare and production economy. Therefore, a number of strategies have been developed to eliminate or control the risk of infectious disease development.

The immune response of the animals is the major defense mechanism against infection. When the balance between immunity and infection is disturbed disease may develop. The severity of disease outbreaks may also be influenced by the environment, management procedures, hygiene level, and the nutritional status of animals.

Methods for Reduction of Infectious Diseases

Infectious disease limiting systems may be categorized into two main types:

1. Disease control systems that rely on a balance between infection, immunity, and management resulting in the absence of clinical disease.
2. Disease eradication systems that rely on the absence of specified infectious agents.

The first category includes changes in management, such as improved hygiene, immunization programs, and treatment with antibiotics. Hygiene measures and immunization are relevant for all types of infection, whether it is viral, bacterial, or parasitic in nature, whereas treatment with antibiotics is directed only against bacterial infections.

The second category includes specific pathogen free (SPF) production, national eradication campaigns, and programs for elimination of infections from herds.

Independent of disease control strategy a strict biosecurity program should be established in order to keep new infections out of herds. This is important for infections, which are endemic to a given region, and for transboundary infections that may spread from other parts of the world.

Infectious Disease Control

Infectious disease control is defined as an effort to live with the pathogens in a balance with immunity and management. This means that the infection may still be present in a subclinical form, but a mixture of immunity, antibiotic treatments, and low infection pressure will keep it under control.

Vaccination Programs

Vaccination is a commonly used method for the control of infections. Most often vaccination will protect against clinical disease, but not prevent animals from becoming infected carriers. In pig production there are three main vaccination strategies:

1. protection of piglets by immunization of sows and transfer of passive immunity via colostrum,
2. vaccination of individual animals in order to induce immunity to subsequent infection, and
3. vaccination of breeding animals in order to induce immunity to infections, which may impair the reproductive performance.

Typical vaccines in group 1 include *Escherichia coli* neonatal diarrhea, erysipelas, necrotizing clostridial enteritis, porcine reproductive and respiratory syndrome (PRRS), and postweaning multisystemic wasting syndrome (PMWS). Typical group 2 vaccines include pleuropneumonia, *Lawsonia intracellularis*, *Haemophilus parasuis*, PMWS, and PRRS. Typical group 3 vaccines include Porcine Parvovirus, PRRS, and PMWS.

Antibiotic Treatment Programs

Treatment with antibiotics is another important method for control of infections in pig herds.

For many years antibiotic growth promoters, in so-called subtherapeutic doses, were included in feed in order to enhance growth and control subclinical disease. The European Union banned this use in the year 2006, due to risk of resistance development and transfer of bacterial resistance to human bacterial pathogens. Antibiotic growth promoters are still used in other parts of the world, although the use is controversial.

Therapeutic treatment programs in pig herds must be based on a precise diagnosis, including herd history, clinical signs, and laboratory findings. Depending on the prevalence of disease and the risk of spread, it may be decided to use batch medication or individual animal treatment. It is important to follow approved guidelines for doses and treatment periods in order to reduce development of resistance. Certain antibiotics that are critical for human treatment should be avoided in pig production. These antibiotics include Quinolones and Cephalosporins. Development of resistance against these first-line antibiotics in zoonotic bacteria, such as *salmonella* and *campylobacter*, may cause life-threatening treatment failure in humans.

The main routes of administration of antibiotic compounds to pigs are medicated water or feed and individual

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animal injections. An advantage of injections is that the individual animal dose is well defined. The disadvantage of this administration is the amount of work involved, and the diagnostic difficulties in identification of animals suffering from subclinical infections.

Water and feed medication programs will result in a much more variable dosing of the individual pigs due to variation in disease severity, mobility, and social rank. Unfortunately, the most severely affected animals tend to stop eating and eventually also drinking, which means that the diseased animals will receive lower doses than the healthy ones.

Infectious Disease Elimination

National Eradication Programs

Several important transboundary pig diseases have been eradicated nationwide, especially in Europe and the United States. Such infections include classical and African swine fever, foot-and-mouth disease, and Aujeszky's disease. More recently Enzootic pneumonia caused by *Mycoplasma hyopneumoniae* has been eradicated from countries like Switzerland, Finland, and Norway. So far, the important viral infection PRRS has never been eradicated when established in a country. However, Sweden succeeded in stamping out the first outbreaks, and thereby prevented further spread of the disease in the country.

Successful national programs require that spread and transmission of infections is arrested and that diagnostic procedures for correct distinction between infected and non-infected herds are available. Strategies for national eradication include 'stamping out' where all animals in a positive herds are culled, or 'test and slaughter' where single animals are tested and culled. Stamping out has been used in Europe for infections such as foot-and-mouth disease and classical swine fever. Test and slaughter has been used for eradication of Aujeszky's disease. Eradication programs with culling and slaughtering of large numbers of animals will probably not be acceptable for the public in the future. Therefore, more focus is put on vaccination strategies where vaccination zones are used to create barriers for spread of infection. For this purpose so-called DIVA (differentiating infected from vaccinated animals) vaccines are useful in eradication programs because they allow destruction of infected animals while animals protected by vaccines may be slaughtered and consumed.

Eradication Programs at the Herd Level

Individual herd owners may decide to eradicate infections due to economic losses or welfare problems. In herds selling breeding animals, a high health status is particularly relevant. It is important to consider the risk of reintroduction before investing time and money in a herdwise eradication program. Herds located downwind within a distance of 2–3 km should be considered a risk factor for windborne infections such as *M. hyopneumoniae* and PRRS.

Eradication with Total Depopulation

Eradication based on total depopulation may be achieved by removal of all animals followed by cleaning, disinfection, and an empty period of 2–3 weeks. After this down period new animals with a well-defined SPF status may be inserted. By this method it is possible to obtain freedom from several infections in a single process.

Eradication with Remaining Breeding Stock

More recently, protocols for eradication of infections where the breeding animals remain at the farm during the program have been developed. The advantage of such programs is that the period with economic losses due to reduced production is shorter.

Eradication of *M. hyopneumoniae* has been a model for eradication programs where the breeding animals are retained on the farm. Eradication of this infection is facilitated by a strong immunity that develops in convalescent animals which in practice means that they clear the infection. The initial programs were developed in small herds in Switzerland in the 1970s. In Denmark the same principles have been used in herds with 1000 sows or more.

The original principles for herd eradication consisted of:

1. stabilization of herd by vaccination or development of natural immunity,
2. removal of all animals younger than 10 months,
3. two weeks farrowing stop,
4. cleaning and disinfection of infected premises, and
5. medication of remaining animals >10 months.

During the years these principles have been simplified, and it has been shown that eradication may be successful even without removal of piglets from the farrowing unit.

At present eradication herdwise programs with success rates between 80% and 100% exist for *M. hyopneumoniae*, PRRS, *Brachyspira hyodysenteriae*, and Mange (*Sarcoptes scabei*) in Denmark. Programs with higher risk of failure have been developed for *Actinobacillus pleuropneumoniae*, toxigenic *Pasteurella multocida*, and *Lawsonia intracellularis*.

Specific Pathogen Free Production

The term SPF is an abbreviation for 'specific pathogen free.' 'Specific' means that certain well-defined pathogens among numerous more or less well-defined causes of disease are included. 'Pathogen free' means that herds are free, not only from clinical disease or subclinical infection, but also from the infectious pathogen as such. The number of pathogens that a given SPF herd is free from may differ according to the ambitions of the farmer. SPF production may be used for all animal species, but it has been developed for pig production in particular.

The early SPF techniques were inspired from research on cesarean sections and germ-free rearing of pigs. This research showed that freedom from pathogens in pig populations could be maintained after establishment of a clean source.

The first commercial farms were established in the 1950s in the United States and since then several systems have been developed in North America and Europe. The Danish SPF program was established in 1968 and is, by far, the largest

system with 250 breeding and multiplying herds and 3500 herds producing pigs for slaughter. The impact of SPF production on Danish pig production and export is huge because more than 75% of the sows delivering slaughter pigs and more than 90% of the breeding and multiplying animals have SPF status.

The advantage of SPF production is that animals with a guaranteed freedom from specific infections may be obtained. Freedom from infection and subsequent disease leads to increased growth rate and feed conversion ratio and reduced mortality. This leads to improved economic results and to a more stable production. Improved animal welfare and reduced antibiotic consumption are additional benefits from improved health status.

SPF production is based on the following principles:

1. Herds are established after careful cleaning and disinfection.
2. A strict biosecurity program preventing reintroduction is established.
3. Transport of animals between herds is carried out in special trucks with filtered air-inlets.
4. Openness about information on reintroductions of infections.
5. Monitoring by farmers, vets, and by laboratory testing.
6. Quarantine of visitors and vets coming from herds with lower health status.

Specific Pathogen Free Diseases

It is expensive to monitor, test, and declare freedom of pathogens. Therefore, it must be carefully considered which pathogens are relevant to include in the SPF system. Only infections with considerable economic impact and a well-defined and preferably cheap diagnosis should be included. It is also important that the infections are predominately transmitted by pigs. Infections transmitted by humans, rodents, feed, or bedding should be avoided. In Table 1 the infections included in the Danish SPF system are given.

A 'perfect' disease in an SPF program has a well-defined causal pathogen that may be laboratory confirmed by low-cost diagnostic tests with a high sensitivity and specificity. Such tests are most often serological testing of blood samples or automated polymerase chain reaction (PCR) tests.

Biosecurity and Introduction of Infections

Strict rules on biosecurity are cornerstones in SPF production, and have been advantageous for pig production in general. Biosecurity rules of the Danish SPF system are presented in Table 2.

Although biosecurity rules are enforced, approximately 20% of Danish SPF herds will experience introductions of unwanted infections each year (Figure 1). The highest number of introductions is experienced for *M. hyopneumoniae* and PRRS. It is well known that these infections are spreading by wind over distances as far as 2–3 km, and it is, therefore, assumed that the main route of infection is airborne from infected herds in the neighborhood.

Table 1 Major infections in pig herds that may be controlled by specific pathogen free production

- *Mycoplasma hyopneumoniae*
- *Actinobacillus pleuropneumoniae*
- Porcine reproductive and respiratory syndrome virus
- Toxigenic *Pasteurella multocida*
- *Brachyspira hyodysenteriae*
- *Haematopinus suis*
- *Sarcoptes scabiei*

Table 2 Biosecurity specifications and procedures in the Danish specific pathogen free (SPF) system

Entry of animals
Semen from controlled boar studs
Pigs from herds with equal or higher SPF status
Piglets from non-SPF herds after C-section
An 8-week quarantine period for pigs before entry in breeding herds
Only neutered cats from urban communities
Pest controlled properly
Bird entry avoided
Entry of humans
Twelve hours quarantine after contact with pigs of lower health status
No quarantine after contact with pigs of equal or higher SPF status
Change of footwear
Change of cloth to the level of underwear
Washing of hands
Minimum distance to neighboring herds
100 m for production herds
500 m for breeding and multiplying herds
Perimeter of production facilities
Well-defined borders of the production area
Entrance must be marked with SPF status
All external doors must be locked up
Special compartments for pigs leaving the farm
No direct contact between trucks and the production facilities
Safe procedures for entry of feed and bedding
Transport of pigs between herds
Only approved transporters with especially designed trucks
Air inlet filters to avoid infection by air under transport
Health monitoring in SPF herds
Clinical inspection
Fifteen weeks intervals in production herds
Monthly intervals in breeding herds
Testing for Ap, Myc, and PRRS in blood samples
Yearly in production herds
Monthly in breeding herds
Clinical suspicion of infection
Six weeks investing period
Openness to the public
All information on SPF status may be accessed on www.spf-sus.dk

Actinobacillus pleuropneumoniae is also among the more prevalent causes of infections in the Danish SPF program. Although this infection may also spread by airborne transmission over short distances, it is believed that this is rarely the case. The monitoring of this infection by blood testing is complex because more than 12 serotypes exit, which may partly contribute to the spread of this infection.

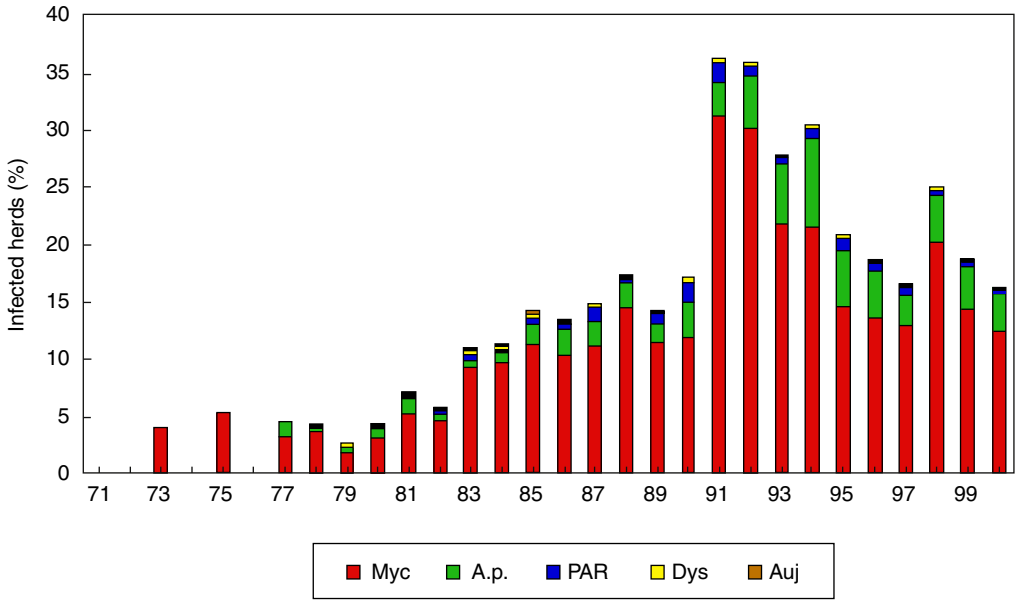

Figure 1 Infection episodes in Danish SPF system, including approximately 3500 production and breeding herds in the period 1985–2000.

Infections with *B. hyodysenteriae*, *Sarcoptes scabei*, and toxigenic *P. multocida* are very infrequent in the Danish SPF system.

Conclusions

Infectious disease reduction in pig herds may be based on a control strategy (living with the infection) or an eradication strategy (SPF). The strategy must be chosen by individual farmers, regions, or nations based on the pig density in areas, the structure of the pig production, and the cost and consequences of disease.

The advantage of the control strategy is that a balance between infection, immunity, and management may be obtained leaving the herd well protected against new infections. The disadvantages are that this strategy may require permanent costs for vaccination, treatment, and eventually disease outbreaks when the balance is disturbed.

The advantage of the eradication strategy is that once the infection is totally eliminated, there will be no more cost or losses as a result of the corresponding disease. This is a very rewarding situation. Disadvantages include that monitoring efforts may be costly and that such herds always are at risk of introduction of the infections they are free from, in particular those that may be transmitted through air.

See also: Foodborne Zoonoses. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Beta-Agonists; Bovine and Porcine Somatotropin; Meat Production in Organic Farming. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms; Potential Environmental and Wildlife Sources of Microorganisms in Meat. Parasites Present in Meat and Viscera of Land Farmed Animals. Preslaughter Handling: Welfare Including Housing Conditions; Welfare of Animals. Quality Management: Farm Level: Pork Quality. Risk Analysis and Quantitative Risk Management

Further Reading

Harris, D.K., Alexander, T.J.L., 1999. Methods of disease control. In: Straw, B.E., D'Allaire, S., Mengeling, W., Taylor, D.J. (Eds.), Diseases of Swine, eight ed. Oxford: Blackwell Science, pp. 1077–1110.

Jorsal, S.E., Thomsen, B.L., 1988. A Cox regression analysis of risk factors related to *Mycoplasma suipneumoniae* reinfection in Danish SPF herds. Acta Veterinaria Scandinavica Supplementum 84, 436–438.

Keller, H., 1973. 10 Jahre Herdensanierung mit Specifisch-Pathogenfreien (SPF) Schweinen. Erfahrungen aus dem Zentrum Zürich. Venia legendi veterinariae Thesis, University of Zürich.

Kristensen, C.S., Bøtner, A., Takai, H., Nielsen, J.P., Jorsal, S.E., 2004. Experimental airborne transmission of PRRS virus. Veterinary Microbiology 99, 197–202.

Masserey-Wullschleger, Y., Maurer, T., 1998. Die Flächendeckenden Sanierungen von Schweinezucht- und Mastbetrieben in Grossraum Burgdorf sowie im Luzerner Michelsamt. Dr. med. vet. Thesis, University of Zürich.

Petersen, H.H., Ersbøll, A., Nielsen, E.O., *et al.*, 2008. Risk factors for high serum haptoglobin concentration in finishing pigs. Veterinary Record 162 (12), 377–382.

Sørensen, V., 1997. Evaluation of laboratory diagnostic assays for monitoring respiratory diseases in pigs. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Stärk, K.D.C., Nicolet, J., Frey, J., 1998. Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. Applied and Environmental Microbiology 64 (2), 532–535.

Young, G.A., 1964. SPF swine. Advances in Veterinary Science 9, 61–112.

Zhuang, Q., 2002. Epidemiological investigation of risk factor for infection with respiratory diseases in Danish genetic and production (SPF) herds. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Exotic and other Species

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Glossary

Carcass weight The weight of an animal that remains after slaughter once the head, feet, skin, and internal organs have been removed.
Cottage industry A small-scale industry, often home based rather than factory based, with relatively few employees or a limited customer base and low economic impact.
Doe An adult female in some animal species.
Dressing percentage/dress out The percentage of an animal's live weight that becomes the carcass weight at slaughter, determined by dividing carcass weight by live weight and multiplying by 100.
Extensive production/farming An agricultural production system which, relative to the land area being farmed, is characteriseized by small capital and labor inputs, as well as limited reliance on technologies (e.g., pesticides and fertiliseizers).
Feral Refers to an animal that has changed from being domesticated to being wild or untamed.
Ghee A class of clarified butter that originated from South Asia.
Hind or Doe A female deer, especially a red deer.
Inanition A lack of adequate nutrition or food (starvation).
Intensive production/farming An agricultural production system which, relative to the land area being farmed, is characteriseized by high capital and labor inputs, as well as substantial use of technologies (including pesticides and fertiliseizers).
Kit or kitten A young rabbit.
Parturition The act of giving birth.
Pâté de foie gras A food product made from the livers of ducks or geese that have been specially fattened, typically by force feeding.
Poikilothermic A term used to refer to an organism whose internal temperature varies considerably.
Rut The mating season of ruminant animals.
Stag or Buck A male deer, especially used when referring to large deer species.
Ungulate A mammal with hooves.
Velvet antler Refers to the entire cartilaginous antler (in a precalcified stage) of certain deer species that is used in a dried and powdered form in many traditional Chinese medicines.
Venison The meat of a game animal.
Weaner A young animal that has been weaned from mother's milk and has been introduced to an adult diet.

Introduction

With the rapid global advances in transportation and infrastructure, individuals from different ethnic groups frequently find themselves in regions of the world which are far from home. Nonetheless, such individuals often retain the desire to consume those foods with which they are traditionally accustomed. To be able to address these desires, a more formal scientific approach is required for the production of the specific species comprising this ethnic food, which includes aspects such as genetic selection (for specific paternal or maternal characteristics), advanced nutritional interventions, as well as disease control. However, it is not only the production systems that need to be of a first-world standard, but also the entire supply chain leading to the market. The latter may include the supply of live animals that would need to pass through a suitable abattoir and processing plant, as well as the transport and distribution of the resultant products to the end consumer – who may be local or international. Consequently, the production of many exotic species has developed in two different directions. The first direction follows older traditional systems, where small-scale production and low numbers of certain species are maintained. Meat is a secondary product in such cases and the products are generally sold locally and for cash. The second direction involves the production of exotic species in a more scientific manner, with the supply of meat frequently being the main output.

Species that are well-established as meat producers include birds (ratites and game birds), buffalo, camels, deer, game animals (ungulates and other African wildlife species), goats, rabbits, rodents, reptiles, kangaroos, and yaks. With some species, meat may be a lower-valued coproduct of other activities or from the sale of alternative items derived from the animals, such as hides, fur, or oil. For example, crocodile farms usually acquire the majority of their revenue from the public display of the animals in pens and from the sale of the skins. Depending on market conditions, rabbit and possum fur can be an economically important coproduct and antler velvet can provide deer farmers with approximately one-third of their annual revenue. The oil pressed from the preen gland of the emu can be more valuable than the meat. Water buffaloes are kept in some countries mainly for draught purposes, with milk production being of secondary importance and the meat serving only as a supplementary product when there is surplus stock or when the animals have to be culled. Goats represent an important food source in many poorer communities in

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Africa. Typically, several goats will be kept per household, one or more of which may be slaughtered for meat consumption on a special occasion or as the family's needs dictate. In such countries, livestock are often used for draught, as an investment, and as a source of manure, rather than as a primary source of meat. In other regions of Africa, multispecies game reserves and combined livestock/game enterprises are replacing monocultures of domestic livestock. Consumptive practices (culling by hunting) are often combined with nonconsumptive options (tourism) on such wildlife ranches, because the returns from meat production alone normally do not justify the substantial investment in fencing required to contain the animals on the reserve. Nonetheless, with the growth in consumer awareness on the health and ethical aspects linked to production practices, a market has grown for meat products that are perceived to be derived from animals raised as 'natural,' 'wild,' or 'free range.' Against this backdrop, game (wildlife) species from South Africa and Namibia have been harvested in a sustainable manner and exported worldwide.

Birds

Of the birds that are well established as meat producers, the ratites (large, flightless birds) and game birds are among the most important.

Living ratites include the ostrich (Struthionidae) of Africa, the rhea (Rheidae) of South America, the emu and cassowaries (Casuariidae) of Australasia, and the kiwis (Apterygidae) of New Zealand. Ratite species are closely related and share common physical characteristics, such as underdeveloped pectoral muscles, the lack of well-developed wings, and keeled sterna, which prevent them from flying, and an adaption to rely on their strong leg muscles for locomotion.

Of all the ratites, ostriches are produced in the largest numbers. Although there have been numerous attempts at rearing emu (mainly in Australia and New Zealand, but also in India and the Americas), the lack of a suitable market for its products has largely hindered the growth of this industry. The female emu normally lays between 5 and 15 eggs in a season. The adult birds are omnivorous and can be fed on lucerne pellets supplemented with grain, whereas the chicks are usually started on a grain-based crumb. Where the primary purpose is to produce emu meat, the birds can be slaughtered as 1 year olds. Nevertheless, the low muscle yields limit the suitability of this species for commercial meat production. Furthermore, emu feathers and skin are of little commercial value. There has, however, been some interest in the medicinal properties of the oil derived from the retroperitoneal and subcutaneous adipose tissue sites of the emu. The kiwi and rhea species are bred artificially primarily for conservation purposes.

The capture and domestication of ostriches for feather production began in the mid-1860s in the Karoo and Eastern Cape regions of South Africa. The income generated by feather production stimulated the rapid development of ostrich farming in this country (mainly around the Oudtshoorn area in the Little Karoo), which reached a climax in the early twentieth century just before the onset of World War I. In 1913, farmed ostrich numbers were approximated at 770 000; however, this number dropped rapidly to 23 000 by 1930 due to the collapse of the market for ostrich feathers. Feathers are now a secondary product of ostrich farming, whereas leather and meat has gained greater prominence since the 1980s. The current income proportions generated from ostrich meat, leather, and feathers in South Africa are 60%, 30%, and 10%, respectively.

An indication of how rapidly the ostrich industry developed in South Africa and also worldwide can be attained by considering global ostrich slaughter figures over the past two decades. In 1993, 152 000 ostriches were slaughtered in South Africa, which accounted for 84.5% of the global ostrich production. In 2002, however, the 340 000 ostrich slaughtered in South Africa constituted only 60.7% of the global ostrich-slaughter market, due primarily to competing ostrich production in Europe, Australia, Namibia, and China.

The increasing demand for ostrich meat throughout the world is mainly due to the low intramuscular fat content and the favorable fatty acid profile. Most of the marketable meat components of the ostrich carcass are concentrated in the posterior limbs and these are normally sold as individual muscles, such as the Musculus iliofibularis (fan fillet), M. iliofemoralis (side strip), M. iliotibialis cranalis, M. femorotibialis accessorius, M. fibularis longus, M. flexor cruris lateralis, M. obturatorius medialis, M. gastrocnemius (big drum), and the M. iliotibialis lateralis.

The ideal habitat for ostriches is the open, short-grass plains and semidesert areas of Africa, but they also inhabit the hot deserts of the Western Sahara and Namibia. They tend to avoid dense woods and tall grass areas. Although little is known about their natural diets, ostriches are believed to feed on low-growing, green vegetation such as forbs, green grasses, seeds, berries, and succulents in arid, semidesert to grassland environments. The mating season of ostriches in the Little Karoo is normally between June and January, and this is also the egg-laying period for female ostriches. A good producing hen will lay 8–10 eggs per month. Once the breeding season has begun, eggs are collected every day, preferably early in the morning or in the late afternoons. The eggs then undergo a process of sterilization and preparation to enter an artificial incubator in the next few days. Almost all commercial ostrich farms in South Africa use artificial incubators to increase hatching numbers.

In South Africa, the three phases of ostrich rearing between hatch and slaughter include: immediately posthatch, rearing to 4–6 months of age, and finishing (most often in feedlots). Posthatch, the chicks are usually maintained indoors for 2–7 days in a temperature-controlled (30 °C) room or enclosure with adequate ventilation. Thereafter, rearing can be conducted in two ways, namely, foster- or artificial rearing. In the former case, young ostriches are raised by experienced breeders that serve as foster parents and each parent is able to shelter 1015 young birds on irrigated lucerne pastures. Alternatively, farmers that have yearling hens as foster parents may move the young birds to a shelter at night (with adequate enclosure and heat) and return them to the foster parents the following morning. In the case of artificial rearing, chicks are maintained in groups of 30–50 in a temperature-controlled building in cold weather and/or at night. These buildings are

usually coupled with an outside run or small paddock of kikuyu grass or lucerne, where the birds are placed during the day. The birds stay in-house at night for the first 2 weeks posthatch. As they become better adapted to regulating their body temperatures, they are kept in the paddocks at night, provided with shelter. The growing birds are kept under such conditions until 16 weeks of age. During the finishing phase of rearing, the ostriches spend 7–8 months in a feedlot or on lucerne and natural veldt camps. In a feedlot, there is zero-grazing and the birds are dependent on a grower ration and chopped lucerne for nutrition. Other ingredients used in ostrich feeds include maize, soybean, and sunflower oilcake, lupines such as full-fat canola and canola oilcake, fishmeal, hay, and salt bush. However, birds are able to graze when kept on lucerne or natural veldt and receive a grower ration as a supplementary feed. In the Little Karoo, approximately 80% of the ostriches destined for slaughter are reared in feedlots from 3 months of age, whereas the remainder are raised on pastures. Ostriches in South Africa are usually slaughtered at an age of 8–14 months in order to obtain high-quality meat, optimal leather quality, and skin size and a single feather harvest, and at this age can yield up to 35 kg of meat from a 55 kg carcass.

The game birds, classified broadly as land fowls and water fowls, have long been hunted for recreational reasons. They, however, also represent an important food source due to their ability to endure continuous harvesting. Game bird meat forms an integral part of the culinary customs of numerous countries, such as Britain and France. Species of the following orders are regarded as game birds: Galliformes (including guinea fowls, partridges, quails, francolins, and pheasants), Anseriformes (including ducks and geese), Columbiformes (including doves and pigeons), Pterocliformes (including sandgrouse species), and Charadriiformes (including snipes).

Game bird hunting is believed to have originated with the ancient Egyptians, who used falcons, dogs, and other weapons and techniques in order to capture the birds. With the invention of the shotgun, game bird hunting later developed into a major sport in Europe, with many traditional, prestige, and ethical aspects involved. The game bird hunting industry contributes to the economy in the UK by providing employment for approximately 26 000 individuals and generating an annual revenue of €0.5 billion. Game bird hunting is also popular in the USA, where more than 38 million game birds are shot each year.

Captive-bred birds form a key part of the international industry, especially in Europe, where large numbers of game birds are bred on an annual basis specially to be released into the wild. In the UK, these captive-bred birds predominantly include pheasants, although red-legged partridges, gray partridges, and ducks are also reared for this purpose. The UK Game Farmers Association estimates that around 20–30 million birds are reared for release into the wild each year. Apart from the financial advantages afforded to landowners by this practice, it is also believed to reduce pressure on wild game bird populations.

Goose production is well established as a cottage industry in many parts of the world, whereas in other regions it has developed into a large-scale business venture. In France, Muscovy ducks and European geese are used for producing *pâté de foie gras*. On many farms, this involves force-feeding the birds with a tube 2–3 times daily while they are caged in a cramming shed for the purpose of producing an enlarged fatty liver. Forced feeding is considered unethical treatment and is illegal in several countries. Goose farming is also well developed in central and eastern Europe, where Rhenish and Italian breeds are well represented, along with the Muscovy duck. The guinea fowl (*Numeda meleagris*) is another game bird that may be farmed as a cottage industry in Africa or may be produced under intensive production systems similar to those used for broiler production. In Africa, both live guinea fowls and their eggs are traded, whereas the meat is produced under more formal marketing systems in Europe. Squats (pigeons) are also produced in numerous countries as part of the cottage industry. Pigeons are grown in pens or cages on rooftops in urban areas of Asia.

Buffalo

Water buffaloes are large animals belonging to the family Bovidae, which are an important livestock species in southern Asia, as well as in South America, southern Europe, and northern Africa. It is estimated that there are approximately 158 million water buffalo in the world, 97% of which inhabit tropical and subtropical parts of Asia, where there is at least one buffalo for every three heads of cattle. Water buffaloes are particularly suited to the marshland conditions in East and Southeast Asia, but high mortality due to calf drowning can occur during the wet season.

The classification of the water buffalo currently remains unresolved. Certain authorities list a single species, *Bubalus bubalis*, with three subspecies: the river buffalo (*Bu. bubalis bubalis*), the swamp buffalo or carabao (*Bu. bubalis carabanesis*), and the wild water buffalo or arni (*Bu. bubalis arnee*). Others consider these to be closely related, but separate species. In 2003, the International Commission on Zoological Nomenclature ruled in favor of classing the wild buffalo as a separate taxon. As a result, the wild forms are now usually referred to as *Bu. arnee* and the domestic forms as *Bu. bubalis*. The former usage, however, remains acceptable for those authors who regard them as conspecific. Both the river buffalo and swamp buffalo are considered to be derived from the wild water buffalo, being the products of thousands of years of selective breeding in Asia. The river buffalo has 50 chromosomes, whereas the swamp buffalo has 48. The two do not readily interbreed, although fertile offspring can occur. Buffalo–cattle hybrids have not been observed to date, and the embryos of such hybrids reportedly do not reach maturity in laboratory experiments.

In paddy-farming regions, swamp buffalo are traditionally raised for draught purposes, whereas river buffalo are kept for fresh milk and ghee production. At present, there is no organized buffalo meat industry. Rather, the animals are slaughtered for meat consumption once they reach the end of their working lives and are often in a poor condition when this occurs. Male calves that are not needed as replacements are not always used for meat production and the surplus stock are normally allowed to die from inanition. Water buffalo feature as a source of meat in countries neighboring the Caspian and Black Seas, where they are slaughtered at approximately

18 months of age at 300–360 kg live weight and dress out at approximately 55%.

Camelids

Camelids belong to the biological family Camelidae, which comprises the genera *Camelus* (including true camel species), *Lama* (including the guanaco and Ilama), and *Vicugna* (including the vicuña and alpaca). The term 'camel' is, however, often broadly used to describe all of the aforementioned camel-like animals. Of the true camel species, the one-humped dromedary or Arabian camel (*Camelus dromedarius*) represent approximately 91% of all the camels found, whereas the two-humped Bactrian camels (*Cam. bacterium*) make up the remainder. Some authorities, such as the International Union for Conservation of Nature (IUCN), apply the binomial name *Cam. ferus* to the wild forms of Bactrian camel, while reserving the name *Cam. bactrianus* for the domestic forms. Although there have been attempts at crossing the dromedary and Bactrian camels, weak and infertile offspring have most often resulted.

Of the estimated 15 million camels in the world, approximately 80% are found in Africa, with the greatest concentration being kept as dairy animals in Somalia. Dromedaries occupy the northern half of Africa, the Arabian peninsula, Iraq, Iran, Afghanistan, Turkmenistan, Pakistan, and western India, where they are used primarily for milk production. Bactrian camels are found to the north of this range, extending into western China. They are kept in considerable numbers in Kazakhstan, Uzbekistan, and Turkmenistan for the purposes of milk, meat, hair, and hide production. A population of approximately 0.2 million largely feral dromedaries inhabit Australia, from where live camels are exported as breeding stock or for the supply of the Middle East meat market.

Camels exhibit a slower growth rate than cattle. The live weights of yearlings can reach 200 kg and these weights can increase under good conditions to 350–400 kg by the end of the second year. Compensatory growth can occur up to the age of 3.5–4 years and the camels reach mature body size at approximately 8 years. They have a lower reproductive rate and higher calf mortality compared to other livestock; thus, young females are usually retained for breeding. Within Africa and the Middle East, many of the animals presented for slaughter are cull breeding stock.

Because the camels are frequently found in harsh environments, the meat is of particular value in the dry seasons when beef is in short supply. However, the demand for camel meat generally exceeds supply and the meat from young stock is particularly sought after. In the eastern region of Ethiopia, camel meat is regarded as high quality and socially acceptable. The dromedary dresses out at approximately 56% of live body weight (average slaughter weight of mature, fattened desert camels is 456 kg) and 64% of empty body weight, yielding 56% meat, 19% bone, and 13.7% fat. In comparison to other red meats (beef, lamb, goat, and chicken), dromedary meat has been found to contain more moisture, less fat, less ash, and similar protein contents.

The majority of the camel's fat reserves are deposited in the hump, which constitutes approximately 8% of the carcass weight (1–5% of live weight). It is thought that the hump serves to insulate the camel from solar radiation, because fat conducts heat more slowly than water. Analysis of the adipose tissue of the hump has revealed that this contains approximately 84% lipid, 139 mg cholesterol per 100 g (wet weight), and 166 mg cholesterol per 100 g lipid. The hump frequently forms part of the sirloin cut and can result in the latter having a high lipid content (49% fat).

Of the genera *Lama* and *Vicugna*, the llama (*Lama glama*) and alpaca (*Vicugna pacos*) are domesticated, whereas the guanaco (*L. guanicoe*) and vicuña (*V. vicugna*) are wild, and commercial farming of the latter two remains limited. The llama is produced for both its meat and fiber, whereas the alpaca is primarily farmed for its fiber. Numerous attempts have been made to cross the alpaca with the llama in order to improve the quantity, but not necessarily the quality of this highly priced species.

Young (9–12 month old) male llamas reared in Chile are often heavier than their same-aged female counterparts (104 kg vs. 68 kg, respectively), but gender differences become less pronounced in older (>3 years) animals (101 kg vs. 105 kg, respectively). Male llamas have slightly heavier dressing percentages (approximately 56%) than females (approximately 54%). However, llamas reared extensively in the mountains of Peru are generally lighter and have lower dressing percentages than those from Chile, likely due to genetic differences between the two populations. Alpaca from Peru are smaller (46.1 kg) than the llama from the same region, although they have a higher dressing percentage (53%). When farmed, fiber, and meat are the most important commodities obtained from the wild guanaco. Adult guanaco weigh 88–120 kg, with little differences in gender, and dress out at approximately 60%. The wild vicuña is the smallest of the camelids, but produces the finest and most valuable wool.

Deer

Deer are ruminant mammals belonging to the family Cervidae. Of the cervid species farmed in Europe, the red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) are the most common. The national deer herd numbers in the UK are estimated at approximately 36 000, and have been relatively stable since 1995. Deer production is a very well-established industry in New Zealand, developing from the point where deer were regarded as pests, to original capturing of wild animals (a pioneer's business) and finally to an important export industry. Red deer comprise approximately 85% of the total farmed deer numbers in New Zealand. Approximately 680 000 yearlings, hinds, and stags are slaughtered annually and more than 90% of the products (venison, velvet antler, and coproducts) are exported. Venison from red deer is high in protein and has a low fat content. Red deer have been genetically selected for large antlers (mainly for velvet production), in combination with docility and fast growth rate.

Like most game species, deer require high fencing to prevent escape, which adds to the establishment costs of a deer farm. Grazing management is controlled in a similar manner as for sheep, because both are bottom-grazers that select the shorter grass species. Most farms breed and rear deer calves to

slaughter, rather than selling the calves to finishing units as weaners. The calves are usually sold for slaughter as yearlings and are transported to abattoirs in specialized deer trucks. Deer can be flighty animals and experience is required for their handling. Breeding stags also show aggression toward humans, particularly during the rut.

The most common deer species farmed in the USA and Canada are the elk or wapiti (*Cer. canadensis*), fallow deer, sika deer (*Cer. nippon*), the chital or axis deer (*Axis axis*), and the white-tailed deer (*Odocoileus virginianus*). The elk is among the largest deer species in the world, whereas the latter species are all considerably smaller in size. The sika deer is probably the best example of a deer species that has been rescued from the brink of extinction and is now farmed commercially for velvet and meat production. Reserves of the wild animals were depleted at the turn of the twentieth century by hunting for their valuable antler velvet. The last populations inhabited the Primorski territory of the former Soviet Union (now the Union of Soviet Socialist Republic (USSR)), with a smaller number in the Far East. Stocks in the USSR were protected and multiplied, and then distributed to New Zealand, Germany, and Denmark for farming and parkland purposes.

The Caspian red deer (*Cer. elaphus maral*), often referred to as the Maral or Noble deer, is one of the most recent species to be domesticated. They are valued mainly for the antler velvet, which is used in the manufacture of pantocrine (a natural drug). The mature stags weigh up to 400 kg, thus have the potential to produce large carcasses for the meat market.

Reindeer or caribou (*Rangifer tarandus*), thought to be one of the first species to be domesticated, are reared today primarily for their meat, antlers, fur, and hides and, to a lesser degree, for milk and transportation. Today, this species is found almost exclusively in the Northern Hemisphere, where there are more than 4 million reindeer (approximately 75% of these are domesticated and 25% are wild). The reindeer are likely to have been tamed in herds rather than individually, from which practices evolved the almost unique method of rangeland reindeer management. Reindeer production, particularly by the indigenous Sami people of the Arctic, changed from an intensive herding system to a more extensive system using modern aids such as trucks, helicopters, motorbikes, and snowmobiles to aid with herding. Reindeer are now mainly kept in free-range systems, not behind fences, and are dependent on the natural pastures of the mountain tundra and forests for food. Their predominant food sources include lichen, browse (especially from shrub willow and birch), sedges, herbs, and grasses. In winter, the reindeer forage by scraping away the snow and lichen comprises approximately 70% of the feed. As a ruminant feed, lichen is relatively rich in carbohydrate and low in protein.

The rutting season of the reindeer is between September and October and pregnancy lasts approximately 7.5 months. Fawns born in April and May put on approximately 60% of their mature body weight during the first summer, but stop growing during their first winter. Puberty is reached by the time of the next rut. Common reindeer breeds include the Chukotka and Nentsi. The Chukotka is well established in the Chukotka, Kamchatka, and Yakutia regions of the tundra, and the less numerous Even and Evenk breeds are found toward the south. The Chukotka has a long body with relatively short legs and the carcass weight of a large stag is typically 60 kg. Nentsi are located on the Arctic coast to the east of the Ural Mountains. Although slightly lighter in weight than the Chukotka, larger Nentsi strains exist on some of the Arctic Islands.

Game Animals

It is estimated that over 25% of the surface area of South Africa that is fit for agricultural practices is currently utilized for some type of wildlife production (either formal National and Provincial game parks or private game farms). Game animals in this country serve for both nonconsumptive purposes (tourism and photographic safaris) and consumptive purposes (hunting safaris and harvesting for export). African ungulates hold particular potential for both of the aforementioned purposes. The animals harvested commercially are mainly the springbok (*Antidorcas marsupialis*) (>80%), blesbok (*Damaliscus pygargus phillipsi*), and kudu (*Tragelaphus strepsiceros*). Other species such as zebra (*Equus burchelli*), blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), and gemsbok (*Oryx gazelle*) are exported in smaller numbers.

There are two game production systems currently practiced in Africa. The first of these involves intensive farming (using modern livestock procedures), where game stud breeders produce highly priced, rare species for other producers or for the hunting market. The second system involves farmers who combine game farming with traditional livestock farming to produce animals for either the hunting industry (>1 million animals hunted per annum) or for harvesting for the export market. It is generally accepted that the animals are enclosed in areas that are large enough not to impede their natural movement, and that the animals can be classified as being organically raised. Not all game species require high fencing to maintain them in their enclosures. Springbok and blesbok, for example, are contained by standard sheep fencing. There are well-developed procedures for the harvesting, processing, and exporting of game meat into the European Union and Asia.

Goats

Domestic goats (*Capra aegagrus hircus*), a member of the family Bovidae, are believed to have descended from the wild goat or bezoar (*Cap. aegagrus*) of southwest Asia and Eastern Europe. The domestication of these small ruminants over 9000 years ago led to numerous phenotypic changes and the development of multiple goat breeds, which soon became widely distributed around the world due to their great ability to adapt to varying environmental conditions. Global goat numbers are estimated at approximately 869.1 million, almost 60% of which are found in Asia and 24% in Africa, where the ratio of sheep to goats is approximately equal. In countries such as India, Pakistan, and Nigeria, goats outnumber sheep by almost two to one.

Goat farming represents a small niche in developed countries, but contributes substantially to the livelihoods of rural populations in developing countries across the globe. Goats may be farmed for their meat, milk, hides, fiber, and manure.

Most of the world's goat meat is supplied by Asia (71%) and Africa (22%). Goat meat is recognized for its low fat content.

The production of goats may be extensive (reliant on goat grazing on large areas) or intensive (farming on small plots with daily management), for subsistence or for profit. Although not customary, goats can be finished off in feedlots before slaughter. In subSaharan Africa, goats are typically kept by crop farmers, who allow them to browse the scrub bushland. In more productive regions, they are tethered on pasture and kept in kraals or huts at night. The interchange of breeding stock may occur when people look after the animals of neighbors. The goat population in parts of Africa is often infested with the tapeworm *Stilesia hepatica*, which occupies the bile duct, but does not appear to cause jaundice.

Although a limited number of breeds have been improved for meat production, specific meat breeds have been developed in South Africa and the USA. The most well-known improved meat breed is the Boer goat from South Africa, which was bred following scientific selection criteria to improve carcass conformation. The Boer goat has now been exported and is being produced in the Americas, Australia, New Zealand, and China. In Australia, large numbers of feral goats are harvested for export and for local meat markets.

Rabbits

Rabbits comprise eight genera in the family Leporidae and are found in several parts of the world. They are simple-stomached, herbivorous animals with a relatively fast growth rate and high feed conversion efficiency. Leporids such as the European rabbit (*Oryctolagus cuniculus*), as well as the hares (*Lepus* spp.), are an important food meat in Europe, South and North America, and certain parts of the Middle East. In addition, the wool and skin/fur of rabbits is of commercial importance. Rabbit production is particularly well developed in China, where approximately 700 000 tons is produced per annum. Within Europe, rabbit production is strongest in Italy (230 000 tons per annum), Spain (74 161 tons per annum), and France (51 400 tons per annum). Today, fresh rabbit meat is sold in the butcheries and markets of some countries, whereas certain supermarkets sell frozen rabbit meat. The meat is highly palatable, high in protein and low in calories, fat, and cholesterol.

When rabbits first became available for sale to the public, many rabbit farms started to increase in size to accommodate the demand for the meat. Not only did the number of does increase, but also the breeds, housing conditions, feeding, and management techniques were developed accordingly. Nonetheless, the industry was badly affected by myxomatosis in the 1950s and 1960s, whereas the spread of rabbit calicivirus, which originated in China, had a detrimental effect in the 1990s. Coccidiosis and cysticercosis have also been difficult to control.

Rabbit farms are classified as small, medium, and large. Regardless of the size, however, the main goal of rabbit farmers is usually to improve the reproductive performance of rabbit does. Stocks of lower productivity are improved by crossing with intensive breeds. A better nutritional status of both fetuses and suckling kits has a positive effect on their later productivity. The gestation period of rabbits is 30–32 days. Does are not prone to lactational anoestrus and can be remated shortly after parturition. Under intensive conditions, a 42 day reproductive rhythm (remating 11 days after parturition) is practiced. Under extensive conditions, the 18 or 25 day mating interval with 35–42 day weaning is more suitable. On small farms, natural mating is favored, whereas artificial insemination is commonly employed on large farms. The number of doe teats and reproductive ability are positively correlated. Does with 10 teats kindle higher numbers of kits (5–10% more) than does with 8 teats. Synthetic rabbit breeds may have 10–12 teats and cross-fostering is common in large breeding units.

In most commercial rabbitries, the animals are housed in flat-decked or stepped wire cages, with the provision of nesting boxes for kindling and for the protection of the young rabbits. Complete pelleted feeds form the main part of the rations, and automated feeding and watering equipment are utilized.

Intensive selected rabbit breeds are most suitable for medium and large rabbit farms due to their good reproductive and productive performance, as well as good maternal ability. Medium-weight breeds (4.0–5.4 kg at maturity) and heavyweight breeds (6.3–7.2 kg at maturity) are most suited for meat production. The New Zealand White and Californian are the leading commercial breeds, with a high proportion of muscle for their size. Synthetic rabbit breeds originated from the crossing of two or more intensive breeds to introduce higher reproductive and productive characteristics, greater adaptability to different environmental conditions or traits preferred by certain groups of consumers. The Californian is the best known of these synthetic breeds, which originated from the crossing of the Himalayan and Chinchilla rabbit breeds, with subsequent crossing of the offspring therefrom with New Zealand Whites.

Certain native rabbit breeds are used in countries with extreme climatic conditions and, even if their reproductive performance is suboptimal, they are well adapted to the local environment and keeping conditions. Colored breeds are kept in some countries to produce meat for own consumption or special markets. Some colored breeds are used in Europe in organic farming or alternative systems. The productivity of colored breeds is between those of native and intensive breeds, and their reproductive ability is sufficiently suitable to make them common on small farms. For backyard rabbit husbandry or home meat production, breed choice is not of critical importance. Rather, under such circumstances, inexpensiveness and resistance to the environment and diseases may be the more important characteristics. The Angora is the primary rabbit breed for wool production, whereas the Rex and Satin breeds have been raised for their pelts for the fur market.

Rodents

Rodentia make up the largest order of mammals, comprising over 440 genera and over 2000 different species. The five main families within this order are Muridae (rats and mice), Sciuridae (squirrels), Echimyidae (spiny rats), Heteromyidae (pocket mice and kangaroo rats), and Dipodidae (jerboas and jumping mice). The Muridae represent approximately 66% of all the rodent taxa. Representatives of the Rodentia are

widespread and, with the exception of Antarctica, occur on all continents and on many oceanic islands. In a number of these regions, rodents are prized items in the diets of local people, with approximately 89 species serving as a food source.

Apart from their ability to survive on diverse diets, the success of rodents is attributed to their large litter sizes, short gestation periods, and early sexual maturity. All of these characteristics make them ideal meat producers. The guinea pig (*Cavia porcellus*), for example, has been a stable meat source for many of the poorest people in the Andes for over 3000 years and 20 000 tons of meat (64 million edible carcasses) is produced from this species annually. Farm-raised guinea pigs have a dressing percentage of approximately 65% and the meat contains approximately 21% protein and 8% fat.

Four types of rodents have production potential in Latin America, namely, the capybara (*Hydrochoerus hydrochaeris*), paca (*Cuniculus* spp.), agouti (*Dasyprocta* spp.) and the coypu or nutria (*Myocastor coypus*). The capybara is believed to have been domesticated in Brazil as early as AD 1565. This species is the largest of the Rodentia and adults typically weigh 35–66 kg. Licensed ranches in Venezuela harvest approximately 85 000 capybara per year. Although capybara meat is considered to be very tasty, most recipes for its preparation require the removal of fat by successive stages of boiling in fresh water. Capybara meat is also traditionally processed in Columbia and Venezuela by salting and drying. Of the closely related pacas and agoutis, the former is particularly regarded as a luxury food source by inhabitants of Guyana, Mexico, Trinidad, and Nicaragua. Coypu are native to South America, but also exist today in Europe, Asia, and North America, where they may be commercially farmed. They are fairly large semi-aquatic rodents (adult weights of 5–9 kg); however, they do show sexual dimorphism and the males are generally heavier than the females. Although the marketing of coypu meat has shown varied success, fresh, frozen, and processed products are now appearing on the commercial market in Uruguay. Coypu has a carcass yield of 52% relative to live weight, resulting in the following meat yields (after cutting up the eviscerated carcass): back (25.0%), two hind legs (23.7%), two front legs (14.7%), and belly flaps (12.9%). The remainder (23.7%) is referred to as 'small meat.' The meat from farm-reared coypu has a lipid content of 1.4–1.8%, and a cholesterol content of 70.1–72.7 mg 100 g^{-1}wet tissue.

Cane rats or grasscutters (*Thryonomys swinderianus* and *T. gregorianus*) are widely eaten in some African countries, but are also regarded as pests on many crops. Research has been carried out over the past 15 years to select stocks for improved adaptability to a restricted life in captivity, as well as to develop rearing programs in rural and periurban areas of Africa. In West Africa, approximately 80 million cane rats are hunted per year, with an equivalent of 300 000 metric tons of meat. With a carcass yield of approximately 64%, comprising 22% protein and 4% fat, these species are highly sought after for meat production in Africa.

Reptiles

Reptiles belong to the class Reptilia, within which the four recognized orders include the Crocodilia (crocodiles, alligators, gavials, and caimans), Squamata (lizards and snakes), Testudines (tortoises, turtles, and terrapins), and Sphenodontia (tuataras). Humans have exploited reptiles in many global regions and for many centuries, mostly as a food source. This, coupled with the use of their skins and their incorporation into many traditional Asian medicines, has led to the overexploitation of certain species. Turtles presently suffer the greatest human predation, particularly in China, where many local populations are now depleted. The exploitation of snakes for food and for medicinal purposes, also mostly in China, has raised concerns for those species involved in the trade. In addition, tegu lizards (*Tupinambis* spp.) have been exploited in Paraguay mainly for their skins, but this has not been on a sustainable basis. Crocodilian species were hunted extensively during the 1950s and 1960s, largely for their skins, and the decimation of many populations resulted in the institution of worldwide conservation and management efforts. In 1975, all populations of the Nile or common crocodile (*Crocodylus niloticus*) were listed by Convention on International Trade of Endangered Wild Fauna and Flora (CITES) in Appendix I, which includes species that are threatened with extinction and for which commercial harvesting and trade is generally prohibited. Between 1983 and 2004, a number of African countries successfully transformed their national *Cro. niloticus* populations from Appendix I to Appendix II, with the latter including species which are not necessarily considered to be threatened, but for which trade is strictly controlled (mostly through quota systems) to prevent overexploitation. The transformation of many of these *Cro. niloticus* populations was largely attributed to the incorporation of ranching into the crocodile management regimes.

Crocodiles have been ranched in Zimbabwe since the early 1960s; however, the number of farms increased during the late 1980s. Lucrative crocodile farms also exist in South Africa, as well as in Asia and Australia. Crocodiles are farmed primarily for their skins, whereas the flesh is a secondary product. The skin represents nearly 20% of the live weight of the Nile crocodile and a dress-out of approximately 56.5% is derived from this species. This dressing percentage is lower than that obtained from the American alligator (*Alligator mississippiensis*) (63.3%) of similar length. The crocodile tail comprises 18% and 33% of the live weight and empty carcass weight, respectively. The carcass weight can be further apportioned as 60.8% lean meat, 12.2% fat, and 26.6% bone. The protein content of raw crocodile meat is approximately 22.1% and the fat content varies with muscle proximity from 0.91% to 2.94%.

With captive crocodile breeding, eggs are regularly harvested from brood stock pens and are thereafter incubated and hatched under controlled conditions (relative humidity of 97–99% and temperature of approximately 31 °C). Crocodiles do not have sex chromosomes. Rather their sex is determined by a process called environmental sex determination (ESD), with the most common form of ESD being temperature-dependent sex determination (TSD). Females are produced at low and high temperatures, whereas males are produced at intermediate temperatures (often called the female–male–female (FMF) pattern). In the wild, nest site selection combined with TSD may enable the female to control the sex of her offspring. The incubation temperature not only influences the sex of the crocodiles, but also the growth rate before and after hatching,

the metabolic rate, optimal thermoregulatory temperatures, and sexual characteristics such as pigmentation pattern and intensity. Commercial breeders may make use of this strategy to ensure that males are predominantly produced. Eggs should hatch within 73 days when the incubation temperature is maintained at 31 °C; however, this may take as long as 95 days. After hatching, average growth rates of 3.1–3.95 mm per day are achieved in commercial production systems. Crocodiles are poikilothermic and are, therefore, reared in heated buildings. Young crocodiles normally reach 1 m in length at 8–10 months, and are then moved to growing or rearing ponds. Crocodiles reared in artificially heated environments (approximately 32 °C) reach a suitable commercial size after 18–30 months (1.5–2.5 years). As crocodiles are mainly produced for their skins, the size (measured across the belly) determines when the reptile is ready for slaughter. Average belly width at slaughter is 27 cm and attained between 24 and 30 months of age.

Kangaroos

Kangaroos are marsupial species of the family Macropodidae, which also includes wallabies, wallaroos, tree kangaroos, pademelons, and forest wallabies. Kangaroos are endemic to Australia and their meat and skins have contributed to the survival of the aboriginal people in this region for tens of thousands of years. Although Australian law prohibits hunting of many of the approximately 50 macropod species, the 3 largest kangaroo species are particularly abundant in rural areas of Australia and are regarded as pests due to their competition with cattle and sheep for valuable pasture resources. As a result, a number of Australian states have determined that kangaroos may be sustainably harvested for commercial purposes, albeit under strict regulatory control. Quota systems determine the maximum number of kangaroos that can be harvested from the wild and the animals may only be killed for commercial use by licensed, skilled marksmen.

The production of kangaroos under intensive conditions has limited feasibility not only due to the high costs of establishing a kangaroo farming enterprise, but also due to the slow growth rate of the animals, their mobility, and ability to jump stock fences, as well as their behavioral patterns which impede mustering or herding. In addition, the market for kangaroo meat and skins can be adequately supplied by the existing rangeland harvesting (hunting).

Approximately 70% of the Australian kangaroo harvest is currently exported, supplying more than 55 countries worldwide. The species that are most commonly harvested for commercial export include the red kangaroo (*Macropus rufus*), eastern gray kangaroo (*M. giganteus*), western gray kangaroo (*M. fuliginosus*), and the common wallaroo or euro (*M. robustus*), with the former three species comprising approximately 90% of the commercial harvest. Meat for human consumption and skins for the leather industry represent the most important kangaroo-derived products. Kangaroo meat is strong in flavor, high in protein, zinc, and iron, while being relatively low in fat (approximately 2%) and cholesterol. Some kangaroo meat may also be processed into pet food.

Yaks

Yaks (*Bos grunniens* or *Bo. mutus*) are used for meat consumption in the mountainous regions of central Asia. They are kept principally for milk and wool production, but there are specialized meat strains that have a relatively large mature body size in Kirgizia. The average birth weight is between 9 and 16 kg and they may be slaughtered at 3 years of age, or when they reach approximately 300 kg live weight. At that weight, they dress out at approximately 57%. The conformation of the yak is quite different from that of cattle. They have a short neck, a thoracic hump, deep chest, short forelegs, and well-developed hindlegs that are adapted to grazing steep slopes. Musk-ox inhabiting the margins of the ice sheet in the northern hemisphere has been used for meat consumption in the past, but numbers are now too low to allow regular cropping. Their unusual feature is their compact but large size.

Various Other Species

A number of other domesticated species are used for meat production, not all of which are farmed. Approximately 470 000 tons of horse meat, derived from domesticated and wild horses, is used annually for human consumption. Both feral and domesticated horses are slaughtered in dedicated abattoirs, and much of the meat is exported to France. The major horse meat consumers are France, Japan, Belgium, Luxembourg, the Netherlands, and Italy. Italy uses horse meat in delicatessen sausages. The major exporting countries are the USA, Argentina, and Canada.

The consumption of frog's legs is popular in many parts of the world. There has been an overall increase in the global trade of frog's legs over the past 20 years, and the industry is valued at approximately $40 million per year. More than 100 000 metric tons of frog's legs were imported between 1996 and 2006 from both farmed and wild sources. France, the USA, Belgium, and Luxembourg are the largest importers of this culinary curiosity.

Meat from dogs is consumed in certain regions of East Asia, and in particular in the Philippines.

At least three species of snail are eaten in Europe as a delicacy: *Helix pomatia*, *H. lucorum*, and *H. aspera maxima*.

See also: Automation in the Meat Industry: Slaughter Line Operation

Further Reading

Barnett, R., 2000. Food for Thought: The Utilization of Wild Meat in Eastern and Southern Africa. Nairobi, Kenya: TRAFFIC East/Southern Africa.

Bothma, J.d.u P., Du Toit, J.G., 2010. Game Ranch Management, fifth ed. Pretoria, South Africa: Van Schaik Publishers.

Deutsch, J., Murakhver, N., 2012. They Eat That?: A Cultural Encyclopaedia of Weird and Exotic Food from Around the World. Santa Barbara, CA: ABC-CLIO.

FAO, 1996. Wildlife Utilization in Latin America: Current Situation and Prospects for Sustainable Management. FAO Conservation Guide – 25. Rome: Food and Agriculture Organization of the United Nations.

Glatz, P., Lunam, C., Malecki, I., 2011. The Welfare of Farmed Ratites. New York: Springer.
Gonzales-Jimenez, E., 1977. The capybara: An indigenous source of meat in tropical America. World Animal Review. (FAO) 21, 24–30.
Hardouin, J., Thys, Ě., Joiris, V., Fielding, D., 2003. Mini-livestock breeding with indigenous species in the tropics. *Livestock Research for Rural Development* 15 (4), Available from: http://www.lrrd.org/lrrd15/4/hard154.htm (accessed 05.03.12).
Hoffman, L.C., 2008. The yield and nutritional value of meat from African ungulates, camelidae, rodents, ratites and reptiles. Meat Science 80, 94–100.
Hoffman, L.C., Wiklund, E., 2006. Game and venison – meat for the modern consumer. Meat Science 74, 197–208.
Johnson, S., Johnson, D., 2009. How to Raise Rabbits. Minneapolis, MN: MBI Publishing.
Mahgoub, O., Kadim, I.T., Webb, E.C., 2011. Goat Meat Production and Quality. Cambridge: CAB International.
Reinken, G., Hartfiel, W., Körner, E., 1990. Deer Farming. Ipswich: Farming Press.
Tetangco, M.H., 1981. Recent Advances in Buffalo Research and Development. Taiwan: Food and Fertilizer Technology Center.
Warkentin, I., Bickford, D., Sodhi, N., Bradshaw, C., 2009. Eating frogs into extinction. Conservation Biology 9, 333–341.
Warwick, C., Frye, F.L., Murphy, J.B., 2001. Health and welfare of captive reptiles. London, UK: Chapman and Hall.

Relevant Websites

www.cites.org
Convention on International Trade of Endangered Wild Fauna and Flora (CITES).
www.daff.gov.au
Department of Agriculture, Fisheries and Forestry (DAFF) (Australia).
www.nda.agric.za
Department of Agriculture, Forestry and Fisheries (DAFF) (South Africa).
www.fao.org
Food and Agriculture Organization of the United Nations (FAO).
www.iucn.org
International Union for Conservation of Nature (IUCN).
www.naweb.iaea.org/nafa/aph/
Joint FAO/IAEA Programme: Animal Production and Health.
www.wessa.org.za
The Wildlife and Environment Society of South Africa (WESSA).
www.traffic.org
TRAFFIC (Wildlife Trade Monitoring Network).
www.usda.gov
United States Department of Agriculture (USDA). Animal Production.

Meat Production in Organic Farming

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Introduction

Worldwide, there has been a significant growth in certified organic agriculture since the 1990s, and this now takes place in more than 100 countries. Organic agriculture has been one of the fastest growing sectors of agricultural production and the global market for organic products tripled from 2000 to 2009. In 2009, countries like Australia, Argentina, the USA, China, Brazil, Spain, India, and Italy each had 10^6 ha or more under organic management. In Europe, the proportion of organically managed land in 17 of its countries exceeds 5%.

Although other large agricultural areas throughout the world are managed without the use of fertilizers, pesticides, or feed additives without being labeled organic, it is important to make a distinction between the two types of farming. Organic agriculture not only is based on minimizing the use of external inputs but also follows a system and process-oriented approach in directly aiming to promote agroecosystem health including biodiversity, biological cycles, soil biological activity, and prevention of animal diseases instead of curing them. Livestock often plays an important role in obtaining some of these principal aims in organic farming. Thus, livestock production, especially ruminant livestock, forms an integral part of many organic farms. However, the market share of organic meat products – in contrast to milk and egg products – is still very limited.

This article highlights some of the problems and challenges as well as some results obtained in organic meat production, but first some basic aspects of certification and regulation are mentioned.

Guidelines and Regulations in Organic Production

The basic principles and standards for organic farming are formulated by The International Federation for Organic Agricultural Movement (IFOAM). This is an international umbrella organization of organic movements in more than 100 countries. These basic principles are not very detailed but form the basis for legal regulation in local, national, or international bodies. The IFOAM basic standards state, in relation to livestock, that the farming practice should facilitate production of high-quality food in sufficient quantities; interaction with natural systems and cycles in a constructive and life-enhancing way; maintenance of genetic diversity of the production system in order to create a harmonious balance between crop production and animal husbandry; and provide all livestock conditions of life with due consideration for the basic aspects of their innate behavior.

To facilitate trade in organic products on an equal basis, as well as consumer protection, more detailed regulations have been set by national organizations such as the European Union (EU) and the US and more lately by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO). The joint FAO/WHO Standard Program and the Codex Alimentarius Commission developed guidelines in 2001 for organic animal production. These guidelines for organic food take into account the current regulations in several countries, EU regulation 834/2007 in particular, as well as private standards applied by producer organizations, especially those based on the IFOAM Basic Standards.

The EU regulation is very detailed and is a good example of how the basic standards of organic farming are implemented. It includes specifications for housing conditions, animal nutrition, and animal breeding, as well as animal care, disease prevention, and veterinary treatment. A key principle is to rely mainly on the management of internal farm resources rather than on external input and, in relation to animal health management, to rely on prevention measures rather than on allopathic medical treatment.

Livestock must be fed on organically produced feedstuffs, preferably from the farm itself. A limited proportion of conventional feedstuffs were permitted within a transitional period but expired on 31 December 2011 (recently postponed by the EU commission). A summary of the special demands is given in **Box 1**.

Disease prevention in organic livestock production is based on the following principles:

- Selection of breeds with abilities to cope with the required conditions, viability, and resistance to disease. Breeds should be selected to avoid specific diseases or health problems that prevail in conventional livestock production. Preference is to be given to indigenous breeds.
- Livestock should be raised in a manner that suits the requirements of the species and promotes a good resistance against disease and infections.
- Application of good quality feeds which, together with application of outdoor areas and grazing, strengthen the natural immune system of animals.
- Securing a suitable space in order to prohibit overcrowding and associated health problems.

Treatment of sick animals should be carried out immediately. Nonallopathic medicine should be chosen before allopathic medicine, if efficient. Preventive treatments with allopathic medicine are not allowed. Medical treatment requires instruction and diagnosis by a veterinarian. The withdrawal time for allopathic medicine is set to twice the length required by veterinary authorities.

In individual countries, the detailed regulations can differ depending on private certification bodies. An example of such

Box 1 Main EU regulations' feeds and feeding

The feeding of young mammals must be based on natural milk, preferably maternal milk, for a minimum period depending on the species

Rearing systems for herbivores are to be based on maximum use of pasturage according to the availability of pastures in the different periods of the year. At least 60% of the dry matter in daily rations is to consist of roughage, fresh or dried fodder, or silage

Roughage, fresh or dried fodder, or silage must be added to the daily ration for pigs and poultry, and for poultry in the fattening period, at least 65% of the feed formula must contain cereals

Only feed materials listed in Annex V of Council Regulation No. 834/2007 whether conventionally or organically produced can be used (a positive list). Furthermore, conventional feed materials of agricultural origin can be used only if they are produced or prepared without the use of chemical solvents. This implies that, for example, soybean meal, the most common protein source in animal nutrition, cannot be used in organic feeds

Antibiotics, coccidiostats, medicinal substances, growth promoters, or any other substance intended to stimulate growth or production shall not be used in animal feeding

No feed components may have been produced with the use of genetically modified organisms (GMOs) or GMO derivatives

Vitamins authorized for conventional animal production under Directive 79/524/EEC must preferably be derived from raw materials occurring naturally in feedstuffs or synthetic vitamins identical to natural vitamins only for monogastric animals

adjustment is the use of antibiotics. In the USA, use of antibiotics in disease treatment excludes the animal from being sold as organic and in Sweden the use of homeopathic medicine cannot be chosen by a veterinarian. Another example is the use of a ring in the sow's nose to prevent rooting. In many countries, this is allowed for environmental reasons, whereas in others (e.g., Soil Association in the UK and Sweden), this is not allowed because it is considered non-acceptable as it prevents the sows from performing their natural behavior.

Pork Production

Some main requirements within the EU as related to pig production are that pigs should have access to grazing whenever weather and ground conditions allow this. If the pigs are housed indoor in barns, access to an outdoor run and special specifications for space requirement (considerably higher than that in a conventional system) are required. The weaning age for piglets should be at least 40 days.

Depending on the prevalent conventional pig production systems, the organic systems vary between different European countries. In Spain, the traditional Dehesa systems have lent themselves to organic conversion fairly easily and the same is true for combined woodland and livestock (silvopastoral) systems in other Mediterranean Countries. Similarly, in the UK, the prevalent outdoor breeding units have been relatively easy to convert to organic production, where farm size and crop rotations have allowed adequate space. In Western Continental Europe, more intensive systems have been converted into organic production by running the breeding stock outdoors during lactation (Figure 1) and keeping finishers in barns allowing pigs access to outdoor pens.

The land required to fulfill the demand for access to outdoor areas differ between countries, often impacted by the local environmental regulation. For example, in Denmark, a farrowing outdoor pen in use during 1 year (supporting 6–7 farrowing with suckling period) requires a size of approximately 1000 m^2 and needs to be free of pigs every second year. Considering the land requirement to be available for pig farming in general due to environmental regulations, this outdoor area for pigs can easily be spaced. In situations where there is no regulation on land related to pig farming, the requirement of outdoor area may be a serious limiting factor. However, a general perception is that the establishment of an outdoor farrowing unit requires far less investment than that of a typical pig building, which must be taken into account for an economic assessment.

Figure 1 In Western continental Europe more intensive systems have been converted into organic production by keeping the SOWs outdoots during lactation.

Good production results in terms of daily gain, and pigs per litter are often obtained in intensive organic systems. However, the longer weaning period means that number of litters per sow and year is lower than that in conventional pig production. Long weaning periods have been hypothesized to impair the welfare of the sow due to heavy weight loss and/or damage to teats and udder. However, Danish experiments showed no difference between a weaning age of 5 and 7 weeks in this matter, probably because the nursing frequency and milk production have already decreased markedly after 5 weeks coinciding with an increase in the piglets' intake of solid feed.

According to surveys from the UK, Denmark, Austria, and the Netherlands, endo- and ectoparasites appear to be the most common concern for organic pig producers. Lameness, injuries, and sunburn are other common problems reported from studies on outdoor pig production. Respiratory diseases, however, seem to be less prevalent in organic than in conventional herds.

Several investigations indicate that growth rate obtained in outdoor systems with finishers on grass can be comparable to the growth rate in indoor production. However, variable feed conversion rates have been obtained. In the summer period, a feed conversion comparable to indoor conditions has been obtained in some investigations, whereas in other periods of the year, or in other investigations, a higher feed consumption per kilogram gain has been reported.

Organic pigs need access to roughage, i.e., grass and grass silage. If high-quality forage is available, pregnant sows are able to cover 50% or more of the daily energy and protein intake by roughage. Lactating sows, however, are able to rely only on fibrous roughage as energy source to a moderate extent if milk production (and the live weight gain of the piglets) is to be maintained. Several investigations have focused on the use of roughage for finishers. For instance, the effect of restricting concentrate on the *ad libitum* intake of clover grass and clover grass silage, as well as on the production results and sensory meat quality, has been investigated.

Restricting concentrate to 70% of *ad libitum* intake on a daily basis resulted in:

- a higher roughage intake (20–30%), but, nevertheless, only amounting 5–6% of total energy intake;
- a lower daily gain (12–16%);
- a lower feed consumption per kilogram gain (10%);
- an increased lean content (1–2%);
- reduced tenderness of the meat; and
- a higher content of polyunsaturated fatty acids in carcass fat depots and meat.

Few direct comparisons between organic and conventional production systems are available and the effects on daily gain and carcass quality reported in literature are conflicting, probably depending on the exact nature of the feed used, genetic factors, and rearing conditions. Combined effects of rearing the growers with access to more space and organic feedstuffs seem to result in more intramuscular fat, a higher fat thickness, and more dark colored meat. In addition, use of more oil-rich ingredients for amino acid supply than used in conventional production may result in an increased level of polyunsaturates in fat, which, in turn, may impair the technological product quality but might improve the quality from a human health perspective.

Cattle and Sheep Production

The importance and characteristics of organic beef production vary widely in different countries. In countries where organic dairy production is highly developed, the major contribution to organic beef production originates from dairy bull calves and culled cows. In other countries, the major contribution comes from extensive suckler herds.

Dairy Cattle

Organic standards require group-housing systems and adequate bedding and tend to limit stocking rates in dairy production. Milk yield per cow is often lowered due to a lower feed intake as a result of the restriction on which feed is to be used. It has been hypothesized that the restriction in feed intake might result in an increased risk of metabolic disorder and poor fertility because the same high-merit genotypes for milk production are often used in conventional and organic production. However, a number of investigations have not been able to demonstrate this. The overall picture is that no well-documented differences in reproduction and health problems (as related to mastitis, milk fever, ketosis, and lameness) exist for dairy cattle.

Beef Production

The intensive rearing of bulls is very limited in organic production. The requirement of 60% forage in the diet makes it very difficult to obtain good production results. Bulls older than 1 year can be housed in a stable with access to an outdoor run throughout the year, but this often puts high costs on the production.

In some countries, the bull calves from organic dairy breeds for these reasons are not raised as organic but sold for conventional rearing (or harvested very young). In other countries, there is tradition for grass-based rearing systems for steers, which fits very well the organic production conditions.

The main concern in such systems regarding production and health are related to infection with endoparasites because no prophylactic use of antiparasiticides is allowed in organic production. Important measures in endoparasite control include pasture management, low stocking density, and early- and late-season feed supplementation.

Extensive Suckler Beef and Sheep Production

In general, extensive suckler and sheep production complies with the aims and requirements in organic production to a wide extent. The organic producers often do not consider animal health problems in such systems significant. External parasites, diarrhea, and mineral deficiencies, although receiving low rankings, are considered the most important conditions in young beef stock.

Although organic dairy sheep production is rapidly growing in the Mediterranean countries, the bulk of organic sheep production in Europe is lamb production in France and the UK. In these countries, similar ranges of lamb mortality, lambing percentage, and ewe replacement rates as in corresponding conventional systems have been reported.

As in beef systems, restrictions on internal and external parasites control with conventional prophylactic antiparasiticides have been recognized as a major constraint to sheep health management. Therefore, establishing proper pasture management options is essential. A key issue here is a better understanding of the prospect of taking advantage of the content of condensed tannins in certain legumes and herbs for the prevention of infection and/or losses as a result of infection with gastrointestinal nematodes.

Broiler Production

For poultry production, the implementation of the organic ideals in the EU regulations includes, among others, a

maximum flock size of 3000 and 4800 for layers and broilers, respectively. These flock sizes are well below flock sizes normally seen in conventional poultry production but still much higher than what can be considered 'natural' flock sizes. The birds should have access to a hen yard corresponding to at least 4 m^2 per laying hen/broiler; coccidiostats cannot be included in the feed; and beak trimming is not allowed.

In organic broiler production, coccidiosis is no longer a major problem due to the fact that vaccination is now allowed. However, prophylactic uses of anticoccidials is banned, but recently, focus on medical plants such as *Artemisia annua* has received attention as supplement to the feed to prevent or control coccidiosis in free-range broilers. The effect of medicinal plants on, for example, parasitic infections such as coccidiosis in poultry still needs to be investigated further.

In those countries where free-range layer flocks have been common, establishment of organic egg production has been relatively rapid, whereas organic broiler production has been much slower to develop, possibly due to the considerable higher price for organic broilers compared with that of conventional broiler products. As the difference in price between organic and conventional is particularly high in broiler products, this might probably deter consumers from buying organic broilers, unless quality of the product is correspondingly higher than that of the conventional broiler products.

According to the EU legislation, ages at slaughter for broilers depend on the growth rate and whether the parent stock is organic or not. In general, the use of slow-growing strains is preferred due to the fact that high growth rates often are positively correlated with different kinds of health problems such as dermal lesions and gait abnormalities. However, the regulation seems paradoxical in the way that a higher harvest age is required for fast-growing than for slow-growing broiler strains in order to motivate producers to use slow-growing strains. Broilers should be of at least 81 days if they are not slow growing, whereas slow-growing broilers can be harvested at 70 days of age. If the parent stock, in addition to the slow growth, is organic, then there is no age limit. In practice, there might be a risk that the use of broiler genotypes with high growth rates compromises animal welfare, which is in contrast to consumer expectation for organically produced broilers.

In the original form, the EU regulations for organic broiler production have been very much inspired by the French Label Rouge concept for broilers, despite the fact that Label Rouge not necessarily needs to be organic. However, the Label Rouge concept still maintains the demand for very slow growing, hardy broiler breeds, which are harvested between 81 and 110 days of age, whereas the EU regulations for organic broiler production allow another definition on slow-growing broilers, which, in practice, means that a slow-growing broiler will still grow faster than broiler strains in the French Label Rouge system. This also means that organic broiler production may vary between the EU member states. For example, organic producers of broilers in France, until now, use similar broiler genotypes as those used in the Label Rouge concept and other free-range systems, whereas other countries, such as Denmark and Sweden, use broiler strains with faster growth that have lower harvest ages and weights. These strains are even sometimes restricted in protein in the diet as they otherwise would grow too fast. This feeding strategy can, in some cases, lead to feather pecking or even cannibalism among the broilers and increase cost of production.

Product Quality

The term product quality as seen from the consumer's perspective covers a range of topics from nutritional, sensory, and functional properties of the product to food safety and the perception of the production methods used in the production process. Because trade with organic products is predominantly market driven, the attitudes and expectations of consumers are very important. Many studies and surveys carried out in Europe have shown that consumers choose to buy organic food because they think that this food is safer and organic production practices are better for the environment and animal welfare.

Nutritional, sensory, and functional properties of meat are very much dependent on the very specific feeding that takes place and the genotypes of the animals. Because these conditions vary much within organic production and within conventional production, the results of different comparisons vary accordingly and there is no evidence of essential and consistent differences in flavor or nutritional qualities between organic products and conventional ones. However, due to a more pronounced reliance on homegrown feed in organic production – a tendency to use other genotypes/strains that may be more adopted to the local environmental – and a less developed framework for management of traditional carcass quality parameters, the variation in organic products is more pronounced, and it is more difficult to obtain a defined carcass quality beforehand.

Perceptions of meat quality of consumers are, among others, strongly related to the geographical and cultural background, and consumers often prefer the meat quality they are familiar with. It has been suggested that long-term exposure to, for example, conventional broiler meat may be an obstacle to the liking of meat from other broiler products. Thus, conventional broiler meat is traditionally very tender, whereas meat from slow-growing broiler breeds used in organic systems may be less tender. However, some studies indicate that some slow-growing broiler genotypes reared in extensive organic production systems develop more tender and juicy meat when slaughter age is close to or has reached sexual maturity. In addition, new studies indicate that other sensory attributes like some taste and aroma attributes are more important to assessors in a sensory panel. Slow-growing broiler breeds with higher harvest ages are often associated with more intensive flavors, which many people perceive positively.

In regard to consumers' health, no epidemiological studies are available. With respect to chemicals, organic agriculture differs from conventional agriculture as it refrains from using synthetic agricultural inputs such as synthetic pesticides, herbicides, fertilizers, fungicides, veterinary drugs (antibiotics and growth promoters), synthetic preservatives, and additives. Thus, potential hazards posed by synthetic input residues are prevented to the extent possible.

Studies carried out to investigate the relative presence of pesticide residues in organic as opposed to conventional products show lower presence of pesticide residues in organic food, although organic food may not be defined as pesticide free. However, the health risk related to pesticides and veterinary drugs are generally considered much lower as compared with microbiological hazards. Also, there is no clear evidence to indicate that organic food is more prone to mycotoxin contamination than conventional food, and there is no evidence, at present, to support the assertion that organic animal food is more or less microbiologically safer than conventional food.

In conclusion, the most central quality parameter is the way of production, which offers some animal welfare and environmental benefits. The quality of individual products per se will be determined by the individual producer and processor.

Conclusion and Perspectives

Comparative production, product quality, and disease and welfare data from conventionally managed and organic livestock farms are scarce. Owing to their limited nature, results are often contradictory.

However, it appears that there are grounds to conclude that many of the concerns predicting serious health problems in organic livestock production have been overstated. However, the fact that animal health situation does not appear to be consistently better on organic than on conventional farms raises further questions to the extent of management and system changes that are actually implemented on livestock farms during a conversion to organic production.

The rearing of pigs and especially poultry is very different in organic systems compared with conventional ones. It seems that the idea of using better-adapted genotypes in organic production, is not really being explored and implemented.

All livestock production is land based in the sense that land is needed to supply feed for the livestock. However, a challenge for the organic production is that more land is needed to support a given meat output due to lower yield of fodder per hectare and often a slightly lower feed conversion compared with conventional production. Although the higher cost related to this in a farmer's perspective is compensated by a higher selling price, which in many cases, in fact, results in a higher net return to the farmer, the increased land use is a concern in a resource perspective.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment. Meat, Animal, Poultry and Fish Production and Management: Poultry. Nutrition of Meat Animals: Pigs; Poultry. Preslaughter Handling: Welfare Including Housing Conditions; Welfare of Animals. Species of Meat Animals: Cattle; Pigs; Poultry

Further Reading

Beharrell, B., MacFie, J.H., 1999. Consumer attitudes to organic foods. British Food Journal 93, 25–30.
Blair, R., 2007. Nutrition and Feeding of Organic Pigs. Oxfordshire, UK: CABI.
Blair, R., 2008. Nutrition and Feeding of Organic Poultry. Oxfordshire, UK: CABI.
Blair, R., 2011. Nutrition and Feeding of Organic Cattle. Oxfordshire, UK: CABI.
CEC, 2007. Council Regulation no 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing regulation no 2092/91. Committee of the European Communities, Brussel.
Hansen, L.L., Claudi-Magnussen, C., Jensen, S.K., Andersen, H.J., 2006. Effect of organic pig production systems on performance and meat quality. Meat Science 74, 605–615.
Hermansen, J.E., 2003. Organic livestock production systems and appropriate development in relation to public expectation. Livestock Production Science 80, 3–15.
Horsted, K., Allesen-Holm, B.H., Hermansen, J.E., 2010. The effect of breed and feed-type on the sensory profile of breast meat in male broilers reared in an organic free-range system. British Poultry Science 51, 515–524.
Horsted, K., Allesen-Holm, B.H., Hermansen, J.E., Kongsted, A.G., 2012. Sensory profile of breast meat from broilers reared in an organic niche production system and conventional standard broilers. Journal of Agricultural and Food Chemistry 92, 258–265.
Hovi, M., Sundrum, A., Thamsborg, S.M., 2003. Animal health and welfare in organic livestock production in Europe: Current state and future challenges. Livestock Production Science 80, 41–53.
Kongsted, A.G., Claudi-Magnussen, C., Hermansen, J.E., Horsted, K., Andersen, B. H., 2011. Effect of breed on performance and meat quality of first parity sows in a seasonal organic rearing system. Journal of the Science of Food and Agriculture 91, 2882–2887.
Kongsted, A.G., Hermansen, J.E., 2009. Sow body condition at weaning and reproduction performance in organic piglet production. Acta Agriculturae Scandinavica Section A-Animal Science 59, 93–103.
Kouba, M., 2003. Quality of organic animal products. Livestock Production Science 80, 33–40.
Strudsholm, K., Hermansen, J.E., 2005. Performance and carcass quality of fully or partly outdoor reared pigs in organic production. Livestock Production Science 96, 261–268.
Willer, H., Kilcher, L. (Eds.), 2011. The World of Organic Agriculture. Statistics and Emerging Trends 2011. Frick, Switserland: IFOAM, Bonn and FiBL. Available at: www.organic-world.net/yearbook-2011.html (accessed 07.11.13).

Poultry

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Glossary

Broiler A meat chicken usually killed at 5–8 weeks.
Brooding Caring for very young chicks often with a heat source.
Commercial enterprise A poultry farm that produces poultry in large numbers using modern methods.
Formulated feed A diet fed that meets the nutrient needs for meat production.
Grower diet A diet fed to meat chickens between 21 and 39 days or longer.
Hygiene The act of maintaining cleanliness.
Insecticides Chemicals that kill insect pests. It should be used with caution by following the instructions.
Litter A material placed on the floor to soak up excreta from chickens in order to keep the ground dry.
Medication A medicine or the act of treating with medicine.
Starter diet A formulated feed given from day 1 to 21.
Virus Minute organisms that can infect chickens and make them sick or lead to death.
Vitamins The essential organic elements in poultry diets for chick growth.

Chicken Meat Production Systems

The commercial intensive chicken meat industry is vertically integrated, incorporating company-owned feed mills, breeder farms, hatcheries, and processing plants. In this model chickens are produced under a contract in which the farmer provides the housing, equipment, litter, utilities, and labor, whereas the company provides the chicks, feed, medication, transportation, and technical/veterinary supervision. A modern intensive farm can comprise 8–40 houses, with each house growing 30–45 000 birds per batch, and birds reaching market weight within 5–6 weeks (Figure 1). Each house will grow 5–5.5 batches of birds annually, with each batch separated by a clean out and rest period.

Over the past several years demand for free-range chicken meat has risen markedly in many developed markets and as a result free-range farms have evolved from small mobile or fixed houses producing 500–10 000 birds/batch (Figure 2) to large sheds of more than 2000 m^2 housing approximately 35 000 birds that have access to shaded and vegetated outdoor areas via hatches or popholes that are open during daylight hours when feathering and weather conditions are appropriate.

Intensive meat bird houses are clear-span structures with litter on the floor and birds running free; cage systems are uncommon in meat bird production. The types of housing for growing meat birds includes controlled-environment houses that are designed to exhaust different volumes of air through the house depending on the weight and density of birds and internal temperature and humidity. Modern houses have full automation that links fans, evaporative coolers, and heaters to appropriate sensors to maintain the required house environment. Some intensive systems do not use evaporative cooling or heaters to control temperatures but do control ventilation.

Figure 1 Aerial view of commercial broiler enterprise. Courtesy of Australian Chicken Meat Federation (ACMF).

Figure 2 Meat birds foraging on free range broiler farm. Courtesy of Australian Chicken Meat Federation (ACMF).

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Older free-range chicken meat houses where birds are given access to forage pastures (Figure 2) are often naturally ventilated, relying on the farmers' experience to use heaters or fans to control the house environment. More recent free-range houses incorporate automatic mechanical ventilation and heating. Organic free-range production systems often require that meat birds not have access to forage areas where manufactured fertilizers, herbicides, or insecticides have been used, and not be provided with supplementary feed that contains synthetic additives.

The main management concerns and activities in intensive meat chicken production systems are biosecurity (keeping diseases out), preparing the house for placing chicks, assessing and monitoring chick and bird quality and health during their brooding and growing periods, vaccination, waterer and water management, ventilation, lighting, feeding, catching and transport, dead bird and waste disposal, and improving carcass quality.

Free-Range Systems

The free-range house provides dry litter, food, water, and protection from predators. Incandescent or fluorescent light (up to 12 h per day) is provided to birds to supplement the natural day length according to market specification for the product. Stocking rate in the house and for the range is defined by the prevailing Code of Practice, or determined by members of the local and national free-range associations in conjunction with welfare groups and government authorities. Chickens are reared on litter mostly in houses with open-sides or hatch doors, using gas or electric brooders, and birds will not leave the house to forage for 2 or 3 weeks until they are fully feathered and weather conditions are appropriate.

Free-range allows birds to forage (Figure 3) but there are risks to bird health and welfare, such as predation, fear of predation, exposure, diseases, cannibalism, and poor pasture management.

Adequate natural or artificial overhead cover is provided in the foraging areas to offer birds conditions akin to their native natural habitat, protection from overhead predators (e.g., hawks), and shelter from extreme weather conditions. Pasture is normally rested from poultry for 2 months between each batch and every third year in order to prevent parasitic build up and allow revegetation. If the free-range system is also an organic system, forage may need to be grown without use of manufactured fertilizers, herbicides, and insecticides.

In some organic systems, no synthetic additives are permitted in their manufactured feed and birds supplement their diet by foraging outdoors. 'Organic' is a term defined by law in some countries and organic meat producers are governed by a strict set of guidelines, relating to registration and certification, permitted and nonpermitted ingredients, the environment and conservation, processing and packing.

Sourcing breeds that are suitable for organic or free-range conditions can be difficult due to the underdeveloped nature of these systems, resulting in the use of breeds intended for intensive production. Slaughter age for organic birds may be 8–12 weeks but most modern breeds develop musculoskeletal problems at that age. There are some producers using slower-growing meat strains under contract to poultry companies; these modern strains of birds are often more active and grow slower in free-range systems and it is often claimed they develop a more distinctive meat flavor from consuming flavor-producing grasses, herbs, and insects.

Free-range foraging areas must provide continuous vegetation, shade, and protection from weather and predators, and must drain adequately. Portable houses must be continually moved. Grain crops, legume pastures, grasses, and herbs are good sources of forage for birds. Birds are reared in batches and processing commences by removing the most advanced birds at 5 weeks of age and then finishes with removal of the remaining birds at approximately 8–12 weeks, depending on the market requirement.

Intensive Systems

Housing

For intensive housing of meat birds, considerable thought and planning are required to establish a house and its surrounding infrastructure (Figure 4). Suitable sites must comply with local planning rules, and consideration has to be given to driveways, loading area, feed silos, work room, water supply, power and water supply, electrical systems, lighting,

Figure 3 Free range meat birds resting under shade of tree. Courtesy of Australian Chicken Meat Federation (ACMF).

Figure 4 Intensive chicken meat house. Courtesy of Australian Chicken Meat Federation (ACMF).

building type, insulation, cooling pads, roof system, curtains, sidewall inlets, placement of doors and ramps, computer systems with back-up alarm warning systems, back-up generators, feeders, waterers, heaters, and fans. There are three common house types: naturally ventilated, fan-assisted, and fully controlled environmental houses. Fully controlled environmental houses are designed to exhaust between 0.1 and 12 m^3/h/kg average bird weight of air through a house, and have full automation that links fans to sensors to maintain the required shed environment. Producers increasingly use computerized systems to remotely check and change settings in houses. Modern intensive houses are approximately 16 m wide and can range in length from 95 to 235 m. Optimum length for efficient use of fans, good air movement patterns, and effective evaporative cooling is 10 times the width of the house. Beyond a width to length ratio of 1:12, the ventilation system can be more effective if the house length is divided into half and fans placed at both ends with inlets in the center or vice versa.

Environmental control and automated systems must be adequately alarmed and there must be appropriate back-up systems. For example, a water tank, or an alternative water supply, sufficient for a minimum of 24 h should be made available and the system must include a reliable back-up power supply for force-ventilated houses.

Many older houses are being renovated to eliminate air leaks, upgrade insulation, or to install modern tunnel ventilation and evaporative cooling systems. Many growers are eliminating energy-wasting curtains in favor of solid sidewalls with insulated panel ventilation shutters. To minimize the consequences of back-up generator failure, panels are being installed that can either be easily removed, moved to one side or lifted up to allow natural ventilation of the birds, but the safest and most common option on new farms is a second back-up generator.

Insulation

The value of insulation is generally recognized in cold climates, where it can reduce heat loss by conduction and help minimize fuel usage. However, in warm climates insulation also protects birds from heat stress caused by solar heat gain through the roof. Under-roof insulation is essential in both open-sided and fan-ventilated houses. Even with the best ventilation systems, uninsulated houses can suffer mortality rates of 10–15% or more during times of extreme heat. Types of insulation used in poultry houses include spray-on polyurethane foam, blanket batts, polystyrene sheeting, shredded paper or blown-in mineral wool or fiberglass. External reflective roof coatings or white paints can also provide some benefit. Insulated panel is most commonly used for modern poultry houses and provides the most effective insulation. These panels comprise sheets of insulation covered on both sides by painted metal.

Ventilation

Ventilation requirements for birds change during the growing period, which has an influence on the way a house is ventilated. Most modern houses have several different ventilation systems. The two most common are exhaust fans and sidewall curtains.

Adequate ventilation is vital at all times to provide the birds with a constant and uniform supply of fresh air, and to remove moisture, gases and dust, all of which can carry microorganisms that are pathogenic to birds. Although high air speeds are needed to keep birds cool in summer, the system must be capable of reducing these speeds in cold weather to protect the birds from chilling, while still expelling pollutants. Mixing fans can reduce heating costs by moving the air in the house to achieve a warmer floor and uniform air temperature. The most important indicators used to assess environmental conditions within a house are odor and litter moisture. To maintain an appropriate environment for the birds, the farmer must take into consideration the number and speed of fans, temperature, and humidity. Noxious gases including ammonia, carbon dioxide, carbon monoxide, and hydrogen sulfide need to be kept below 20, 3000, 50, and 5 ppm, respectively, for bird and worker comfort and health.

Types of fan ventilation systems

Fans ventilate poultry houses by either pushing air in (positive pressure), or pulling air out (negative pressure) (Figure 5). A poultry house should be airtight to reduce air leakages and light leakages that can occur through damaged or loose curtains, or joining gaps in sidewalls, end walls, and ceilings. A 30% saving in fuel costs can result by improving house tightness, tested by conducting static pressure measurements. Polyurethane spray-on foam is often used to repair cracks and gaps in houses.

Tunnel ventilation

Tunnel ventilation systems use large exhaust fans to pull air into the house through inlets at one end, often through evaporative cooling pads, then down the length of the house, and out through the fans at the opposite end (Figure 6). When the fans are on, the air moving over the birds helps to keep the birds cool, with the effect increased when the evaporative pads are in use. Tunnel ventilation that incorporates a good minimum ventilation system is more efficient than the fully controlled-environment housing.

Figure 5 Tunnel ventilation fans. Courtesy of Australian Chicken Meat Federation (ACMF).

Figure 6 Meat chickens in tunnel ventilated house. Courtesy of Australian Chicken Meat Federation (ACMF).

Minimum ventilation

Minimum ventilation is used when the temperature outside is more than 1–2 °C lower than that required by chickens for optimum growth and feed conversion. It uses extraction fans to bring fresh air into the house via sidewall inlets at a low rate, which is sufficient to maintain the air quality while retaining enough of the heat generated by the chickens to keep the desired house temperature.

Natural ventilation

Some older houses without fans rely on natural air currents for ventilation through sidewall curtains or shutters. Natural ventilation can be used for growing chickens (3–6 weeks of age) when the ambient temperature is between 16 and 20 °C. The curtains or shutters are opened manually or adjusted automatically with a controller in response to internal temperature.

Heating and cooling

Forced-air furnaces and radiant heating are the two common methods of providing heat to young chicks. Hot air rises and ceiling fans are used to reduce the temperature differential between the floor and the ceiling in the house. Sidewall inlets (minivents) are used to direct air from outside toward the ceiling and this movement then pushes the hot air produced by the furnace to the floor. Radiant heaters provide heat in the form of infrared light to the floor instead of the air. Chicks can locate their desired temperature by moving to the appropriate area of the floor.

Figure 7 Cooling pads and pump. Courtesy of Australian Chicken Meat Federation (ACMF).

The most effective cooling systems are those that work using the air intakes and cool the whole house, such as foggers, misters, and evaporative cooling pads (Figure 7). The pads comprise perforated absorbent materials with water running through them. Orientation and spacing of buildings is another important consideration to reduce the overall heat load. Planting trees around the facility also provides shade and has other benefits such as providing a visual barrier.

Usually chicks are restricted to 30–50% of the house in the first 2 weeks. For newly hatched chicks, the temperature below which they cannot metabolize sufficiently is approximately 26 °C; thus there is a need to provide artificial brooding temperatures of 30–32 °C. This is gradually reduced as the target temperature for older birds lowers to approximately 21 °C. High temperatures (>35 °C) can present serious problems for meat birds, especially when interacting with other factors such as high humidity and high stocking density. As the birds get older, they are more susceptible to heat and cooling becomes more important. For example, 6-week-old meat birds commence panting at 23–26 °C and experience considerable heat stress at 27–29 °C. The ways in which birds can lose heat include (1) standing erect with the wings held away from the body and raising the scapular (shoulder) feathers, (2) vibrating the floor of the mouth cavity ('gular flutter'), and (3) panting. During hot weather, foggers, misters, and evaporative cooling can very effectively reduce heat stress on birds.

Feeding system

Bulk feed storage bins (silos) (Figure 8) are located outside the house and a centerless auger or dragline moves feed to the mechanical feeders that generally consist of 2–3 lines of feeders. There are two main automated feeding systems: pan

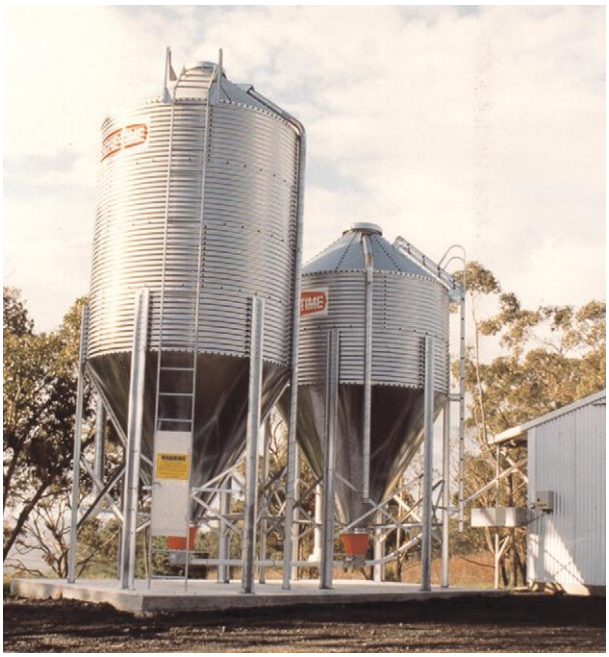

Figure 8 Bulk feed storage bins (silos). Courtesy of Australian Chicken Meat Federation (ACMF).

feeders (1 pan/65 birds) and tube feeders (1 tube/70 birds). The height of the feeders can be altered manually using feeder winches or remotely using switches. Before placement of chicks the feeders are located at ground level, with paper on the ground next to the feeders, and with a crumble feed on the paper for the first few days. This encourages birds to eat and locate feeders. At all stages of production, birds should receive diets with appropriate nutrients and particle size. Feed delivery equipment must be properly maintained and kept at the correct height, with the lip or trough of the pan level with the bird's back. The feed level within each pan is adjusted according to the age of the bird and feed requirements by using a feed flow adjustment fitted to each pan. At the end of the growing period, feeders are raised to the ceiling to allow catching and clean out. Manual feeding systems are more commonly used in older free-range systems, although farmers with newer or fixed free-range housing will use automatic feeding systems.

Drinking system

Birds must have continuous access to good quality water for optimum growth and efficiency. There are four types of waterers; nipple (with or without a drip cup); cup; bell (automatic circular waterer), and trough waterers. Troughs run lengthwise in the house and bell waterers are similar in principle to troughs. Birds drink from cup waterers by activating a small trigger that releases water into the bottom of the cup or from nipple waterers by reaching up and activating a trigger pin that releases water into the bird's mouth. To maintain the correct water pressure, a regulator is used in the drinking line and cable winches enable adjustment of the waterer height as the bird ages. Pressure in the nipple drinker system is reduced in the first few days to encourage chicks to peck at the drops on the nipple. Lighting is kept bright for the first few days to encourage birds to find water and learn to drink. Problems for waterers include air locks, leaks, uneven floors, and contaminants. Water meters can be used as a management tool to measure water intake, which can help diagnose production and health problems. Drinking water should be filtered unless prefiltered mains water is in use and kept cool in hot weather.

Lighting

There are usually two lines of dimmable lighting fixtures in a house, arranged so that light intensity at floor level is at least 20 lx for the first 3 days and 5–10 lx thereafter. The primary aim of artificial control of day length for meat birds is to increase growth rate by increasing feeding time. The lighting program determines when and for how long birds have access to the feeders. In recent years many poultry producers have changed from incandescent lamps to more energy-efficient and longer-lasting fluorescent lamps. Lighting programs vary widely from company to company and depend on the strain of bird used, housing type (naturally ventilated versus controlled environment), geographical location, and season. A small dark period is sometimes used to adapt birds to darkness in the event of a power failure. In some instances meat birds are generally given restricted access to feed to slow growth, in order to avoid physiological problems (e.g., leg weakness and heart attack) in their growing cycle. This is achieved by providing birds with a period of 4–14 h of darkness each day. To reduce power costs and improve feed conversion, intermittent lighting programs consisting of varying periods of light and darkness can be used. These light regimes tend to disrupt labor management due to the short periods of light/dark cycles and can only be used in lightproof housing. In dark-curtain tunnel houses light levels can be kept very low during the black-out periods.

Litter

Litter management is important for producing high-quality birds and for minimizing health problems. Respiratory infections resulting in lung lesions are increased by contaminants in the air; the most important being dust, bacteria, and ammonia. New poultry housing technology, such as nipple waterers with splash cups, mechanical ventilation, and moisture sensors that continually monitor litter moisture levels are providing the ability to more closely regulate litter moisture by varying air exchange rates in poultry houses. Dry litter certainly leads to easier handling at the end of the batch and less odor problems. It is common practice for broiler companies to include enzymes (mainly xylanase and lipase) in diets to minimize the possibility of wet droppings and to reduce excessive litter moisture.

A variety of litter materials are used, including wood shavings or sawdust, chopped straw, shredded paper, rice hulls, and sand. Collection by a contractor or an agreement with a fresh litter supplier or neighbor wanting manure as fertilizer for crop, viticulture and horticulture production is the normal means of disposing used litter. However, there is concern regarding heavy metals and pathogens in litter along with risk of excessive nutrients causing environmental harm, particularly to waterways. Alternative methods of utilizing the spent litter are via composting or burning. Poultry litter can be incinerated in furnaces to generate electricity either on-farm or

on a large scale at a regional location subject to local regulations concerning emissions.

The most environmentally friendly approach is to use an integrated biosystem. Methane can be generated using anaerobic digestion technology and utilized to generate power or heat, depending on economic feasibility to run such systems. *Gasification technology is* also being considered using *incomplete combustion* of litter *in a limited oxygen environment.* Stormwater *or* wastewater from house cleaning can be recycled after chlorination for use in cooling pads and for washing out.

Dust is uncomfortable for staff and birds and high levels can also lead to equipment failure and/or electrical fires. Factors that influence airborne dust levels include litter composition and moisture levels, bird activity, stocking density, and the amount of dust on surfaces and floors. Particles that affect health are generally in the invisible range (dried feces, feathers, skin, and litter) and in birds particles of 3–7 μm are deposited in the anterior region of the respiratory system whereas smaller particles are deposited throughout the rest of the respiratory system. Their adverse effects arise because they carry or incorporate bacteria, fungi, and gases. Planting trees around broiler houses has been demonstrated to effectively reduce pollutant emission and improve odor dispersion. The trees filter dust, feathers, odor, and noise and direct the pollution plumes upwards and improve dispersion. Vegetation cover around broiler farms also provides shade on buildings resulting in reduced thermal load on the sheds and a reduction in the electricity and water costs to cool the sheds in summer. Tree barriers placed in front of outgoing air plumes (especially from tunnel-ventilated sheds) can reduce the likelihood of cross-infection via airflow between different sheds, as airborne dust and pathogens attached to the dust particles will be filtered out by the barriers on impact.

House and bird management

A clean house before chick placement is an essential management task that impacts on both production and welfare. A clean house reduces levels of pests, parasites, rodents, insects, mites, roundworms, coccidia, other microbial pathogens, and dust. Grass and lawn areas surrounding the house should be cut regularly and unwanted equipment discarded to reduce build up of vermin. In commercial production, a contract cleaning crew is often used to clean and sanitize the house. After each batch of chickens, old litter is removed from the shed and cobwebs and dust are washed off walls and ceilings. An appropriate disinfectant is then used on walls and equipment. Cresols are often used as floor disinfectants, chlorine compounds for relatively clean surfaces, synthetic phenols for the floor and equipment, and quaternary ammonia compounds for walls. All feed residues are removed from the shed and the drinking lines flushed and sanitized.

Birds are placed within 8 h of hatching, depending on the distance of farms from the hatchery. Day-old birds are delicate and require gentle handling at placement. They are normally placed close to the waterer lines to encourage them to locate water as quickly as possible (Figure 9). A skilled farmer can tell from the appearance of birds whether they are healthy. Careful observation of birds in the first few days is essential to ensure their health and make sure that an appropriate thermal environment is provided. Maintaining a high-health status is essential and vaccination programs are used to prevent some diseases. During the first week there are normally 4–6 inspections per day and 2–3 daily inspections thereafter.

Figure 9 Day old chicks next to drinkers. Courtesy of Australian Chicken Meat Federation (ACMF).

To ensure that body weight is meeting the target for both growth and evenness, a sample of birds is weighed regularly after week one to determine the coefficient of variation, which is an indicator of the uniformity of the flock. An electronic scale with a weighing platform is located in the shed and records the weight of birds automatically when they stand or sit on the platform.

Recommended stocking densities for broilers vary around the world, with a range from 28 kg m^{-2} in summer to 40 kg m^{-2} in winter depending on the capability of the ventilation system. A high stocking density may place physical restriction upon movement; distance traveled decreases with age and with increasing stocking density.

Removal and correct disposal of dead/culled birds are important to prevent build up of pathogenic microorganisms, transmission of disease to healthy birds, and odors. Common methods for disposal include contained composting onsite or freezing and subsequent regular collection for off-farm disposal, burial in properly covered pits, or on-farm incineration. Composting is done in rotary or sealed containers, which are aerated mechanically, or in piles of litter.

Broilers are often caught at night for slaughter the following day. A pick-up crew comprises 6–10 catchers and a forklift driver, who load up to 7000 birds in containers onto a semi-trailer. Feeder and drinker lines are raised, lights are dimmed to keep the birds calm, and portable netting may be used to contain and minimize disturbance to birds. The catchers pick-up birds, hold them by one or both legs, and place them in containers. Some companies utilize mechanical harvesters to automatically catch birds and transfer them to containers. The lines that are raised depend on the house layout and the percentage of birds taken for processing. If it is a small pick-up, some farms may raise the center line of feeders and take the birds from the center of the house. If it is a single sex pick-up in a house where male and females are grown in separate halves of the house, the feeders and waterers in that half of the house will be raised. It is common practice to pick-up birds by 5 a.m. and to transport birds to the processing plant within 2 h; however, if the weather is hot, pick-up should be finished by dawn and birds transported immediately.

The major risk to birds from the time of pick-up are overheating or chilling. In hot weather, this is minimized by pick-ups occurring mostly in the late afternoon/evening/night when the thermal load is reduced. There are some recommendations for fully enclosed, ventilated, and alarmed trucks to allow birds

to be safely transported during the day in cold or hot weather without exceeding or going below their thermal limits.

Conclusion

The intensive chicken meat industry uses modern technology in all aspects of housing and management to produce chicken meat at least cost. Alternative production systems use some of the technical developments made in poultry housing to improve bird management and reduce cost of producing free-range meat chickens.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Growth of Meat Animals: Growth Patterns. Manure/Waste Management: Manure Management; Waste Management in Europe. Meat, Animal, Poultry and Fish Production and Management: Disease Control and Specific Pathogen Free Pig Production; Meat Production in Organic Farming. Nutrition of Meat Animals: Poultry. Preslaughter Handling: Preslaughter Handling; Welfare Including Housing Conditions; Welfare of Animals. Professional Organizations. Species of Meat Animals: Meat Animals, Origin and Domestication; Poultry

Further Reading

Appleby, M.C., Hughes, B.O., Elson, H.A., 1992. Poultry Production Systems. Behaviour, Management and Welfare. Wallingford, CT: CAB International.

Barnett, J.L., Glatz, P.C., Almond, A., *et al*., 2001. A Welfare Audit for the Chicken Meat Industry. Canberra: Rural Industries Research and Development Corporation.

Bell, D.D., Weaver, W.D., 2001. Commercial Chicken Meat and Egg Production, fifth ed. Los Angeles, CA: Kluwer Publishers.

Bottcher, R.W., Baughman, G.R., Magura, J.R., 1996. Field measurements of fan speed and power use in poultry houses. Journal of Applied Poultry Research 5 (1), 56–62.

Bottcher, R.W., Czarick, M., Lacy, M.P., Baughman, G.R., 1992. Evaporative efficiency of a fogging fan for poultry. Applied Engineering in Agriculture 8 (6), 855–860.

Food and Agriculture Organization of the United Nations, 1996. Biogas technology: A training manual for extension. Consolidated Management Services Nepal (P) Ltd. Available at: http://www.betuco.be/biogaz/Biogas%20fao.pdf (accessed 26.07.13).

Kilgour, R., Dalton, D.C., 1984. Livestock Behaviour: A Practical Guide. London: Granada Publishing Ltd.

Leonard, J.J., Feddes, J.J.R., McQuitty, J.B., 1984. Air quality in commercial broiler housing. Canadian Journal Engineering 26 (1), 65–70.

Nahm, K.H., Nahm, B.A., 2004. Poultry Production and Waste Management. Korea: Yu Han Publishing Co. ISBN: 89-7722-623-6.

Poultry Science Association, 2009. Research demonstrates effectiveness of trees and shrubs in reducing odours, dust and ammonia from poultry farms. Available at: http://www.poultryscience.org/pr092809.asp (accessed 26.07.13).

Weaver, W.D., Meijerhof, R., 1991. The effect of different levels of relative humidity and air movement on litter condition, ammonia levels, growth and carcass quality for broiler chickens. Poultry Science 70, 746–755.

Weeks, C.A., Nicol, C.J., Sherwin, C.M., Kestin, S.C., 1994. Comparison of the behaviour of broiler chickens in indoor and free-range environments. Animal Welfare 3, 179–192.

Wood-Gush, D.G.M., 1971. The Behaviour of the Domestic Fowl. London: Heinemann Educational Books Ltd.

Relevant Websites

http://www.poultryhub.org/production/husbandry-management/housing-environment/
Australian Poultry CRC Poultry Hub.

http://www.poultryhub.org/production/husbandry-management/poultry-behaviour/
Australian Poultry CRC Poultry Hub.

http://www.poultryhub.org/production/husbandry-management/the-principles-of-poultry-husbandry/
Australian Poultry CRC Poultry Hub.

http://www.poultryhub.org/production/husbandry-management/welfare/auditing-animal-welfare/
Australian Poultry CRC Poultry Hub.

http://www.poultryhub.org/production/husbandry-management/welfare/welfare-standards-for-the-chicken-meat-industry/
Australian Poultry CRC Poultry Hub.

Red Meat Animals

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Glossary

Common Agricultural Policy (CAP) It is a system of agricultural subsidies existing in the European Union and available to all Member States (28 countries as of 2013).
Cysticercosis Parasitic disease caused by the larval stage of some cestodes of the *Taenia* genus.
Food and Agriculture Organization of the United Nations (FAO) A United Nations' agency aiming to improve food security, food safety, and reducing rural poverty.
Maasai Seminomadic ethnic group that is found in areas of Kenya and Tanzania.
Outdoor systems Production system also described as extensive, which requires lower capital investment for housing and where food producing animals are allowed outside access, including contact with soil and growing plants.
Pastoralism A production system widely used in areas with a low availability of natural resources (water and pastures), and usually has a nomadic component.
Trichinellosis Zoonotic parasitic disease caused by encysted larvae of *Trichinella spiralis* in striated muscle in humans.
World Organisation for Animal Health (OIE) An intergovernmental organization responsible for improving animal health, animal welfare, food safety, and biodiversity worldwide.

Introduction

The production systems currently in use for raising animals for meat production are not homogeneous across the world. Differences can be found among countries, and even among regions of a country. This variability is not influenced by a single common cause, but it is multifactorial (geography, climate, culture, and political factors).

Because some animal species are more suitable for specific terrain conditions than others, and there are space restrictions for animal production (competition for human living space, crop production, etc.), geographic conditions are relevant when analyzing production systems. For example, Argentina (pampa region) and Brazil (central states) have large and fertile areas that are used for extensive production of cattle (Table 1). However, small countries, such as the Polynesian islands, due to the limited areas available for animal breeding, rely mostly in raising pigs and small ruminants (Table 1). The same is true for some Mediterranean countries where their abrupt terrain is more adequate for small ruminants than cattle (Table 1).

Climate can also be a driver in the animal production method chosen and in the species more suitable to be raised. For example, countries located in North Africa will be able to raise mostly sheep and goats, which are considered to be more 'rustic' animals and will be able to better endure the harsh environmental conditions of the Sahara (Table 1). However, the Sahel region has higher annual precipitations, and hence grassing areas can be found that can support cattle as well as, in general, a higher number of livestock (Table 1).

Cultural factors play an enormous role in the way in which animals will be raised and in how animal products will be used. Certainly, raising cattle, pigs, or small ruminants is performed mostly with the intention of obtaining a food product for human consumption (meat or milk) as a result, but the fact that an animal will be raised for production does not necessarily mean that it is intended to be harvested.

An example of cultural differences in animal production is India, where cattle are considered sacred for the Hindu religion. Cattle in India is used mostly for milk production, and most of the owners will have only a small number of animals. Additionally, several states in India have outlawed the opening of cattle slaughterhouses. Nevertheless, in India there are a large number of people who do not profess Hindu religion and they will be open to beef consumption. This poses a problem with animal welfare, as cattle will have to stand long hours of transport in order to reach states where cattle abattoirs are allowed to exist. At the same time, because cattle have not been bred with the intention of meat production, meat quality and meat yield are poor, resulting in a low economic return.

Pig production is another example where cultural factors influence animal production. In pigs, genetic selection has improved their feed conversion, and most of the commercial production will be driven by intensive indoor production systems. However, though pigs can get a commercial weight faster than cattle and have a more desirable meat yield, due to religious factors, pig breeding is almost inexistent in some areas, such as North Africa and Middle East countries (Table 1).

Political factors have also influenced animal production. For example, the Common Agricultural Policy of the European Union (EU), as well as the subsidies given by the New Zealand (NZ) government in the 1970s, did encourage animal production and increased competitiveness of the EU and NZ in the world meat market. Though some countries still give economic incentives, these have been reduced or eliminated in the EU and NZ, pushing producers to increase profitability by

Table 1 Number of livestock (cattle, pigs, sheep, and goats) per geographical area

Area	*Cattle*	*Pigs*	*Sheep*	*Goats*
Africa (total)	246 721 712	32 216 091	255 481 282	276 684 030
Eastern Africa	130 312 626	10 785 915	59 674 832	95 540 636
Middle Africa	22 394 370	6 187 100	9 662 960	25 441 400
Northern Africa	10 355 730	29 450	62 121 630	18 708 480
Southern Africa	20 063 300	1 780 070	28 687 800	11 430 050
Western Africa	63 595 686	13 433 556	95 334 060	125 563 464
America (total)	515 593 103	162 355 973	93 101 675	37 678 479
North America	104 838 100	79 146 800	6 379 050	3 026 350
Central America	46 871 360	20 485 690	8 874 658	9 193 360
Caribbean	9 224 838	3 556 306	2 655 547	3 699 205
South America	354 658 805	59 167 177	75 192 420	21 759 564
Asia (total)	477 129 242	575 936 637	463 575 597	539 178 357
Central Asia	20 822 930	1 527 896	48 885 570	10 749 749
Eastern Asia	93 526 802	491 255 327	154 691 619	162 025 240
Southern Asia	292 366 400	10 710 500	168 534 330	309 719 910
South Eastern Asia	49 075 922	71 544 760	12 327 735	27 690 537
Western Asia	21 337 188	898 154	79 136 343	28 992 921
Europe (total)	121 203 563	187 357 088	127 306 839	17 072 238
Eastern Europe	39 584 812	54 749 843	33 522 108	4 722 128
Northern Europe	22 720 931	24 309 500	40 281 360	203 720
Southern Europe	17 054 195	43 970 557	42 071 687	10 023 611
Western Europe	41 843 625	64 327 188	11 431 684	2 122 779
Oceania (total)	39 260 755	5 178 398	104 247 240	4 917 080
Australia/NZ	38 527 100	2 611 998	104 231 100	4 585 970
Melanesia	670 500	2 113 000	15 700	289 200
Micronesia	14 140	53 800	–	4800
Polynesia	49 015	399 600	440	37 110
World total	1 399 908 375	963 044 187	1 043 712 633	875 530 184

Source: FAOSTATS (2013) FAO. Available at: http://faostat3.fao.org/home/index.html (accessed 15.04.13).

genetic selection or changes in management. Political decisions restricting markets can also affect production, as it happened with Argentina's restriction to beef exports in the middle of the last decade, which resulted in a change of use of land with farmers stopping beef production, thereby reducing the Argentinian cattle stock due to lower returns they received in the market.

Animal production is closely related to food security, and that concept is linked to animal welfare, animal health, and food safety. Hence, although some countries or economic blocks (such as the EU) have a strong legislation on the protection of animal welfare, these regulations are always linked to the production of safe and affordable food from animal origin for human consumption.

According to the World Organisation for Animal Health,

> "an animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress."

That definition also includes that

> "good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing."

Overall, animal welfare is a very important factor to consider for every different animal production system because high levels of welfare will reflect in a better production output. But, to decide what is appropriate welfare is not always easy and it will depend on the way in which all aspects of animal production are assessed. For example, arguably in developing countries where pastoralist production systems are in use, animal welfare might have lower standards than in developed countries because animals may not be provided with the same high level of biosecurity or animal health preventative management measures (i.e., vaccination). Nevertheless, pastoralism might allow animals to express more of their natural behavior and have lower levels of stress than animals raised in intensive production units.

Cattle Production Systems

Cattle for meat production are bred mostly in extensive production systems (Figures 1 and 2), in areas with availability of large grazing lands. Some countries use intensive systems, or a combination of both, intensive and extensive systems, where feedlots are normally used for the finishing of the animals after raising them in pastures (Figure 3).

Though most of the beef cattle are bred in extensive systems, it does not mean that beef cattle are raised only in large

Figure 1 Beef cattle grazing in the Peak District, Derbyshire, UK.

Figure 2 Beef cattle (Angus) grazing in the Ňuble province, Chile.

Figure 3 Beef cattle (Longhorn), Peak District, Derbyshire, UK.

herds. Beef production is largely intensified or concentrated in many countries, and still traditional production systems are in use (Figure 4).

Figure 4 Cattle in a traditional production system, Linares, Maule, Chile.

Table 2 Animals (head)

Rank	*Cattle*	*Pigs*	*Sheep*	*Goat*
1	Brazil	China	China	India
2	India	USA	India	China
3	USA	Brazil	Australia	Pakistan
4	China	Vietnam	Iran	Nigeria
5	Ethiopia	Germany	Nigeria	Iran
6	Argentina	Spain	UK	Ethiopia
7	Pakistan	Russia	New Zealand	Indonesia
8	Mexico	Mexico	Pakistan	Mali
9	Colombia	France	Ethiopia	Mongolia
10	Australia	Poland	South Africa	Tanzania

The total number of cattle kept in the world is over a billion animals (Table 1). Regarding distribution of cattle, the Americas have the largest proportion (36% of world total), followed by Asia (34%), Africa (17.6%), Europe (8.65%), and Oceania (2.8%) (Table 1). In fact, cattle distribution is not homogenous, and only 10 countries (6 of them in the Americas) concentrate nearly 60% of the estimated world cattle population (Table 2).

Cattle, nevertheless, are not necessarily bred for meat consumption, because in some countries cattle could have a higher economic value in dairy production, or as working animals (transport/field) (Figure 5). Nevertheless, at the end of their production life most of the dairy and working cattle will also be used for meat consumption. But, as that is a secondary use for these animals, management, feeding, genetic selection, etc. are not focusing in getting high-value meat or high yields. A clear example of that is India, which possess the world's second largest cattle population (Table 2); however, the level of beef production in India (world number 11) is below Germany (world number 10), even though the latter has a cattle herd size equivalent to only 6% of the one in India. One of the reasons for that differential is because a large segment of Indian population professes Hinduism, a religion that considers cattle as sacred, meaning that beef consumption is forbidden. Cattle in India are used mostly for milk production

and for work, and though some beef is produced for local consumption, most of it will go for the export market albeit. However, owing to the previously described factors, it will have poorer yields when compared to net beef producing countries.

Brazil, Argentina, and Uruguay are some of the most important players in beef international trade, and although part of their agricultural sector still rely on animal work, a large percentage of their economy depends on exports of agricultural products, including beef. Hence, nutrition, farm biosecurity, animal health and welfare, as well as traceability are of great relevance for them. Cattle in these countries are raised mostly in extensive systems. But, although the availability of large grassing areas gives them an advantage by allowing them to sustain large herds, for a long time they were subjected to restrictions for international trade due to problems to control notifiable diseases (specifically foot and mouth disease (FMD)), and lack of traceability. Nowadays, the progress made in herd health status, the negligible risk status for some zoonotic diseases, as well as improvements in management and animal welfare have allowed these countries to open new markets.

There are threats for beef-producing and -exporting countries. One of the most critical is the competition for the use of land. The demand for bioenergy production has resulted in many areas previously used for grazing cattle to be turned instead into areas for crops, hence reducing the animal load that the countries can sustain. The use of grains for bioenergy also pushes higher the prices of animal feeding, affecting especially intensive production systems. All that has translated into a reduced world livestock number, lower returns for the beef farming sector, and higher meat prices for consumers.

Finally, pastoralism is still an important production system used in large areas, particularly in developing countries. More than half of the cattle in Africa is located in Eastern Africa (Tables 1 and 2), where pastoralism is one of the most common cattle production systems. However, as for the case of India, a large proportion of their cattle is not necessarily kept for beef production. The reason for keeping large herds relies on cultural factors, as for some communities, such as the Maasai, ownership of cattle represents a symbol of status; hence the larger the herd, the more important the person will be in his community.

Owing to reduced human intervention when compared with commercial beef production systems, pastoralism arguably can offer a better state of welfare for the animals. However, animals in pastoralist systems are also more susceptible to environmental changes, such as droughts, which regularly affect the East Africa region (as in 2010–11). These systems are also exposed to a higher risk of diseases because of lack of immunization or due to exposure to wild reservoirs which maintain the disease (i.e., FMD in wilder beast and buffalo in some areas of Africa).

As a result, animals that do go to beef market might not have adequate conformation, have a poor yield, or they might have a high percentage of rejections during postmortem inspection.

Regardless of the problems previously described, without a doubt, pastoralism is essential for food security in isolated, impoverished communities. Keeping cattle allows them to self-sustain and gives them potentially a source of extra income in case of need. Lack of quality and safety, however, mean that cattle raised in this system cannot currently become an important player in the international beef market.

Sheep and Goats Production Systems

As for beef cattle, most of the small ruminant production is carried out in extensive systems. Sheep production for meat is especially found in extensive systems and can be found either in large grazing units (Figure 6), or small-/medium-size units (Figure 7). Regarding goat production, most of it is done in small units (mostly for milk production) and in pastoralist systems.

The total number of small ruminants in the world is approximately 2 billion animals (Table 1). Regarding distribution of sheep, Asia has the largest number (44.4%), followed by Africa (24.5%), Europe (12.2%), Oceania (10%), and the America (8.9%). For goats, Asia also has the largest number of animals (65.6%), followed by Africa (31.6%), the Americas (4.3%), Europe (1.9%), and Oceania (0.6%).

Figure 5 Cattle as working animals in Chennai, Tamil Nadu, India.

Figure 6 Sheep grazing in the Peak District, Derbyshire, UK.

Figure 7 Sheep in the Arauco province, Chile.

From the species covered in this article, the combined number of small ruminants (sheep and goats) represents the largest number of food producing red meat animals worldwide (Table 1). Owing to their more resistant nature to environmental changes and lower nutritional requirements, compared with other farm animal species, small ruminants are mostly kept in developing countries where environmental conditions are less suitable for keeping cattle, access for feeding for an intensive system is more difficult, or there are cultural issues for pig production.

Although most of the small ruminants are farmed in extensive systems, there are a large number of sheep and goats that are raised in pastoralist systems in Asia and Africa (Tables 1 and 2). In these areas, most of the small ruminants are kept as a source of milk. The production of meat is usually the result of the slaughtering of old females and males, which are surplus to the dairy herds.

Although mostly not produced in a commercial setting, sheep and goats kept in pastoral systems provide a steady source of protein for the diet of a large percentage of impoverished and transient population. This is particularly important when considering goats in Africa, where they represent, proportionally, the largest share of food-producing animals for the continent (34% of the red meat animals species in Africa are goats (Table 1)), and there is approximately one goat for every four people.

Traditional sheep and goat breeds in Africa show more resistance to the mostly dry environmental conditions where they are bred than their European counterparts. However, as with other species, a pastoralist system does have constraints in animal health, nutrition, and management. Certainly, in order to increase the availability of food for these communities as well as to increase the chances to access the markets and get better returns, genetic improvement would be needed; however, genetic improvement on its own is not enough when the other limitations still exist.

Meat production from small ruminants as a surplus of dairy production is not only restricted to pastoralist systems but it is also true for some developed countries that rely mostly on intensive and semiintensive systems for small ruminants' production. Here, housing, general management, feeding, and genetic improvement are essential. An example is Cyprus, where climatic conditions and landscape do not provide natural grass areas. In that country, the agricultural sector focuses in milk production in order to provide fresh liquid milk for human consumption as well as for cheese production for local consumption and exports. Because of cultural preferences, in Cyprus, approximately a third of the raw milk produced comes from sheep and goats. Males are used for meat and are normally harvested at a more tender age (3–5 months) than in meat production systems. Genetic selection and management interventions target better milk yields rather than meat production.

Table 3 Indigenous production of meat

Rank	*Beef*	*Pork*	*Lamb*	*Goat meat*
1	USA	China	China	China
2	Brazil	USA	Australia	India
3	China	Germany	New Zealand	Nigeria
4	Argentina	Spain	UK	Pakistan
5	Australia	Brazil	India	Bangladesh
6	Mexico	Vietnam	Turkey	Iran
7	France	Canada	Syria	Mali
8	Russia	Russia	Algeria	Indonesia
9	Canada	Netherlands	Russia	Niger
10	Germany	France	Nigeria	Ethiopia

However, Australia, NZ, and the UK are three of the most important players in lamb production at world level (Tables 2 and 3). They have large sheep herds that are intended for meat production, and use mostly extensive sheep farming methods, with large herds, and high seasonality for their production. In the past, the profitability of sheep farmers in these countries depended largely on wool prices, but current meat prices are the drivers for sheep farming. Genetic selection, nutrition, innovation, and animal health among other factors, have been pivotal for them to keep competitiveness in the international meat market.

Although developed countries using extensive and intensive commercial production systems do not face as many challenges as pastoralist systems, because they can control better animal health risks, environmental impact, such as CO_2 print, presents new constraints. Small ruminants are not as efficient producing meat as pigs and poultry, meaning that, for similar meat yields, CO_2 production in sheep farming is higher. That is particularly critical when legislation in developed countries is targeting the reduction of CO_2 emissions, and consumers are willing to pay for environmental friendly produce.

Pig Production Systems

Pork has become a very popular meat and a very good source of animal protein for human diets. A factor associated with pork becoming so relevant in the international meat trade is that pig production cycles are shorter than the ones for cattle and small ruminants, meaning that production improvements related to changes in management and genetics can be obtained faster.

The total number of pigs in the world is approximately 1 billion animals (Table 1). Regarding distribution of pigs, Asia has the largest proportion (59.8%), followed by Europe (19.5%), the Americas (16.9%), Africa (3.3%), and Oceania (0.5%).

Several developed countries are currently using outdoor systems (Figure 8) for pig production. However, most of the world's commercial pig production is carried out in intensive large indoor units (Figures 9 and 10). Also, it is possible to find small producers, either owning small commercial units or subsistence farmers owning backyard pigs.

As with cattle and beef production, the world distribution of pig population and pork consumption is influenced by several factors, with religion being one of the most relevant. For some religious groups, pigs are regarded as 'dirty animals,' hence in some countries pork is a forbidden meat for the majority of the population. Pigs are only bred for meat production, and so the relevance of pig breeding in areas where pork is not consumed is either low or negligible.

The relevance of religion in the world distribution of pig farming can be appreciated in Table 1. The North African region accounts only for 0.09% of the total African domestic pig population. In Asia, the continent that accounts for more than half of the world pig population, the production concentrations is in the Eastern and South Eastern subregions, whereas regions with a majority of Muslim population, such as Western Asia, accounts only for 0.15%, and Central Asia represents 0.26% of the total for the continent.

Although low in numbers, pig farmers can be found in countries with a majority of Muslim population. However, pig production in these areas is generally, but not always, confined to small communities from other religious groups, with pigs bred in small, mostly backyard production units. The method of breeding pigs in these regions and the limited resources available can have a detrimental effect on animal health and welfare and also affect human health. For example, in these countries, there is a lack of veterinary professionals trained or with experience in treating/identifying pig diseases, there is not enough expertise in order to identify management problems and suggest solutions. They also lack medicines and vaccines specifically to be used in pigs; they have problems with biosecurity and poor access to genetic improvement. Also, the higher level of exposure of pigs to zoonotic diseases, linked to the lack of official ante- and postmortem inspection (home killing), can result in a higher risk of human foodborne outbreaks.

Problems in pig production related to animal health and welfare are not restricted to countries where pigs are not raised commercially. China, the world's largest pig producer, has been involved in several problems in the present year (2013). Pork has been sold from carcasses that were intended to be collected by the government for disposal after dying on farm; at the same time approximately 20 000 pig carcasses were found floating in Huangpu river in Shanghai. These situations do not only result in human health risks, but also generate public scare, and affect the pig industry as a whole as the consumer reduces the demand for pork. Additionally, it does raise concerns about the morality of pig industry and the intensive production system.

Out of the 10 countries with the largest pig herds in the world, 5 are the EU member states (Table 2); the same applies to the ranking of the largest pork producers (Table 3). These

Figure 8 Outdoor pig fattening unit, Parral, Chile.

Figure 9 Indoor pig fattening unit, O'Higgins region, Chile.

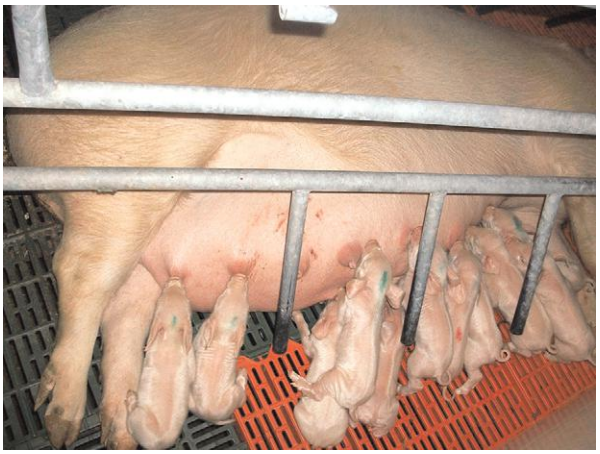

Figure 10 Sow and piglets in intensive pig production, O'Higgins region, Chile.

countries use intensive pig production systems either indoor or outdoor. The high level of biosecurity used and the strength of their veterinary services has successfully helped to eradicate most of the diseases that produce a negative impact in international trade, animal health, and human health. Diseases, such as trichinellosis and cysticercosis, do represent a negligible risk in these systems, and new cases mostly appear only in impoverished areas where backyard pigs are still kept, or associated with wild boar hunting.

To protect animal health and welfare current the EU legislation has strict requirements for breeding pigs in intensive systems. For example, piglets cannot be weaned before 28 days of birth, and males cannot be castrated without using anesthesia and veterinary supervision in piglets older than 7 days. Additionally, from 1 January 2013 there is an EU-wide ban on the keeping of sows in close-confinement stalls from 4 weeks after service.

Higher pig welfare standards result in lower loses due to diseases and a better final product. However, every intervention in the system does have an associated cost. These costs make the production of pork more expensive in highly regulated countries or economic blocks as the EU than in countries where strong legislation in animal welfare might not be available. Additionally, there is a high cost burden associated with feeding costs, and, as it has been discussed earlier in this article, the current high price for grains affects intensive production systems.

Overall, due to genetic selection, pig farming is a very efficient way to produce meat for human consumption. There are threats that can affect the industry, such as the use of grains in pig feeding when these could be used for human diet otherwise. However, the flexibility and variety of systems that can be used in pig farming in order to fit the local resources, as well as the public demands, make this species a very good choice regarding food security.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Growth of Meat Animals: Growth Patterns. Meat, Animal, Poultry and Fish Production and Management: Meat Production in Organic Farming. Preslaughter Handling: Welfare Including Housing Conditions. Quality Management: Farm Level: Pork Quality; Farm Level: Safety and Quality of Beef. Species of Meat Animals: Cattle; Meat Animals, Origin and Domestication; Pigs; Sheep and Goats

Further Reading

Ademosun, A.A., 1994. Constraints and prospects for small ruminant research and development in Africa. In: Lebbie, S.H.B., Rey, B., Irungu E.K. (Eds.), Small Ruminant Research and Development in Africa. Proceedings of the Second Biennial Conference of the African Small Ruminant Research Network AICC, Arusha, Tanzania 7–11 December 1992. The Technical Centre for Agricultural and Rural Co-operation. Addis Ababa: International Livestock Centre for Africa.

EC, 2008. Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs. Official Journal of the European Union L 47, 5.

EFSA, 2012. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA Journal 10 (3), 2597.

FAOSTATS, 2013. FAO. Available at: http://faostat3.fao.org/home/index.html (accessed 15.04.13).

Gathogo, S.M., Kuria, J.K.N., Ombui, J.N., 2012. Prevalence of bovine tuberculosis in slaughter cattle in Kenya: A postmortem, microbiological and DNA molecular study. Tropical Animal Health and Production 44 (7), 1739–1744.

Kaiman, J., 2013. China arrests 900 in fake meat scandal. The guardian (online) 3 May. Available at: http://www.theguardian.com/world/2013/may/03/china-arrests-fake-meat-scandal (accessed 01.06.13).

Karlikaya, G., Çiftçi, B., Bulut, H., Orken, C., Tireli, H., 2005. Neurotrichinosis. A case report from Turkey. Journal of Neurological Sciences 22 (1), 75–78.

Mavrogenis, A.P., Antoniades, N.Y., Hooper, R.W., 2006. The Damascus (Shami) goat of Cyprus. Animal Genetic Resources Information 38 (2), 57–65.

Ministry of Agriculture and Forestry, 2009. Meat: The Future. Opportunities and Challenges for the New Zealand Sheep Meat and Beef Sector Over the Next 10 to 15 Years. Wellington: Ministry of Agriculture and Forestry.

OECD/FAO, 2013a. OECD–FAO agricultural outlook 2011–2020.

OECD/FAO, 2013b. OECD–FAO agricultural outlook 2013–2022.

OIE, 2012. Introduction to the recommendations for animal welfare. In: Terrestrial Animal Code (Chapter 7.1).

OIE, 2013a. Resolution N 17: Recognition of the foot and mouth disease status of member countries. 81st General Session May 2013.

OIE, 2013b. Resolution N 20: Recognition of the bovine spongiform encephalopathy risk status of member countries. 81st General Session May 2013.

Pozio, E., 2007. World distribution of *Trichinella* spp. infections in animals and humans. Veterinary Parasitology 149, 3–21.

United Stated Department of Agriculture (USDA), 2013. Livestock and poultry: World markets and trade. Available at: http://www.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf (accessed 01.06.13).

Relevant Websites

http://www.bpex.org.uk/
British Pork Executive (BPEX).

http://www.eblex.org.uk/
English Beef and Lamb Executive (EBLEX).

http://faostat.fao.org/site/569/default.aspx#ancor
FAOSTATS. Food and Agriculture Organization of the United Nations (FAO).

http://www.fao.org/livestock-environment/en/
Food and Agriculture Organization of the United Nations (FAO).

http://www.ers.usda.gov/topics/animal-products/animal-production-marketing-issues.aspx#.Uoz8HvlSjec
United States Department of Agriculture (USDA).

MEAT-BORNE HAZARDS, CONCEPTS AND METHODS FOR MITIGATING RISKS RELATED TO

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Glossary

Attribute Characteristic that can be quantified during an evaluation of a surveillance system. Examples of attributes include cost, benefit, sensitivity, and timeliness.

Coverage Proportion of the population of interest (e.g., cattle on farms in a certain region, or beef carcasses at an abattoir) that is included in the surveillance activity. A high coverage is particularly important in surveillance for the early detection of exotic or new (emerging) diseases.

Evaluation Systematic and objective assessment of the relevance, adequacy, progress, efficiency, effectiveness, and impact of a course of actions, in relation to objectives and taking into account the resources used.

Hazard A pathogen, substance, or activity that has the potential to lead to adverse health consequences. In this article, only hazards relating to meat are considered. Negative health consequences in both livestock and consumers are relevant.

Representativeness Extent to which features of the population of interest are reflected in the surveillance data that are collected. Features may include: herd size; herd type (e.g., breeding, fattening, milk, and meat); age; sex; and location. A surveillance system that is representative accurately describes the distribution of a hazard in the population by place and animal. Bias reduces as representativeness increases.

Risk Probability of occurrence of an adverse event and the magnitude of the resulting consequences.

Risk-based surveillance Use of information about the probability of occurrence and the magnitude of the biological and economic consequence of health hazards to plan, design, or interpret the results obtained from surveillance systems.

Sanitary status The infection or disease status of a country, compartment, or region according to international standards and relevant for international trade. In principle, only animals and goods from areas of the same status can be traded or from units of higher status to lower status, but not the other way round.

Surveillance Resource-using activity involving the ongoing collection, collation, analysis, and communication of health-related events in order to inform decision making and to trigger targeted intervention, typically involving a defined threshold for action, with the aim to offset negative effects.

Introduction

Meat-borne hazards can have a range of negative effects. Depending on the biology and epidemiology of the hazard, they can cause subclinical or clinical disease in livestock and subsequent losses due to suboptimal growth or production. Hazards can also reduce the suitability of meat for certain products during processing or lead to cross-contamination as a consequence of hygiene failures. Perhaps most importantly, meat-borne hazards can cause illness in consumers ranging from subclinical infection to severe disease and even death. Pathways of infection might be complex if other foods are contaminated and result in indirect infection.

In order to assess such negative effects, risk analysis has become the method of choice for industries and governments, particularly in the context of international trade. A key input into the risk assessment component of risk analysis are data originating from monitoring and surveillance activities. Knowledge of the pathways of pathogen transmission and factors affecting pathogen survival, growth, and spread are essential if effective risk management strategies are to be developed. Risk management can involve a range of interventions targeted at pathogen reduction, inactivation, or elimination. To assess the success and viability of risk mitigation, surveillance and intervention have to be considered jointly because both are resource-using activities. Therefore, the most efficient balance of surveillance and intervention will include consideration of both the effectiveness and the economic aspects of a strategy.

In this article, concepts and methods of mitigating risks related to meat-borne hazards will be described and discussed. This will cover both surveillance as well as intervention. Hazards are highly diverse. Individual hazards, including microbiological hazards such as *Salmonella* or chemical hazards such as dioxin, are covered in more detail in separate articles. Here, we are considering the generic concepts and methods used in the design, implementation, and evaluation of hazard mitigation. We will use selected examples to illustrate how these may be applied in the context of meat-borne hazards.

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Concepts of Hazard Mitigation

Mitigation = Surveillance + Intervention

Owing to the link between surveillance and intervention, decisions need to be made as to how best to divide resources between them. Limited budgets mean that resources spent on surveillance will not be available for interventions and vice versa. To dedicate more efforts to surveillance might be desirable as it may lead to early detection and, therefore, minimize costs related to interventions. Alternatively, surveillance may be costly, for example, if a hazard is very rare, and it may be preferable to save resources for interventions if and when needed. For both surveillance and intervention, there will be several options for the design and selection of technology. In the selection of the most suitable components, both effectiveness and costs need to be taken into account. Such considerations require the use of economic concepts.

A second consideration relates to the mitigation objective. Although it may be feasible to achieve a significant reduction in the risk related to a meat-borne hazard, to eliminate it entirely from the production chain might be unrealistic. For example, hazards such as *Campylobacter*, which have environmental or wildlife reservoirs, will be expensive if not impossible to eliminate. The choice of objective of mitigation, therefore, will also have to consider the biology of a hazard together with the feasibility of surveillance and the efficacy of interventions.

Responsibilities

Mitigation programs for meat-borne hazards can be managed and implemented at different levels. For some hazards, surveillance and intervention are government-led. This is typically the case for hazards of public health significance, either because of their frequent occurrence (e.g., heavy metals) or because of their severe health consequences (e.g., prions). For such hazards, mitigation will be predefined and compulsory and the responsibility for design and implementation lies with government. Depending on government structures, some mitigation activities may be delegated to health services or industry. For example, *Salmonella* surveillance in pigs is coordinated and implemented by industry in several European countries, with targets provided by governments. In case of under-performance of such programs, penalties will be implemented by governments.

Mitigation for less severe hazards is often the responsibility of meat producers themselves. Typically, such hazards pose a smaller risk to public health, are less easily transmitted by meat, or the consequences of exposure are less significant (or uncertain). For example, paratuberculosis has been discussed as a possible public health hazard but due to continuing uncertainties, mitigation programs are currently mainly industry-led. Similarly, risks related to antimicrobial resistance continue to be debated and mitigation in most countries is currently mainly based on codes of practices rather than compulsory legal interventions.

Legal Frameworks and Standards

Compulsory hazard mitigation programs require a legal basis to achieve the desired coverage. This is provided by the competent authorities in a country, typically the Ministry of Agriculture and the Ministry of Health. Legislation is also developed in an international context because meat is often traded freely between countries. In order to assure quality and safety of products, international standards are developed by the Codex Alimentarius Commission and the World Organisation of Animal Health (OIE) on behalf of the World Trade Organization. These standards define minimum levels of surveillance and the required and permitted intervention options. They also define the minimum sanitary status required for a country, compartment or zone to participate in international trade. Risk analysis is the method of choice used to establish such standards.

Both public health and animal health concerns can trigger trade restrictions. For example, foot-and-mouth disease (FMD) is highly contagious for cattle and pigs but not a zoonosis. As the virus can survive in carcasses, trade from infected zones or countries is restricted because of the animal health risks. Another example is *Salmonella* in poultry meat. Some countries have eliminated this pathogen from their poultry chains. To prevent new incursions and to assure public health, imported poultry is also required to be *Salmonella*-free.

Possibly the most stringent and comprehensive international standards ever developed in relation to meat relate to the control of prions in beef (Box 1). The relevant articles of the Animal Health Code developed by the OIE provide detailed requirements for both surveillance as well as intervention activities.

Surveillance Systems

Level of Surveillance and Mitigation

Surveillance can be implemented at local, regional, or even global level. This will be closely related to who will use the surveillance information and the anticipated method(s) of

Box 1

Bovine spongiform encephalopathy (BSE) is a severe degenerative disease of ruminants caused by prions. The disease was first identified in the late 1980s and subsequently led to major trade restrictions of beef which still persist in some regions. Clinical signs are not highly specific and cases can only be confirmed postmortem. Owing to the low incidence of BSE, risk-based surveillance designs are used. These focus on high-risk population strata such as fallen stock, emergency slaughter stock, and suspect cases. Because BSE is zoonotic, severe trade restrictions apply to countries where it is present. A system for risk classification of countries is used. The risk category is also relevant for setting surveillance targets. A point system was developed in order to encourage countries to test high-risk animals. Control of BSE focusses on removal of central nervous system material from carcasses at slaughter and incineration at high temperature and under pressure. Owing to uncertainties of complete removal of high-risk material, the feeding of animal-derived proteins was banned in many countries. However, with a decreasing number of BSE cases, discussions have begun to relax some of the testing and prevention measures because maintaining this level of mitigation is costly and appears no longer justified in all countries.

intervention. If the intervention decision is made at the farm or local level, surveillance should also be focused there. However, many mitigation decisions relating to meat-borne hazards are taken at regional or national level because of public health relevance or the risk of meat-borne spread to susceptible livestock.

International surveillance programs have gained particular attention recently following several multicountry outbreaks. For example, livestock feed was contaminated repeatedly with dioxin in Europe due to manufacturing errors. This incident led to contaminated meat and milk being marketed in many countries and a subsequent multicountry recall. Such events highlight the international connectedness of food and feed systems and the potential consequences of mistakes in one country on consumers elsewhere. As such incidents are also very costly and damage consumer confidence, more intensive surveillance programs were developed using harmonized methods. Surveillance standards developed by international organizations (see Section Legal Frameworks and Standards) as well as industry standards have an important role in preventing the spread of meat-borne hazards through trade.

Objectives of Surveillance

Surveillance for meat-borne hazards may have one or more objectives. Depending on the prevalence of a certain hazard, the objective of surveillance may be to identify cases to instigate a control measure, to demonstrate continuing absence of the hazard, to detect emergence of a new hazard as early as possible, or to estimate the current prevalence or changes in prevalence over time (temporal differences) or between locations (spatial differences). The design of the surveillance system will vary according to the respective objective.

Surveillance Activities

At the beginning of all surveillance is case definition. The case definition can be based on clinical or pathological signs or syndromes, which will typically be considered a 'suspect case.' Suspect cases in animals might be detected at farms, veterinary practices, abattoirs, or markets. Suspect cases in humans are detected by family practitioners or hospital workers. When the case definition is unspecific and describes a clinical syndrome rather than a case of a specific disease, it is referred to as 'syndromic surveillance.' This approach increases the sensitivity but results in decreased specificity. This is desirable when the surveillance objective is early detection.

Suspect cases are subject to laboratory confirmation using the most suitable test or combination of tests. The choice of test technology will be related to the objective of surveillance. For example, antibody-based tests are often not the optimal choice for early detection because of the delay in antibody production in many diseases. Antigen-based testing might be more suitable for such an objective.

Once a case is detected, a process for reporting is required. In the case of compulsory mitigation, a legal basis will define notification responsibilities. Typically, anyone owning or caring for animals is responsible for notification. The decision to notify requires basic knowledge of what reportable diseases might look like. It is, therefore, dependent on disease awareness, which is generally accepted to be the weakest link in surveillance based on case reporting. Educational programs are often used to increase disease awareness.

Reported cases can trigger mitigation steps or contribute to regular disease statistics that are used to document progress of intervention programs. Surveillance results are frequently communicated via the internet, but printed reports remain common. For some diseases, international reporting is also required. The OIE published regular reports on their website within the World Animal Health Information Database system.

Approaches to Surveillance

Surveillance can be classified according to the approach taken to case detection. As described in Section Surveillance Activities, case reporting is very common. This is also referred to as 'passive' surveillance because the central unit responsible for data collection and decision making is taking a passive stance waiting for reports to arrive. Alternatively, 'active' surveillance can be implemented by designing specific mechanisms for case searching and enhanced detection. This can, for example, consist of specific surveys that are implemented regularly. Passive and active surveillance can both be applied to all eligible units (e.g., farms, animals, and villages) or to a sample of units. When sampling is applied, measures should be taken to minimize undesired bias.

Surveillance Designs

The design of a surveillance program will require the specification of many aspects including: the surveillance objective; case definition; sampling approach; sampling frame; sample size (if applicable); methods of data capture, management, security, and confidentiality; statistical analysis; and reporting and communication channels. Potential interventions resulting from detected cases should also be defined and the necessary legal basis assured.

In some programs, the hazard is predefined, but in others there may be choices. For example, when conducting surveillance for antimicrobial resistance, a large number of pathogen-resistance–host combinations exist. The concept of risk as used in risk analysis – i.e., the probability of occurrence and the magnitude of the consequences – can be used to set priorities in such a situation. This is referred to as risk-based surveillance. Information on risk factors is systematically used to target sampling and testing activities to strata of the target population where the chances of detecting the hazard are highest. Risk factors might relate to husbandry, location or management practices, depending on the epidemiology of a hazard. See Box 2 for an example.

The objective of risk-based designs is to increase surveillance efficiency, i.e., the amount of information obtained per resource input. This means that the same efficacy should be achieved with reduced costs or an increased efficacy with the same costs. This approach has become increasingly popular over the last few years and development of enhanced methods is continuing. Risk-based approaches are also used in

Box 2

Salmonella surveillance in pig populations was systematically introduced in countries with major pig industries to prevent human salmonellosis and to maintain market confidence. Most *Salmonella* infections in pigs other than cases caused by *Salmonella choleraesuis* are subclinical. A surveillance system based on sampling pigs at harvest is commonly used following a program first developed in Denmark. A risk-based sampling approach is used such that sampling intensity is based on previous test results and the size and types of farms. Interventions against *Salmonella* in pigs consist of a combination of general measures, such as better biosecurity, changes in diet, and improved pig flow and hygiene. Specific measures such as vaccination are not yet available. When targets for *Salmonella* counts are not achieved, carcasses from affected pigs can be decontaminated or the meat can be heat treated. Trade of such meat may be restricted, mainly based on private standards.

Box 3

Avian influenza (AI) is a disease of poultry caused by viral strains of low or high pathogenicity. Some strains are zoonotic. Surveillance is required by international standards and involves systematic sampling and testing of poultry as well as reporting of increased mortality in poultry. A risk-based approach can be used by increasing sampling on farms in areas with large wild bird populations or farms with imperfect biosecurity, such as free-range poultry farms. Highly pathogenic and zoonotic strains of AI are subject to stamping-out strategies. For other strains, hygiene and general containment is applied. Improved biosecurity is an important mitigation tool. Vaccination is also available. The occurrence of AI in wild birds (often without clinical signs) and the emergence of novel strains in wildlife reservoirs pose a considerable challenge for the prevention and containment of outbreaks in poultry. Surveillance is, therefore, often extended to wild bird populations by sampling birds that are trapped, hunted, or found dead.

Table 1 Examples of combinations of surveillance approaches and designs that can be used to detect meat-borne hazards

Approach taken to surveillance	*Surveillance design*	
	Conventional surveillance	*Risk-based surveillance*
Passive	Case reporting of suspected cases Syndromic surveillance	Case reporting within risk strata Syndromic surveillance within risk strata
Active	Complete population screening (census) Random sampling	Targeted screening of and within risk strata Random sampling within risk strata

the context of inspection. Combinations of surveillance approaches and designs are shown in Table 1.

While not all surveillance designs are suitable for every surveillance objective, more than one may be applicable in any given situation. In some instances, a combination of different surveillance approaches might be the most effective and efficient way to achieve the objective. The term 'surveillance system' is used to refer to surveillance that consists of more than one component (surveillance activity), combining different approaches and designs. For example, surveillance of diseases that involve wildlife often includes several surveillance components (Box 3).

Methods for Assessing Surveillance

The key attributes of surveillance programs are their sensitivity, specificity, and representativeness. Depending on the objective of a surveillance program, the primary attribute of concern might be the probability of detecting a case (sensitivity) or the precision of the estimated level of occurrence of a disease (incidence or prevalence).

There in an increasing need for methods to assess complex surveillance systems, to establish equivalence across surveillance designs, and to identify the most effective designs. Scenario-tree analysis has been developed to assist such decisions. This quantitative approach can deal with a range of surveillance system components with varying designs. Each 'branch' in the scenario tree represents a specific design. The output of the tree is the probability of detecting a case. It is, therefore, particularly suitable for programs that are used for demonstrating freedom from infection. Scenario-tree analysis has been widely used to assess the performance of risk-based surveillance in comparison to conventional designs.

Interventions for Hazard Reduction

Endpoints

If interventions are to be applied when cases are identified, various options exist depending on the aim of intervention. The objective might be to eliminate the hazard from all animals and units in a population and thereby eliminate all related economic losses. Alternatively, the objective might be to reduce losses by reducing the frequency of occurrence or preventing severe cases. The decision regarding the endpoint will depend on the legislation, which will specify elimination targets for certain hazards. For example, brucellosis is subject to elimination objectives in many countries. However, when cases occur frequently, elimination might not be economically feasible and a reduction target might be more realistic. This is the case in many countries where brucellosis in wildlife is still common. In a situation where a hazard is generally absent (i.e., surveillance is targeted at early detection), an identification and elimination strategy is typically used. This is the case for all so-called exotic diseases. The diseases grouped in this category will depend on the sanitary status of the country or region. Huge progress toward areawide elimination of several diseases has been made, most notably the global eradication of rinderpest in 2011. For zoonotic diseases with severe consequences for humans, elimination is also desirable. An example for such a hazard is BSE for which all countries have an elimination target.

Intervention Options

Intervention strategies might form part of either preventive or corrective action, and might be specific or nonspecific for a particular hazard (Table 2).

When effective vaccination is available, this might be an economic prevention strategy in regions of significant infection pressure and for pathogens that cause substantial losses. However, diagnostic tests are often unable to distinguish infected from vaccinated animals. New vaccine technologies increasingly allow for this distinction. Vaccination has been successfully used in major eradication campaigns against diseases such as classical swine fever and FMD (see Box 4). As such programs progress, vaccination is discontinued to allow for ultimate eradication of the hazard.

Specific pathogen free programs might offer another solution. This has been very successfully used in pig industries around the world, sometimes involving entire regions and even countries (e.g., elimination of specified respiratory hazards from pigs in Switzerland). For endemic diseases, a test-and-remove strategy can be used to eliminate individual infected animals. This is used in some countries for tuberculosis control in cattle.

When there are no specific intervention strategies available, management changes might offer a solution. For example, biosecurity can be increased to reduce infection pressure. The highest risk of hazard introduction is related to replacement stock. This risk can be managed by purchasing only from certified sources, by using testing and quarantines or by operating a closed herd. Biosecurity is not only relevant to control access of visitors and livestock, but also vermin. For example, some hazards can be physically carried by insects and rodents. Measures targeted at such pathways are sometimes summarized under 'good farming practice.' This also includes hygienic management of pasture, slurry, and feed. Early separation of young stock from infected dams is effective for some pathogens. Unspecific use of antimicrobials is not considered best practice as the negative impact on resistance situation generally outweighs the benefits.

For some hazards, dietary changes may also be an option. This is most significant for feed-borne hazards, including *Salmonella*, prions or environmental contaminants, where changes in feed manufacturing and storage have significant impact. For some hazards, dietary changes can also impact the survival of hazards in the digestive tract and thereby reduce infection pressure indirectly. This is being used to reduce the occurrence of *Salmonella* in pigs. In this field, more research is needed to assess the efficacy of feed additives, such as the use of pre- or probiotics, phyotherapy, or immunomodulators.

Table 2 Examples of combinations of intervention strategies that can be used to reduce the impact of meat-borne hazards

	Specific for a hazard	*Not specific*
Preventive	Specific pathogen free animal production	Biosecurity and good farming practice
	Vaccination	Early weaning
Corrective	Treatment	General antibiotic use
	Test-and-kill	Improved husbandry and redesign of buildings
	Eradication	Cleaning and disinfection and restocking

Box 4

FMD is a highly contagious disease affecting cloven-hoofed animals. Owing to the reliable occurrence of characteristic lesions, surveillance is mainly based on clinical surveillance. In sheep, however, clinical signs might be mild. This led to a major outbreak of FMD in the United Kingdom and subsequently other European countries in 2001 causing losses of over £8 billion (~€12 billion or US$12 billion). The most widely used intervention strategies are vaccination in endemic areas and stamping-out in free areas. Vaccination is challenging due to the circulation of different serotypes and the antigenic change of viruses. Owing to the significant difference in the occurrence of FMD, severe trade restrictions are applied between countries and regions of different sanitary status. Some discussions have started to consider possibilities for a global eradication of FMD. However, costs and technical challenges of such a project appear prohibitive at the moment.

Integrated Control along the Food Chain

For some hazards, interventions in primary production, i.e., on the farm, may not be the most effective or efficient option. Alternatives are interventions at harvest and along the food chain. Often, combinations of interventions are also useful. Options for controlling meat-borne hazards can be applied at slaughter. For example, when hazards are located in specific parts of the carcass, these can be trimmed out and destroyed. This is used for BSE. Hazards that cause specific lesions detectable at harvest can also be eliminated from the food chain by removing affected organs or carcasses. This is used, for example, as part of tuberculosis control.

Freezing can inactivate some hazards, including some parasites. However, control at source on the farm should still be considered as decontamination steps might not be sufficient to sufficiently reduce the public health threat. Heat treatment is another effective measure to prevent human infection. In regions where food-borne hazards are known to be prevalent and elimination is not feasible, measures along the food chain are the only option. Ultimately, safe storage, handling, and hygienic preparation of meat by consumers are extremely important and effective measures to prevent food-borne disease. It is, therefore, important to emphasize the responsibility of consumers and to increase their awareness and knowledge of meat-borne diseases.

Economics of Hazard Mitigation

Costs of Surveillance

In order to conduct surveillance that is useful, a range of activities is required, which all incur costs. This includes the costs of investigation activities (e.g., veterinary time and travel), sampling, sample shipment and analysis, data capture, statistical analysis, reporting, and communication. Unfortunately,

data detailing the cost of surveillance are often not readily available because many activities are conducted as part of routine tasks and not easily specified or associated with specific hazards. For example, a meat inspector often collects a wide range of surveillance data on several meat-borne hazards simultaneously. How to divide the cost of manpower between different surveillance programs is not trivial.

Cost of surveillance is related to coverage. The larger the sample size, the higher the costs. Sampling is, therefore, an intuitive cost saving mechanism when compared to surveillance of entire populations. The latter is, however, still very commonly used in clinical surveillance. The assumption is that clinical observation of livestock is part of due care expected from farmers and herders. In case of unusual observations, compulsory reporting and follow-up investigations by veterinary services provide the most efficient and, therefore, most widely used surveillance design world wide. However, underreporting due to real or perceived negative economic consequences and stigmatization of affected farms is a major weakness in all general reporting systems.

Costs of Intervention

Costs of intervention vary greatly. Although generic measures such as improved biosecurity are effective for a range of hazards and can be considered part of 'good farming practice,' measures such as vaccination have a specific effect and are, therefore, comparatively more expensive. The most expensive intervention is likely to be a strategy where a pathogen is eliminated using drastic measures, such as slaughtering all animals ('stamping-out') and replacing them with healthy ones. Such interventions incur not only direct costs but also indirect costs related to loss of production during periods when farm buildings are cleaned and disinfected or a farm is subject to movement restrictions as well as loss of market shares. The latter is particularly critical in a global market. A disease outbreak in one region, such as AI in Southeast Asia, can lead to permanent loss of market to another continent.

Trade-offs and Optimization

Nobody would implement any mitigation if there were only costs. Of course there are also benefits, but these are sometimes difficult to quantify because they might be incurred not only by the individuals who implement mitigation, but by a wider group, leading to so-called externalities. Over the last decades, resources available for mitigation have become increasingly limited emphasizing the need for improved efficiency of surveillance and interventions. This has emphasized the need for trade-offs and for more integrated consideration of surveillance and intervention in economic frameworks. However, capacity to conduct such analyses is still limited in veterinary services and animal industries around the world.

Who Should Pay for Mitigation?

There are many different systems for sharing costs of surveillance and interventions among participants of meat-production chains. Solutions vary according to hazard types with highly contagious and zoonotic hazards being mostly covered by governments (e.g., tuberculosis and FMD) while less contagious or endemic hazards are left to industry to manage. Sometimes, governments set mitigation objectives, but technical solutions for efficient surveillance and intervention are developed by the industry (e.g., *Salmonella* mitigation in pigs in Denmark).

Evaluation of Mitigation

Evaluation has been defined as a systematic and objective assessment of the relevance, adequacy, progress, efficiency, effectiveness, and impact of a course of actions. In this context, we consider actions related to surveillance and mitigation. Such an assessment should be linked to the intended objectives of a program as well as to the resources used. Systematic evaluation of programs and policies has become increasingly popular as a consequence of reduced budgets. For the evaluation of surveillance, a flexible framework with specific guidance has recently been developed, including relevant surveillance attributes and illustrative examples. In addition to attributes that are regularly used to evaluate surveillance such as sensitivity, specificity, and bias, additional attributes such as feasibility, coverage, and costs are used. A similar approach is applicable to the evaluation of disease control. Ideally, surveillance and intervention targeted at a specific hazard should be evaluated jointly. Such examples are, however, currently rare.

Future Directions

Challenges for the future include improving the efficiency of surveillance and control of meat-borne hazards. Risk-based designs are likely to be increasingly applied to achieve this. Evaluation of surveillance and intervention programs should be conducted regularly to enable continued improvements to be made to the process. The ever-increasing human population and the associated demand for more safe meat means that mitigating risks relating to meat-borne hazards will remain important for a long time to come.

See also: Animal Health Risk Analysis. Economics: Meat Business and Public Policy. Microbiological Safety of Meat: Pathogenic *Escherichia coli*; Prions; *Salmonella* spp. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Risk Analysis and Quantitative Risk Management

Further Reading

Alban, L., Stärk, K.D.C., 2005. Where should the effort be put to reduce the *Salmonella* prevalence in the slaughtered swine carcass effectively? Preventive Veterinary Medicine 68, 63–79.

Blickenstorfer, S., Schwermer, H., Engels, M., *et al*., 2011. Using scenario tree modelling for targeted herd sampling to substantiate freedom from disease. Biomedical Central Veterinary Research 7, 49. doi:10.1186/1746-6148-7-49.

Clements, A.C.A., Pfeiffer, D.U., 2009. Emerging viral zoonoses: Frameworks for spatial and spatiotemporal risk assessment and resource planning. Veterinary Journal 182, 21–30.

Drewe, J.A., Hoinville, L.J., Cook, A.J.C., Floyd, T., Stärk, K.D.C., 2012. Evaluation of animal and public health surveillance systems: A systematic review. Epidemiology and Infection 140, 575–590.

Häsler, B., Howe, K.S., Stärk, K.D.C., 2011. Conceptualising the technical relationship of animal disease surveillance to intervention and mitigation as a basis for economic analysis. Biomedical Central Health Services Research 11, 225. doi:10.1186/1472-6963-11-225.

Howe, K.S., Häsler, B., Stärk, K.D.C., 2012. Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. Epidemiology and Infection 141, 91–101.

Salman, M.D., 2008. Animal Disease Surveillance and Survey Systems: Methods and Applications. Ames, Iowa: Blackwell Publishing Company, p. 222.

Snow, L.C., Newson, S.E., Musgrove, A.J., *et al.*, 2007. Risk-based surveillance for H5N1 avian influenza virus in wild birds in Great Britain. Veterinary Record 161, 775–781.

Stärk, K.D.C., Regula, G., Hernandez, J., *et al.*, 2006. Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: Review of current approaches. Biomedical Central Health Services Research 6, 20. doi:10.1186/1472-6963-6-20.

Relevant Websites

www.codexalimentarius.net
Codex Alimentarius Commission.

http://www.rvc.ac.uk/SERVAL
SERVAL.

http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home
World Animal Health Information Database (WAHID).

www.oie.org
World Organisation for Animal Health.

www.wto.org
World Trade Organisation.

MEAT MARKETING

Contents

Cold Chain

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Glossary

Conduction, thermal A heat transfer mechanism through a solid material/medium in which kinetic energy is transmitted by the particles of the material from particle to particle without gross displacement of the particles.

Convection, thermal A heat transfer mechanism through a liquid or gas by means of circulating currents caused by changes in density.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Pasteurization A form of heat treatment that kills certain vegetative bacteria and/or spoilage organisms in milk and other foods. Temperatures below 100 °C are used.

Radiation, thermal A heat transfer mechanism in which electromagnetic radiation is generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emits thermal radiation.

Refrigeration May be defined as the process of removing heat from any substance to (1) render colder – reduce temperature, (2) change its state – for example, water to ice, (3) maintain its state – preserving foods, storing ice.

Water activity (a_w) A measure of the available water in a substance. 'High a_w' foods support bacterial growth; 'low a_w' foods do not. This is not the same as water content. Some foods with a high water content have a relatively low a_w because the water is bound up with dissolved salts or sugar, for example, jam.

Introduction

To provide safe meat and meat products of high organoleptic quality, attention must be paid to every aspect of the cold chain. The process commences with the initial chilling and continues through to the storage of the chilled retail portion or meat product within the home. Within the cold chain are processes such as primary and secondary chilling, freezing, thawing, and tempering, where the aim is to change the average temperature of the meat. In other processes, such as chilled and frozen storage, transport, and retail display, maintaining the temperature of the meat is the prime aim.

Failure to understand the needs of each process results in excessive weight loss, higher energy use, reduced shelf life, or a deterioration in product quality.

Refrigeration is the prime process controlling the growth of pathogenic and spoilage microorganisms on meat. It is therefore very important to understand the refrigeration methodology that is required in each stage of the cold chain and the interaction between the stages.

Removing the required amount of heat from a carcass is a difficult, time-consuming operation but critical to the operation of the cold chain. Most of the subsequent processes are designed to maintain not to reduce temperature. As a meat product moves along the cold chain it becomes increasingly difficult to control and maintain its temperature. Temperatures of bulk packs of chilled product in large storerooms are far less sensitive to small heat inputs than single consumer packs in transport, open display cases, or under domestic refrigeration.

Elements of The Cold Chain

Primary Chilling

The rate of heat removal and the resulting rate of temperature reduction at the surface and within the carcass has a substantial influence on the weight loss, storage life, and eating quality of the meat produced. EU regulations require that all meat temperatures within the carcass must be reduced below 7 °C (3 °C for poultry) before the carcass is further processed or moved from the chiller. Similar legislation is applied in many other parts of the world. Careful control is required to achieve conditions that will reduce the carcass temperature in the designed time cycle. This has to be carried out in the most economic manner taking into account weight loss and energy consumption.

Conventional Chilling

The majority of carcass meat is chilled in conventional air chill rooms nominally operating at one or sometimes two conditions during the chilling cycle. Similar methods are normally used for poultry carcasses that are sold in a fresh state, i.e., chilled. Immersion chilling is sometimes used for poultry that is subsequently frozen, and spray/evaporative chilling has been used on beef, pork, and poultry carcasses.

Air temperature, air velocity, and to a limited extent relative humidity are the environmental factors that affect the cooling time of meat carcasses and sides. Cooling rate will also be a function of the weight and fat cover of a given carcass.

Air temperature is the single most important factor controlling chilling time. For example, with beef sides, cooling in air blowing at 3 ms^{-1} using a constant 4 °C compared with 0 °C will increase the time to reach 7 °C in the deep leg of a 100 kg side from 20.3 to 27.7 h (a 36% increase). With an air velocity of 0.5 ms^{-1} using a constant 4 °C compared with 0 °C to cool a 220 kg side will increase the time from 45.9 to 68.3 h (a 49% increase). In systems designed to produce fully chilled sides, with average meat temperatures of 2–4 °C, the requirement for low air temperatures becomes even more important. In air at 0 °C and 3 ms^{-1} it will take approximately 48 h to reduce the average temperature of a 220 kg side to 4 °C. In air at 4 °C it will take an indefinite amount of time.

Increasing the air velocity during chilling produces a substantial reduction in chilling times at low air velocities, but similar increases at higher velocities have a much smaller effect. At 0 °C a four-fold increase in air velocity from 0.5 to 2 ms^{-1} results in an 8 h reduction in chilling time to 7 °C for a 140 kg beef side, but requires a 64-fold increase in fan power. In most practical situations with large carcasses it is doubtful whether an air velocity greater than 1 ms^{-1} can be justified.

Decreasing relative humidity has been shown to produce a slight reduction in chilling time due to increased evaporative cooling from the carcass surface. However, unless water is added to the surface of the carcass, any increase in the rate of evaporation will be directly reflected in a higher weight loss.

In some beef, pork, and poultry chilling systems refrigerated water sprays are often used in conjunction with air chilling. The sprays are not applied continuously but in short bursts, for example, 90 s at 15 min intervals for the first 8 h in beef systems, 5 and 15 min after the start of poultry chilling. The process increases the rate of evaporative heat loss but, by replacing the water lost, reduces the overall weight loss. Although often called evaporative air chilling, it should not be confused with true evaporative chilling carried out in a vacuum chamber as used with freshly harvested leafy vegetables and salad crops. Experiments have been carried out on the chilling of poultry carcasses in a vacuum chamber and these achieved cooling rates similar to immersion systems. However, the 5% weight loss was considered too high for industrial use.

In practice, it is difficult to separate the effect of carcass weight from fat cover on chilling time. Heavy carcasses and sides tend to have more fat cover than lighter carcasses and sides. For example, little difference has been observed in the cooling times of 30 kg ram carcasses with high and low fat thicknesses. However, when the fat was completely stripped from the muscle, the cooling time to 7 °C was reduced from 10 to 8 h.

The chilling time of lean, light (16.8 kg) lambs can be under half that of 26.8 kg lambs with a much thicker fat covering. In industry, it would not be unusual for a chilling system to contain lamb carcasses covering this range of weights and fat covers. The design and operation of such a system must therefore be a compromise between overly long chilling periods for the smaller carcasses and under cooling of the larger carcasses.

Chilling rates in immersion systems are a function of the cooling medium used, its temperature, the size of the carcass being chilled, and whether it is wrapped or unwrapped. In static systems immersion in slush ice is far more effective than water immersion at the same temperature. Mechanical movement or agitation of the immersion liquid can substantially reduce chilling times. In all cases unwrapped carcasses cool far faster than those that are wrapped. In some cases there is a doubling in the cooling time. Smaller wrapped broilers will cool in 50–67% of the time required for larger wrapped fowls under the same conditions. This is most probably due to the immersion liquid penetrating the cavity and substantially reducing the effective meat thickness.

If specified cooling schedules are to be attained, refrigeration machinery must be designed to meet the required heat extraction rate at all times during the chilling cycle. Heat enters a meat chill room via open doors, from personnel, through the insulation, from lights and cooling fans, and from the cooling carcasses or sides. The product load, i.e., heat released from the meat, is the major component of the total heat to be extracted from a fully loaded chill room.

The rate of heat release from a single side or carcass varies with time. It is at a peak immediately after loading, and then falls rapidly. The peak value is primarily a function of the environmental conditions during chilling and the rate at which hot meat is introduced into the chill room. Increasing air velocity, decreasing air temperature, or shortening loading time increases the peak heat load. There is a four-fold difference in peak load between a beef chill room operating at 8 °C and 0.5 ms^{-1} loaded for more than 8 h and the same room operating at 0 °C and 3 ms^{-1} and loaded for more than 2 h.

Accelerated Chilling

Using conventional single-stage chilling regimes only relatively lean, light beef sides can be cooled to 7 °C in the deep tissue during a 24 h operating cycle, while evaporative losses are of the order of 2%. Pork is conventionally cooled overnight, whereas lamb can be transported on the same day as slaughter. There is considerable interest in methods of shortening cooling times and reducing evaporative weight loss. All accelerated cooling systems are likely to be more expensive to install and operate than conventional plants. Therefore, to be cost-effective they must offer substantial savings in terms of increased throughput and/or higher yields of saleable meat.

Most accelerated chilling systems rely on the maintenance of very low temperatures (−15 to −70 °C) during the initial stages of the chilling process, by either powerful mechanical refrigeration plant or cryogenic liquids. Because any substantial freezing would produce increased drip loss on final cutting, accelerated systems only maintain very low temperatures during the first few hours of the chilling process. One or more successive stages at progressively higher temperatures are employed, with the final stage at or above 0 °C to remove the last of the heat or to allow for temperature equalization.

Freezing

Meat for industrial processing is usually frozen in the form of carcasses, quarters, or boned out primals in 25 kg cartons. Most bulk meat, consumer portions, and meat products are frozen in air blast freezers. Some small individuals items, i.e., beef burgers, may be frozen in cryogenic tunnels, and a small amount of offal and other meat is frozen in plate freezers. It is not unusual for meat to be frozen twice before it reaches the consumer. During industrial processing frozen raw material is often thawed or tempered before being turned into meat-based products such as pies, convenience meals, and burgers or consumer portions such as fillets, and steaks. These consumer-sized portions are often refrozen before storage, distribution, and sale.

The need for fast freezing to maximize the quality of frozen meat is increasingly open to argument. Fast freezing may well be the most economical practical process, and it is very likely to reduce weight loss from unwrapped meat. Fast freezing produces smaller ice crystals than the slower processes. However, the differences in crystal size and composition do not translate to effects that can be detected by taste panels or quantitative sensory methods.

For example, in one study on hamburgers the effects of three different freezing methods, spiral freezing, cryogenic freezing (liquid nitrogen), and impingement freezing, were compared. The parameters studied were appearance, dehydration during freezing, cooking losses, meat structure by microscopic analysis, and sensory properties by sensory analysis. The time required to freeze a 10 mm thick 80 g hamburger from +4 °C to −18 °C ranged from less than 3 to more than 22 min. Weight loss in the slow process was more than three times than that in the fast process. Ice crystals were significantly larger in hamburgers frozen in 22 compared with 3 min. However, no significant difference could be seen in cooking losses. Sensory analysis revealed no difference in eating quality between the three freezing methods, even after 2 months of storage. In studies with aged beef steak no differences were found in the texture or other organoleptic qualities of the final cooked steak between frozen (to −7 °C in 1.5 or 35 h) and unfrozen steaks.

Thawing and Tempering

Although thawing is often considered as simply the reverse of freezing, there are important differences between the two operations. The majority of the microorganisms that cause spoilage or food poisoning are found on exposed surfaces. During freezing, surface temperatures are rapidly reduced to levels that slow and then suspend microbial growth. In the thawing operation, surface temperatures rise rapidly to temperatures at which growth can recommence. These temperatures are maintained for the duration of thawing, which for large slow-thawing materials, or for uncontrolled or high-temperature thawing, can result in unacceptable spoilage at the surface before center regions have thawed.

Another difference is that thawing is inherently slower than freezing. The thermal conductivity of thawed meat is approximately one-third of that of frozen meat. This means that as thawing progresses, a layer of poor thermal conductivity is formed at the surface that acts, in effect, as insulation. The freezing process in general suspends the growth of, but does not destroy, microorganisms. Above −12 °C, microorganisms begin to grow again. This limits the temperatures that can be used to thaw, as high air temperatures will promote rapid microbial growth on the surface of the food.

Air is used in the vast majority of thawing/tempering applications. Air thawers are very flexible and may be used to thaw any size of meat cut from whole carcasses to individual steaks. Use of still air is limited to thin products; otherwise thawing times are excessively long. Although little or no equipment is needed, considerable space is required to lay out individual items of product. Moving air is more commonly used, providing more rapid heat transfer as well as improved control of temperature and humidity. Two-stage air thawing with a high initial air temperature followed by a second stage at an air temperature below 10 °C has also been used. The duration of the high-temperature stage is limited to 1 or 2 h to avoid excessive bacterial growth, but the increase in heat input during this time considerably reduces the overall process time.

Immersion in liquid media allows much more rapid heat transfer, especially if pumped or agitated to avoid temperature stratification in the liquid and grouping together of products. Thawing times are therefore greatly reduced. Practical limitations are that boxes and other packaging (unless vacuum-pack or shrink-wrap) must be removed before immersion. In addition, bulk blocks are liable to break up, leaching from product surfaces can lead to poor appearance, and frequent changing of water for hygiene reasons requires disposal or treatment of large quantities of effluent.

Vacuum thawing transfers latent heat of condensation of steam onto product surfaces at low pressure and temperature. For example, if a pressure of 1704 Nm^{-2} (0.017 atm) is maintained, steam can be generated at 15 °C and will condense at this temperature onto the frozen product surfaces. This ensures that although large amounts of latent heat are

added, the product will not rise above 15 °C. The process is rapid, but evacuation to subatmospheric pressure restricts it to batch operation. This process is more effective for thin products where the heat released into the surface is quickly conducted through the product.

Microwave systems have been successfully used to temper meat, i.e., raise the temperature from −2 to −5 °C, but not to fully thaw it, i.e., raise the temperature above 0 °C. Potentially very rapid, the application of microwaves is limited by thermal instability and penetration depth. Instability results from preferential absorption of energy by warmer sections and by different ingredients, such as fat. Warmer sections may be present at the start of the process; for example, the surface temperature may be warmer than the middle, or they may be produced during the process, such as energy being absorbed at the surface rather than penetrating all of the product. In the extreme, such warming can lead to some parts of the food being cooked whereas others remain frozen. These problems, as well as the capital cost of equipment, have greatly limited commercial use.

Secondary Chilling

Any handling operation, such as cutting, wrapping, mincing, or dicing, will add heat to the previously chilled meat. It is important that a secondary chilling operation be carried out to remove this added heat before the product is bulk packed.

The aim of any precooking process for chilled foods is to ensure the destruction of the vegetative stages of pathogenic microorganisms. However, there is always the possibility that the cooking process will not kill some microorganisms that produce spores or that the food will become recontaminated. Therefore, the temperature of the food should be rapidly reduced to below 7 °C after cooking to prevent multiplication. In addition to the microbiological factors, rapid reduction in product temperature aids retention of nutrients, which is vital in systems such as cook–chill that are often used for the preparation of meals for the elderly, the infirm, and young people.

The majority of companies rely on air blast cooling systems for the chilling of precooked products or ready meals. In batch systems the products, packs, or trays of cooked material are placed directly on racks in the chiller or on trolleys that can be wheeled into the chiller when fully loaded. Continuous systems range from trolleys pulled through tunnels to conveyerized spiral or tunnel air blast chillers.

Some meals and products are chilled using cryogenic tunnels; however, care must be taken to avoid surface freezing. Sous-vide and other imperviously packed products are often chilled by immersion in cooled water or other suitable liquid. With some cooked products such as large hams in molds and sausages, chlorinated water sprays can be used in the initial stages of cooling. Increasingly, pie and ready meal fillings are pressure-cooked and vacuum-cooled. With many products an initial cooling stage using ambient air can often substantially reduce the refrigeration load in the cooling system.

Chilled and Frozen Storage

After chilling, carcass meat is often stored for a period, ranging from a few hours to two weeks, unwrapped in a chill storage room. Meat primals and consumer cuts are often placed, wrapped or unwrapped, in trays or on racks in similar rooms. Low temperatures, minimal air movements, and high relative humidities should be maintained around unwrapped meat in order to maximize storage life and minimize weight loss. With wrapped and unwrapped meat low velocities are also desirable to minimize energy consumption. Many storage rooms are designed and constructed with little regard for air distribution and localized velocities over products. Horizontal throw refrigeration coils are often mounted in the free space above racks or rails of product, and no attempt is made to minimize the air velocity over the surface of unwrapped meat. Using a false ceiling or other form of ducting to distribute the air throughout the storage room can substantially reduce variations in velocity and temperature. Maximum air velocities of 0.75 and 0.4 ms^{-1} were measured over products in two storerooms having 30 cm deep false ceilings with slits to distribute the air throughout the rooms. The maximum temperature distribution measured within the rooms was less than 2 °C. Using air socks, improved air distributions can be maintained with localized velocities not exceeding 0.2 ms^{-1}.

Increasingly, red meat is aged for 4 weeks and longer to improve its texture and taste. There are two methods of aging: unwrapped on the bone (dry aging) or as vacuum-packed primals (wet aging). In both cases aging is best achieved by maintaining the stores at a temperature close to, but not below, the initial freezing point of the meat. There has also been increased use of controlled-atmosphere retail packs to extend the display life of meat. Because the packs tend to be large and insulate the products, effective precooling before packaging is especially important if product quality is to be maintained.

The frozen storage life of meat is limited by quality changes, primarily rancidity development in the fat. The rate of rancidity development is mainly a function of the feeding of the animal, the treatment of the meat before freezing, the type of packaging, lighting, and the storage temperature.

Transport

Developments in temperature-controlled transportation systems for products have been one of the main factors leading to the rapid expansion of the chilled food market. The sea transportation of chilled meat from Australasia to European and other distant markets, and road transportation of chilled products throughout Europe, the US and the Middle East is now in common practice. Air freighting was initially used for high-value perishable products such as strawberries, asparagus, and live lobsters. However, it is now increasingly used to provide consumers with a year-round supply of once only seasonal products.

Effective temperature control during transportation is becoming increasingly important as the shelf life of products is extended and legislation increased. Shipboard transportation of chilled vacuum-packed primals to distant markets is now common practice. However, to achieve the required shelf life, meat temperatures have to be maintained at −1±0.5 °C to avoid bacterial growth or freezing.

Retail Display

Retail display cabinets for meat and meat products are designed to display wrapped or unwrapped chilled product, or frozen wrapped product. The retail display of refrigerated food is probably still the weakest link in the cold chain. It is also very energy-hungry, consuming approximately 50% of all the energy used on refrigeration in a typical cold chain. The required retail display life and consequent environmental conditions for wrapped chilled products differ from those for unwrapped products. The desired display life for wrapped meat and meat products ranges from a few days to many weeks and is primarily limited by microbiological considerations. Retailers of unwrapped meat and delicatessen products normally require a display life of one working day. In this case display life is limited by dehydration leading to changes in the appearance.

Chilled Wrapped Meat

To achieve the display life of days to weeks required for wrapped chilled meat the product should be maintained at a temperature as close to its initial freezing point (approximately −1.5 °C) as possible. Air movement and relative humidity have little effect on the display life of a wrapped product, but the degree of temperature control can be important, especially with transparent, controlled-atmosphere packs.

To maintain product temperatures below 0 °C the air off the coil must typically be −4 °C and any ingress of humid air from within the store will quickly cause the coil to ice up. Frequent defrosts are often required, and even in a well-maintained unit the cabinet temperature will then rise to 10–12 °C and the product by at least 3 °C. External factors such as the store ambient temperature, the siting of the cabinet, and poor pretreatment and placement of products substantially affect cabinet performance. Warm and humid ambient air and loading with insufficiently cooled products can also overload the refrigeration system. Even if the food is at its correct temperature, uneven loading or too much product can disturb the airflow patterns and destroy the insulating layer of cooled air surrounding the product.

Chilled Unwrapped Meat

Changes in appearance are normally the main criteria that limit the display life of unwrapped foods, with the consumer selecting newly loaded product in preference to that displayed for some time. Deterioration in appearance has been related to the degree of dehydration in red meat and is likely to similarly occur in other foods. Apart from any relationship to appearance, weight loss is of considerable importance in its own right because most meats and meat products are sold by weight.

Changes in relative humidity (RH) have a substantial effect on weight loss, with a reduction from 95 to 40% increasing weight loss by a factor of between 14 and 18. Raising the air velocity from 0.1 to 0.5 ms^{-1} has little effect on weight loss at 95% RH but increases the loss by a factor of between 2 and 2.4 at 60% RH. Temperature changes from 2 to 6 °C have a far smaller effect on weight loss than the changes in either relative humidity or velocity. Fluctuations in temperature or relative humidity have little effect on weight loss, and any apparent effect is caused by changes in the mean conditions.

Frozen Display

All frozen meat and meat products are wrapped before they are placed in retail display. The principal reason for packaging meat during frozen display is to minimize moisture loss. Moisture loss causes deleterious effects on the texture, flavor, and color of the meat. As long as the display keeps the temperature of the meat below −12 °C there will be no microbial problems. Changes to the color of the surface of the meat normally limit the frozen display life. Low temperatures, minimal temperature fluctuations, and low illumination levels will maximize display life.

Domestic Handling

After a chilled or frozen product is removed from a retail display cabinet it is outside a refrigerated environment while it is carried around the store and then transported home for further storage. In the home it may be left in ambient conditions or stored in the refrigerator/freezer until required.

In the past two decades there have been at least 15 surveys of temperatures in domestic refrigerators around the world. The results are all very similar, with overall mean temperatures in domestic refrigerators ranging from 4.0 to 7.4 °C, and maximum temperatures from 8 to 20.7 °C. These results are very worrying because they imply that the average temperature of at least 50% of domestic refrigerators is above 4.5 °C, around the world. When one looks at the percentage of temperatures measured that were above set points the results are even more worrying. In the last French and UK studies 80% and 70%, respectively, of the temperatures were above 5 °C and in Greek work 50% were above 9 °C. Comparable studies have not been carried out on domestic freezers.

See also: Modeling in Meat Science: Refrigeration. Physical Measurements: Temperature Measurement. Refrigeration and Freezing Technology: Freezing and Product Quality; Thawing

Further Reading

Brown, M., 2008. Chilled Foods: A Comprehensive Guide, third ed. Cambridge, UK: Woodhead Publishing Limited.

Cutting, C.L., 1972. Meat Chilling – Why & How? MRI Symposium No. 2. Langford, Bristol, UK: Agricultural Research Council, Meat Research Institute.

Cutting, C.L., 1974. Meat Freezing – Why & How? MRI Symposium No. 3. Langford, Bristol, UK: Agricultural Research Council, Meat Research Institute.

International Institute of Refrigeration,1986 Recent advances and developments in the refrigeration of meat chilling, Proceedings of the Conference of: IIR Commission C2, Bristol (UK). International Institute of Refrigeration, Paris, France.

International Institute of Refrigeration, 1995. Guide to Refrigerated Transport. Paris, France: International Institute of Refrigeration.

International Institute of Refrigeration, 2000. Recommendations for Chilled Storage of Perishable Produce. Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 2001. Rapid cooling of food. Proceedings of The Conference of: IIR Commission C2, Bristol (UK). Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 2006. Recommendations for the Processing and Handling of Frozen Foods. Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 2010. Sustainability & the cold chain. Proceedings of the Conference of: IIR Commissions B1, B2, C2, D1 and D2. Cambridge (UK). Paris, France: International Institute of Refrigeration.
James, S.J., Evans, J.E., James, C., 2008. A review of the performance of domestic refrigerators. Journal of Food Engineering 87, 2–10.
James, S.J., James, C., 2002. Meat Refrigeration. Cambridge, UK: Woodhead Publishing Limited ISBN 1 85573 442 7.
James, S.J., James, C., 2010. The food cold-chain and climate change. Food Research International 43 (7), 1944–1956.
James, C., Vincent, C., de Andrade Lima, T.I., James, S.J., 2006. The primary chilling of poultry carcasses – a review. International Journal of Refrigeration 29 (6), 847–862.
Kerry, J.P., Kerry, J.F., 2011. Processed Meats: Improving Safety, Nutrition and Quality. Cambridge, UK: Woodhead Publishing Ltd.
Mena, C., Stevens, G., 2010. Delivering Performance in Food Supply Chains. Cambridge, UK: Woodhead Publishing Ltd.
Sun, D., 2012. Handbook of Frozen Food Processing and Packaging. Boca Raton, FL: CRC Press/Taylor & Francis.
Swain, M.J., Evans, J.A., James, S.J., 2009. Energy consumption in the UK food chill chain – primary chilling. Food Manufacturing Efficiency 2, 25–33.
Visakh, P.M., Thomas, S., Iturriaga, L.B., 2008. Freezing of fish. In: Evans, J.A. (Ed.), Frozen food science and technology. Oxford, UK: Blackwell Publishing Ltd., pp. 151–164.

Relevant Websites

http://www.ecff.net/
Official Site for The European Chilled Food Federation.
http://www.fao.org/
Official Site for The Food and Agriculture Organization of The United Nations.
http://www.iifiir.org/
Official Site for The International Institute of Refrigeration.
http://www.chilledfood.org/
Official Site for The UK Chilled Food Association.

Market Requirements and Specifications

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Glossary

AUS-MEAT The authority for uniform specification of meat and livestock in Australia.

Bovine spongiform encephalopathy (BSE) A fatal neurodegenerative disease in cattle commonly known as mad cow disease.

Credence attributes The product attributes that have a value but cannot be evaluated by a consumer even after 'consumption,' for example, animal welfare standards or environmental impact of the production method.

Equivalence One of the three objectives of the World Trade Organization (WTO) agreement on the application of sanitary and phytosanitary measures (SPS Agreement 1995). It is based on accepting that different sanitary measures can achieve the same level of protection and helps ensure that imported meat meets all safety standards applicable in the importing country.

(S)EUROP A European Union classification system that grades beef or lamb carcasses on the basis of conformation and fat scores.

Harmonization One of the three objectives of the WTO agreement on the application of sanitary and phytosanitary measures (SPS Agreement 1995). It relates to the establishment, recognition, and application of common SPS measures by different members. By harmonizing SPS measures with international standards, food safety and animal health protection can be achieved without unduly restricting international trade.

Institutional meat purchase specifications Specifications for meat and meat products are developed and maintained by the US Department of Agriculture. They are developed to provide a standardized system to describe traded meat products.

***In vitro* meat** An animal flesh product that has been grown in a culture and was never part of a complete, living animal.

Mechanically separated meat (MSM) Meat produced by forcing beef, pork, or poultry through a sieve or a similar device under high pressure to separate the bone from the edible meat tissue.

SPS Agreement 1995 The WTO agreement on the application of sanitary and phytosanitary measures.

World Organisation for Animal Health (formerly the Office International des Epizooties (OIE)) The world organization for animal health recognized by the WTO agreement on the application of sanitary and phytosanitary measures (SPS Agreement 1995).

Introduction

Market requirements are specified for several reasons ranging from ensuring food safety and animal health to facilitating trade and achieving market differentiation. The rationale for developing specifications influences their complexity as well as the requirements of the systems required for their verification. The reasons for developing market specifications, the main specification attributes, and methods of assessing adherence to specifications are elaborated below. A conclusion section outlines some recent developments in market specifications as a result of technological developments and changing market requirements.

Reasons for Specifications

Food Safety and Animal Health and Welfare

Specifications relating to sanitary measures are important to protect consumer well-being as well as animal health. They can also play a critical role in shaping meat trade. Given the increasingly global nature of food supply (and associated food safety and animal health implications), it is important that these specifications are in place at an international level.

An international agreement was put in place in 1995 called the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement 1995). The agreement is based on three key principles:

- Science-based risk assessment. This is a very important principle from a trade perspective; the science requirements of the agreement have minimized its potential to be used as a protectionist instrument to support domestic producers.
- Equivalence. The concept of equivalence is based on accepting that different sanitary measures can achieve the same level of protection and helps ensure that imported meat meets all safety standards applicable in the importing country.
- Harmonization. This relates to the establishment, recognition, and application of common SPS measures by different members. By harmonizing SPS measures with international standards, food safety and animal health protection can be achieved without unduly restricting international trade.

Although technical and sanitary measures may have common objectives, such as protecting human and animal health and minimizing market failure, operationalization at national level can result in trade issues due to heterogeneous requirements. Reasons for countries adopting different forms of regulations to reach similar objectives include differences in the way the product is defined and characterized, or it may be due to requirements about the production process, requirements on conformity assessment, etc.

Within the agreement, sanitary measures related to meat include hygienic, veterinary, and sanitary requirements for the maintenance and use of meat-processing enterprises. Specifications relate to construction of the plant, its operation, as well as handling of animals during transport and lairage.

Sanitary measures are important for domestic products as well as imported products. Hence, national and regional regulations exist for domestically produced meat. For example, implementation of an hazard analysis critical control point system is mandatory for food production establishments in regions such as the EU and the USA and is generally treated as a precondition of trade. Maximum permissible residue levels (e.g., of nitrofuran and nitrofurazon) in meat and freedom from contamination by relevant microbiological species (e.g., *Escherichia coli*, *Salmonella* spp., *Listeria* spp., etc.) are also specified by various national authorities.

Animal welfare is an important aspect of ensuring food safety due to the close links between animal welfare, animal health, and foodborne diseases. Hence, specifications in relation to animal welfare are often mandatory in nature. However, the link between animal welfare and product quality also means these specifications are important in terms of ensuring consumer satisfaction. For example, pigs slaughtered immediately on arrival have a tendency to produce PSE (pale soft exudative) meat and therefore for animal welfare and meat quality reasons pigs should have a rest period between transport and slaughter. At the EU level, there are directives and legislation in place to cover a range of animal species and welfare-affecting issues, including production methods, transport, and activities at the time of stunning and slaughter. Specific directives cover the protection of individual animal categories, such as calves, pigs, and laying hens. Other international organizations have also issued recommendations and guidelines concerning animal welfare, such as the World Organisation for Animal Health and the Council of Europe. These are not mandatory in nature.

Trade Facilitation

Trade is facilitated by having a common language through which products can be compared and, ultimately, prices can be negotiated. Having commonly understood product specifications means that buyers know what they are getting for a certain price and sellers know what they have to produce to achieve a certain price. For trade in carcasses, the relationship between certain animal characteristics and yield of saleable meat (and thereby profitability) are the basis of product specifications. In the EU, the relationship between conformation and fat level with yield of saleable meat is the basis of the (S)EUROP grid applied to cattle and sheep. Leaner animals with good conformation, for example, continental-type breeds such as Charolais and Limousin, are predicted to have higher yields of saleable meat. The grid consists of a 5-point scale in which each conformation and fat class is subdivided into low, medium, and high classes resulting in 15 classes (some countries have further subdivisions, for example, the UK has a 7-point scale for fatness). It was introduced, in 1981, to facilitate the application of a community scale for classifying adult bovine animals. It has since been extended to sheep and is used as a legal EU requirement for beef (but not sheep) price reporting.

The United Nations has been active in developing an 'international language' to facilitate trade use between buyer and seller and has developed standards for meat that moves beyond the carcass. It describes meat items commonly traded internationally and defines a coding system for communication and electronic trade. It has standards for beef, veal, pork, sheep, goat, llama, alpaca, chicken, and turkey. The standards generally relate to raw (unprocessed) meat. Requirements relating to food standardization and veterinary control are not addressed; they are presumed to be addressed by national or international legislation or requirements of the importing country. Specifications relate to cutting, trimming, and boning of cuts; refrigeration processes and appropriate internal temperatures reflecting whether the product is chilled, frozen, or deep-frozen; traceability; production and feed system; slaughtering system; fat limitations on certain cuts; labeling; and third-party conformity assessments. Localized systems for describing traded meat products are also available, for example, from AUS-MEAT in Australia and the Institutional Meat Purchase Specifications for meat and meat products in the USA.

Classifications based on the EUROP grid in particular, and also on the UN standards, do not provide any indication of the quality of meat from a consumer's perspective. The EUROP system has been criticized as just being a way to pay farmers and being of little value after the payment point. Thus, it is argued that payment for animals based on such a system does not adequately support the needs of modern meat processing. The lack of noninvasive methods to predict eating quality has hindered the development of payment methods for livestock based on eating quality. A number of schemes have been developed recently that attempt to predict eating quality based on various production and processing attributes. One such scheme has been developed by Meat Standards Australia. This grading system for beef, lamb, and sheep meat predicts tenderness and flavor using factors such as breeding, nutrition, environment, cooking length, and cooking method.

Product Differentiation

From a marketing perspective, the main reason for developing specifications is to be able to use key characteristics of production and processing (cues) as a basis for communicating quality attributes to consumers and hence differentiate the product from competitors. This changes the focus of the sale away from being based on the cheapest supplier and away from a 'commodity' product perspective. Such cues are needed because many quality attributes are experiential, i.e., cannot be determined until after purchase, for example, taste and tenderness. Therefore, specifications need to be developed that can provide easily identifiable quality cues for consumers. For example, color, fat levels, cut, trim level, and meat juiciness are used as quality cues for consumer demand attributes such as tenderness, taste, and juiciness. Credence attributes are another important consumer-related specification issue. Credence attributes are attributes that cannot be observed by the consumer at the point of sale or after consumption. Examples in the context of meat include organic, free range, and quality

assured. Providing quality assurance to consumers is an important source of differentiation in many developed markets following bovine spongiform encephalopathy (BSE), the more recent horse meat scandal in processed beef products and other food-related scares. These schemes have generally been related to food safety and traceability, underpinned by national animal movement schemes. However, many have expanded to include sensory attributes, animal welfare, and other credence-type attributes. Consumer interest in products with such attributes is growing with the environmental impact of meat production (e.g., carbon footprint) and its local provenance currently of interest in some markets (e.g., Canada's largest food retailer Loblaws is highlighting the sourcing of Ontario beef and is implementing a Grown Close to Home™ program). Growing consumer interest in carbon reduction due to increased government and media attention to climate change and its causes is one of the drivers of this.

Specifications developed for food safety and animal health reasons tend to be mandatory in nature and may range from national to transnational in scope. Specifications relating to trade may be mandatory or voluntary depending on the context; however, specifications for market differentiation reasons tend to be voluntary. The latter are generally retailer specific; however, national and regional schemes also exist where there is an export market focus.

Specification Attributes

In addition to sanitary, animal welfare, and other regulations that are clearly defined and mandatory in most countries, voluntary specifications relating to production and processing characteristics are specified in most markets. These are primarily based on factors known to influence eating quality and include production and processing factors. Although significant progress has been made in developing effective processing specifications to enhance quality and consistency of the final product for the consumer, the control and manipulation of animal variability is not as well understood. Furthermore, postslaughter factors are known to have a high influence on meat-eating quality because the main determinants of meat tenderness are the shortening of the muscle fibers and the extent of proteolysis of key structural proteins. Both can be optimized using electrical stimulation (and can even ensure that tenderness of *Bos indicus*-type cattle matches that of British and Continental breeds). Hence, it is considered that the postslaughter specifications are particularly important in presenting a product of acceptable quality to the consumer. This section outlines the main animal production and processing characteristics that may be specified in different markets.

Animal Production Characteristics

The final characteristics of the product are determined by the interaction of an animal's genotype with its environment (i.e., phenotype); hence, genetic as well as environmental characteristics may be specified. Specifications for live animals may include breed, sex (including whether male animals are castrated or not), age, and weight (live or dead weight) at slaughter and animal welfare practices including parasitic dosing, housing and feeding (including age at weaning) regimens, and healthcare. Breed, sex, age, and weight at slaughter influence fat and conformation levels and ultimately saleable meat yield and yield of cuts of various quality, and quality in terms of taste and tenderness. For example, Blonde d'Aquitine animals produce a higher yield of saleable meat because of lower fat levels and better conformation than other breeds. Age at slaughter is reported to effect beef tenderness; however, the impact of breed on meat quality is not clear because of the interfering influences within and between breeds, such as feed, growth rates, etc. Many of these factors are interrelated: for a given age at slaughter, bulls tend to be heavier; however, heifers of a European continental breed, for example, Charolais, may be heavier than bulls of one of the British breeds, for example, Hereford. Age at slaughter is also a food safety issue. For example, animals more than 30 months of age are not allowed into the food chain in the UK because of concerns about BSE. However, animals more than 30 months of age are allowed into the food chain in other EU countries if they have been tested free of BSE. Animal welfare practices influence consumer acceptability of the product and a priori knowledge can also bias sensory perceptions in favor of the product deemed to be from systems with higher animal welfare and greater environmental concern. However, feeding regimen also influences carcass and meat quality. Too low feeding levels increase age to slaughter, decrease the amount of intramuscular fat, darken the muscle color in the case of beef, and decrease the palatability of the meat. Too high feeding levels provide proper contents of intramuscular fat and good palatability of the meat but carcasses tend to be too fat, which has a negative effect on marketing and processing. In addition to the level of feeding, composition of the diet influences fat and muscle color. Pink beef with white fat can be produced by feeding a diet including maize silage, whereas beef that is deep red with yellow fat can be achieved on a grass-based diet. Moreover, the high level of genetically modified protein sources in animal feed has resulted in a specification that requires genetically modified feed to be excluded from an animal's diet for a specified period before slaughter in some markets.

Processing Characteristics

Specifications for carcasses include product attributes such as weight of carcass, conformation, fat levels and color of fat and muscle, and process attributes such as slaughtering, maturing/aging procedures, cutting, and packing. Process attributes are specified because they are important environmental variables that influence the final quality of the product, saleable yield, and ultimately profitability.

The most common slaughtering method is the captive bolt. However, meat destined for certain markets may require specific slaughter methods. For example, meat for the Muslim market must be from cattle slaughtered by cutting the throat to allow the meat to be sold as halal. Electrical stunning can be used. Electrical stimulation during the stunning or bleeding period can improve tenderness, so some multiple retailers specify this.

The aging process/conditioning has a significant impact on tenderness as the natural enzymes in meat (e.g., calpains)

tenderize the meat over time. In general, extending the postmortem aging period improves tenderness. Under normal industrial conditions, aging period varies by animal species with 3–5 days being the norm for pigs, 7–10 days for sheep, and 10–21 days for cattle. Moreover, the chilling regimen can influence the aging process, as activity of the enzymes is influenced by temperature. The way the carcass is suspended during the aging process also influences tenderness. For example, pelvic suspension improves tenderness and is a specification used by many Irish and UK multiple retailers. Furthermore, the addition of tenderizers (enzymes), such as papain, helps to break down the connective tissue and make the meat tender; however, this is not a commonly used specification. In practice the length of the aging period is often determined by geographical proximity of the market (meat for local markets needs to be tender within a few days, whereas meat for the export market may have the advantage of several weeks of chilled storage in which aging can take place) with other means used to achieve tenderness within the given time limit.

Cutting style affects yield and cutting time and thus profitability. Cutting specifications vary by anatomical separation location (e.g., the rib number at which the animal is quartered) and/or the level of external fat trim (whether or not various fat deposits are left on the carcass). Primal and further processed cuts are obtained in accordance with different cutting specifications. Industry cutting specifications are produced by a number of agencies. For example, in the USA, the Institution Meat Purchase Specifications/National Association of Meat Purveyors Specifications number may be specified to avoid the need to specify cutting procedures in detail. Different cuts are specified according to intrinsic characteristics of the muscle, because for example, fiber structures may be an important source of variation in meat-eating quality. BSE has influenced cutting specifications within the EU as all beef animals more than 12 months of age at slaughter are required to have their spinal column removed. This has resulted in a new outlet for animals less than 12 months of age, which is aimed at the 'T-bone market.'

The level of processing to some extent determines packing specifications. Primal cuts tend to be vacuum packaged and retail cuts packed in modified atmosphere packaging; however, other forms of packing are also available. Other quality specifications at this stage include level of marbling, total bacterial counts, bruising, pH, drip, and shelf life.

In an attempt at differentiation, some breed-specific specifications have been developed, for example, Certified Hereford Beef Specification, and some production systems based on geography and traditional methods are protected within Europe. Examples of the latter include products that use Protected Geographical Indications (PGI), for example, Ternera de Navarra (Spain) and Connemara Hill Lamb (Ireland).

In addition to the above, to comply with food safety and other requirements, modern meat production specifications include a requirement to maintain records of all activities along the chain.

Assessment of Adherence to Product Specifications

Adherence to specifications can be objectively determined in some instances; however, an element of subjectivity exists for others. This is not surprising as quality is an important factor in determining many specifications and quality encompasses both objective (e.g., carcass weight and organoleptic measures) and subjective elements (e.g., about production methods). Visual inspection is common for examining some aspects of the physical product, such as fat and conformation. However, other attributes require auditing, certification, and evaluations. For example, feeding regime and parasitic dosing regimes require detailed records, which may have to be verified by a third party. Signed declarations from producers/processors to agree to a code of practice covering issues such as stockmanship, animal welfare, feeding, etc. are fundamental to quality assurance and other credence attributes-based schemes. Random farm/abattoir visits by an independent agency are also part of the process.

Objectivity is important for all specifications but particularly for specifications that determine price. The EUROP classification scheme for beef and sheep was entirely subjective when first implemented as it was generally carried out by visual appraisal by trained graders. This often resulted in mistrust between farmers and abattoirs and between abattoirs and retailers. More recently mechanical grading systems have been developed and implemented. These are now widespread in Australia and some parts of Europe. Ireland was the first EU country to get authorization for mechanical grading and has 24 systems installed in export factories. France now have a significant number of systems, Denmark and Germany have a small number of systems, and a few countries (the UK, Spain, Sweden, and Norway) have done, or are in the process of doing, trials. These systems are generally based on video imaging analysis (VIA). They use one or more digital cameras, lights, and a large numbers of measurements at specific points of a completely still carcass to assess shape and coloration, and hence predict conformation, fat cover, and meat yield using algorithms. Such systems also have limitations with an argument that VIA is not very good for fat assessment or for assessing damaged carcasses (e.g., those damaged during hide removal). Methods to address these limitations include augmenting the system with other systems, such as near-infrared spectroscopy in the case of the fat limitation, paying based on a batch average, or engaging a human grader for the damaged carcass.

The range of objective measurements is increasing in availability and prevalence, (e.g., mechanical grading on the (S)EUROP grid and testing using single-nucleotide polymorphisms (SNPs) for PGI status). Moreover, measurement is becoming easier with the use of noninvasive live animal and carcass measurements to predict key attributes. For example, ultrasound measurements of live animals close to slaughter can be used to predict retail beef yield and thus avoid the need to individually weigh cuts. Bioelectrical impedence of primal cuts can be used to determine total skeletal muscle and total skeletal fat-free muscle weight.

Recent Developments

Technological advances result in the need to develop new specifications to protect consumer health, ensure animal welfare, etc. Such advances in the context of meat include farm animal cloning, advances in mechanical separation of meat, and

the development of *in vitro* meat production systems. Specifications in these areas are often limited by the systems that are available to assess adherence to specifications. For example, with regard to cloning, although the European Commission has proposed introducing a 5-year temporary suspension of cloning or importing of clones for food, a practical test does not exist to determine whether an animal has been produced as a result of cloning technology or natural reproduction.

Although mechanically separated meat (MSM) has been available for some time, issues around its safety arose in the 1980s and resulted in restrictions around how much can be used and the type of products in which it can be used. Clear regulations exist around how it may be described on food labels, for example, it may not be labeled as 'meat' but must be labeled as 'mechanically separated' pork, chicken, or turkey in the ingredients statement. Products such as hot dogs may contain no more than 20% mechanically separated pork. Concerns about the potential for spinal cord being mixed up with the rest of the meat during the mechanical separation process have resulted in restrictions around its application. For example, mechanically separated beef is considered inedible in the USA and is not permitted in any processed products. Concerns also exist about the calcium content of MSM so that MSM specification may require that the calcium content is not significantly higher than that of minced meat. Specifications relating to microbiological criteria are also important in the context of MSM.

In vitro meat is not commercially available; however, recent developments indicate that it could be available as a meat ingredient in the not too distant future. Although sensory and visual aspects are not clear, such a product could be attractive to consumers for animal welfare and environmental reasons. Specifications on its production will be considerably different to meat produced naturally and could include specifications relating to growth medium, origin of the 'starter' culture, etc. It could also result in a market specification regarding the origin of the meat, i.e., *in vivo* or *in vitro*.

Continued developments may be expected in this area driven by an increasingly demanding consumer (particularly regarding credence attributes) as well as technological advances in measurement techniques to verify specifications. However, fundamental to the development of a definitive, objective list of market specifications will be improved understanding of the relationship between production and processing conditions and quality and saleable yield as well as more research that identifies various noninvasive predictors of eating quality and meat yield.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era. Carcass Chilling and Boning. Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Color and Pigment; Palatability; pH Measurement; Water-Holding Capacity. Classification of Carcasses: Beef Carcass Classification and Grading; Pig Carcass Classification. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycogen; Rigor Mortis, Cold, and Rigor Shortening; Slaughter-Line Operation and Pig Meat Quality. Cooking of Meat: Flavor Development; Heat Processing Methods. Cutting and Boning: Hot Boning of Meat. Electrical Stimulation. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Meat Marketing: Cold Chain; Transport of Meat and Meat Products. Meat Pricing Systems. Mechanically Recovered Meat. Microbial Contamination: Microbial Contamination of Fresh Meat. Modeling in Meat Science: Meat Quality. Muscle Fiber Types and Meat Quality. On-Line Measurement of Meat Composition. On-Line Measurement of Meat Quality. Preslaughter Handling: Preslaughter Handling. Quality Management: Abattoirs and Processing Plants. Refrigeration and Freezing Technology: Principles. Religious Slaughter. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production. Sensory and Meat Quality, Optimization of. Slaughter, Ethics, and the Law. Tenderizing Mechanisms: Chemical; Mechanical

Further Reading

Allen, P., Finnerty, N., 2001. Mechanical Grading of Beef Carcasses. Ireland: Teagasc ISBN 1 84170 262 5.

Datar, I., Betti, M., 2010. Possibilities for an *in vitro* meat production system. Innovative Food Science and Emerging Technologies 11, 13–22.

Díez, J., Albertí, P., Ripoll, G., *et al*., 2006. Using machine learning procedures to ascertain the influence of beef carcass profiles on carcass conformation scores. Meat Science 73, 109–115.

Gursansky, B., O'Halloran, J.M., Egan, A., Devine, C.E., 2010. Tenderness enhancement of *Bos indicus* and *Bos taurus* cattle through electrical stimulation. Meat Science 86, 635–641.

Joint Research Centre of the EU Commission, 2010. Evaluation of the livestock sector contribution to the EU Greenhouse Gas (GHG) emissions (GGELS). Italy: Joint Research Centre of the EU Commission. Available at: http://ec.europa.eu/agriculture/analysis/external/livestock-gas/full_text_en.pdf (accessed 06.09.13).

Negrini, R, Nicoloso, L., Crepaldi, P., *et al*., 2008. Traceability of four European Protected Geographic Indication (PGI) beef products using single nucleotide polymorphisms (SNP) and Bayesian statistics. Meat Science 80, 1212–1217.

Schlueter, S.W., Wieck, C., Heckelei, T., 2009. Regulatory policies in meat trade: Is there evidence for least trade distorting sanitary regulations? American Journal of Agricultural Economics 91 (5), 1484–1490.

Thompson, J., 2002. Managing meat tenderness. Meat Science 62, 295–308.

Thornton, P.K., 2010. Livestock production: Recent trends, future prospects. Philosophical Transactions of the Royal Society of Biological Sciences 365, 2853–2867.

Transport of Meat and Meat Products

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Glossary

Conduction, thermal. Mechanism for heat transfer The process of heat transfer through a solid material/medium in which kinetic energy is transmitted by the particles of the material from particle to particle without gross displacement of the particles.

Convection, thermal. Mechanism for heat transfer The process of heat transfer through a liquid or gas by means of circulating currents caused by changes in density.

Eutectic solution Relating to or denoting a mixture of substances (in fixed proportions) that melts and freezes at a single temperature that is lower than the melting points of the separate constituents or of any other mixture of them, for example brine (salt and water). Eutectic refrigeration systems utilize the latent heat within a frozen eutectic solution to provide heat extraction and maintain temperatures.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Pasteurization A form of heat treatment that kills certain vegetative bacteria and/or spoilage organisms in milk and other foods. Temperatures below 100 °C are used.

Radiation, thermal Mechanism for heat transfer. Electromagnetic radiation generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emits thermal radiation.

Refrigeration May be defined as the process of removing heat from any substance to: (1) render colder – reduce temperature, (2) change its state – for example, water to ice, (3) maintain its state – preserving foods, storing ice.

Water activity (aw) A measure of the available water in a substance. 'High-aw' foods support bacterial growth; 'low aw' do not. This is not the same as water content. Some foods with a high water content have a relatively low aw because the water is bound up with dissolved salts or sugar, for example, jam.

Introduction

Worldwide transportation of chilled and frozen meat by sea, land, and air is a well-established practice. Developments in frozen transport in the nineteenth century established the international meat market. In 1877 a cargo of frozen meat was sent from Buenos Aires to France. The following year 5000 frozen mutton carcasses were transported from Paraguay to France. In 1880 the Strathleven arrived in London with a cargo of 40 ton of frozen Australia beef, and the Dunedin followed in 1882 with mutton, lamb, and pork from New Zealand; by 1910 the UK was importing 600 000 ton of frozen meat. Further developments in temperature control have established sea transportation of chilled meat from Australia and New Zealand to European and other distant markets, and road transportation of chilled products throughout Europe, the Middle East, and the United Sates is now common practice. Air freighting is increasingly being used for high-value perishable meat products such as venison and Wagyu beef.

In the UK, transportation is considered to be the third highest sector in the food cold chain in terms of energy consumed by refrigeration systems. Under European Union legislation red meat (beef, lamb, and pork) must be kept below 7 °C, poultry below 4 °C, and offal below 3 °C during transportation.

It is particularly important that the meat is at the correct temperature before loading because the refrigeration systems used in most transport containers are not designed to extract heat from the load but to maintain the temperature of the load. In the large containers used for long-distance transportation meat temperature can be kept within ±0.5 °C of the set point. With this degree of temperature control transportation times of 8–14 weeks (for vacuum-packed meats stored at −1.5 °C) can be carried out and still retain a sufficient chilled storage life for retail display.

There are substantial difficulties in maintaining the temperature of refrigerated meats transported in small refrigerated vehicles that conduct multidrop deliveries to retail stores and caterers. During any one delivery run, the chilled product can be subjected to as many as fifty door openings, where there is heat ingress directly from outside and from personnel entering to select and remove product. The design of the refrigeration system has to allow for extensive differences in load distribution, dependent on different delivery rounds, days of the week, and the removal of product during a delivery run.

Sea Transport

Historically it was the need to preserve meat during sea transport that led to the development of mechanical refrigeration and the modern international trade in foodstuffs. Recent developments in temperature control, packaging, and controlled atmospheres have substantially increased the range of foods that can be transported around the world in a chilled condition. With conventional vacuum packing it is difficult to achieve a shelf life in excess of 12 weeks with beef and 8 weeks for lamb. Controlled/modified atmospheric packaging can extend this by many weeks. A shelf life of up to 14 weeks at −1.5 °C can now be achieved with lamb from New Zealand.

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Table 1 Expected maximum transport duration for meat shipments for a reasonable retail display/shelf life

	Vacuum pack (0 °C; weeks)	*Vacuum pack (−1.5 °C; weeks)*
Pork	6	8
Lamb	7	10
Beef	10	14

These cuts are individually packed in evacuated bags of linear polyethylene, and then placed in a foil laminate bag that is gas flushed and filled with a volume of CO_2 approximately equal to that of the meat. Similar storage lives are currently being achieved with beef primals transported from Australia and South Africa to the EU.

Assuming good standards of preparation and prompt cooling the times given in Table 1 can be used as approximate guidelines for long-distance meat shipment. These times rely on the meat being at or below the storage temperature before loading. The 2- to 4-week advantage of transporting meat at −1.5 rather than 0 °C is lost if the meat is loaded at a temperature above 0 °C. Cooling in the center of a load of meat is very slow and the meat will be well into its journey before the desired temperature is achieved.

Most International Organization for Standardization (ISO) containers for food transport are either 6 or 12 m long, hold up to 26 ton of product, and can be 'insulated' or 'refrigerated.' The refrigerated containers incorporate insulation and have refrigeration units built into their structure. The units usually operate electrically, either from an external power supply on board the ship or on the dock, or from a generator on a road vehicle. Insulated containers either utilize plug-type refrigeration units or may be connected directly to an air-handling system in a ship's hold or at the docks. Close temperature control is most easily achieved in containers that are placed in insulated holds and connected to the ship's refrigeration system. However, suitable refrigeration facilities must be available for any overland sections of the journey. When the containers are fully loaded and the cooled air is forced uniformly through the spaces between cartons, the maximum difference between delivery and return air can be less than 0.8 °C. The entire product in a container can be maintained to within ±1.0 °C of the set point.

Refrigerated containers are easier to transport overland than the insulated types, but often have to be carried on deck when shipped because of problems in operating the refrigeration units within closed holds. Therefore, they are subjected to much higher ambient temperatures on board ship and consequently larger heat gains that make it far more difficult to control product temperatures.

For bulk transportation of frozen meat refrigerated cargo ships are commonly used. Frozen meat is generally stored and transported at −18 °C or below. Unlike chilled meat, small temperature changes during loading and unloading can be tolerated with frozen meat.

Air Transport

Perishable goods, food, and ornamental plants making up 80% by volume is one of the largest air cargo sectors, accounting for 14% by volume of total global air freight. Reports in 2005 highlighted perishables as one of the fastest growing air cargo sectors at approximately 10% per year, expecting volumes to amount to approximately 10 million tones per year by 2010. Although air freighting of foods offers a rapid method of serving distant markets, there are many problems because the product is usually unprotected by refrigeration for much of its journey. Up to 80% of the total journey time is made up of waiting on the tarmac and transport to and from the airport. During flight the hold is normally between 15 and 20 °C. Perishable cargo is usually carried in standard containers, sometimes with an insulating lining and/or dry ice, but is often unprotected on aircraft pallets.

Studies in Australia have led to the following recommendations for air transport of chilled foods:

1. Insulated containers should always be used to reduce heat gain.
2. Product should always be precooled and held at the required temperature until loading.
3. With products that deteriorate after any surface freezing dry ice should not be used.
4. Containers should be filled to capacity.
5. A thermograph should accompany each consignment.

Overland Transport

Overland transportation systems range from 12 m refrigerated containers for long-distance road or rail movement of bulk chilled or frozen products, to small uninsulated vans supplying food to local retail outlets or even directly to the consumer. Some of the first refrigerated road and rail vehicles for chilled product were cooled by air that was circulated by free or forced systems, over large containers of ice. Similar systems using solid carbon dioxide as the refrigerant have also been used for cooling of transport vehicles. However, most overland vehicles for long-distance transport are now mechanically refrigerated.

There can be a substantial difference in the ability of transport systems to cool warm meat. One particular study in the USA, for example, has shown rail containers to be more effective than road containers at reducing temperature in meat should it be loaded warm. In the study deep temperature in beef sides and quarters at the time of their loading into transport vehicles ranged from 6 to 18 °C. Maximum surface temperatures were also high and ranged from 0.5 to 6.5 °C. In rail wagons the surface temperature declined during the first 24 h and was subsequently maintained at a temperature of 0 ±1 °C. In the road vehicles the surface temperature fell slowly during the whole journey and had not attained a steady minimum value when unloaded. On average the deep temperature of sides in rail wagons reached 1 °C after 72 h. Temperatures in quarters in road vehicles were still above 2 °C after 120 h. Journey times varied from 3.8 to 6.7 days, and it was calculated that during that time pseudomonads could proliferate by between 8 and 22 generations in the meat transported by road. Adequate air movement through the load in the rail vehicles produced even temperature distributions.

In general, it is not advisable to rely on product cooling during transportation. However, in the Netherlands 'in

transport cooling' is used as an integral part of a processing system for pork carcasses, which allows the carcasses to be dispatched on the same day the animals are killed.

Types of Refrigeration System

The majority of current road transport vehicles for chilled foods are refrigerated using either mechanical, eutectic plates or liquid nitrogen cooling systems. The use of photovoltaics (solar power) to power mechanical units has been pioneered in some countries.

Mechanical Units

Many types of independent engine and/or electric motor-driven mechanical refrigeration units are available for trucks or trailers. One of the most common is a self-contained 'plug' unit that mounts in an opening provided in the front wall of the vehicle. The condensing section is on the outside and the evaporator on the inside of the unit, separated by an insulated section that fits into the gap in the wall. Units have one or two compressors, depending on their capacity, and these can be belt driven from the vehicle but are usually driven directly from an auxiliary engine. This engine may use petrol from the vehicle's supply, an independent tank, or liquid petroleum gas. Many are equipped with an additional electric motor for standby use or for quiet running, for example, when parked or on a ferry.

Irrespective of the type of refrigeration equipment used the product will not be maintained at its desired temperature during transportation unless it is surrounded by air or surfaces at or below that temperature. This is usually achieved by a system that circulates air, either forced or by gravity, around the load. Inadequate air distribution is probably the principle cause of product deterioration and loss of shelf life during transport. Conventional forced air units usually discharge air over the stacked or suspended products either directly from the evaporator or through ducts toward the rear cargo doors. Because air takes the path of least resistance it circulates through the channels that have the largest cross-sectional area. These channels tend to be around rather than through the product. If the products have been cooled to the correct temperature before loading and do not generate heat then they only have to be isolated from external heat ingress. Surrounding them with a blanket of cooled air achieves this purpose. Care has to be taken during loading to avoid any product contact with the inner surfaces of the vehicle because this would allow heat ingress during transport. Many trucks are now being constructed with an inner skin that forms a return air duct along the side walls and floor, with the refrigerated air being supplied via a ceiling duct.

The application of photovoltaics to refrigeration for the distribution of chilled supermarket produce has been pioneered in some countries such as the UK. In 1997 Sainsbury's, a major UK supermarket chain, commissioned the world's first solar-powered refrigerated trailer. Operating in the UK, it was stated that 'During most of the year there has been an excess of solar energy over daily demand.' However, few commercial systems are currently available, and it is possible that the high capital cost of current PV systems is limiting adoption. It is anticipated that with time these costs should come down and payback times shorten.

Eutectic Plates

Eutectic plate cooling systems are used in refrigerated vehicles serving local distribution chains. The system works on the principle of latent heat. The eutectic plate consists of a coil, through which a primary refrigerant can be passed, mounted inside a thin tank filled with a eutectic solution. The eutectic solution is frozen by the refrigeration system in a static, or overnight, situation. During the working day no power is required, the cooling being provided by the melting of the frozen eutectic plate. A significant amount of refrigeration power is created as the eutectic melts, due to latent heat, while keeping a constant temperature in the container. Standard eutectic solutions freeze at temperatures between −3 and −50 °C. A number of these plates are mounted on the walls and ceilings or used as shelves or compartment dividers in the vehicles. Two methods are commonly used for charging up the plates: (1) when the vehicle is in the depot the solutions are frozen by coupling the plates to stationary refrigeration plants via flexible pipes and (2) a condensing unit on the vehicle is driven by an auxiliary drive when the vehicle is in use and an electric motor when stationary.

To provide the required cooling capacity, the plates should be mounted so that air can circulate freely over both sides and over the product. Most systems rely on gravity circulation but some are equipped with fans, ducts, and dampers for temperature control.

Eutectic systems are chosen for the simplicity, low maintenance, and quietness of their operation but can suffer from poor temperature control.

Liquid Nitrogen

A typical liquid nitrogen system consists of an insulated liquid nitrogen storage tank connected to a spray bar that runs along the ceiling of the transport vehicle. Liquid nitrogen is released into the spray bar via a thermostatically controlled valve and vaporizes instantly as it enters the body of the vehicle. The air is then cooled directly utilizing the change in the latent and sensible heat of the liquid nitrogen. Once the required air temperature has been reached the valve shuts off the flow of liquid nitrogen and the temperature is subsequently controlled by intermittent injections of liquid nitrogen.

Many advantages are claimed for liquid nitrogen transport systems, among them their simplicity, low capital cost, and silent operation. It is also claimed that long hauls can be carried out, vehicles are available that will maintain a chilled cargo at 3 °C for 50 h after a single charge of liquid nitrogen, and overall costs are comparable with mechanical systems.

Problems Particular to Local Delivery Vehicles

In a 1970–71 UK survey of vehicles used to transfer chilled meat from small abattoirs to shops, almost 70% were

unrefrigerated and 20% had no insulation. Since that time the intensifying demand from legislation and retailers for lower delivery temperatures has put increasing pressure on fleet operators to improve temperature control. However, there are substantial difficulties in maintaining the temperature of chilled foods transported in small refrigerated vehicles that conduct multidrop deliveries to retail stores and caterers. The vehicles have to carry a wide range of products and operate under diverse ambient conditions. As already mentioned, during any single delivery run, the chilled product can be subjected to as many as fifty door openings, during which there is heat ingress directly from outside and from personnel entering to select and remove product. The design of the refrigeration system has to allow for extensive differences in load distribution, dependent on different delivery rounds, days of the week, and the removal of product during a delivery run. A refrigeration system's ability to respond to sudden demands for increased refrigeration is often restricted by the power available from the vehicle. All these problems combine to produce a complex interactive system.

Throughout the world sales of chilled foods are expanding. In the meat industry the traditional range of pies, sausages, and cooked meats has been rapidly expanded with the addition of many ready meal-type products. Traditional meat product manufacturers are now aiming to further extend their range of products to include items such as gourmet-style meals without artificial colors, flavors, or preservatives. Retailers are discovering that considerable quality and economic advantages can be derived from maintaining chilled products at temperatures far closer to their initial freezing point. Increasingly, fleet operators will be forced to deliver chilled meats at temperatures between 0 and 2 °C.

The rise in supermarket home delivery services, where there are requirements for mixed loads of product that may each require different storage temperatures, is introducing a new complexity to local land delivery.

Design and Operation of Local Distribution Vehicles

Refrigerated vehicles are developed and tested in carefully controlled conditions. Owing to the large number of interacting variables, as many as possible of these variables are held constant during the tests. These tests do not provide van manufacturers with data on food temperatures and van performance during commercial operation. Additionally, van operators need to know in advance whether a particular van, on a particular round, under given ambient conditions, will be able to deliver food at the correct temperature. To overcome these problems computer prediction programes such as 'CoolVan' have been produced that allow the systematic alteration of one or more variables while simulating the operation of a vehicle in a complex, realistic way.

In the following section, data from the verified model illustrate the effects of some of these variables while operating a small refrigerated delivery van over a realistic journey, during which it stops and starts, the door opens and closes, the ambient conditions vary, the refrigeration system operates under its own control, and food is removed from the vehicle. The van modeled was a 3-ton vehicle, typical of the type used for the delivery of cooked meat products. It consisted of an insulated box with a single door at the rear. A nominally 2.8 kW refrigeration unit was mounted in a pod on the front bulkhead of the van over the driver's cab. Inside, the van was equipped with racking for carrying trays of products and a reinforced floor. The type of journey for which these vehicles are used ranges from a single bulk delivery to a regional depot or supermarket, to multiple deliveries to up to 40 separate shops in a region during 1 day. The details of the standard journey used to compare factors are shown in Table 2.

Table 2 Standard delivery van schedule for assessment of effect of van and operational variables

Variable	*Value*
Mean daily temperature	16 °C
Maximum daily temperature	28 °C
Relative humidity	60%
Cloud cover	None
Month	June
Latitude	53° N
Start time	07:30
Journey length	321 km
Number of stops	20
Time halted at each stop	5 min
Time the door is open at each stop	2 min
Door protection	Strip curtains
Average speed between stops	32 km h^{-1}
Weight of food at start of journey	1000 kg[a]
Initial van temperature	4 °C
Thermostat switch-on temperature	5 °C
Thermostat switch-off temperature	3 °C

[a]An equal amount of food is delivered at each stop and no food is left at the end.

Van Insulation

To maintain meat temperatures below 5 °C in an uninsulated van the refrigeration system has to have an extraction capacity of over 10 kW. However, only a small thickness of insulation is required to greatly reduce the amount of heat that has to be extracted, the amount decreasing with the reciprocal of the thickness of insulation (Figure 1). Loading a van with precooled meat reduces the total amount of heat that has to be extracted during a journey. The load acts as a heat sink, consequently modifying the action of the thermostat and reducing the running time of the refrigeration system and the heat extracted by it. For an insulation of 75 mm thickness (which is typical for a commercial vehicle) loading the van reduced the average heat extraction rate by 26%. Increasing the insulation thickness from 75 to 100 mm produced a 33% reduction in the average extraction power required.

Infiltration

Poor maintenance and customization can have a significant effect on heat infiltration. A comparison of two vehicles, one a well-sealed van with a close-fitting door and effective door seals, the other a poorly maintained one with several gaps and

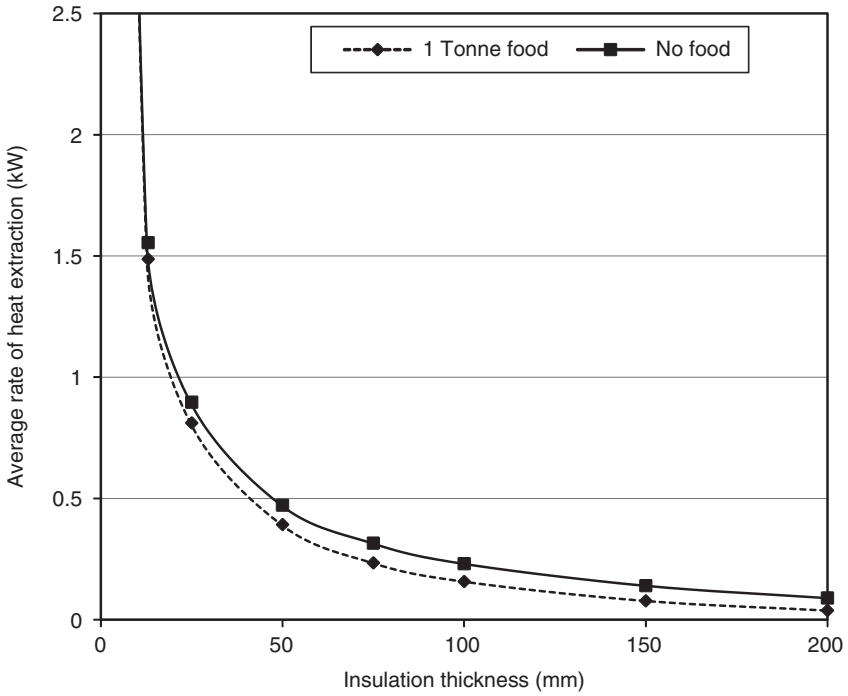

Figure 1 The effect of the thickness of insulation of a refrigerated delivery vehicle, with and without a food load, on the average rate of heat extracted by the refrigeration plant over a standard journey (details of journey shown in **Table 2**).

holes through the insulation and poor door seals, showed a difference in measured heat infiltration of 86%. However, infiltration during the time that the door is closed is a relatively small proportion of the total refrigeration load. Consequently, the vehicle, fitted with a nominal 2 kW cooling system, was found to keep the meat temperature below 5 °C during the journey.

Door Openings

The heat extracted from a closed van is very small; however, opening the door greatly increases the heat load. In vans where the engine drives the refrigeration system this extra heat must be removed during the period when the van is moving. Several factors interact when the number of door openings increases. The complete journey takes longer, and during the extended journey the ambient temperature and the solar radiation on the van is different from the early part of the journey. If the length of time that the door is left open at each stop is also increased from 5 to 10 min then the temperature of the air around the food in the van increases further during each stop (Figure 2). The refrigeration plant therefore operates at a higher evaporating temperature (and hence it has greater capacity) when reducing the temperature once the doors are closed and the vehicle starts moving again. The time during which the refrigeration plant can run remains the same as the number of drops increases, and therefore the rate of heat extraction increases approximately linearly with the number of stops.

The fitting of plastic strip curtains across the doorways can substantially reduce heat gain and consequently the heat that has to be extracted (Figure 2). However, in practice they are often damaged or tied aside because they restrict free access.

Initial Food Temperature

Delivery vans are designed to transport precooled meat. If the meat is loaded at 7 °C rather than 0 °C the heat extracted by the refrigeration system will be 7 times greater. In the predicted case, shown in Figure 3, the meat was assumed to spread out over the shelves of the van and thus cooled down quickly. If the meat were stacked with little or no air circulation through it then the heat extracted would be less, but the meat would remain warm.

Length of Journey

As the length of the journey gets shorter while the number of drops remains the same the heat entering the van during the stops must be extracted in shorter time intervals between each stop. The rate of heat extraction therefore varies inversely with the length of the journey (Figure 4). It is easier to maintain food temperatures on long journeys than when there are a large number of stops with little time spent traveling between each stop.

Combination of Factors

It is when a number of factors combine that problems occur and the temperature of the meat cannot be maintained. If an

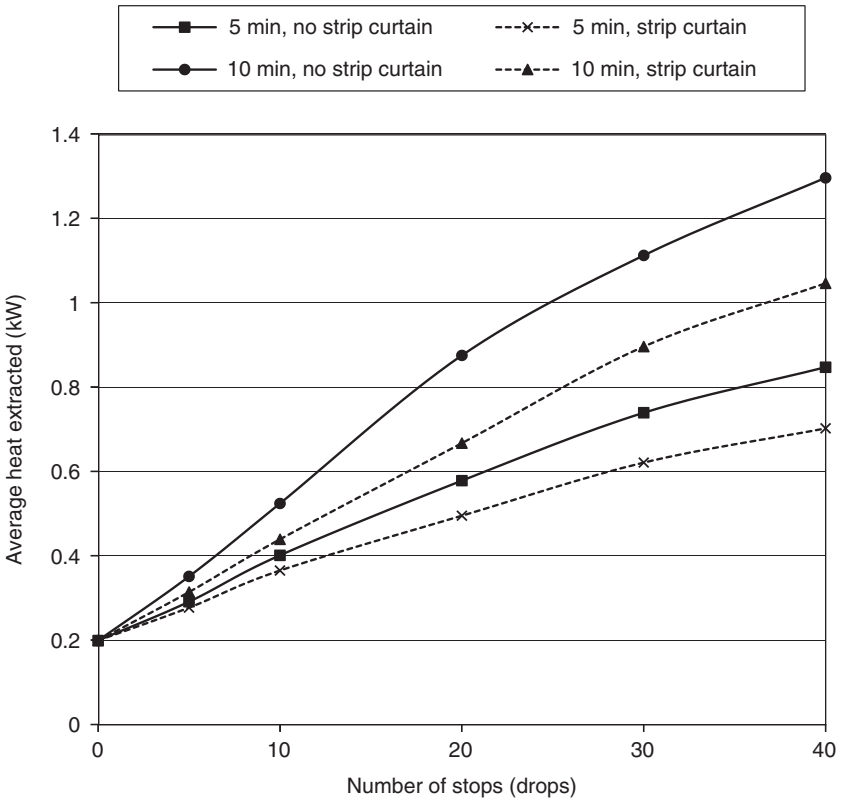

Figure 2 The effect of the number of stops (drops), and time of the stop, a refrigerated delivery vehicle makes, with and without a strip curtain, on the average rate of heat extracted by the refrigeration plant, averaged over the periods when the vehicle is moving, over a standard journey (details of journey shown in **Table 2**).

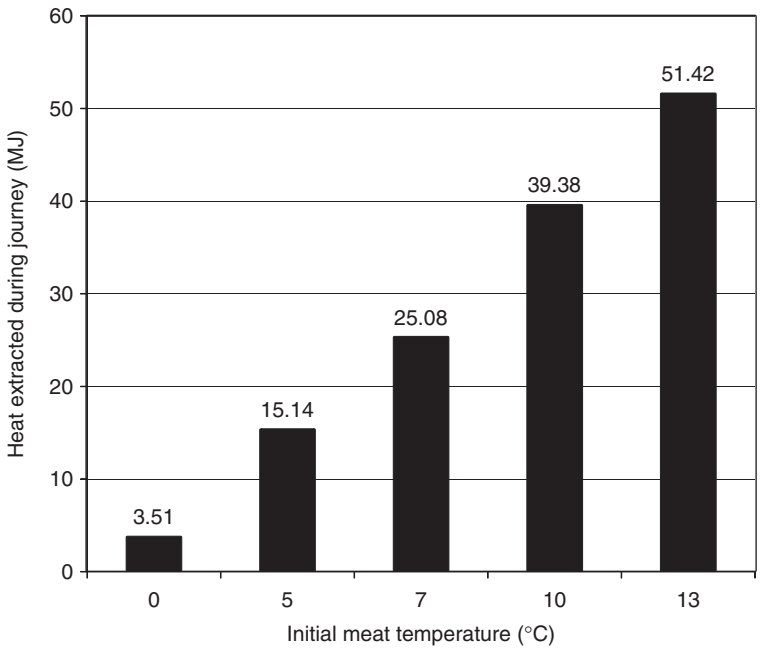

Figure 3 The effect of different initial product temperatures loaded on to a refrigerated delivery vehicle on the heat extracted by the refrigeration plant over a standard journey (details of journey shown in **Table 2**).

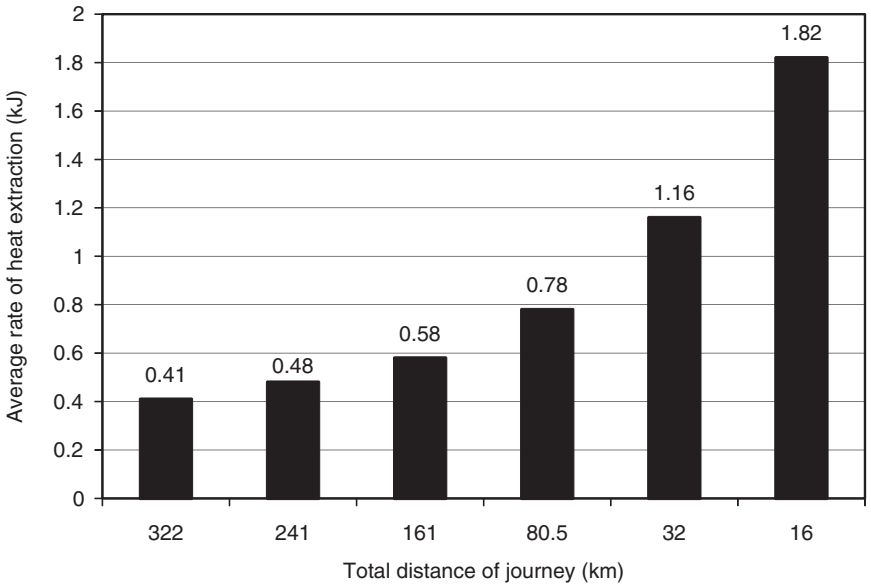

Figure 4 The effect of the total distance of a journey on the average rate of heat extracted by the refrigeration plant of a refrigerated delivery vehicle, averaged over the periods when the vehicle is moving (details of journey shown in **Table 2**).

old van were used for the standard journey described in this section and the van stopped for 10 min at each delivery with the door open for 5 of those minutes, with strip curtains in place, then the meat temperature would be 5.1 °C at the end of the journey. If the number of stops increased to 30 and the length of the journey decreased to 100 miles then it would increase to 6.4 °C. In these journeys, the refrigeration plant is not running long enough when the van is moving to remove all the heat entering the van when it has stopped.

If strip curtains were fitted over the door (and used) but the door was left open for the full 10 min that the van was stopped at each delivery, then the meat temperature at the end of the run would increase to 7.2 °C. If the operator then reduced the amount of food carried on the van to an initial load of 250 kg, the final food temperature would be 8.0 °C. This is because when the air temperature in the van increases above the food temperature the food starts to cool the air and acts as a store of refrigeration capacity. If less food is placed in the van then there is a smaller store of refrigeration capacity and temperatures increase. If the strips are not used over the door opening then the food temperature increases even more to 9.4 °C. Moreover, if the journey is started later in the day, when the ambient temperature has warmed up, then the situation deteriorates even further.

To try to rectify matters operators have moved to more powerful refrigeration units, or to eutectic systems, and they have also considered vans where the refrigeration system is driven by its own engine so that it can operate when the van is stationary. If such a system were used then it would have to have a capacity in excess of 4 kW if it were to maintain the food temperature to a maximum of 5 °C during the last journey described above. Eutectic systems have the advantage that they can deliver cooling while the van is stationary. However, their storage capacity is limited and a eutectic system would need a large number of beams (the thermal storage units) to maintain temperature during the above combinations.

See also: Modeling in Meat Science: Refrigeration. Physical Measurements: Temperature Measurement. Refrigeration and Freezing Technology: Applications; Freezing and Product Quality; Thawing

Further Reading

Brown, M., 2008. Chilled Foods: A Comprehensive Guide, third ed. Cambridge, UK: Woodhead Publishing Limited.
Critchell, J.T., Raymond, J., 1912. A History of the Frozen Meat Trade. London, UK: Constable & Company.
Cutting, C.L., 1972. Meat Chilling – Why & How? Langford, Bristol, UK: Agricultural Research Council, Meat Research Institute MRI Symposium No. 2.
Cutting, C.L., 1974. Meat Freezing – Why & How? Langford, Bristol, UK: Agricultural Research Council, Meat Research Institute MRI Symposium No. 3.
Gigiel, A.J., 1997. Predicting food temperatures in refrigerated transport. The Proceedings of the Institute of Refrigeration. Vol. 93, Session 1996–97. Carshalton, UK: The Institute of Refridgeration, pp. 76–89.
Gill, C.O., Phillips, D.M., 1993. The efficiency of storage during distant continental transportation of beef sides and quarters. Food Research International 26, 239–245.
Heap, R.D., 1997. Chilling during transport, including control. Proceedings of the World Congress on Food Hygiene. The Hague, The Netherlands: Wageningen Academic Publisher, pp. 51–55.
International Institute of Refrigeration, 1995. Guide to Refrigerated Transport. Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 1998. Refrigerated transport, storage and retail display. Proceedings of the Conference of: IIR Commissions D2/3, With D1, Cambridge, UK. Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 2001. Rapid cooling of food. Proceedings of the Conference of: IIR Commission C2, Bristol (UK). Paris, France: International Institute of Refrigeration.
International Institute of Refrigeration, 2010. Sustainability & the cold chain. Proceedings of the Conference of: IIR Commissions B1, B2, C2, D1 and D2, Cambridge (UK). Paris, France: International Institute of Refrigeration.

James, S.J., James, C., 2002. Meat Refrigeration. Cambridge, UK: Woodhead Publishing Limited ISBN 1 85573 442 7.
James, S.J., James, C., 2010. The food cold-chain and climate change. Food Research International 43 (7), 1944–1956.
James, S.J., James, C., Evans, J.A., 2006. Modelling of food transportation systems – A review. International Journal of Refrigeration 29, 847–862.
Sharp, A.K., 1988. Air freight of perishable product. In: Refrigeration for Food and People, Proceedings of the Conference of: IIR Commissions C2, D1, D2/3, E1, Brisbane, Australia. Paris, France: International Institute of Refrigeration, pp. 219–224.
Smith, B.K., 1986. Liquid nitrogen in-transit refrigeration. In: Recent Advances and Developments in the Refrigeration of Meat Chilling, Proceedings of the Conference of: IIR Commission C2, Bristol, UK. Paris, France: International Institute of Refrigeration, pp. 383–390.

Relevant Websites

http://www.ecff.net/
Official site for the European Chilled Food Federation.
http://www.fao.org/
Official site for the Food and Agriculture Organization of the United Nations.
http://www.iifiir.org/
Official site for the International Institute of Refrigeration.
http://www.chilledfood.org/
Official site for the UK Chilled Food Association.

Wet Markets

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Introduction

The traditional system of retail food marketing in Asian countries is the wet market. A wet market is a combination of poultry farmers' markets, fishmongers, and open-air butcher shops that are about the size of a large supermarket.

A wet market gets its name from floors that are soaked from being continually washed down; washed because fish intestines and blood from butchering chickens and ducks in the poultry stalls become putrid if left around for very long, and also because a wet floor is considered better than a bloody and smelly one.

Wet markets are normally divided into distinct sections: live poultry, meat (pork, beef, and mutton), fish and seafood, eggs, fruit, vegetables, rice, and dry grocery items. Naturally, the wettest parts of the wet markets are the poultry and fish sections. Stalls in the wet markets are relatively small, rarely more than a couple of square meters, and are separated by narrow aisles (Figures 1–3).

Wet market stalls are restricted in what they are permitted to sell. For instance, if the stalls are licensed to sell vegetables, they sell just that. The same goes for stalls that sell meat, fish, or poultry.

Wet markets normally operate from very early hours of morning until noon. Some wet markets, for example those in Hong Kong, also operate in late afternoon to cater to office workers who normally do some quick shopping for fresh foods before they head home to prepare dinner for their families.

Meat Stalls

In Muslim countries, such as Indonesia and Malaysia, and in multiracial societies, such as Singapore, Islamic teaching that Muslims must not come into physical contact with pigs and pork dictates the meat stalls in these countries. Hence, the meat stalls are of two distinct types: those that sell only pork, and those that sell beef and/or mutton. These two types of stalls are physically segregated in different parts of the wet markets.

In the meat stalls, large slabs of meat hang from metal hooks. Pig heads, tails, and entrails are also on display. The butchers are skillful in deboning the half carcasses or quarters that they have taken delivery (nonrefrigerated) from the abattoirs. They chop and slice selections of meat with huge metal cleavers according to the requirement of their customers.

Figure 1 A wet market Tekka Center, Singapore.

Poultry Stalls

Traditionally, live poultry (chickens, ducks, and pigeons) are kept in multitier cages for customers to inspect and choose. For a nominal fee, the vendors will slaughter the poultry

Figure 2 Indoor markets also exist under shopping areas such as this one under Lok Fu Shopping Center (Hong Kong).

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Figure 3 A wet market in Hong Kong.

selected and purchased by the customers. Bleeding, scalding, defeathering and cleaning of the entrails, and dressing the carcasses are speedily carried out within a confined space of a couple of square meters.

Poultry droppings, feathers, and blood that inevitably splash all over the floors and walls create a high risk health environment for both poultry and humans. A perfunctory hosing down of the floors, walls, and empty cages at closing hours, without thorough cleaning and disinfecting, does not remove the disease-causing microorganisms in the fecal materials and caked blood that have accumulated over months and years.

Wet Market Strengths

Wet markets are conveniently located within residential areas. Perishable products sold in the wet markets are perceived by customers to be fresher than those offered at the supermarkets. Asian chefs are known to take pride in choosing the best cuts of meat and the healthiest chickens that are fresh from the wet markets to prepare their signature dishes.

Furthermore, in order to buy the freshest supplies to cook for their family meals, many housewives, such as those in Hong Kong and other Asian cities, go to the wet markets twice a day, firstly in the morning, and again in the late afternoon.

Customers are allowed to touch and smell the products, and to inspect the live poultry before deciding on their purchases. It is also customary for customers to bargain with the stall vendors for discounts on their purchases. Often, stall vendors also give credit and keep special cuts of meat for their regular customers.

Wet Market Weaknesses

As the space allocated to each stall is limited to a couple of square meters, the stalls in the wet markets tend to be cluttered. Furthermore, the standard of food hygiene tends to be poor and the level of cleanliness low. In addition, shopping hours in wet markets are restricted, which is the main drawback for individuals who work.

Market Forces

According to market surveys carried out by Anderson Consulting in 2000 on understanding shopping trends in wet markets in Southeast Asia, Hong Kong, and Korea, wet markets' share of retail grocery sales ranges from 60% in Singapore, 70% in Malaysia and Thailand, 80% in Indonesia to 90% in Hong Kong.

According to another report (dated March 2003) by AC Nielsen Marketing Research, between 80% and 90% of shoppers visit wet markets on a regular basis in Indonesia, Korea, Malaysia, Singapore, Thailand, and the Philippines. In Indonesia, the wet market is the main store-type frequented by nearly one-third of all shoppers. Wet markets are also the most popular store-type that meet shoppers' daily need for fresh meat, fruit, and vegetables. In Thailand and Indonesia, shoppers frequent the wet markets at least once every other day, five times more frequently than they visit the supermarkets. Fewer than 30% of shoppers turn to supermarkets for their fresh food requirements. Two out of three respondents in Singapore said they go to the wet markets seven times a month.

According to a report by the Foreign Agriculture Service of the US Department of Agriculture, there are 564 000 wet markets in Thailand for a population of 60 million. Figures for other Asian countries are not available. It is conceivable that figures for other Asian countries, if available, will be comparable to that in Thailand. This is because wet markets can be found in every village, township, and city, catering to the populations' daily need for fresh foods.

In an estimate by the Food and Agriculture Organization of the United Nations, more than 90% of the meat in Asia is marketed 'warm,' that is, without refrigeration. Pigs and cattle are slaughtered during the night and their 'warm' meat reaches wholesale and retail markets in the early morning and thereafter, the customers' kitchen, all within a day.

At first glance, wet markets may give the impression of being unhygienic. However, if properly organized, especially under the supervision of municipal authorities in accordance with a system of licensing of the store holders and products offered for sale, wet markets are generally recognized to be an acceptable marketing system well suited for the relatively poorer infrastructure in underdeveloped/developing countries. Wet markets are a good and viable source for fresh foods at affordable prices. Thorough cooking of food by consumers helps to ensure that food is safe.

It should be noted that the method of 'warm' meat distribution and marketing described above (i.e., without refrigeration) works well only where transport distance is short – it requires abattoirs to be located near urban centers where wet markets are located to ensure freshness of the meat.

Public Health Hazards

In view of the less than desirable hygiene conditions in many of the traditional wet markets, it is only a matter of time before

serious public health problems will occur. In fact, a series of widely publicized food-poisoning outbreaks in China in recent years has tarnished the image of neighborhood wet markets, resulting in consumers' perception that the meat sold in such markets is not clean and safe. The perception stems from the manner in which the meat is sold: the unprotected 'warm' meat is exposed to dust and other pollutants, and attracts flies, rodents, and other vermin. However, there is substantial upgrading of conditions in wet markets.

In Hong Kong, people traditionally buy their chickens live from dedicated poultry markets, where the selected poultry are freshly killed. Avian influenza virus (H5N1), also known as bird flu, showed up in Hong Kong in 1997 and was quickly traced to the crowded poultry markets. The virus killed 6 people and infected 18 others before it was contained, after which some 1.5 million chickens had to be humanely destroyed by the health authority. It was the first time in medical history that an influenza virus was known to have transmitted directly from chickens to humans. The influenza virus returned in 1999, resulting in 1.2 million chickens being destroyed. Since then, the virus has returned periodically, albeit on smaller scales.

A recent review and some studies have suggested that Chinese wet markets would be unique epicenters for transmission of potential viral pathogens, as new genes could be acquired or existing genes modified through mechanisms such as genetic reassortment, recombination, and mutation. Because wet markets are at close proximity to humans, there is the possibility of a high transmission efficiency of viruses to humans.

The Hong Kong authority considered how best to change the traditional way in which poultry are kept, slaughtered, and sold in the poultry markets. A proposed policy option considered by the authority was the centralized slaughter of poultry, banning slaughter of poultry in wet markets. Implementation of this proposal ushered in a new era of poultry hygiene practices in Hong Kong. In fact, a model of this seemingly revolutionary practice was already in place in Singapore.

Modern Wet Markets

Long before the unfortunate avian influenza outbreaks in Hong Kong occurred and the attendant public health implications were recognized, the Singapore government in 1993 banned the unhygienic practice of keeping and slaughtering poultry in the wet markets. Poultry stallholders and live poultry wholesale importers were encouraged to set up mechanized poultry slaughterhouses. There are now 10 chicken and 4 duck slaughterhouses strategically located in different parts of the island. The poultry stalls in the wet markets now sell chilled and dressed whole chickens and ducks supplied by these slaughterhouses. The poultry sold are also individually tagged with the name of the slaughterhouses and the date of slaughter to facilitate trace-back and enforcement of the Sale of Food Act.

After the initial outbreak of avian influenza in Hong Kong in 1999, the authorities introduced new strict guidelines on the handling and slaughtering of poultry in the wet markets. This included a requirement that chickens and ducks be kept and slaughtered separately. This technical requirement takes into consideration the nature of the avian influenza virus, which is carried naturally in ducks, and which can be passed on to chickens and humans, causing serious disease. It is interesting to note that, since 1993, chickens and ducks in Singapore have been slaughtered in separate licensed premises, a regulatory requirement premised on poultry slaughter hygiene and public health considerations.

After several years of preparation and intense discussion with, and education of, the stallholders in the wet markets, the Singapore government in 2000 implemented the cold chain system for the distribution and marketing of meat and poultry. All vendors of pork, chicken, beef, and mutton in the wet markets are now required to install display chillers to keep the meat and poultry at a constant temperature of 4 °C. This marked the disappearance of 'warm' meat from the wet markets.

New-generation wet markets in Singapore and elsewhere are better in design and construction and are spacious, bright, and well ventilated. There is also proper floor drainage resulting in dry surfaces. The health authorities also conduct regular inspections ensuring a clean and healthy environment (Figures 4 and 5).

The Future

Despite all the shortcomings of wet markets and the aggressive expansion of international supermarket chains, traditional wet markets will likely continue to exist in Asia. In fact, the wet markets have changed with the times. It has been reported that beef exporters in the United States have in recent years been very successful in penetrating the low and middle market segments in China and several Southeast Asian countries by supplying meat cuts such as short plates and chucks to the wet markets. This augurs well for the future of wet markets as a recognized channel for distribution and sale of meat in Asian countries.

Figure 4 Modernized wet markets are housed in full-structure buildings although there are numerous street-level wet markets still in use throughout Asia. Multiple floors make good use of limited commercial space.

Figure 5 Indoor markets are generally more organized than outdoors in Hong Kong.

An uninterrupted cold chain for perishable meat and poultry from the production sources (abattoirs and import consignments) to the stalls in wet markets will ensure food safety practices and enhance the image of this colorful, traditional system of meat marketing in Asia. This is already happening in Singapore, where the government is building more wet markets in new public residential areas to meet popular demand from the constituents. With other countries capitalizing on the experience of Singapore in the management of modern wet markets, the decline in the number of wet markets can be slowed, as has been reported in some Asian cities.

See also: Ethnic Meat Products: China and Southeast Asia. Foodborne Zoonoses. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Meat Marketing: Cold Chain; Market Requirements and Specifications. Parasites Present in Meat and Viscera of Land Farmed Animals. Sensory and Meat Quality, Optimization of

Further Reading

AC Nielsen, 2003. Growth of Modern Trade Takes its Toll in Traditional Stores in Singapore.

AC Nielsen Research, 2002. Shopper Trends Asia Special.

Anderson Consulting, 2000. Understanding shopping at wet markets in South-east Asia, Hong Kong and Korea. Fresh Food Conference. Bangkok, Thailand: CIES and USDA.

Asian Productivity Organization, 1990. Marketing Systems for Farm Products in Asia and the Pacific. Tokyo: Asian Productivity Organization.

Chen, Y., Liang, W., Yang, S., *et al.*, 2013. Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: Clinical analysis and characterisation of viral genome. Lancet 381 (9881), 1916–1925. doi:10.1016/S0140-6736(13)60903-4.

FAO, 1998. Proceedings of the Regional Workshop On Area-Wide Integration of Crop Livestock Activities, 18–20 June. Bangkok: FAO Regional Office.

Ho, H.F., 1994. Poultry slaughter goes hi-tech. Singapore Primary Production Department occasional publication. Agro Vision 1994 (3), 10–11.

USDA, 2002. FAS Report TH 1129: Large Chains Drive Thailand's Retail Food Sector. Washington, DC: USDA.

Woo, P.C., Lau, S.K., Yuen, K.Y., 2006. Infectious diseases emerging from Chinese wet-markets: Zoonotic origins of severe respiratory viral infections. Current Opinion Infectious Diseases 19 (5), 401–407.

Relevant Websites

http://en.wikipedia.org/wiki/Wet_market

http://www.strippedpixel.com/bowrington-road-wet-market/

MEAT PRICING SYSTEMS

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Glossary

Consumption The purchase of goods or services that individuals directly benefit from and do not subsequently sell to others.

Demand A term used by economists describing what quantity of goods or services buyers are willing to purchase at varied purchase prices.

Price discovery A process of determining the price of a good in the marketplace through the iterative interactions of sellers and buyers.

Supply A term used by economists describing what quantity of goods or services sellers are willing to produce at varied sales prices.

Utility A term used by economists to represent preferences over a set of goods or services.

Introduction

The retail meat supply chain is characterized by a constantly changing complex of product offerings. Accordingly, many different prices and valuations persist in the meat business. A consumer purchases a meat package only if the posted price does not exceed the value presented by the product. Economists regularly use a measure called 'utility' to explain observed consumer decision making. Ultimately, consumers will distribute their limited food budget across multiple food items in a manner that the last item purchased of each product contributes the same utility. The price of a meat package plays a critical role in guiding which consumers purchase the product. Similarly, meat prices guide how much of the product is ultimately provided by the industry. More broadly, meat prices serve an economic role in signaling how limited resources should be allocated both by the supplying industry and the consuming public. Recognizing the broader roles of price, product pricing, and price discovery as critical mechanisms to initiate and coordinate actions throughout the meat supply chain is critical for understanding the everchanging meat business.

Price Discovery

The ongoing process of an industry searching for the price level equating the quantity that suppliers offer for sale at a schedule of alternative prices (supply curve) and the quantity consumers are willing to purchase at alternative prices (demand curve) is referred to as price discovery. The price discovery process is dynamic and imprecise as neither consuming nor producing parties have complete information on factors influencing demand and supply. A sound understanding of how the meat price discovery process incorporates changing demand and supply factors in order to find a price level that clears the market for different meat products is important.

Figure 1 shows the US inflation-adjusted retail Choice beef and pork prices from 1980 to 2010. The presented prices are adjusted for inflation in the US, and a similar adjustment to prices in other meat-producing countries would be needed to allow an examination of underlying demand and supply factors without confounding this with general inflation. The general trend of declining beef and pork prices in the US in the 1980s and 1990s is evident. Additional analysis is needed to identify if decreasing demand or increasing supply is the underlying driver of these declining prices. Figure 2 suggests that increasing beef and pork supplies are not the driver of declining retail prices. Per capita retail beef consumption has dropped sharply since 1980. Although not drastic, per capita retail pork consumption has dropped considerably. In many years, particularly in the 1980s and 1990s, both real prices and per capita consumption were declining for Choice beef. This indicates that the US consumers were placing notably lower value on Choice beef over this period. Not surprisingly, this reduced retail beef demand was the trigger for a host of changes in the US beef industry, including a notable downsizing of the beef cow herd.

Figure 3 presents a demand index for Choice beef starting with 1980 as the base year. The beef demand index measures vertical shifts in beef demand over time. One way to understand the index is to note that creating the beef index involves calculating the real beef price that one would expect to observe if beef demand was the same as that experienced in the

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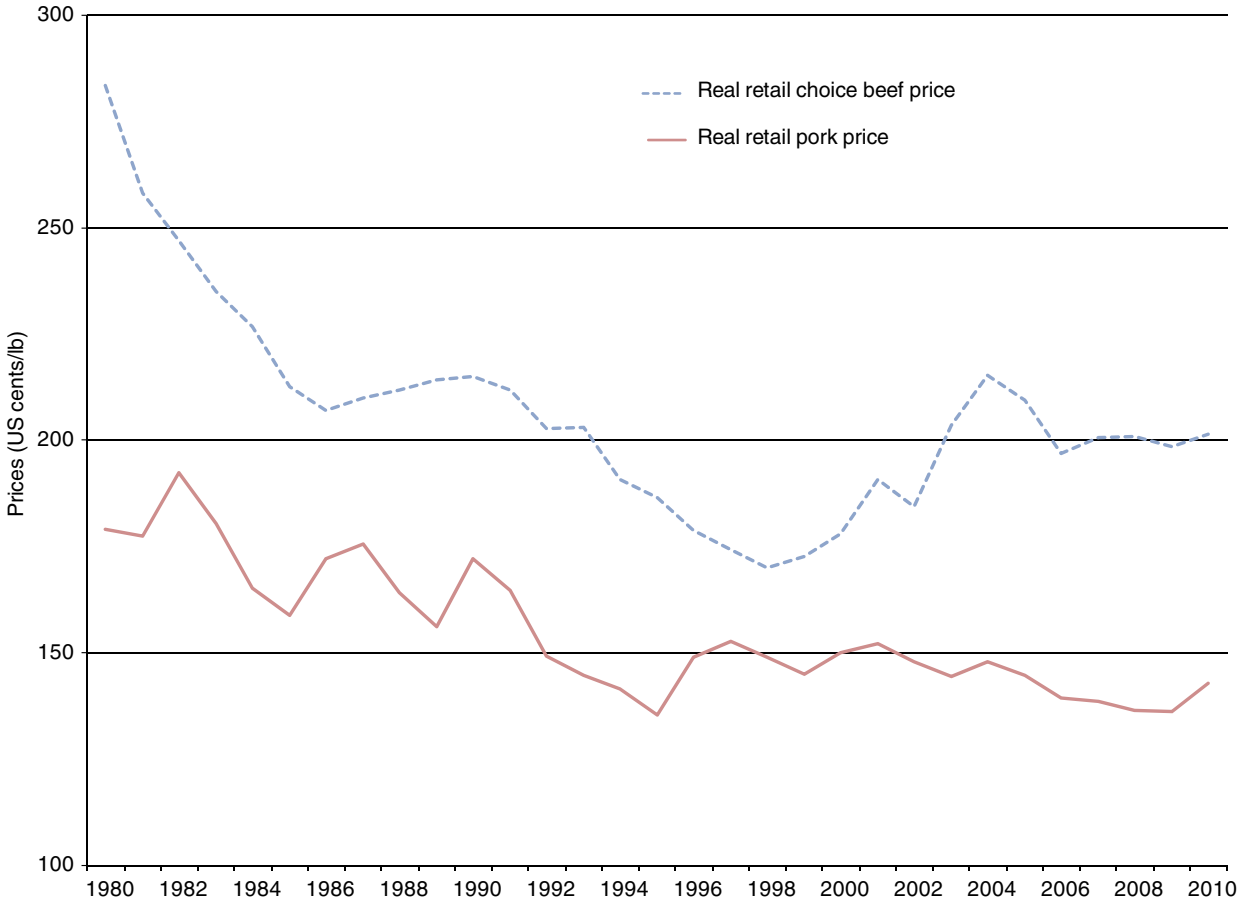

Figure 1 US inflation-adjusted (Consumer Price Index, 1982–84 = 100) retail prices for Choice beef and pork, 1980–2010.

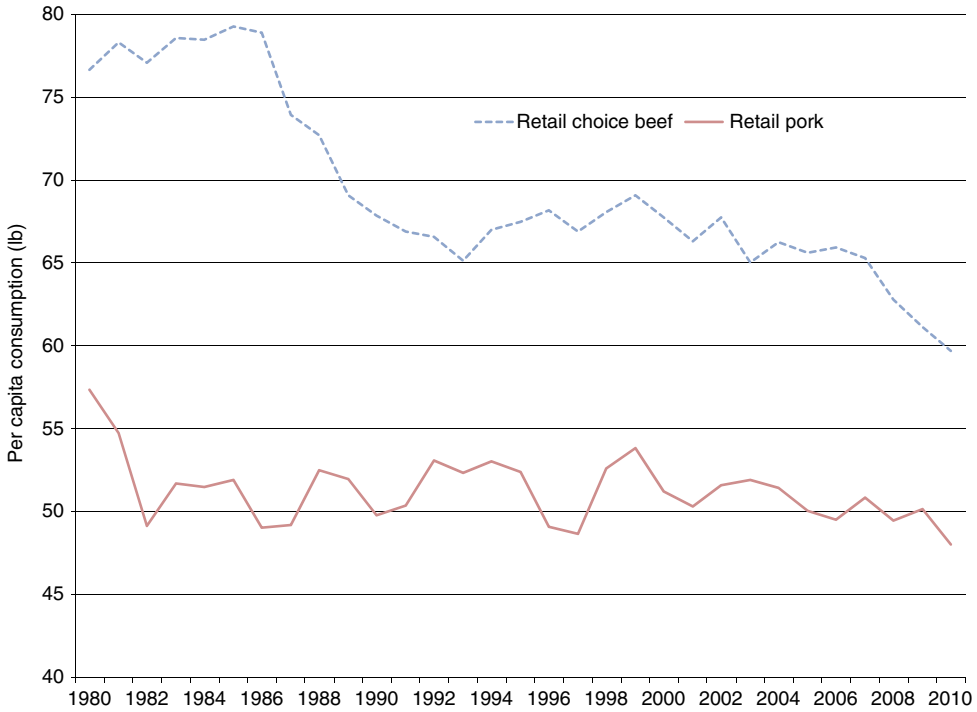

Figure 2 US per capita consumption in retail weights for Choice beef and pork, 1980–2010.

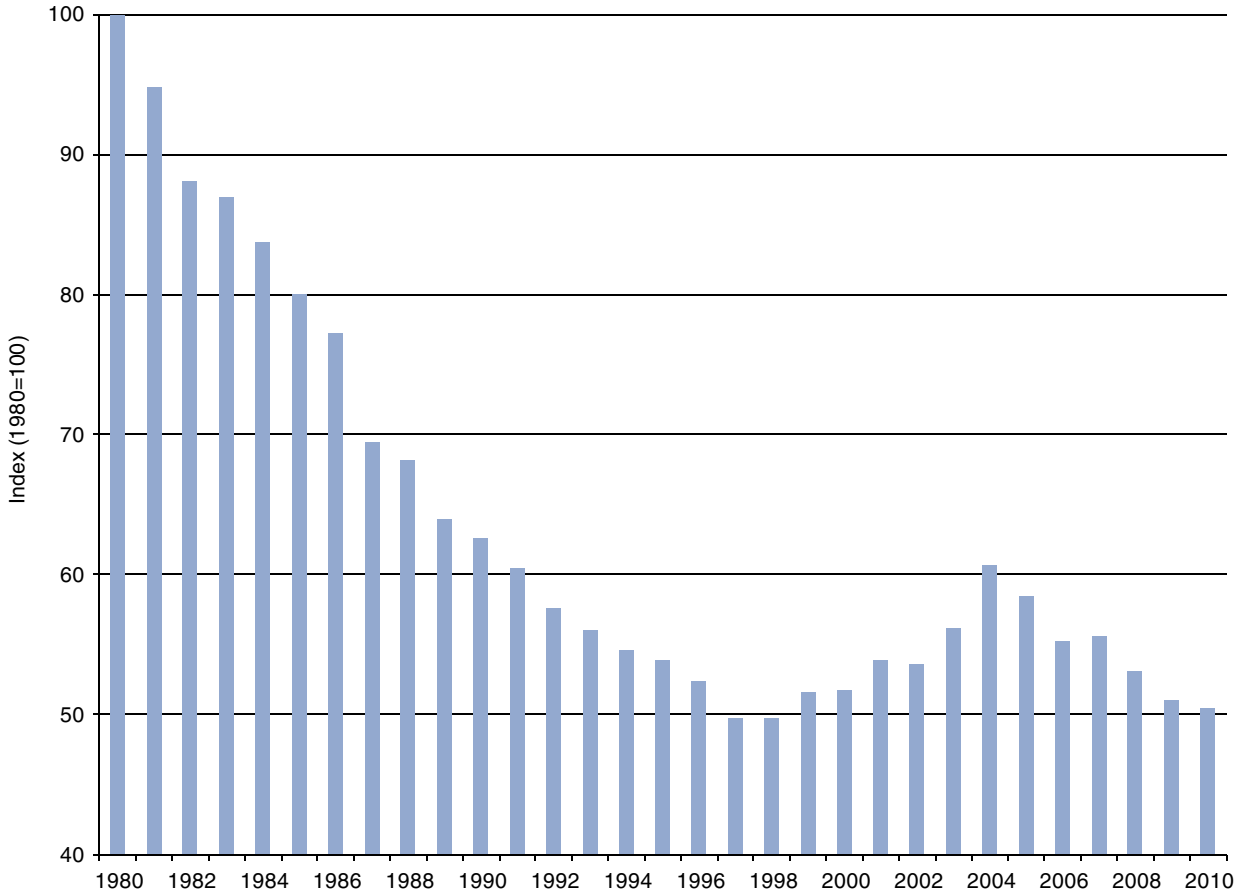

Figure 3 US Choice beef demand index, 1980–2010.

base year. With this constant demand, expected beef price is compared to the price actually transpiring in the marketplace in order to indicate changes in underlying demand. For instance, the minimum beef demand index value of 49.7 in 1998 suggests beef retail prices were 50.3% lower in 1998 than they would have been if beef demand was at its 1980 level. This example highlights that beef demand is not per capita beef consumption. Per capita consumption is simply domestic beef disappearance (net volume of domestic production, cold storage adjustments, and international trade) divided by resident population and it provides little information regarding beef demand when considered independently from prices. This example estimate of 50.3% quantifies the magnitude of demand reduction experienced by the industry between 1980 and 1998. In fact, for 18 consecutive years, beef demand declined, which presented substantial problems and resulted in downsizing of the beef industry. Although no 'magic bullet' or single dominant driver exists, a number of factors including consumer incomes, relative meat prices, food safety recalls, health and nutrition information, and product convenience influence meat demand.

Figure 4 presents a similar demand index for retail pork. The cumulative demand decline was less in magnitude for pork than for beef. However, the pattern of reduced retail prices and per capita consumption also applies to pork. In both cases, the declines suggested by calculated demand indices, particularly between 1980 and the mid- to late 1990s, indicate that the US consumers placed notably lower values on pork and beef. Deeper examination of why these demand problems developed in the US markets can provide important inferences for any beef- and pork-producing country that may face similar consumer demand concerns. Moreover, understanding the success of various response attempts by the US industries to remedy these concerns is useful both for industry leaders in the US and other producing countries.

Figure 5 shows a trend of increasing the total US per capita red meat and poultry since 1980. This largely corresponds to expanding consumption of poultry products. The observation of declining beef and pork consumption in a period of expanding total meat consumption and declining beef and pork retail prices suggests that consumers have placed higher relative value on available poultry products than on beef and pork products over this time period. Questions regarding these trends are regularly asked by industry leaders and policy makers, who at times have suggested attempting to 'solve the problem' by regulating price discovery processes throughout the US meat supply chain. To understand the impact of these regulation issues, consideration of meat prices needs to be further expanded to what economists refer to as 'pricing efficiency.'

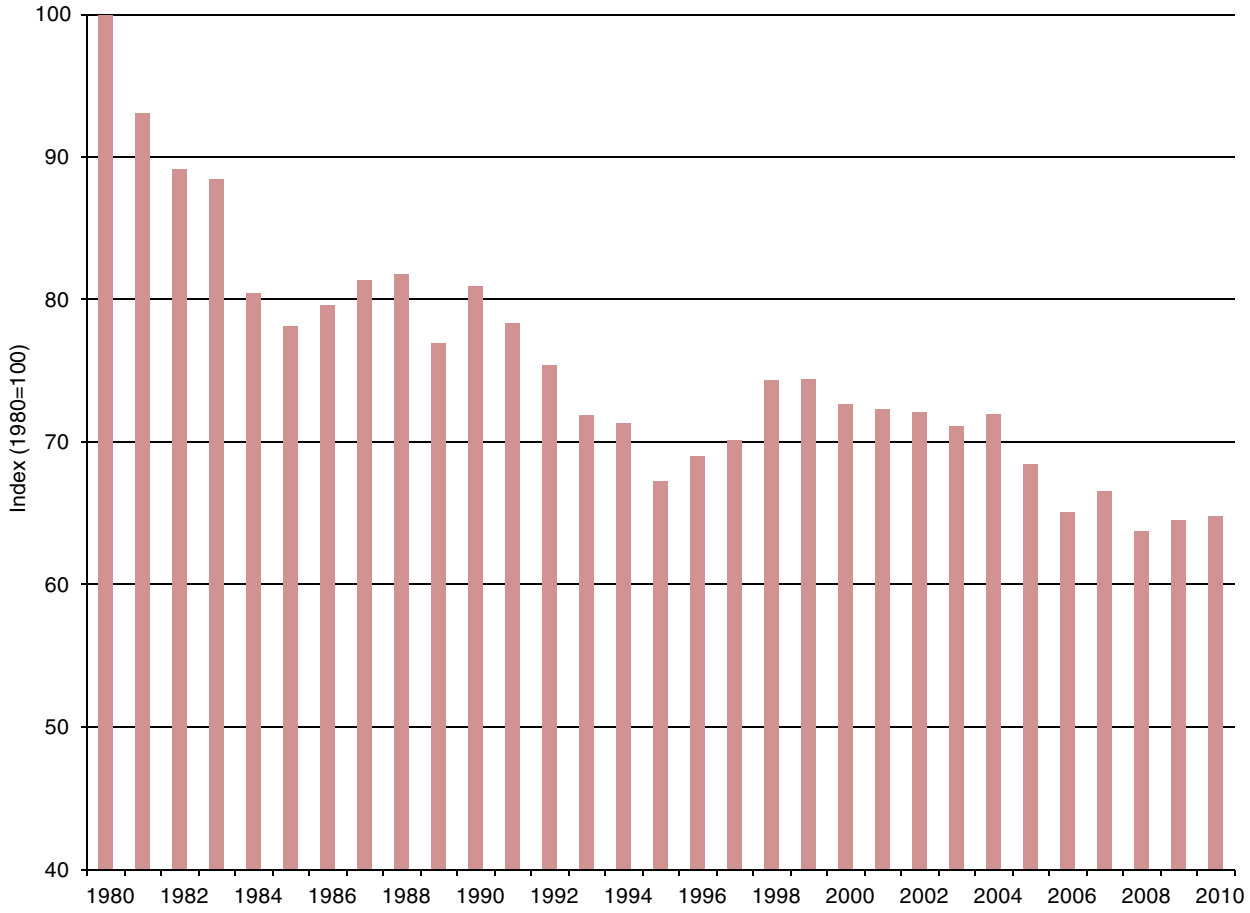

Figure 4 US pork demand index, 1980–2010.

Pricing Efficiency

The general concept of pricing efficiency refers to the effectiveness of a marketing system to clearly communicate price signals to market participants. Consumers send signals by their purchasing decisions regarding what they prefer and what they are willing to pay for different meat products. For instance, consumers may pay a price premium for meat products of a particular quality grade, brand, or carrying a desired alternative attribute. Similarly, producers throughout the meat supply chain send signals regarding what they are willing to provide to consumers at alternative prices through their production decisions. This interactive signaling process between consumers and suppliers is crucially important to effectively align scarce resources. This process is even more important in the meat industry where multiple sectors of production (e.g., cow calf, stocker, feedlot, processing, and retail in the beef industry) are involved in complicating preference and pricing signals, that is, multisector industries such as those characterizing livestock production require more extensive pricing systems and communication of pricing signals than sole-sector industries.

The price signaling processes in the meat supply chain will work effectively only if price premiums and discounts are clearly associated with different meat product types and attributes that are of interest to consumers. Some attributes such as portion size are easily observed by consumers, such that signals are effortlessly and cleanly distributed throughout the meat supply chain. Other attributes such as tenderness or food safety are typically not directly apparent from visual inspection of a product by consumers. If these attributes are important to consumer purchasing decisions, they must be measured, tracked, and conveyed on retail meat products. Several countries have systems in place providing quality grade information about meat products. The success of these systems in sufficiently conveying price signals underlying consumer purchasing decisions has been debated. For instance, the US beef quality grades are based largely on marbling and do not directly measure product tenderness. This is an example of a partial and incomplete signal that conveys imperfect information to market participants. Arguably, the above-mentioned declines in the US beef demand were at least exacerbated by this less than fully efficiency pricing system where communication of consumer desires and corresponding changes in supplied product attributes are not completely achieved.

A fair assessment of any pricing system must recognize that no panacea exists because meeting a threshold of 100% complete and accurate price signaling is impossible. Moreover, at some point, the benefits of additional enhancement in

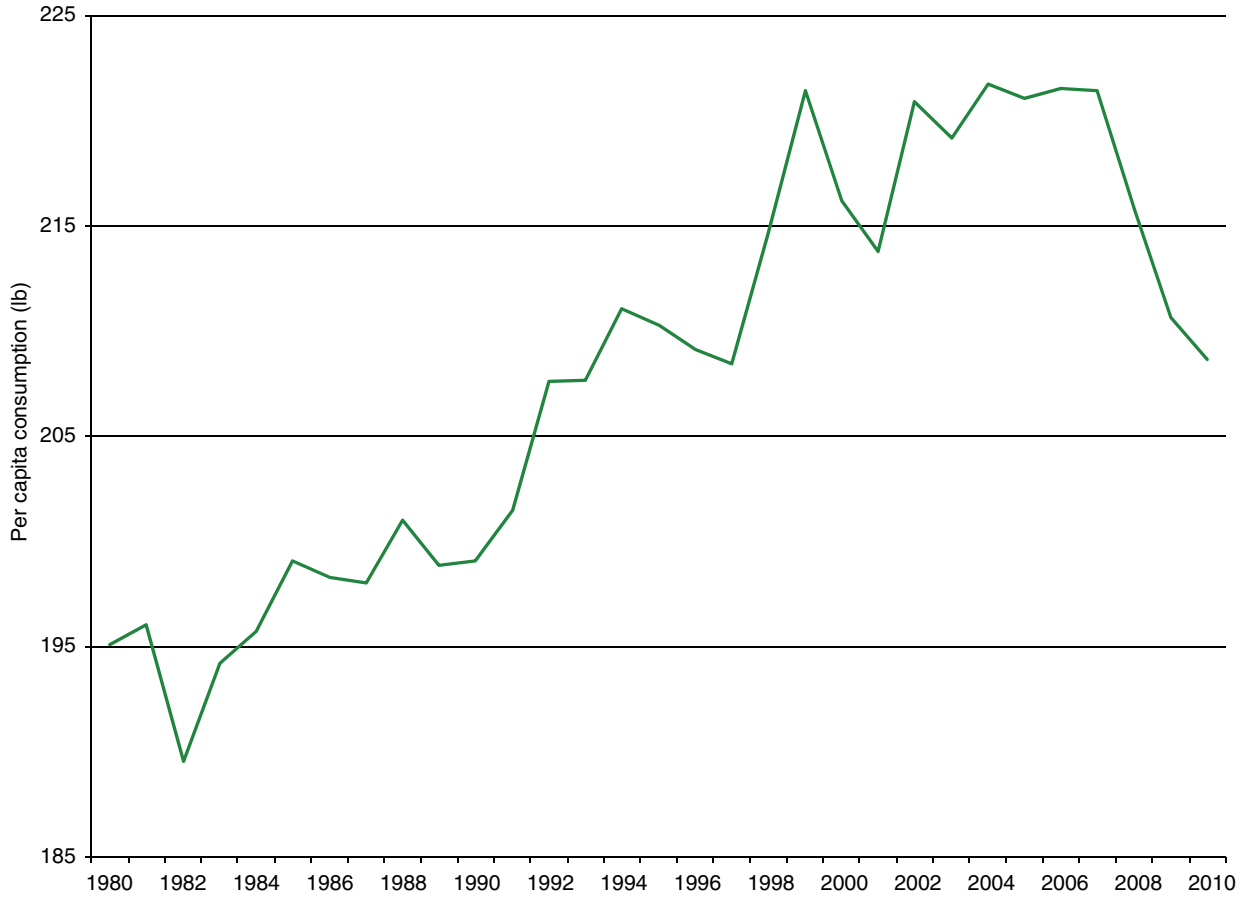

Figure 5 US total red meat and poultry per capita consumption in retail weights, 1980–2010.

pricing efficiency are no longer sufficient to cover the costs of their provision. This benefit–cost assessment process is ongoing both within meat industries and in policy discussions. These discussions, in turn, have led to a host of changes including a shift toward more vertically integrated systems away from systems comprised of separate, individual profit-maximizing centers spanning market segments. Historically, the US beef system has been less vertically integrated and less coordinated than pork and particularly poultry industries. However, over the past two decades, the beef industry has increasingly adopted use of contracts, marketing arrangements, and vertical alliances in order to improve vertical coordination. Similarly, the pork industry has moved rapidly to a model similar to the poultry industry where most production now occurs in vertically integrated systems.

The movement toward increased vertically integrated meat-producing systems has largely occurred because previously existing systems composed mainly of more independently operating businesses which failed to provide sufficient pricing efficiency. Price signals from traditional systems were basically unable to convey consumer desires regarding particular meat product attributes. This, in turn, left suppliers without clear information needed to assess whether the price they would receive for a product carrying a different set of attributes would sufficiently cover the associated increases in production costs. Ultimately, this type of disconnect leads to outcomes including those noted above regarding substantial demand declines. Although adoption of vertically integrated systems more likely provides more efficient pricing systems by more closely aligning and conveying information across market segments in the meat industry, this adjustment is ongoing and presents new challenges for public policy regarding meat pricing.

Meat Pricing Systems in Transition

The increased emphasis and use of contracts, marketing arrangements, and vertical alliances characterizing increasingly vertically integrated meat supply chains alters the role of prices. For instance, provision of many meat product attributes desired by consumers requires substantial adjustments not just in day-to-day operations and variable cost aspects of meat production but also in significant capital investments altering an operation's fixed costs. As an example, the extent to which meat tenderness can be impacted in live animals includes not only possible adjustments in feeding regimens (e.g., altering the diet and hence variable costs) but may also require changes in animal genetics (e.g., changing breed composition and hence fixed-cost investments in more expensive breeding stock). This adjustment toward increased focus on fixed-cost-altering investments highlights the changing relative role of

traditional commodity product price signals. Many contracts, marketing arrangements, and vertical alliances include nonprice aspects of coordination but importantly incorporate price components typically in the form of a price grid of premiums and discounts over a base price. However, this base price is frequently still established by transactions in the industry segments that have not moved toward additional vertical integration. Recognizing both the increased share of production occurring in vertically integrated systems and the associated reliance on price signals from outside the vertically integrated system highlights a controversial issue in the US meat supply chain. The same type of industry maturation and adjustments in pricing system requirements and roles can be expected in other meat-producing countries, particularly in countries that repeat the US case of delayed implementation of more effective pricing systems.

The retail meat case available to consumers can be characterized as increasingly complex as it offers an expanding line of product types, forms, and characteristics largely aimed at better meeting diverse consumer preferences. Perhaps the most significant of these changes, and the broader movement away from commodity-oriented pricing systems, is the substantial growth in branded products. For instance, between 2004 and 2010, the portion of retail beef products branded in the US increased from 42% to 63%. Besides expansion of branded product lines, consumers now have access to 'convenience' products that may be precooked, have been 'naturally raised,' are enhanced with flavor additives, or contain a host of other claims and attributes. This expansion in product offerings reflects the meat industry's attempt to enhance consumer demand in response to the previously noted demand struggles.

Similarly, the role of exports is increasing in many of the world's largest meat-producing countries which add further complexity to the global retail meat case. The impacts of consumers in various countries valuing products differently results in meat not being exported in carcass proportions. For instance, a notably larger portion of beef livers than ground beef products are exported from the US. This results in the meat case presented in two countries to vary notably and presents both opportunities and challenges to the livestock industry supplying these varied retail outlets.

One broad implication of these increasing meat pricing complexities is that analysts are faced by an exponentially more challenging task. For instance, the need and feasibility of scanner data-based analyses has rapidly developed. As an example, recent scanner-data based research suggests that consumers have paid more than \$5 lb^{-1} premiums for branded steak products. Only a few years ago, the ability to conduct similar microlevel research was constrained to consumer survey approaches. However, in the future, accurate identification and understanding of consumer meat preferences will increasingly rely on studies informed by scanner data and related household-level data sources that reflect actual purchasing behavior in notably more detail than previously available. Given the traditional reliance on national, aggregate disappearance-data-based analyses, which mask over variations in product types, forms, and attributes will increasingly present both notable challenges and promising opportunities for researchers and industry leaders.

Public Policy Issues in Meat Pricing Systems

The growing importance of base livestock prices as inputs into arrangements of vertically integrated systems and associated concerns with possible market power of industry segments characterized by fewer and larger entities have resulted in several public policy issues. For instance, the United States Department of Agriculture (USDA) has a long history of reporting cash prices from direct trade of slaughter hogs with similar practices in place for other US livestock. However, the portion of slaughter hogs marketed in negotiated cash markets has fallen from 43% in 1997 to less than 5% in 2010. The declining market share of livestock being transacted in cash markets has led to numerous calls for new regulation and industry adjustment. These and other related concerns ultimately led to mandatory price reporting of prices in live hogs, fed cattle, boxed beef, lamb, and boxed lamb markets under the authority of the US Livestock Mandatory Reporting Act of 1999. These mandatory price reporting systems have recently been reauthorized through 2015 and now include wholesale pork products. Broadly speaking, the introduction of corresponding mandatory price reports by the USDA has altered the pricing efficiency of the US meat industry by providing information, which in some cases at least partially replaces previously reported prices and in other cases provides entirely new insights to the industry. Although assessing the net benefit/cost of these reports is beyond the scope of this discussion, an appreciation of several aspects is needed, including the public and private industry costs underlying generation of these reports, the provision of new information such as wholesale primal level prices previously not reported publically, and the value of these reports in supplementing insights obtained directly from the reduced set of direct cash market transactions.

A related policy issue pertains to the actions of meat processors, including a particularly focused discussion on the impact of various livestock procurement procedures. Several producer groups have expressed concern that processor procurement strategies have resulted in detrimental impacts on cash market prices. This concern has led to notable public interest in the issue including an extensive Congressionally funded study completed in 2007. This interest has persisted with the USDA proposing changes, which ultimately were largely not implemented at the time of this writing, as recently as 2012. Given the underlying changes in industry structure that are expected to continue, there will remain a complex set of public policy issues to be considered and debated regarding meat pricing systems well into the future. Moreover, similar discussions can be expected in other meat-producing countries that may have similar experiences to the US and yet have alternative histories of public price information provision and expectations.

Meat Pricing Systems of the Future

Looking ahead, several issues from the past will remain worthy of focus in the future. The ability of increasingly vertically integrated systems in the US to provide efficient pricing systems will be debated for years to come and arguably the

verdict is yet to be determined as adjustments remain well underway. More broadly, the extent to which similar moves to vertically integrated systems and development of altered price discovery mechanisms occurs in other meat-producing countries is interesting to consider. As meat industries worldwide attempt to profitably operate in an increasingly complex environment characterized by higher costs of production and increased overall social interest in their production practices, at a minimum the relative success of different meat-producing countries and industries will be sensitive to the success in implementing and adopting efficient pricing systems. The lessons learned from notably delayed action in the case of the US beef industry can be very instructive for industries worldwide. This process can only be expected to be of increasing importance as multitudes of new meat consumers enter the global marketplace in coming years. Similarly, as industries outside of the developed world continue to develop, mature prudent attention is encouraged to the experiences of those in the developed world which have been briefly highlighted here, that is, although much more is known about pricing systems in the developed world, application to other countries (and even species not directly mentioned here) is encouraged. Moreover, deeper recognition of how consumer preferences vary across meat product types, attributes, and cultures (e.g., consider different values of beef tongue in Japanese and the US markets) will only exacerbate the need for more detailed and complex pricing systems and general mechanisms for information exchange to be implemented. Ultimately, the viability of meat-producing industries hinges on the ability to produce what consumers desire in order to properly align activities within supply chains, to efficiently offer these desired products, and to remain keenly aware of how 'answers' to these challenges dynamically change over time. The ability of the global meat supply chain to meet this challenge will largely determine its economic success.

See also: Economics: Meat Business and Public Policy

Further Reading

Franken, J.R., Parcell, J.L., Tonsor, G.T., 2011. Impact of Mandatory Price Reporting on Hog Market Integration. Journal of Agricultural and Applied Economics 43, 229–241.

Muth, M.K., Liu, Y., Koontz, S.R., Lawrence, J.D., 2008. Differences in Prices and Price Risk Across Alternative Marketing Arrangements Used in the Fed Cattle Industry. Journal of Agricultural and Resource Economics 33, 118–135.

National Meat Case Study, 2010. A Snapshot of Today's Retail Meat Case: 2010 National Meat Case Study Executive Summary. Available at: http://www.beefretail.org/CMDocs/BeefRetail/research/2010NationalMeatCaseStudy.pdf

Parcell, J.L., Schroeder, T.C., Tonsor, G.T., 2009. Wholesale Pork Price Reporting Analysis. Available at: http://www.agmanager.info/livestock/marketing/PorkPrice/PorkPrice_FinalReport.pdf (accessed 3.10.13).

Plain, R., 2011. U.S. Market Hog Sales, 2002–2010. Available at: http://agebb.missouri.edu/mkt/vertstud10.htm (accessed 3.10.13).

Schroeder, T.C., Tonsor, G.T., 2012. International cattle ID and traceability: competitive implications for the US. Food Policy 31, 31–40.

Schulz, L.L., Schroeder, T.C., White, K., 2012. Value of beef steak branding: hedonic analysis of retail scanner data. Agricultural and Resource Economics Review 41, 260–273.

Tomek, W.G., Robinson, K.L., 2003. Agricultural Product Prices, fourth ed. Ithaca: Cornell University Press.

Tonsor, G.T., Mintert, J., Schroeder, T.C., 2010. U.S. Meat Demand: Household Dynamics and Media Information Impacts. Journal of Agricultural and Resource Economics 35, 1–17.

Relevant Websites

http://www.agmanager.info/livestock/marketing/default.asp

The Department of Agricultural Economics at Kansas State University maintains a website with a host of charts and data summaries, as well as white papers, fact sheets, and related resources which may be of interest to readers.

http://www.lmic.info/

The Livestock Marketing Information Center's website contains a host of useful data summaries and tabulations for production, price, trade, etc. of livestock and meat directly germane to this chapter.

http://www.usda.gov/wps/portal/usda/usdahome

The United States Department of Agriculture's Agricultural Marketing Service, Economic Research Service, Foreign Agricultural Service, Grain Inspection Packers and Stockyards Administration, and National Agricultural Statistics Service agencies provide a multitude of reports that may be of interest to readers.

MEAT RESEARCH INSTITUTIONS

RG Kauffman, University of Wisconsin, Madison, WI, USA

Introduction

Meat science and muscle biology research efforts and interests worldwide have existed only to a significant extent in the past century. These interests developed rapidly as knowledge about using domesticated animals for food increased. Many individuals, through their academic backgrounds of biochemistry, microbiology, histology, anatomy, physics, physiology, and mathematics, have studied muscle and related tissues and how they can be used for food. These scientists were either focused on the animal and food sciences or associated with private meat companies; all were attempting to improve fresh meat and find better ways to preserve, store, and process meat into consumer products.

From the initial scientific efforts by individuals, groups of individuals evolved and began to collaborate in investigations that required expertise from multiple disciplines. Some problems, such as meat tenderness, required sophisticated biological and physical approaches, whereas others, such as methods of cooking, packaging, and storage, required technological and engineering inquiries. The former have been arbitrarily identified as being more basic in nature, whereas the latter more applied. Many of the problems required combinations of these two approaches, and to resolve them in the most satisfactory way, group efforts prevailed. Therefore, individual scientists began cooperating for such complex investigations, each pursuing that part of the problem in which their expertise was required. From this obvious need, university, government, and industry organizations established groups, laboratories, sections, or independent institutes to launch complex research programs, all focused on learning more about muscle and related animal tissues (fat, bone, viscera, skin, etc.). They varied in size from just a few scientists to many, depending on need and financial support. Even though most investigations have been of biological or technological nature, some have required socioeconomic expertise (such as learning about consumer acceptance and marketing strategies).

When groups of scientists were formally assembled, they usually included some scientists trained in more basic disciplines and others in more applied ones. This combination of

Table 1 National meat research institutions (19)

Country	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
Argentina	Instituto Tecnologia Agropeduaria INTA	Centro di Agroindustria, INTA, CC 77, B1708WAB Moron, Pica, Buenos Aires, Argentina	Safety, biochemistry, physical analysis, processing, quality, new products, microbiology, and lipids	Beef	Large
Australia	Food Science Australia (previously CSIRO)	Box 3312, Tinalpa DC, Queensland 4173, Australia or Private Bag 16, Werribee, Melbourne, VIC 3030, Australia	Equipment automation, new products, quality, safety, and microbiology	Beef, lamb, pork, poultry, and fish	Large
Brazil	Centro de Tecnologia de Carnes (CTC)	do Instituto Tecnologia de Alimenteos, Av. Brasil 2880, Campinas, São Paulo, Brazil	Quality, slaughter techniques, packaging and irradiation, safety, new products, and nutrition	Pork and beef	Large
Canada	Agri-Food Canada	Lacombe Research Center, 6000 C & E Trail, Lacombe, AB T4L 1W1, Canada	Quality, safety, packaging, and processing	Pork and beef	Large
Denmark	Danish Meat Research Institute	Slagteriernes Forskningsinstitut Maglegaardsvej 2, DK-4000, Roskilde, Denmark and www.dmri.dk	Safety, microbiology, automation, quality, processing, composition, environment, and fresh meat evaluation	Pork, beef, and veal	Large

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Table 1 Continued

Country	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
Denmark	Ministry of Food, Agriculture and Fisheries: Danish Institute of Agricultural Sciences	Research Center Foulum, PO Box 39, DK 8830, Tjele, Denmark and www.agrsci.dk	Proteins, quality, composition, organoleptics, nutrition, by-products, processing, and food engineering	Pork, beef, and nutrition	Large
France	Station de Recherche sur la Viande, INRA	De Theix, 63122 Saint Gene, Champanella, France	Tenderness, quality, microbiology, and safety	Beef and pork	Large
Germany	Federal Center for Meat Research	Institute for Chemistry and Physics, EC Baumannstrasse 20, Kulmbach, D-95326, Germany	Quality, composition, biochemistry, water holding, microbiology, safety, and nutrition and health	Beef and pork	Large
Hungary	Hungarian Meat Research Institute	Gubacsi ut 6/b Budapest, Hungary	Safety, health, nutrition, composition, fermentation, nitrosamines, and microbiology	Pork and beef	Medium
Ireland	National Food Center	Teagasc, Agriculture and Food Development Authority, Dublin 15, Ireland	Safety, microbiology, residue detection, new products, and processing	Pork, beef, lamb, and poultry	Large
New Zealand	Ruakura Research Center	East St., Private Bag 3123, Hamilton, New Zealand	Quality, composition, processing, refrigeration, and proteins	Lamb, beef, veal, venison, poultry, and fish	Medium
Norway	Norwegian Food Research Institute	OSLOVN 1, 1430 Aas, Norway	Quality, safety, tenderness, processing, packaging, and consumer acceptance	Pork and beef	Large
Poland	Meat & Fat Research Institute	4190 Warszawa, ul. Jubilerska, 4, Poland	Cooking, quality, processing, safety, composition, and health	Pork, beef, and lamb	Medium
Switzerland	Swiss Veterinary Office	Chemistry Section, Schwarzenburgstrasse 161, CH-3003, Bern, Switzerland	Residues and additives and composition	All species, including wild game	Medium
USA	United States Department of Agriculture-Athens	Russell Research Center 950 College Station Road, Athens, GA 30605–2720, USA	Growth, lipids, and safety	Beef, pork, lamb, poultry, and fish	Large
USA	United States Department of Agriculture-Beltsville	Meat Science Research Lab, Bldg. 201, BARC-East, 10300 Baltimore Avenue, Beltsville, MD 20705, USA	Quality, safety, processing, and organoleptics	Beef, pork, lamb, and poultry	Large
USA	United States Department of Agriculture-Clay Center	Box 166-Spur 18D, Clay Center, NE 68933, USA	Organoleptics, quality, composition, and proteins	Beef, pork, and lamb	Large
Finland	Finnish Meat Research Institute	Luukkaankatu 8, Hämeenlinna Box 56, FIN-13101 HML, Finland	Processing, fresh meat, safety, quality, and stress	Pork, venison, beef, poultry, and fish	Large
Netherlands	Research Institute of Animal Production	Box 65, 8200AB, Leylstadt, The Netherlands	Fresh meat, processing, stress, growth, quality, composition, safety, and biochemistry	Pork, beef, lamb, poultry, and veal	Large

Table 2 Provincial (state and/or city) meat research institutions (78)

Country	*Province (state and/or city)*	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
Australia	Armidale	University of New England	Animal Science Meat Science, NSW 2351, Armidale, Australia	Quality, composition, and safety	Beef and lamb	Small
Australia	Victoria	Victoria University of Technology	W008, Box 14428, Melbourne City, MC 8001, Australia	Quality, safety, and processing	Beef and lamb	Small
Austria	Wien	Institute of Meat Hygiene, Meat Technology and Food Science	University of Veterinary Medicine-Vienna, Veterinärplatz 1, 1210 Wien, Austria	Safety, quality, and microbiology	Beef, pork, poultry, fish, and lamb	Large
Belgium	Ghent	University of Ghent	Animal Production, Research Center for Animal Feeding and Meat Science, Proefhoevestraat 10, B-9090 Melle, Belgium	Quality, safety, processing, lipids, nutrition, and composition	Pork, beef, and lamb	Small
Canada	Alberta	University of Alberta	Agricultural Food and Nutritional Science, 4–10 Ag/For Center, Edmonton, AB T6G 2P5, Canada	Quality, safety, processing, composition, growth, and genetics	Beef, pork, and poultry lamb	Small
Canada	Ontario-Guelph	University of Guelph	Animal and Poultry Science Meat Science Laboratory Guelph, ON N1G 2W7, Canada	Processing, quality, safety, instrument techniques, and color	Pork, beef, and poultry	Medium
Canada	Saskatchewan	University of Saskatchewan	Applied Microbiology and Food Science, Saskatchewan Food Product Innv, 51 Campus Drive, Saskatoon SK, S7N 5A8, Canada	Safety, quality, processing, and microbiology	Beef, pork poultry, and fish	Small
Chile	Valdivia	Universidad Austral de Chile	Campus Isla Teja, Box 567 Valdivia, Chile	Processing, safety, and quality	Beef, pork poultry, and fish	Small
China	Beijing	China Meat Research Center	70, Yangqiao, Yongdingmen Wai, Beijing 100075 PR, China	Processing, flavor, and new products	Pork, fish, and poultry	Large
China	Nanjing	Nanjing Agricultural University	Weigang, Nanjing 210095, China	Quality, processing, growth, and safety	Beef, pork, and poultry	Small
Costa Rica	San José	Escuela de Agricultura de la Region Tropical Humeda	Apartado 4442–1000, San José, Costa Rica	Processing, quality, composition, and safety	Pork, beef, poultry, and fish	Small
Czech Republic	Prague	Institute of Chemical Technology	Food Preservation and Meat Technology, VSCHT Praha, Czech Republic	Processing and preservation	Pork, beef, and poultry	Medium
Denmark	Copenhagen	Royal Veterinary & Agricultural University of Copenhagen	Dairy and Food Science Rolighedsvej 30, DK-1958, Frederiksberg C, Denmark and www.mli.kvl.dk	Proteins, lipids, quality, microbiology, and processing	Pork and poultry	Medium
Finland	Helsinki	University of Helsinki	Food Technology, Vikki E, Box 27, FIN-00014, Helsinki, Finland	Quality, welfare, glycogen, minerals, fermentation, water holding, and processing	Pork and beef	Medium
Ireland	Cork	University College Cork	Food Science, College Road, Cork, Ireland	Microbiology, processing, quality, safety, and organoleptics	Beef, pork, and poultry, lamb, and fish	Medium
Italy	Parma	Universita di Parma	Dipartimento di Produzion Animali, Biotecnologie Veterinerie, Qualita e. Sicurezza degli Alimenti, 43100 Parma, Italy	Quality, processing, new products, color, safety, lipids, and health	Pork, beef, and poultry	Medium

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Table 2 Continued

Country	*Province (state and/or city)*	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
Japan	Fukuoka	Kyushu University	Laboratory of Chemistry and Technology of Animal Products, Graduate School of Agriculture, 6–10–1 Hakozaki, Higashi-Ward, Fukuoka 812–8581, Japan	Processing, nutrition, growth, and biochemistry	Pork, beef, poultry, and fish	Small
Japan	Sapporo	Hokkaido University	Meat Science Laboratory Sapporo, 060–8589, Japan	Tenderness, biochemistry, and aging	Pork and poultry	Small
Korea, South	Gyeongsang	Gyeongsang National University	Animal Science, Meat Science Laboratory, Gaja-Dong 900, Chinju, Gyeongsang 660–701, South Korea	Quality, safety, processing, instruments, and composition	Pork, beef, poultry, and fish	Small
Korea, South	Kyunggi-do	Seoul National University	Animal Science and Technology, Suwon, Kyunggi-do 441–744, South Korea	Quality, safety, proteins, microbiology, and processing	Pork, beef, poultry, and fish	Small
Korea, South	Pukkwangju	Chonnam National University	Animal Science, Box 205 Pukkwangju 500–600, South Korea	Quality, safety composition, and processing	Pork, fish, poultry, and beef	Small
Netherlands	Utrecht	Utrecht University	Science of Food of Animal Origin, Box 80175, 3508 TD Utrecht, The Netherlands	Microbiology, safety, quality, histology, and processing	Pork, beef, veal, lamb, and poultry	Medium
New Zealand	Palmerston North	Massey University	Institute of Food, Nutrition and Human Health, Private Bag 11–222, Palmerston North, New Zealand	Processing, quality, safety, biochemistry, and microbiology	Lamb, beef, pork, poultry, and fish	Small
Norway	Aas	Agriculture University of Norway	Animal Science, Box 5025N-1432 AAS, Norway	Quality, safety, and processing	Pork, beef, poultry, and fish	Small
Poland	Poznan	Agricultural University of Poznan	Ul. Wojska Polskiego 31, Institute of Meat Technology, Poznan 60–624, Poland	Proteins, quality, processing, safety, and biochemistry	Pork, beef, poultry, and fish	Medium
Puerto Rico	Mayaguez	University of Puerto Rico	Box 9030, College of Agricultural Science Mayaguez PR 00681–9030, Puerto Rico	Processing, safety, and quality	Pork, fish, beef, and poultry	Small
South Africa	Pretoria	University of Pretoria	Animal and Wildlife Science Pretoria 1, South Africa	Composition, quality, safety, and growth	Beef, pork, lamb, fish, and wildlife	Small
Spain	Madrid	University Complutense de Madrid	Nutricion Y Bromatologia III, Facultad de Veterinaria, 28040 Madrid, Spain	Processing, safety, and quality	Beef, pork, poultry, and fish	Small
Spain	Valencia	Instituto de Agroquimica y Technologia	De Apartado 73, 46100 Burjasot, Valencia, Spain	Quality, safety, and processing	Beef, pork, poultry, and fish	Small
Sweden	Uppsala	Swedish University of Agricultural Sciences	Food Science, Box 7051, SE-75007, Uppsala, Sweden	Quality, genetics, processing, and organoleptics	Pork, beef, fish, poultry, and reindeer	Medium
Taiwan	Taichung	Tunghai University	Food Science, No. 181, Section 3, Taichung-Kan Road, Taichung 407–04, Taiwan	Processing, safety, quality, and proteins	Pork, fish, and poultry	Small
UK	Bristol	University of Bristol	School of Veterinary Science, Langford House, Langford, Bristol BS4O5DU, UK and www.vetschool.bris.ac.uk/Langford/Langford.html	Safety, proteins, quality, and lipids	Beef, pork, and lamb	Small
UK	Loughborough	University of Nottingham	Food Science, Sutton Bonington, Loughborough LE12 5RD, UK	Biochemistry, microbiology, quality, processing, and safety	Beef, pork, lamb, veal, poultry, and fish	Small

USA	Alabama	Auburn University	Animal Sciences, Ann Upchurch Hall, Auburn University, AL 36849, USA	New products, processing, safety, quality, and composition	Pork, beef, veal, fish, lamb, and poultry	Medium
USA	Alaska	University of Alaska	School of Fisheries and Ocean Sciences, 245 O'Neil Building, Fairbanks, AK 99775–7220, USA	Proteins, processing, quality, and safety	Fish	Small
USA	Arizona	University of Arizona	Muscle Biology Group, 624 Shantz, Tucson, AZ 85721, USA	Proteins, enzymes, quality, and biochemistry	Beef, pork, poultry, fish, and wild game	Medium
USA	Arkansas	University of Arkansas	Animal Science B123, Animal Science Building, Fayetteville, AR 72701, USA	Composition, safety, quality, and processing	Poultry, beef, fish, pork, and lamb	Small
USA	California-Davis	University of California-Davis	Animal Science, 1 Shields Avenue, Davis, CA 95616, USA or Veterinary Medicine, 910 43rd St, Sacramento, CA 95819, USA	Quality, safety, microbiology, processing, proteins, and histochemistry	Beef, pork, poultry, and fish	Medium
USA	Colorado	Colorado State University	Animal Science, Fort Collins, CO 80523–1171, USA	Processing, quality, composition, safety, nutrition, microbiology, and welfare	Beef, pork, lamb, fish, poultry, and wild game	Medium
USA	Connecticut	University of Connecticut	Animal Science, 3636 Horsebarn Road EXT, Storrs CT 06269–4040 USA	Color, quality, processing, proteins, lipids, and organoleptics	Pork, beef, poultry, fish, and veal	Small
USA	Florida	University of Florida	Animal Science Building 459 Box 110910, Gainesville, FL 32611, USA	Quality, safety, composition, and organoleptics processing	Beef, pork, lamb, fish, poultry, and veal	Small
USA	Georgia	University of Georgia	Animal and Dairy Science Complex, 425 River Road or Food Science and Technology, 130 Camp Halinan Road, Athens, GA 30601, USA	Processing, safety, quality, and microbiology	Pork, beef, fish, lamb, and poultry	Medium
USA	Idaho	University of Idaho	Animal and Veterinary Science Moscow, ID 83844–2330, USA	Quality, composition, safety, and processing	Beef, pork, and lamb	Small
USA	Illinois–Urbana	University of Illinois	Food Science and Human Nutrition, 905 South Goodwin Avenue, 399 Bevier Hall, or Animal Sciences, Meat Science Laboratory, 1503S Maryland Drive, Urbana, IL 61801, USA	Color, nutrition, composition, quality, processing, evaluation techniques, and safety	Pork, beef, poultry, fish, and lamb	Medium
USA	Indiana	Purdue University	Animal Sciences, Smith Hall, West Lafayette, IN 47907, USA	Quality, safety, composition, processing, growth, nutrition, proteins, and evaluation methods	Pork, beef, lamb, veal, fish, and poultry	Medium
USA	Iowa	Iowa State University	Animal Science, Kildee Hall, Ames, IA 50011, USA	Processing, safety, irradiation, quality, composition, proteins, lipids, and nutrition	Pork, beef, poultry, lamb, and fish	Medium
USA	Kansas	Kansas State University	Animal Sciences and Industry, Weber Hall, Manhattan, KS 66506–0201, USA	Color, packaging, processing, ethics, quality, safety, composition, and organoleptics	Beef, pork, lamb, fish, poultry, and veal	Medium
USA	Kentucky	University of Kentucky	Animal Sciences, W P Garrigus Building, Lexington, KY 40546–0215, USA	Composition, safety, quality, and processing	Beef, pork, poultry, veal, and lamb	Small
USA	Louisiana	Louisiana State University	Animal Science, South Campus Drive, Baton Rouge, LA 70803–4210, USA	Composition, quality, processing, evaluation techniques, and proteins	Beef, pork, poultry, fish, and lamb	Medium
USA	Maryland	University of Maryland	23311 Swancove, Box 342, College Park, MD 21612, USA	Quality, processing, and safety	Pork, beef, and poultry	Small

(*Continued*)

Table 2 Continued

Country	*Province (state and/or city)*	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
USA	Massachusetts	University of Massachusetts	Food Science, 236 Chenoweth Laboratory, Amherst, MA 01003, USA	Proteins, lipids, and processing	Beef, pork, poultry, and fish	Small
USA	Michigan	Michigan State University	Food Science and Human Nutrition, Anthony Hall, East Lansing, MI 48824–1224, USA	Proteins, nutrition, processing, safety, quality, composition, and organoleptics	Beef, pork, poultry, fish, and lamb	Medium
USA	Minnesota	University of Minnesota	Food Science and Nutrition, ABLMS 1334 Eckles Avenue, Saint Paul, MN 55108–6099, USA	Quality, safety, composition, proteins, and organoleptics	Beef, pork, fish, lamb, and poultry	Medium
USA	Mississippi	Mississippi State University	Animal and Dairy Science, Box 9815, Mississippi State, MS 39762, USA	Quality, safety, composition, and processing	Fish, lamb, beef, pork, and poultry	Small
USA	Missouri	University of Missouri	Food Science and Nutrition 2214 Bluff Boulevard, or Animal Sciences, S138 Animal Science Center Columbia, MO 65211, USA	Consumer acceptance, quality, safety, proteins, processing, and composition	Beef, pork, poultry, lamb, and fish	Medium
USA	Montana	Montana State University	Animal and Range Sciences Linfield Hall, Box 172900, Bozeman, MT 59717–2900, USA	Quality, composition, safety, and processing	Beef, pork, poultry, and lamb	Small
USA	Nebraska	University of Nebraska	Animal Science, Box 830908, Lincoln, NE 685830908, USA	Processing, quality, safety, cookery composition, anatomy, and organoleptics	Beef, pork, lamb, fish, and poultry	Medium
USA	Nevada	University of Nevada	Animal Science, Veterinary Medicine-202, Reno, NV 89557, USA	Proteins, quality, safety, processing, and tenderness	Beef, pork, poultry, lamb, and fish	Small
USA	New Hampshire	University of New Hampshire	Barton Hall, Durham, NH 03824, USA	Processing, safety, and quality	Pork, beef, lamb, fish, and poultry	Small
USA	New Mexico	New Mexico State University	Animal and Range Science, 3255 Hillrise, Las Cruces, NM 88001, USA	Quality, processing, and composition	Beef, lamb, and pork	Small
USA	New York	Cornell University	Animal Science, Morrison Hall, or Food Science, 8 Stocking, Ithaca, NY 14853–7201, USA	Processing, biochemistry, microbiology, safety, quality, and instrument methods	Beef, pork, lamb, veal, fish, and poultry	Medium
USA	North Carolina	North Carolina State University	Scott Hall, Box 7680, Raleigh, NC 27695–7608, USA	Processing, quality, composition, color, emulsions, proteins, and safety	Poultry, beef, pork, fish, and veal	Medium
USA	North Dakota	North Dakota State University	Animal Science, Hultz Hall, Fargo, ND 58105, USA	Composition, quality, processing, and nutrition	Beef, pork, and lamb	Small
USA	Ohio	The Ohio State University	Animal Sciences, 133 Aldrich Road, Columbus, OH 43214, USA	Processing, safety, quality, composition, proteins, and organoleptics	Beef, pork, veal, fish, lamb, and poultry	Medium
USA	Oklahoma	Oklahoma State University	Animal Science, Animal Science Building, Stillwater, OK 74078–0425, USA	Composition, quality, safety, processing, and proteins	Beef, pork, and lamb	Medium
USA	Oregon	Oregon State University	Clark Meat Science Center, Withycombe Hall, Corvallis, OR 97331, USA	Quality, safety, composition, processing, and proteins	Beef, pork, poultry, fish, and lamb	Small

USA	Pennsylvania	Pennsylvania State University	Food Science, Borland Laboratory, University Park, PA 16802, USA	Quality, safety, processing, and composition	Beef, pork, lamb, veal, and poultry	Small
USA	South Carolina	Clemson University	Food Science and Human Nutrition, Box 340371, Poole Agriculture Center, Clemson, SC 29634–0371, USA	Proteins, safety, quality, processing, and composition	Poultry, fish, beef, pork, and lamb	Medium
USA	South Dakota	South Dakota State University	Animal and Range Science, 1904 Morningside Drive, Brookings, SD 57006, USA	Quality, safety, processing, and composition	Beef, pork, poultry, lamb, and veal	Small
USA	Tennessee	University of Tennessee	Food Science and Technology, McLeod Building, Box 1071, Knoxville, TN 37901–1071, USA	Safety, quality, processing, composition, and proteins	Pork, beef, poultry, fish, and veal	Medium
USA	Texas–College Station	Texas A & M University	Animal Science, Kleburg Center, 2471 TAMU College Station, TX 77843–2471, USA	Composition, quality, safety, processing, proteins, nutrition, and organoleptics lipids	Beef, pork, lamb, goat, veal, fish, poultry, and wild game	Medium
USA	Texas-Lubbock	Texas Tech University	Animal Science and Food Technology, Meat Science Laboratory, Box 42162, Lubbock, TX 794091162, USA	Composition, quality, and processing	Beef, pork, and lamb	Medium
USA	Utah-Logan	Utah State University	Nutrition and Food Science 750N. 1200 East, or 8700 Old Main Hill Logan, UT 84322–8700, USA	Color, proteins, safety, processing, nutrition, and growth	Pork, beef, poultry lamb, and fish	Medium
USA	Utah-Provo	Brigham Young University	Animal Science, Provo, UT 84602, USA	Composition, quality, processing, and safety	Beef, pork, lamb, veal, and poultry	Small
USA	Virginia	Virginia Polytechnic Institute & State University	Food Science Building, Blacksburg, VA 24061–0418, USA	Processing, safety, quality, and composition	Beef, pork, fish lamb, and poultry	Small
USA	Washington	Washington State University	Animal Sciences, Clark Hall, Box 646310, Pullman, WA 99164–6310, USA	Quality, safety composition, and processing	Beef, pork, poultry, lamb, and veal	Medium
USA	Wisconsin–Madison	University of Wisconsin	Meat Science and Muscle Biology Laboratory, 1805 Linden Drive, Madison, WI 53706, USA	Proteins, lipids, safety quality, microbiology biochemistry, composition, processing, and organoleptics	Beef, pork, poultry, lamb, fish, and veal	Medium
USA	Wyoming	University of Wyoming	Animal Science, Laramie, WY 82071–3684, USA	Proteins, microbiology, safety, quality processing, composition, and biochemistry,	Beef, pork, lamb, poultry, and wild game	Medium

Table 3 Private industrial meat research institutions (28)

Country–city	*Name*	*Address and/or website*	*Primary subjects*	*Species*	*Size of program*
Brazil–Campinas	Instituto de Tecnologia de Alimentos	Av. Brasil 2880, 13073–001 Campinas – São Paulo, Brazil	Processing, safety, quality, packaging, and new products	Beef, pork, poultry, and fish	Large
Brazil–São Paulo	Centro de Pesquisas Technology	Sadia, Rua Coroados 182, São Paulo 05092–020, Brazil	Processing, safety, quality, sanitation, engineering, and packaging	Pork, beef, poultry, and fish	Large
Mexico–Sonora	Centro de Investigacion en Alimentacion	Km 0.6 Carretera A La Victoria, CP 1735 Hermosillo, Sonora	Quality, safety, and processing	Pork, beef, poultry, and fish	Small
Spain–Valencia	Instituto de Agroquimica y Technologia (CSIC)	Food Science, Apartado 73, 46100 Burjassot, Valencia, Spain	Processing, quality, safety, new products, microbiology, and biochemistry	Pork, beef, poultry, and fish	Medium
Sweden–Goteborg	Institute of Food and Biotechnology	Box 5401, SE-402 29 Goteborg, Sweden	Processing, safety, quality, packaging, and new products	Poultry, beef, pork, and fish	Large
USA–Alsip	Griffith Laboratories	1 Griffith Center, Alsip, IL 60803, USA	Ingredients, safety, processing, and quality	All species	Large
USA–Appleton	American National Can Company	1806 North Edgewood Avenue, Appleton, WI 54914, USA	Packaging, quality, and safety	All species	Medium
USA–Austin	Hormel Foods Corporation	2 Hormel Place Austin, MN 55912–4935, USA	Processing, fresh meat, packaging, quality, safety, by-products, new products, and organoleptics	Pork, beef, poultry, and fish	Large
USA–Austin	Hormel Institute	(Discontinued)	Lipids, fats, oils, nutrition, and by-products	Pork, beef, and poultry	Large
USA–Bedford Park	Heller Seasonings	6363 West 73rd Street, Bedford Park, IL 60638, USA	Ingredients, processing, and safety	All species	Large
USA–Camden	Campbell Soup Co.	Research and Development, Campbell Place, Box 202, Camden, NJ 08103–1799, USA	Processing, new products, safety, quality, composition, and microbiology	Beef, pork, poultry, fish, and lamb	Large
USA–Chicago	Viskase Corporation	6855 West 65th Street, Chicago, IL 60638, USA	Packaging, processing, and safety	Pork, beef, poultry, and fish	Large
USA–Cordova	Sara Lee Corporation	Research and Development, 8000 Centerview Parkway, #400, Cordova, TN 38018, USA or Bil Mar Foods, 8300 96th Avenue, Zeeland, MI 49464, USA	Processing, safety, quality, engineering, and packaging	Beef, pork, poultry, and fish	Large
USA–Downers Grove	Armor Swift-Eckrich	3131 Woodcreek Drive, Downers Grove, IL 60515, USA	Processing, safety, quality, microbiology, packaging, and engineering	Pork, beef, poultry, and veal	Large
USA–Duncan	Cryovac Sealed Air Corporation	100 Rogers Bridge Road, Duncan, SC 29334, USA	Packaging, safety processing, quality, and microbiology	All species	Large

USA–Fort Wayne	Central Soya Company, Inc.	1946 West Cook Road, Fort Wayne, IN 46818, USA	Processing, ingredients, safety, and quality	All species	Large
USA–Gainesville	ABC Research Corporation	3437 SW 24th Avenue, Gainesville, FL 32607, USA	Microbiology and safety	Pork, beef, lamb, fish, poultry, veal, and wild game	Medium
USA–Greenfield	Elanco Animal Health	Research and Development, 2001 West Main Street, Greenfield, IN 46140, USA	Growth, safety, composition, and quality	Pork, poultry, and beef	Large
USA–Lodi	Alkar, Division of DEC International, Inc.	932 Development Drive, Lodi, WI 53555, USA	Processing, smoking and curing, safety, and quality	All species	Medium
USA–Madison	Oscar Mayer Foods Division Kraft Foods	910 Mayer Avenue Box 7188, Madison, WI 53707, USA	Processing, packaging, organoleptics, proteins, lipids, by-products, quality, safety, and new products	Pork, beef, poultry, and fish	Large
USA–Manitowoc	Red Arrow Products Co.	633S. 20th Street, Manitowoc, WI 54221, USA	Smoke processing, and safety	Pork, poultry, beef, and fish	Medium
USA–Neenah	Pechiney Plastics Packaging, Inc.	2301 Industrial Drive, Box 702, Neenah, WI 54957–0702, USA	Packaging and safety	Pork, beef, poultry, and fish	Small
USA–Omaha	ConAgra Corporation	Research and Development, 6 ConAgra Drive, Omaha, NE 68102, USA	Processing, safety, packaging, new products, proteins, and microbiology	Beef, pork, and poultry	Large
USA–Searcy	Land O'Frost, Inc.	911 Hastings Avenue, Box 9158, Searcy, AR 72145, USA	Preservation, processing, and packaging	All species	Large
USA–Springdale	Tyson Foods, Inc.	Research and Development, 2210 Oaklawn Drive, Springdale, AR 72764, USA	Safety, quality, new products, fresh meat, composition, and processing	Poultry, pork, and beef	Large
USA–St. Louis	Monsanto Animal Agriculture	Mail Stop: B2NA, 800N. Lindbergh Boulevard, St. Louis, MO 63167, USA	Growth, safety, composition, quality, and microbiology	Pork, beef, and poultry	Large
USA–St. Louis	Protein Technologies International	Box 88940, St. Louis, MO 63188, USA	Proteins, processing, safety, and quality	Pork, beef, poultry, and fish	Large
USA–Wichita	Excel Corporation	Research and Development, 2901 Mead Street, Wichita, KS 67201, USA	Processing, fresh meats, safety, new products, and packaging	Pork, beef, and poultry	Large

scientific qualifications has proven invaluable to the ultimate success of research. Basic disciplines include subjects such as physiology, biochemistry, biophysics, microbiology, histology and histochemistry and theoretical economics, mathematics, and statistics. Applied subjects include subjects such as processing techniques (emulsification, cookery, curing, etc.), refrigeration and freezing technology, engineering, automation, computer applications (e.g., for traceability), environmental aspects, packaging, nutrition, marketing, consumer preference, slaughter procedures (stunning, electrical stimulation, etc.), sausage manufacturing, and new product formulation, ingredients, etc. At least some of these require a working knowledge of the basic sciences. However, one of the unique characteristics of some research institutions was that they chose to enroll scientists representing several areas of expertise. Through the efforts of research coordinators, the various scientists were organized to cover all aspects of a problem.

Regrettably, some small groups of scientists who function informally will be omitted from this article because they were unintentionally missed in this search. The three tables of institutions that follow have been categorized by scope and size. **Table 1** lists national meat research institutions, **Table 2** covers provincial (state and/or city) institutions, and **Table 3** lists private industrial meat research institutions. Some institutions that no longer exist have been intentionally included because of their significant contributions in the past. The word 'institution' has been interpreted to include any formally organized unit having one or more scientists. Within each category, institutions have been alphabetized by country, province (state and/or city), and institution name. When known, the address and website, primary subjects covered, species of animals used, and size of program as arbitrarily defined by numbers of staff scientists involved (small <3, medium 3–12, and large >12) are included. The size of institution does not reflect in any way the quality or significance of the research accomplished, and it should be understood that most provincial institutions conducting research also provide pedagogical and extension functions, many of which are the primary focus for that institution's existence.

Further Reading

American Meat Science Association, 2001. Who's Who in Meat Science, Membership Directory 2001. Savoy, IL, USA: American Meat Science Association

Honikel, K.O., 2003. Weblinks for Meat Research. Kulmbach, Germany: BAFF. Available at: www.bafleisch.de/weblinks.htm (accessed 03.02.04).

Kinsman, D.M., 1978. International Meat Science Dictionary: A Listing of Meat Research Institutes by Country. Storrs, CT, USA: Animal Industries Department, University of Connecticut, pp. 142–168.

Sleeth, R.B., 2001. Global meat research initiatives. In: Proceedings of the 54th Annual Reciprocal Meat Conference, pp. 1–20. Indianapolis, IN. Savoy, IL, USA: American Meat Science Association.

MEAT SPECIES DETERMINATION

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Glossary

Adulteration The act of adding foreign substances to something that makes it impure.
Authentication The process of determining whether something is what it is declared to be.
Chromatography Separating mixtures of molecules based in a mobile phase through a stationary based on different molecular characteristics.
Comminuted Reduced in size to small particles.
Electrophoresis A technique to move dispersed particles in an electric field and separate them based on different molecular characteristics.
Polymerase chain reaction A technology for amplifying specific nucleic acid sequencing from a few copies to many thousands or millions of copies.

Introduction

Food adulteration refers to the act of intentionally debasing the quality of food by either adding or replacing the food substances with undeclared alternative components, or by the removal of some valuable components. This is usually done to lower the cost or increase the bulk of a given food product. This has been a major concern among consumers as it is often undesirable from an economic, health, religious, or legal standpoint. Meat adulteration in comminuted and highly processed meat products is a widespread practice in some retail markets where meat species with higher commercial value have been replaced or substituted with lower value or undesirable alternatives. In addition, fraudulent adulteration of food products with undeclared components might cause health problems such as allergies in sensitized individuals. The 2012 horse meat scandal in Europe, in which horse meat was found in beef products has raised public concern with respect to meat authentication. Although horse meat is a consumable product in its own right and harmless to human health, the general public responded negatively to the fraudulent meat. Furthermore, several religions have strict dietary laws relating to meat animal species. For Muslims, for example, consumption of pork and other porcine derivatives is prohibited among devotees. Food authentication and, in particular, identification of component species in meat products is, therefore, important in order to control food quality and safety as well as to protect consumers' rights.

The analytical techniques commonly used for meat species identification can be broadly divided into protein-based and deoxyribonucleic acid (DNA)-based techniques. The protein-based methods include immunological assays, electrophoretic, and chromatographic techniques. These methods are fast and easy to perform and the investment in equipment is much less compared to DNA-based methods. The use of these methods for meat species identification is limited when assaying thermally processed foods due to the denaturation of proteins. More recently, DNA-based methods have been used as an alternative to protein analysis in meat species identification as DNA molecules are more stable when compared to proteins and allow analysis of processed and heat-treated products. In addition, DNA molecules are present in most biological tissues and can, therefore, be extracted from a variety of tissues. All these factors make them a good choice for differentiation and identification of components in food. Among the DNA-based methods used in species identification, the polymerase chain reaction (PCR), which involves highly specific amplification of one or more DNA fragments is the most well-developed method due to its simplicity, rapidness, specificity, and sensitivity for species identification in foods. A number of PCR techniques have been used for meat species identification and these include PCR-sequencing, PCR-restriction fragment length polymorphism (PCR-RFLP), PCR with species-specific primers, PCR-random amplified polymorphic DNA (RAPD), and real-time PCR. Protein-based and DNA-based methods currently used in meat species identification are described below.

Protein-Based Methods

Enzyme-Linked Immunosorbent Assays

The enzyme-linked immunosorbent assay (ELISA) is an immunological assay that utilizes an enzyme to detect the presence of an antibody or an antigen in a complex, mixed sample based on the specific binding of the antigen and antibody. Of the different types of ELISA available, the indirect and the sandwich ELISA are the two most commonly used types for meat species identification. The indirect ELISA entails the binding of two antibodies, namely the primary antibody (which is specific to the antigen) and the secondary antibody (which is linked to an enzyme that will produce a chromogenic or fluorescent signal when a substrate is added). In the sandwich ELISA, the antigen is bound between two antibodies, specifically the capture antibody and the detection antibody. The detection antibody can be conjugated to an enzyme that

will lead to the production of a signal when an appropriate substrate is added.

ELISA tests can be used for both the qualitative and quantitative detection of meat species. They have been used to identify meats of different species using antibodies against muscular and serum animal proteins. In addition to qualitative studies, quantitative studies such as the evaluation of levels of pork adulteration in raw ground beef have been reported.

ELISA tests used in meat species identification are highly sensitive and specific, fast and cheap, easy to perform, and do not require expensive laboratory equipment. In addition, a variety of ELISA test kits to detect low levels of contamination by meat animal species, even in highly processed meat and meat products, have been developed. These have been used by many regulatory authorities around the world to detect meat species adulteration. The simplicity and rapidness of ELISA analysis make them a suitable choice for routine analysis of a large number of samples. A major drawback of the ELISA test with respect to meat species identification is that some target proteins denatured during processing might not be detected. This limitation has, nevertheless, been overcome by the development of antibodies against specific thermostable proteins that have been applied in ELISA tests for the detection of pork in heat-treated meat products. Even with these innovations, however, ELISA assays still have disadvantages including the initial difficulty in producing antibodies specific to the target and the instability of the antibodies under extreme pH levels or with high concentrations of salt or solvent. These assays can also be hindered by cross-reactions occurring among closely related species due to the reliance on antibodies against specific protein targets.

Chromatographic Techniques

Chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography have been used for meat species identification by analyzing for specific components or compounds of the meats. These techniques are based on differences in protein, peptide, or amino acid profiles between different meat species.

Chromatographic techniques are highly sensitive and reproducible and are suitable for use in routine analysis in meat authentication. One example of the application of chromatographic techniques for meat authentication is the utilization of HPLC to analyze the ratio of the imidazole dipeptide carnosine and its methylated analog anserine, which differs between different meat species. There are, however, some drawbacks to these methods. The separation of the protein peaks is often unsatisfactory. In addition, tedious extraction of the samples before analysis is required and these methods are, therefore, very time consuming. Furthermore, a single chromatographic method is unable to differentiate more than seven species simultaneously.

Electrophoretic Techniques

Protein electrophoresis is based on the separation of proteins in an electric field that are later visualized using staining. This technique has been applied to meat species identification through the separation of meat components such as sarcoplasmic and myofibrillar proteins. The principle behind these techniques is based on the presumption that every animal species has a specific protein composition that is identical within the species. The conventional electrophoretical methods commonly used in meat species identification include polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate-PAGE (SDS-PAGE), and isoelectric focusing (IEF) techniques. More recently, capillary electrophoresis (CE) has also been used in meat authentication.

In PAGE, the proteins are separated based on the electrical charge and sizes of the protein molecules. Individual proteins move toward the anode or cathode, depending on their charge. In SDS-PAGE, denatured protein molecules with negative charges move toward the anode only and hence they are separated based solely on their molecular weight. In IEF, separation of the proteins is carried out on a gel with a stabilized pH gradient with the proteins separated based on their isoelectric points. These conventional electrophoretical techniques offer cheap, fast, and highly reliable methods for meat species identification. In addition, they do not require a sophisticated equipment or a high degree of technical expertise. Among the electrophoretic techniques SDS-PAGE provides the highest resolution and most reproducible results and has been most effectively used in the detection and identification of foreign meat proteins in protein mixtures. It has also been used to identify meats of a wide range of species including cattle, sheep, lamb, goat, red deer, and rabbit. The major drawback of these electrophoretic methods is that the difference in the protein patterns obtained might not be species-specific as these patterns might be affected by other factors such as age, nutritional stage of animals, effects of stress, and/or other individual differences. In addition, great effort is often required in interpretation of the results. Furthermore, these techniques are unable to differentiate closely related animal species and are also not suitable for examination of samples that have been thermally treated.

More recently, CE systems have been used as an alternative to gel electrophoresis. In this method, electrophoresis is carried out in fine capillary tubes at high speed and under high voltage and the separated proteins quantified using various detectors based on fluorescence, refractive index, ultraviolet absorbance, and mass spectrometry. An example of the use of CE in identifying meat proteins is the SDS-CE method which entails separation of meat proteins based on their molecular size. These methods have been used to detect qualitative and quantitative differences in proteins in raw beef, pork, and turkey meats. In general, CE offers a powerful analytical method for meat authentication due to its high resolving power, high separation efficiency, and ease of automation. In addition, the analytical potential of CE can be further enhanced by combining it with PCR amplification-based techniques. However, the application of CE methods in analysis of complex meat matrices or heat-processed samples is restricted due to its low sensitivity and reproducibility.

Deoxyribonucleic Acid-Based Methods

Polymerase Chain Reaction-Based Methods

PCR-based methods are the simplest DNA-based method to determine the presence of a meat species in food. These

methods are based on specific amplification by oligonucleotides (primers), which hybridize to the targeted flanking regions of the DNA sequence(s) followed by amplification of the fragment and verification of the fragment size using agarose gel electrophoresis. PCR-based methods have been recognized as the most specific and sensitive technique in meat species identification. However, these methods have some disadvantages that limit their use in meat authentication. The high sensitivity of these methods could lead to false positive results, especially under low-stringency conditions, due to the nonspecific amplification of nontarget sequence, the formation of hairpin loops, or the formation of primer dimers, which will result in production of undesired fragments. In addition, the efficiency of these methods will be reduced in the presence of inhibitors sometimes present in meat products. A number of PCR-based methods have been developed for species detection in meat products and some of these are described below.

Polymerase Chain Reaction Sequencing

The PCR-sequencing method involves using a universal primer pair to generate single-band amplification products. The product of the generated PCR amplicon is then sequenced and the resulting sequence is analyzed. The results allow discrimination of even very closely related species by relying on the availability of known sequences for comparison and this method can, therefore, be used for interspecific and intraspecific identification of animal DNA in meat products. PCR-sequencing is a simple and direct method, which results in high amount of information because no complex postanalysis or enzymes are required with this method. This method has been reported to successfully identify a wide range of meat species origin, including differentiating closely related species such as goat and sheep, or cattle and buffalo.

PCR-sequencing has been established as the most accurate method for meat species identification but its application to meat authentication has been limited due to some restrictions. For example, PCR-sequencing is not suitable for analysis of cooked or processed samples with degraded DNA. This method has restricted use in meat adulteration investigations as it is not suitable for analysis of mixed-species meats due to the use of a universal primer pair. Furthermore, the need for expensive laboratory equipment means that this technique has limited use for large-scale screening. The PCR-sequencing method is generally used for verification of results obtained from species-specific and real-time PCR.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

The PCR-RFLP technique is based on sequence variations between different species and is determined by the digestion of selected DNA fragments with one or more selective restriction enzymes. This technique generates species-specific patterns that enable differentiation between meat species, including closely related species.

PCR-RFLP is cost-effective, simple, and suitable for use in routine, large-scale studies. An example of its use is for the detection of pork adulteration of raw meats through analysis of the cytochrome *b* gene of mitochondrial DNA (mtDNA). PCR-RFLP has also been used to trace meat adulteration by game meats that might be present in meat samples. As amplification of large DNA fragments is required for enzymatic restriction in order to generate restriction patterns, PCR-RFLP is not suitable for analysis of processed foods due to DNA denaturation. In addition, this technique is not suitable for the analysis of mixed-species meats, because results might show complex restriction patterns representing all the possible species present in the sample.

Polymerase Chain Reaction with Species-Specific Primers

PCR with species-specific primers is widely applied in meat authentication studies to identify a species using specifically designed oligonucleotides under restrictive PCR conditions. The identification of the target sequence can be verified based on the amplicon size determined through gel electrophoresis without subsequent sequencing or RFLP if the complete sequence of the amplified fragment is known.

PCR with species-specific primers is a simple, fast, specific, and highly sensitive method for species identification. It is, therefore, very useful for routine analysis of a large numbers of samples, including cooked or processed products with highly degraded DNA, and also in the analysis of mixed-species food matrices. Specific primers for a wide range of animal species have been designed and successfully applied to identify the origin of species in comminuted and highly processed meat products. The use of species-specific PCR to detect pork adulteration in fresh or processed meat products has been well documented as adulteration associated with the addition of pork frequently occurs.

A major drawback of species-specific PCR is the presence of false positive results that arise due to cross-homology. This can be overcome by increasing the specificity of the designed primers in order to ensure they will not display a cross-reaction with nontarget species.

Polymerase Chain Reaction-Random Amplified Polymorphic Deoxyribonucleic acid

The PCR-RAPD technique is based on the amplification of DNA fragments using a randomly designed short arbitrary primer that amplifies multiple discrete regions on the genomic DNA under appropriate PCR conditions. This is followed by separation of amplified fragments based on their sizes using gel electrophoresis and samples are identified by comparing the DNA bands of the resulting patterns. This technique is able to detect meat species without prior knowledge of the DNA sequence of the species of interest as long as reference material for comparison is available.

The PCR-RAPD technique has been used in many meat identification studies to differentiate between domestic animals as well as among rare species of animals. Use of this technique has also been made in various authentication studies for identification of many species of animals, usually commercially important or domestic species such as pork, beef, lamb, chicken, and turkey. Furthermore, this technique can be applied to either raw or processed forms of products.

Table 1 Summary of protein-based and DNA-based methods used for species identification

Method	*Principles*	*Advantages*	*Disadvantages*
Protein-based			
1. Enzyme-linked immunosorbent assay (ELISA)	• based on an immunological technique that utilizes an enzyme to detect the presence of an antibody or an antigen in a sample based on specific binding between the two	• sensitive and specific, fast and cheap, easy to perform, and does not require expensive laboratory equipment • a variety of ELISA test kits have been developed	• initial difficulty in production of antibody specific to the target • instability of the antibodies under extreme pH levels or with high concentrations of salt or solvent • hindered by cross-reactions occurring between closely related species
2. Chromatographic techniques	• based on separation by molecular weight of proteins, peptides, or amino acids and producing patterns that differ between meat species	• highly sensitive and reproducible • suitable for routine analysis	• separation of protein peaks is usually unsatisfactory • requirements for tedious extractions and long analysis times • unable to differentiate more than seven species simultaneously
3. Electrophoretic techniques	• based on the separation of proteins in an electric field according to their molecular weight and charge by polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate (SDS)-PAGE, or isoelectric focusing	• cheap, fast, and highly reliable method do not require sophisticated equipment and high degree of technical expertise	• unable to differentiate closely related animal species • not suitable for examining material that has been thermally processed
	• capillary electrophoresis at high speed and under high voltage conditions in fine capillary tubes	• high resolving power, high separation efficiency, and ease of automation • has the potential to be combined with polymerase chain reaction (PCR) amplification	• restricted to analysis of complex meat matrices or heat-processed samples due to its low sensitivity and reproducibility
DNA-based			
1. PCR-sequencing	• based on using a universal primer pair to generate single-band amplification products followed by sequencing of the PCR product	• simple and direct method • known to be the most accurate method in meat species identification	• unsuitable for use in cooked or processed samples • unsuitable for the analysis of mixed-species meats • requires expensive laboratory equipment
2. PCR-restriction fragment length polymorphism (PCR-RFLP)	• based on sequence variation between different species established through digestion of selected DNA fragments with one or more restriction enzymes	• cost friendly, simple, and suitable for routine large-scale studies	• not applicable for processed foods due to DNA destruction • not suitable for the analysis of admixed meats
3. PCR with species-specific primers	• using specifically designed oligonucleotide primers under restrictive PCR conditions	• simple, fast, specific, and highly sensitive • suitable for routine analysis of large samples • can be used to analyze cooked or processed products and mixed-species samples	• false positive results due to cross-homology
4. PCR-random amplified polymorphic DNA (PCR-RAPD)	• based on the amplification of DNA fragments using a short arbitrary primer that amplifies multiple discrete regions on the genomic DNA, followed by separation of amplified fragments based on their sizes using gel electrophoresis	• fast and simple powerful technique in instances where little or no information on the DNA sequence is available	• difficulty in interpretation of the gel images and obtaining reproducible data • unsuitable for use in highly processed meats and meat mixture samples
5. Real-time PCR	• based on direct and independent monitoring of cycle-to-cycle amplification using a fluorescent-labeled signaling probe	• high sensitivity and specificity, larger dynamic detection range, and less carryover contamination • allows the detection of trace amount of different animal species in samples of complex composition • provides both qualitative and quantitative information of the targets	• high cost due to cost derived from the specific fluorescent probes • the design and availability of primers and probes are hard to meet as they must be selected according to very rigid conditions

Major drawbacks of the PCR-RAPD method include difficulty in interpretation of the gel images and also in obtaining reproducible data as the results of the analysis vary depending on PCR conditions and also intraspecies polymorphisms. In addition, its application is restricted in highly processed meat samples due to degradation of DNA and is also not suitable for identification of a target species in meat mixtures.

Real-Time Polymerase Chain Reaction

Most of the conventional PCR techniques only enable qualitative and not quantitative analysis of the animal species in meat samples. Quantification of the adulterate components plays an important role in investigation of meat adulteration because the quantitative results can be used to determine whether the adulterant is added intentionally or due to accidental contamination in the production line. One of the quantification methods commonly used for meat authentication is real-time PCR. In this technique, the cycle-to-cycle amplification is monitored directly and independently using a fluorescent-labeled signaling probe. The intensity of the fluorescent signal is directly correlated to the PCR products amplified in each cycle and enables detection in a real-time format at an early stage.

Real-time PCR allows the detection of trace amounts of different animal species in samples of complex composition. In addition, it is more precise than end point analysis as it provides quantification information of the targets originally present in the sample. Furthermore, real-time PCR offers a wide range of other advantages, including high sensitivity and specificity, larger dynamic detection range, and less carryover contamination. The real-time PCR technique is, therefore, arguably the most effective technique currently used for species identification and quantification in meat authentication.

Real-time PCR assays have been applied to detect a wide range of meat species such as beef, pork, lamb, horse, chicken, turkey, and duck, among others. These techniques have also been used to detect and quantify porcine DNA with a detection limit down to 0.1% porcine tissue in heat-treated meat mixtures, indicating high sensitivity of these techniques for detection and quantification purpose. There are, however, some disadvantages of real-time PCR techniques including high cost due to the requirement for specific fluorescent probes. In addition, the design and availability of primers and probes are hard to meet as they must be selected according to very rigid conditions.

Conclusion

A wide range of techniques are available for meat species identification and meat product authentication. Despite the wide choice of analytical techniques available for meat species identification, all have specific advantages and disadvantages that are summarized in **Table 1**. The selection of a technique for meat species identification will be governed by factors such as sensitivity, cost, reliability, and rapidity. All factors should be considered before selecting a technique for a particular purpose.

See also: Cooking of Meat: Physics and Chemistry. Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Proteomic Technologies and Their Applications in the Meat Industry. Religious Slaughter. Slaughter, Ethics, and the Law. Species of Meat Animals: Cattle; Game and Exotic Animals; Pigs; Poultry; Sheep and Goats

Further Reading

Aida, A.A., Che Man, Y.B., Wong, C.M.V.L., Raha, A.R., Son, R., 2005. Analysis of raw meats and fats of pigs using polymerase chain reaction for halal authentication. Meat Science 69, 47–52.

Asensio, L., González, I., García, T., Martín, R., 2008. Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). Food Control 19, 1–8.

Ballin, N.Z., 2012. Authentication of meat and meat products. Meat Science 86, 577–587.

Fajardo, V., González, I., Rojas, M., García, T., Martín, R., 2010. A review of current PCR-based methodologies for the authentication of meats from game animal species. Trends in Food Science & Technology 21, 408–421.

Frenz, J., Hancock, W.S., 1991. High performance capillary electrophoresis. Trends in Biotechnology 9, 243–250.

Hui, Y.H., Aslhus, J.L., Cocolin, L., *et al.* (Eds.), 2012. Handbook of Meat and Meat Processing. USA: CRC Press.

Martin, D.R., Chan, J., Chiu, J.Y., 1998. Quantitative evaluation of pork adulteration in raw ground beef by radial immunodiffusion and enzyme-linked immunosorbent assay. Journal of Food Protection 61, 1686–1690.

Montiel-Sosa, J., Ruiz-Pesini, E., Montoya, J., *et al.*, 2000. Direct and highly species-specific detection of pork meat and fat in meat products by PCR amplification of mitochondrial DNA. Journal of Agricultural and Food Chemistry 48, 2829–2832.

Nakyinsige, K., Man, Y.B., Sazili, A.Q., 2012. Halal authenticity issues in meat and meat products. Meat Science 91, 207–214.

Relevant Website

http://www.fsis.usda.gov
Federal Meat Inspection Act by FSIS.

MECHANICALLY RECOVERED MEAT

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Glossary

Advanced meat recovery Machinery that removes residual meat from bones after primals have been carved off. In contrast to mechanically separated meat obtained by high pressure, bones remain intact and the miscroscopical structure of the product is that of meat.

Bovine spongiform encephalopathy (BSE) An infectious disease in cattle causing degeneration of the brain and spinal cord. The disease is closely related to spongiform encephalopathies in other animal species and humans. There is some evidence for transmission to humans via consumption of certain bovine tissues. To control or eradicate BSE, a number of measures have been implemented in pre and postharvest phase.

High pressure mechanically separated meat (MSM) Meat which is recovered from bones in a way that the structure of the bones is altered, and which contains higher amounts of calcium than minced meat. Usually this means pressing of meat from prefragmented bones at 100 bar or more.

Lean finely textured beef (LFTB) A process where trimmings are desinewed, and fat is removed by gentle heating and centrifugation.

Mechanically recovered meat (MRM) The product is obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical force. Depending on the pressure applied, muscle fiber structure may remain or be destroyed. In the European Union, mechanically desinewed meat also falls under MRM definition.

Introduction

Meat removed from bones or fat trimmings by machines is a welcome contribution to the world's food supply. Recovery of meat from bones of filleted fish began in Japan in the late 1940s and increased as the amount of filleted fish increased. Frames from filleting operations increase yield by 10–20% and lobster bodies, crab, and undersized shrimp yield an additional 40%. Meat from underutilized fish that previously was considered too small or bony is also saved. Approximately 27×10^6 kg of imported and domestically produced minced fish was consumed in the US in 2000.

Mechanical recovery of poultry from necks, backs, and other bones with meat attached started in the late 1950s. The number of machines increased as the amount of cut up chicken and turkey increased. It was estimated that mechanically separated poultry accounts for 2–4% of the annual turnover of poultry meat in the European Union (EU).

Removal of beef and pork from irregularly shaped bones began in the 1970s. The vertebral column and other bones with meat still attached after hand boning were used. In 2003, the annual additional yield of beef was approximately 23×10^6 kg in the US. Mechanically recovered pork yield was approximately 68×10^6 kg. Large amounts of meat are also being produced in other countries. Historically, the beef and pork industries relied on skilled employees using hand-held knives to physically cut meat from bone. Power knives, also called Whizard knives, increased speed and efficiency but were still unable to harvest all of the lean for human food, so it became part of rendered products.

In addition to mechanical removal of meat from bones, mechanical recovery of lean from fat also occurs. Partially defatted tissue is derived primarily from beef and pork fatty trimmings. The trimmings contain lean meat that is difficult to recover by hand. They pass through a low-temperature rendering process followed by centrifugation to remove lean from a rendered slurry. The recovered lean contains 7–17% fat.

A modification of this low-temperature (41 °C) rendering and centrifugation process is being used to produce lean containing not more than 6% fat. Sinew and cartilage are first removed by a rotating auger that forces lean and fat through small holes in a sieve. After low-temperature rendering and centrifugation, the finely comminuted product is frozen within 90 s in a thin sheet on a drum and then shipped frozen to meat processors. This process produces large quantities of lean meat used in low-fat ground beef. The product is known as lean finely textured beef (LFTB), or lean beef trimmings. Boneless lean beef trimmings may be treated with ammonium hydroxide or citric acid to reduce microbial counts. High microbial counts and rancidity are potential problems.

This article addresses the history, utilization, composition, safety, and palatability of meat recovered from bone. Meat mechanically recovered from fat and product from automatic deboning machines that use specially designed grinder blades to remove most of the bone and cartilage unintentionally left in hand-boned meat are not addressed in detail.

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Machines that Remove Meat from Bone

Machines that operate on a belt–drum principle, in which the bone is forced against a perforated drum by a rubber belt, were first used to recover meat from fish bones and they are still the most popular for mechanical recovery of fish. Holes in stainless steel drums range from 1 to 10 mm in diameter, but a hole size of 5 mm is most common. Meat passes through holes, while bones and skin stay on the outside of the drum and are ejected through a discharge chute. Following deboning, mince may be refined by passing it through a strainer that removes most bone particles and small pieces of belly lining. Holes in strainers typically range from 1 to 2 mm in diameter. Mince can range from a coarse texture to a fine paste depending on the source, machine type and setting, and processing method.

Another machine breaks up bones and then separates meat by a microgroove principle. Bone and meat are forced against a drum separator that allows meat to pass through microgrooves into the inner portion as broken bone is forced against it.

The auger type of mechanical deboner is also used to a limited extent in the fish industry and is the most popular machine for poultry. These machines use rotating augers inside stainless steel cylinders to force meat through orifices approximately 1 mm in diameter. In this system, bone is retained on the inside and augured out of the end of the cylinder to separate it from meat.

The most popular mechanical recovery systems for beef and pork utilize hydraulically powered presses. The steps are (1) presizing, (2) pressing, and (3) desinewing. Presizing consists of dividing the bones into sections 10–15 mm in length. Bone sections are then pressed at high pressure in a piston-like device with holes in the walls and the pressing head. As bones compress, meat is pushed off the bone, through filters and away from the machine via the product outlet. Compressed bone is then ejected from the chamber and another batch of presized bone enters. Recovered meat is transferred to a desinewing step where it passes between a belt and a drum with holes 1.0–1.3 mm in diameter. Sinew, cartilage, and bone particles are removed at this step and the product is ready for use in ground or processed meat.

Names for Mechanically Recovered Meat and Pertaining Legislation

The terminology of mechanically recovered meat (MRM) is determined, to some extent, by legislation. The EU stresses in its preamble to regulation (EC) 853/2004 that the definition of mechanically separated meat should be broad, covering all methods of mechanical separation, and be flexible in view of technological innovations. The definition given later in this regulation specifies that mechanically separated meat (MSM) is obtained from flesh-bearing bones or poultry carcasses by mechanical means altering the muscle fiber structure, meat obtained from 'soft' separators or mechanically desinewed meat (sometimes also termed 'Baader meat,' according to the brand name of commonly used machinery for that purpose) was for several years not considered as MSM. In 2010, it was decided that mechanically desinewed meat should fall under the MSM definition (EU COM(2010) 704 final). This standpoint is debatable in a view of improvements in low-pressure MSM technology. Rules for the use of MSM include: MSM must not be produced from ruminants; the addition of MSM from pork or chicken to meat products must be declared on the label. MSM with calcium contents execceding 100 mg per 100 g is considered 'high pressure' MSM, which can be used only for heated meat products.

In the US, meat recovered from bones by means of advanced meat recovery machinery can be labeled similar to hand-deboned meat, for example, beef/pork trimmings or ground beef/pork. The bones must remain intact during the process, and the maximum calcium content of the product is 150 mg per 100 g. Differences in texture and consistency of meat from press machines when compared to auger machines provided further justification for defining the advanced recovery product as meat. However, products with higher calcium levels have to be labeled 'mechanically separated' beef/pork. In contrast, for MSM produced by forcing bones with attached edible meat under high pressure through a sieve or similar device to separate the bone from the edible meat tissues, Food Safety and Inspection Service (FSIS) established a standard of identity for the food product and restrictions were made on how much can be used and the type of products in which it can be used. These restrictions were based on concerns for limited intake of certain components in MSM, like calcium. To protect consumers against bovine spongiform encephalopathy (BSE), beef mechanically separated from bones is considered inedible and is prohibited for use as human food. However, mechanically separated pork is permitted and must be labeled as such in the ingredients statement. LFTB, i.e., beef recovered from fatty trimmings under gentle heating and centrifugation, followed by acid or alkaline treatment und freezing, has commonly been added to ground beef. Recently, consumer concerns about LFTB triggered a heavy debate in the US. There are some pejorative terms used for such product, although its composition makes it nutritive food. Mechanically deboned chicken or mechanically deboned turkey is common names. Until 1995, these items were labeled as chicken or turkey when used in processed meat such as frankfurters in the US. In 1995, the FSIS of the US Department of Agriculture (USDA) amended the regulations to prescribe a definition and standard of identity and composition for poultry product resulting from mechanical separation of carcasses and parts. Requiring 'mechanically separated' on the label ensured that poultry products distributed to consumers were not labeled in a false or misleading manner. Other names that have been used for mechanically removed poultry meat include comminuted poultry, finely comminuted poultry, and finely ground poultry. The terms 'finely ground,' 'ground,' 'finely comminuted,' and 'comminuted' have also been applied to poultry products produced by hand boning.

'Minced' is the most common term for fish that has been mechanically recovered. The name is also used for meat separated from whole fish and bony fillets. In the EU, this product must be termed mechanically separated fish.

The terminology for MRM and the use of special labeling is being debated in many parts of the world. Those who support calling the product meat (i.e., pork) say that the

proximate composition is similar to that of meat and that it looks and tastes like meat. Those who prefer a specific name say that another name will help ensure that products distributed to consumers are not labeled in a false or misleading manner and are not misbranded. Concerns about false labeling relate to presence of nonmuscle tissue, for example, bone marrow and bone particles.

Uses for Mechanically Recovered Meat

Minced fish is usually frozen and then thawed and used in formulated sea foods including fish sticks, fish cakes, nuggets and added-value or specialty products such as chowders, patés, fish balls, and gefilte fish. Washed minced fish that has been blended with stabilizing ingredients is called surimi and is used in popular items such as imitation crab. Other fabricated products also use surimi as a key ingredient to improve texture.

Mechanically separated poultry exceeds 318×10^6 kg annually in the US. Approximately 182×10^6 kg is used in sausages such as frankfurters and bologna, and approximately 136×10^6 kg is used in products such as chicken patties, nuggets, and poultry rolls. In some countries, sausages have mechanically separated chicken or turkey along with beef and pork as ingredients.

In 1995, when the final rule requiring 'mechanically separated chicken' or 'mechanically separated turkey' to appear on the label was implemented, the USDA-FSIS stated that 529 labels for mechanically separated poultry existed. They estimated that labels for products containing mechanically separated poultry such as frankfurters, chilli, bologna, poultry, baby foods, chicken nuggets, or patties would number approximately 5000. This low-cost meat source has led to poultry meat products being more cost effective in the marketplace.

Mechanically recovered pork is used extensively in sausages and in many items where ground pork is used. In some countries, mechanically recovered pork must be indicated in the labeling. In other countries, only the word 'pork' needs to appear on the label.

Mechanically recovered beef is often blended with hand-boned beef. A smaller portion is sold as standalone product for use in jerky, taco meat, and pizza toppings. Uses for mechanically recovered beef and pork from hydraulic press machines change as government regulations change, but decreased costs associated with mechanical recovery increase consumer demand. The need to maximize meat yields while minimizing waste, and a reduction in repetitive motion stress-related injuries, are other reasons why mechanical recovery of meat continues to increase.

Composition

Most fish mince have a similar moisture, protein, and ash content to that of hand-boned fillets, but total fat content is slightly higher. Calcium also is higher in most minced fish, reflecting the presence of bone. Minces made from different parts of fish vary in bone content. In one study, cod bones from fillets produced 84 mg bone per kg of mince; trimmings produced 272 mg kg^{-1}, frames produced 1736 mg kg^{-1}; and backbones produced 4050 mg kg^{-1}. Some residual bones in mince from fillets of gutted whole fish are more than 6 mm long. Use of a drum with smaller perforations reduces bone size but also yields a mince of finer texture. People dislike fatty fish such as herring and mackerel because of the large numbers of small bones remaining in the hand-boned fillets. However, mince made from these fish is relatively free from bones so mince does not always contain more bone than hand-boned fish.

Tissues associated with bone may alter the composition of minced fish. When blood-rich tissues from kidney are included among raw materials, the resulting mince is higher in iron. The increase has not been a concern. Sardines contain 2.4 mg iron per 100 g, which is more iron than is found in most minced fish.

Incorporation of cryoprotectants such as sucrose, sorbitol, and phosphates allows mince to keep better than mince without additives. Washed mince is called surimi. Surimi often contains 3–4% sugar, 4–5% sorbitol, and 0.1–0.3% sodium phosphate and is almost fat free with cholesterol contents of 10–15 mg per 85 g serving. Because most of the fat is washed out of surimi during processing, ω-3 fatty acids also are removed, limiting the health benefits associated with eating fish.

Variations in composition of mechanically separated poultry result from factors such as the age of the bird, the proportion of bone and fat in the material being deboned, the type of machine, and whether the material being deboned is raw or cooked. Skin-on poultry always contains more fat before and after mechanical recovery than poultry with skin removed because most fat in poultry is associated with skin (Table 1). An indication how the proximate composition varies between the different carcass parts of chicken and about the proximated composition of turkey frames is given in Table 2.

The cholesterol content of mechanically separated poultry is higher than that of hand-boned poultry. The increase in cholesterol is associated with increases in fat, marrow, kidneys, and other tissues. Spinal cord extracted during mechanical separation of necks and backs also plays a role because the cholesterol content of spinal cord is 40 times higher (2420 mg per 100 g) than that of in lean. Inclusion of spinal cord material into mechanically separated poultry explains a cholesterol content in excess of the combined values for other tissues. In the US, the FSIS decided that cholesterol was not an issue in mechanically separated poultry as this product is primarily used as an ingredient in further-processed products where cholesterol content is declared on the label anyway. People concerned about limiting cholesterol uptake would be able to make educated decisions based on labeling.

The connective tissue content of mechanically separated poultry is lower than in hand-boned meat from comparable parts because the high tensile strength prevents its extrusion with the meat. An exception is found in cooked mechanically separated poultry because collagen is gelatinized during cooking, allowing it to be extruded.

Calcium in mechanically recovered poultry is higher than in most hand-boned products because of bone particles. Calcium, by USDA regulation, does not exceed 0.235% in MRM

Table 1 Composition of hand-boned and mechanically recovered poultry

Nutrient	Hand-boned, no skin		Mechanically recovered			
	Breast[a]	Leg[a]	Broiler backs and necks		Mature hens	
			With skin	Without skin	With skin	Without skin
Water (g per 100 g)	74.8	76.1	62.7	69.3	69.8	70.9
Protein (g per 100 g)	23.1	20.1	11.4	13.8	20.4	20.4
Fat (g per 100 g)	1.2	3.8	24.7	15.5	9.1	7.5
Ash (g per 100 g)	1.0	0.9	1.0	1.0	1.3	1.3
Calcium (mg per 100 g)	11	11	118	133	112	130
Iron (mg per 100 g)	0.7	1.0	1.6	1.7	1.3	1.3
Cholesterol (mg per 100 g)	58	80	140	120	122	110

[a]Reproduced from Posati, L.P., 1979. Composition of Foods, Agriculture Handbook 8–5. Washington, DC: USDA Consumer and USDA Consumer and Food Economics Institute, pp. 88, 106.

Table 2 Differences in the composition of mechanically separated poultry meat according to species or carcass parts used

Species or carcass part	Protein (g per 100 g)	Moisture (g per 100 g)	Fat (g per 100 g)
Chicken backs and necks	9.3–14.5	63.4–66.6	14.4–27.2
Chicken backs	13.2	62.4	21.1
Skinless necks	15.3	76.7	7.9
Spent layers	13.9–14.2	60.1–65.1	18.3–26.2
Cooked spent layers	18.3	63.2	16.5
Turkey frames	12.8–15.5	70.6–73.7	12.7–14.4

Source: Reproduced with permission from Froning, G.W., 1981. Mechanical deboning of poultry and fish. Advances in Food Research 27, 109–147.

from mature poultry or 0.175% when it is made from other poultry. These calcium values are intended to limit bone content to 1%. Iron in mechanically recovered poultry is higher than in hand-boned poultry, reflecting incorporation of marrow and other organs containing iron.

The compositions of mechanically recovered beef and pork from systems that involve presizing, pressing, and desinewing are shown in Table 3. The composition of hand-boned beef is based on a composite of trimmed retail cuts. Mechanically recovered beef figures are averages from numerous studies. It is clearly not possible to conduct analytical tests for moisture, protein, fat, and ash and to determine whether the product being analyzed is handboned or mechanically recovered. In addition to similar average values, there is a great deal of variation in proximate composition within both products.

Calcium and iron are higher in mechanically recovered red meat than in hand-boned meat because bone particles and marrow are present in mechanically recovered products. Calcium and iron deficiencies exist in many diets, so the presence of these minerals in greater amounts is nutritionally advantageous for most people. Hand-boned red meat contains approximately 0.01% calcium. MRM may contain up to 0.15% calcium by USDA regulations.

The iron content of mechanically recovered beef and pork is consistently higher than it is in hand-boned beef and pork. Some of the increase is a result of the incorporation of red marrow during pressing. Because iron in marrow varies inversely with fat content, some have suggested that the ratio of iron to protein in MRM would be a better indication of the amount of marrow present than iron alone. However, iron or iron:protein ratios may not give the correct marrow content for MRM because an increase in iron content of meat occurs even when marrow is not present. One reason for the increased iron concentration of MRM is that much of the collagen, which is almost devoid of iron, is removed. In one study, MRM contained 1.2% collagen, while comparable hand-boned meat contained 4.8%. Another reason for increased iron levels in MRM is that pressing lean remaining with the bone removes water-soluble pigments that are high in iron.

In general, cholesterol in mechanically recovered beef is higher than in hand-boned beef and at least some of the increase is due to marrow. Variability in the cholesterol content of marrow makes it impractical to use cholesterol levels as an

Table 3 Composition of hand-boned and mechanically recovered pork and beef

Nutrient[a]	Hand-boned		Mechanically recovered	
	Pork[a]	Beef[b]	Pork	Beef
Water (g per 100 g)	61.0	59.2	66.5	63.0
Protein (g per 100 g)	17.0	17.8	15.4	16.4
Fat (g per 100 g)	20.8	21.9	17.3	20.0
Ash (g per 100 g)	0.9	0.8	1.1	1.1
Calcium (mg per 100 g)	5	7	106	115
Iron (mg per 100 g)	0.8	1.9	3.0	5.6
Cholesterol (mg per 100 g)	74	69	126	115

[a]Reproduced from Anderson, B.A., 1983. Composition of Foods, Agriculture Handbook 8–10. Washington, DC: USDA Consumer Nutrition Division, p. 29.
[b]Reproduced from Anderson, B.A., Lauderdale, J.L., Hoke, I.M., 1986. Composition of Foods, Agriculture Handbook 8–13. Washington, DC: USDA, Consumer Monitoring Division, p. 37.

indicator of the amount of marrow present in MRM. In the US, beef bones must have all spinal cord removed before pressing. Therefore, the higher cholesterol content of mechanically recovered beef does not reflect spinal cord contamination.

Safety

Although a large volume of data relating to chemical composition, microbiology, and toxicology of fish is available, very few safety concerns that relate only to minced fish have surfaced. Sanitation requirements that apply to whole gutted fish or fish fillets also apply to mince. Bone fragments in minced fish are of minimal concern because some minced fish contains less bone than hand-boned fillets.

Several safety concerns were raised before the final rule for mechanically separated poultry was adopted by USDA in 1995. One of these concerns related to bone particle size. The conclusion was that bone particles up to 2 mm in mechanically separated poultry do not present a health hazard. There is debate about the use of mechanically separated poultry in baby food because more fluoride is found in bones than in muscle. Some believe that fluoride contributed by foods made with mechanically separated chicken, when combined with other sources of fluoride exposure, could increase the risk of mild dental fluorosis in children less than 8 years of age. However, no documented cases of fluoride problems related to chicken or turkey in baby food are available and no restriction has been placed on the use of mechanically separated poultry in baby food. Other concerns about increased calcium from bone and increased cholesterol from marrow and spinal cord are fully disclosed in nutrition facts on labels. These are nutrition concerns and are not safety related.

Because there are microbiological concerns related to the production and use of mechanically recovered fish, poultry, and red meat, some countries have adopted specific regulations relating to source of bones, the anatomical regions from which they come, and the temperature and time under which they can be held before mechanical recovery of lean. Handling of mechanically recovered products, including room temperature, chilling, and freezing, and their use have also been specified.

Countries where BSE exists have ended mechanical recovery of beef from bones (high pressure), because of traces of the spinal cord, which has been shown to be infectious, could be present. BSE causes the variant Creutzfeld–Jakob disease. With the exception of BSE, mechanically recovered beef, like pork, poultry, and fish, offers no unique safety problems.

Palatability

Data relating to palatability of MRM centers on texture, color, and flavor. Texture is finely comminuted or pasty. Color is redder, reflecting the incorporation of heme pigments from blood and kidney in fish and poultry, and from marrow in beef and pork. Flavor changes relate to increased oxidation of fat, resulting in lower storage stability. Washing removes fat, blood, pigments, and odorous substances. It is used to produce white and attractive surimi products such as imitation crab. Washing also improves storage stability by removing enzymes and fat that result in oxidation and rancidity. Many of the problems related to oxidation in poultry and red meat are overcome commercially by rapid chilling or freezing and by using MRM in products immediately after it is produced.

The popularity of fish sticks and imitation crab from mechanically recovered fish, of frankfurters and nuggets from mechanically recovered poultry, and of ground beef and sausages from mechanically recovered red meat attest to the desirable palatability attributes of these products. Nevertheless, some trained panels have reported differences in sensory characteristics of mechanically recovered products in comparison to their hand-boned counterparts. Because some differences exist and because questions regarding product identity have been raised, mechanically recovered products are not universally accepted. For example, some fast-food restaurants avoid using mechanically recovered beef in their hamburgers.

Mechanically recovered fish and poultry have been in existence longer than mechanically recovered red meat and there is less debate about their identity and use. Because all mechanically recovered products are safe, nutritious, wholesome, and highly palatable, their acceptance and use will continue to increase. More MRM will help feed malnourished, protein-deficient people in developing countries and improve the health of meat plant workers in developed countries by reducing repetitive motion stress that is associated with hand boning.

See also: Automation in the Meat Industry: Cutting and Boning. By-Products: Edible, for Human Consumption. Chemical and Physical Characteristics of Meat: Chemical Composition. Chemistry and Physics of Comminuted Products: Emulsions and Batters. Microbiological Safety of Meat: Prions. Minced Meats. Processing Equipment: Mixing and Cutting Equipment. Slaughter-Line Operation: Cattle. Species of Meat Animals: Pigs; Poultry; Shellfish. Spoilage, Factors Affecting: Microbiological; Oxidative and Enzymatic

Further Reading

Cohen, J.T., Duggar, K., Gray, G.M., *et al.*, 2001. Evaluation of the Potential for Bovine Spongiform Encephalopathy in the United States Boston, MA: Harvard School of Public Health and Tuskegee. Tuskegee, AL: Tuskegee University.

Crosland, A.R., Patterson, R.L.S., Higman, R.C., Stewart, C.A., Hargin, K.D., 1995. Investigation of methods to detect mechanically recovered meat in meat products – I. Chemical composition. Meat Science 40, 289–302.

European Commission, 2010. Communication from the Commission to the European Parliament and the Council. Brussels, on the future necessity and use of mechanically separated meat in the European Union, including the information policy towards consumers COM(2010) 704 final. Available at: http://ec.europa.eu/dgs/health_consumer/docs/msm_report_20101202_en.pdf (accessed 26.10.13).

European Food Safety Authority, 2013. Scientific opinion on the public health risks related to mechanically separated meat (MSM) derived from poultry and swine. EFSA Journal 11 (3), 78 pp.

Field, R.A., 1988. Mechanically separated meat, poultry and fish. In: Pearson, A.M., Dutson, T.R. (Eds.), Edible Meat By-Products, Advances in Meat Research 5. London: Elsevier Applied Science, pp. 83–126.

Field, R.A., 1999. Bone marrow measurements for mechanically recovered products from machines that press bones. Meat Science 51, 205–214.

Froning, G.W., 1981. Mechanical deboning of poultry and fish. Advances in Food Research 27, 109–147.
Froning, G.W., McKee, S.R., 2010. Mechanical separation of poultry meat and its use in products. In: Owens, C.M., Alvardo, C.Z., Sams, A.R. (Eds.), Poultry Meat Processing, pp. 295–309.
Greene, J.L., 2012. Lean finely textured beef: The 'pink slime' controversy. Congressional research service 7–5700. Available at: http://www.fas.org/sgp/crs/misc/R42473.pdf (accessed 26.10.13).
Lee, C.M., 1984. Surimi process technology. Food Technology 38 (11), 69–80.
Meech, M.V., Kirk, R.S., 1986. Chemical characterisation of mechanically recovered meats. Journal of the Association of Public Analysts 24, 13–16.
Regenstein, J.M., Regenstein, C.E., 1991. Introduction to Fish Technology. New York: Van Nostrand Reinhold.
US Department of Agriculture, 1995. Poultry products produced by mechanical separation and products in which such poultry products are used. Federal Register 60, 55961–55983.
US Department of Agriculture, 1998. Meat produced by advanced meat/bone separation machinery and recovery systems. Federal Register 63, 17959–17966.

Relevant Websites

http://www.efsa.europa.eu
European Food Safety Authority (EFSA).
http://europa.eu
European Union (EU).
http://fsis.usda.gov
Food Safety and Inspection Service (FSIS) of the US Department of Agriculture.

MICROBIAL CONTAMINATION

Contents

Decontamination of Fresh Meat

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Glossary

Antimicrobial intervention The application of an antibacterial chemical or temperature treatment to beef surfaces in order to reduce the potential presence of enteric pathogens that may contaminate tissue surfaces during harvesting/processing.

Hot water carcass treatment Treatment of meat surfaces with hot water, typically 80–96 °C, to raise carcass surface temperatures to levels capable of destroying enteric foodborne pathogens.

Organic acid carcass treatment The treatment of meat surfaces with dilute organic acids (lactic, acetic, and citric) in an effort to dramatically reduce the surface pH and destroy enteric foodborne pathogens.

Steam pasteurization A process in which beef carcasses are introduced to a pressurized, closed chamber and sprayed with steam in order to raise the surface to a temperature sufficient to destroy enteric foodborne pathogens.

Steam vacuum A hand-held device consisting of a vacuum wand equipped with a steam nozzle that can deliver a stream of steam to the carcass surface while vacuuming the area.

Introduction

When present on fresh meat products, enteric foodborne pathogens such as Shiga toxin-producing *Escherichia coli* and *Salmonella* can present significant risks to human health. These pathogenic bacteria are considered high priorities for control in food safety programs implemented within the meat processing industry. To address the risk posed by these pathogens, significant research has been directed toward reducing presence on raw meat through the implementation of various decontamination procedures, also known in the industry as antimicrobial interventions.

Currently used methods are typically based on raising the temperature of the meat surface to levels that are lethal for enteric bacterial pathogens or through the application of chemical treatments. The application of more than one treatment is common, often a temperature treatment followed by organic acid spray, in a 'multiple hurdle' approach to reducing contamination.

Temperature-Based Treatments

The use of hot water or steam as a decontamination method has been investigated extensively, and treatment of carcass surfaces with water at temperatures greater than 74 °C are often used as sanitizing interventions. Temperatures used vary between applications and often depend on the temperature of the available hot water supply or the specific spray cabinet used. Bacterial destruction due to hot water treatments is based on a time–temperature relationship and it is recommended that actual bacterial reductions be validated using microbiological testing rather than relying strictly on temperature. The benefit of applying hot water against washing carcasses with warm water was reported in the scientific literature, producing an average reduction of different pathogens on fresh meat of 0.2 $\log_{10}$ cm^{-2} after washing with water at 40 °C and a reduction of 3.1 $\log_{10}$ cm^{-2} after applying water at 80 °C. In addition, several studies have demonstrated that washing carcasses with water at

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temperatures greater than 80 °C will not produce permanent discoloration on the carcass surface. In an early investigation on hot water decontamination, it was reported that beef carcasses treated with a steam and hot water spray (80–96 °C) for 2 min contained significantly lower bacterial numbers than untreated carcasses. A volume of 18.9 L of water was sprayed on each carcass; however, the actual temperature at the carcass surface during the treatment was not indicated. Hot water (80 °C) poured on beef and lamb samples for 10 s was reported to have caused destruction of more than 99% of *E. coli* and *Salmonella* inoculated at levels of 6.5 $\log_{10}$ cm^{-2}. Although the surface tissues of the beef and mutton were not permanently discolored by this treatment, discoloration was seen when water at 90 °C for 120 s was used. In a laboratory evaluation of a hot water cabinet, *E. coli* reductions of 3.0 $\log_{10}$ cm^{-2} were attained on artificially contaminated beef carcass sides treated with hot water that elevated the carcass surface temperature to 83.5 °C for 20 s. Early investigations also reported that lamb carcasses sprayed with hot water at temperatures above 80 °C caused significant decreases (>1.0 $\log_{10}$ cm^{-2}) in aerobic plate counts (APC). In other studies where hot water treatments were evaluated, reductions in coliform or *E. coli* counts of approximately 3.0 $\log_{10}$ cm^{-2} were reported.

Designing an appropriate method to deliver a hot water treatment to a meat surface is of paramount importance for obtaining effective reduction in bacterial populations. A successful treatment relies on the ability of the hot water to raise the temperature on the meat surface to a lethal temperature for a sufficient amount of time in order to cause bacterial destruction (several seconds, depending on the temperature). Further complicating is the design of hot water spray systems, the formation of water droplets provides increased surface area that can allow rapid cooling of water as the spray travels from the nozzle to the carcass surface, resulting in an insufficient temperature increase at the carcass surface. In an early investigation of the effectiveness of hot water treatments, hot beef carcass surface areas were sprayed with 95 °C water in an attempt to raise the carcass surface temperature to 82 °C for approximately 10 s. As a result of this treatment, the bacterial contamination on the carcass surface was reduced significantly. It was reported that problems in applying hot water included obtaining a water spray that would adequately raise the surface temperature of the carcass to a bactericidal level. The volume of the spray and the size of the water droplets were found to have a profound effect on the temperature of the water after leaving the spray nozzle and before contacting the carcass surface. Using a type of nozzle which addressed cooling of hot water due to small droplet size by producing an intact 'fan spray' of hot water, it was reported that hot water sprayed onto different hot carcass surface regions obtained average reductions of initial counts for *E. coli* O157:H7 and *Salmonella* serotype Typhimurium of 3.7 and 3.8 $\log_{10}$ cm^{-2}, respectively. Corresponding reductions for APC and counts of coliforms and thermotolerant coliforms were 2.9, 3.3, and 3.3 $\log_{10}$ cm^{-2}, respectively. In this study, the hot water spray was combined with a previous water wash at 35 °C, which significantly improved the visual quality of the carcass surfaces.

Another heat-based process that gained popularity among meat processors in the 1990s is steam pasteurization. Scientists at Kansas State University assembled an experimental pasteurization chamber for laboratory testing and reported reductions of *E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* by 3.4–3.7 log cycles on surfaces of freshly slaughtered beef carcass surfaces. However, in additional laboratory testing, steam pasteurization alone showed no greater reductions than other treatments such as knife trimming or steam vacuuming. In commercial testing of steam pasteurization in a beef processing plant, carcasses were subjected to a preliminary water wash, and then passed through air blowers to eliminate excessive humidity that would favor steam condensation. The carcasses were then passed through a steam chamber that was supplied by steam from a tank, followed by transfer to another section of the cabinet where cold water was applied. Applying this treatment, it was possible to reduce APCs on carcasses from initial counts of 2.1–2.2 $\log_{10}$ colony forming units (CFU) cm^{-2} to 0.6–0.8 $\log_{10}$ CFU cm^{-2}. Counts of *E. coli* were also reduced from original counts of 0.6–1.5 $\log_{10}$ CFU cm^{-2} to undetectable levels after 6 or 8 s of steam treatment.

In the 1990s, regulations requiring knife trimming of any visible fecal contamination from carcass surfaces resulted in what the industry perceived as excessive and substantial carcass weight loss. Steam vacuuming, a variation on total carcass treatment designed to provide cleaning of isolated surface areas by application of a vacuum supplemented by steam, was introduced as an alternative to knife trimming. The technology was reported to be effective in reducing contamination in some studies; however, others have questioned effectiveness. Nevertheless, steam vacuuming has been implemented in most beef processing plants in the United States.

Chemical Treatments

The application of organic acid rinses containing dilute solutions of acetic, lactic, or citric acids are among the most commonly studied and applied chemical decontamination treatment methods. The antimicrobial properties of organic acids have long been utilized by the food industry for food preservation, and the meat industry also has benefitted from the use of these antimicrobial compounds to decontaminate carcass surfaces. The antibacterial mechanism of organic acids has not been completely described; however, it is widely accepted that the undissociated molecule of the organic acid or ester is responsible for antimicrobial activity. Many weak acids in their undissociated form can penetrate the cell membrane and accumulate in the cytoplasm. If the intracellular pH is higher than the pK_a of the acid, the protonated acid will dissociate, releasing a proton and acidifying the cytoplasm of the microorganism. Some reports indicate that the antimicrobial effect of organic acids may also be due to undissociated molecules, a specific non-pH-related effect of protonated molecules or a simple decrease in pH. An obvious requirement for successful reduction of bacteria on meat surfaces using organic acids is the ability to contact the bacteria on the cell surfaces. If bacteria are hidden in small knife cuts or under tissue and the organic acid cannot contact the cell, the desired antibacterial effect is unlikely.

A concern related to spraying beef carcasses with organic acid is the reported resistance of *E. coli* O157:H7 to low pH environments. However, several studies have indicated that lactic or acetic acid sprays, when applied at 55 °C and at sufficient concentration, can effectively reduce levels of *Salmonella* and *E. coli* O157:H7.

Lactic acid is one of the most commonly used compounds in beef carcass decontamination, and its ability to reduce pathogens or other organisms of fecal origin on beef surfaces has been extensively studied. Most studies on carcass decontamination using lactic acid indicate that this acid exhibits strong antibacterial capacity. A continued antimicrobial effect has also been observed during storage of meat after spraying lactic or acetic acid solutions on hot carcass surfaces. Although the most common application of lactic acid is currently on hot carcass surfaces, it has also been reported that the treatment is effective, even though to a lesser degree, on chilled beef carcass surfaces. In addition, it has been found that APC and *E. coli* counts increased more rapidly on untreated steaks than on steaks treated with acetic or lactic acid.

Chlorine-containing sprays have been extensively investigated for effectiveness in decontaminating meat surfaces. Chlorine dioxide at concentrations of up to 20 ppm was found to be no more effective than water in reducing bacteria of fecal origin on beef carcass tissue. Likewise, no differences in bacterial reductions on meat were reported after spraying tap water or water with 200 ppm sodium hypochlorite. In contrast, other investigators found lower bacterial counts on beef carcasses treated with 200 ppm sodium hypochlorite compared with untreated carcasses. In addition, they reported some continued effect of chlorine during storage of the carcasses. In an investigation conducted at Iowa State University, after inoculating different pathogenic and nonpathogenic organisms onto lean and adipose beef tissue, investigators applied washes containing phosphate buffer, ethanol, sodium chloride, sodium hydroxide, and potassium hydroxide. Phosphate buffer, ethanol, and sodium chloride produced reductions by less than 1 log cycle, whereas sodium and potassium hydroxides effectively reduced the populations of the inoculated bacteria by as much as 4 log cycles.

Acidified sodium chlorite (ASC) solutions have also been evaluated for their ability to reduce the presence of *E. coli* O157:H7 and *S. typhimurium* on beef carcass surfaces. When phosphoric acid was used to acidify sodium chlorite, the resulting ASC solution reduced populations of both pathogens by 3.8–3.9 log cycles, whereas when ASC solutions were prepared by acidifying with citric acid, the reductions obtained ranged from 4.5 to 4.6 log cycles. ASC has been approved as a direct food additive for use in decontamination of poultry and red meats.

Several published studies have indicated that multiple treatments may be required during processing for reducing pathogen contamination, also known as a multiple hurdle treatment. Numerous carcass decontamination methods have been investigated alone and in combination for their potential efficacy in reducing pathogen numbers on meat; however, results and conclusions are varied and often contradictory. For example, one study reported that spraying beef carcasses with 2% lactic acid, steam vacuum, or trimming was ineffective and only steam or hot water treatments were shown to substantially reduce bacterial contamination. The same report indicated spraying carcasses with 200 ppm peroxyacetic acid was likely also ineffective but was difficult to determine due to subsequent steam treatment. Another investigation evaluated the effects of 0.02% peroxyacetic acid, acidified 0.16% sodium chlorite, 2% lactic acid, and 4% lactic acid on chilled beef surfaces and determined that peroxyacetic acid and ASC had negligible effect on coliforms or *E. coli* and were less effective than 4% lactic acid. It was concluded that inconsistent results in evaluating antimicrobial treatments may be attributed to different types of meat surfaces to which they are applied or may reflect the composition of the surface microflora as affected by prior antimicrobial treatments. A later investigation concluded that peroxyacetic acid concentrations up to 600 ppm were ineffective as an antimicrobial treatment applied to chilled inoculated beef carcass surfaces.

Although many options exist for decontamination of meat surfaces, none of the approved interventions is capable of completely eliminating the presence of pathogens. Currently, processors in the meat industry often utilize a variety of redundant intervention technologies in an attempt to reduce risk of foodborne pathogens but are still unable to guarantee complete elimination of bacterial contamination. Proper end user handling of meat products is required for assurance of safety. Research continues to investigate novel interventions in an attempt to assist meat processors minimize or eliminate pathogens such as *E. coli* O157:H7, other Shiga toxin-producing *E. coli*, and salmonellae, as well as unknown and emerging foodborne pathogens.

See also: Microbial Contamination: Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Safety of Meat: Hurdle Technology; Pathogenic *Escherichia coli*; *Salmonella* spp. Spoilage, Factors Affecting: Microbiological

Further Reading

Anderson, M.E., Marshall, R.T., Stringer, W.C., Naumann, H.D., 1977. Combined and individual effects of washing and sanitizing on bacterial counts of meat – a model system. Journal of Food Protection 40, 670–688.

Arthur, T.M., Bosilevac, J.M., Nou, X., *et al.*, 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, Enterobacteriaceae, and *Escherichia coli* O157 at various steps in commercial beef processing plants. Journal of Food Protection 67, 658–665.

Baird Parker, A.C., 1980. Organic acids. In: Silliker, J.H., Elliott, R.P., Baird-Parker, A.C., *et al.*, (Eds.), Microbial Ecology of Foods, vol. I. New York, NY: International Commission on Microbiological Specifications for Foods, Academic Press, pp. 126–135.

Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W., Acuff, G.R., 1998. Comparison of water wash, trimming, and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. Journal of Food Protection 61, 823–828.

Castillo, A., Lucia, L.M., Goodson, K.J., Savell, J.W., Acuff, G.R., 1999. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. Journal of Food Protection 62, 146–151.

Dorsa, W.J., 1997. New and established carcass decontamination procedures commonly used in the beef-processing industry. Journal of Food Protection 60, 1146–1151.

Dorsa, W.J., Cutter, C.N., Siragusa, G.R., 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*. Journal of Food Protection 60, 619–624.

Eklund, T., 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. Journal of Applied Bacteriology 54, 383–389.

Gill, C.O., Landers, C., 2003. Microbiological effects of carcass decontaminating treatments at four beef packing plants. Meat Science 65, 1005–1011.

Hardin, M.D., Acuff, G.R., Lucia, L.M., Oman, J.S., Savell, J.W., 1995. Comparison of methods for contamination removal from beef carcass surfaces. Journal of Food Protection 58, 368–374.

King, D.A., Lucia, L.M., Castillo, A., *et al*., 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella typhimurium* on beef carcass surfaces. Meat Science 69, 401–407.

Nutsch, A.L., Phebus, R.K., Riemann, M.J., *et al*., 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. Journal of Food Protection 60, 485–492.

Padhye, N.V., Doyle, M.P., 1992. *Escherichia coli* O157:H7: Epidemiology, pathogenesis, and methods for detection in foods. Journal of Food Protection 55, 555–565.

Phebus, R.K., Nutsch, A.L., Schafer, D.E., *et al*., 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. Journal of Food Protection 60, 476–484.

Woolthuis, C.H.J., Smulders, F.J.M., 1985. Microbial decontamination of calf carcasses by lactic acid sprays. Journal of Food Protection 48, 832–837.

Relevant Websites

http://www.meatami.com
American Meat Institute.

http://www.bifsco.org
Beef Industry Food Safety Council (BIFSCo).

http://www.bifsco.org
STEC CAP Grant: Controlling Shiga Toxin-Producing *E. coli* to Improve Beef Safety.

Decontamination of Processed Meat

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Glossary

Bacteriocins The proteinaceous substances produced by one kind of bacteria in order to inhibit the growth of a similar or closely related bacterial strain.
Bacteriophages (phages) The viruses that infect bacteria.
Chemical antimicrobials The chemical interventions that include organic acids and chemicals such as ozone.
Extrinsic factors The factors associated with the environment external to the food product.
High-Pressure Processing (HPP) The process of exposing ready-to-eat meat products, such as cold cuts and hams, to very high pressures (5500–9000 bars).
Hurdle approach It is the microbial control where the synergistic effects of the combined treatments is more effective than either treatment used alone.
Intrinsic factors The inherent properties associated with a food product.
Pasteurization The process of heating a food to a specific temperature for a predefined time and then immediately cooling it to slow spoilage. For intervention technologies. pasteurization processes are often designed to reduce the number of microbial pathogens.
Probiotic bacteria These are bacteria (generally lactic acid bacteria), thought to be beneficial to the host.

Introduction

Ready-to-eat (RTE) meat and poultry products that are intended to be consumed without additional preparation by a food establishment or the consumer encompasses a wide variety of products and processes from fully cooked, not shelf stable, or dried to more complex meat processes such as fermentation and dry curing. The types and levels of microorganisms that develop on or in a meat product are influenced by the intrinsic and extrinsic factors of the ingredients, the product and production environment, as well as by the specific growth requirements of the organism. The safety and shelf life of many processed meat products often depend on a combination of several factors that may actually become hurdles that limit microbial growth. All of these variables, the characteristics of the organism(s) of concern, as well as the extrinsic and intrinsic factors that affect their growth, death, and survival must be taken into consideration when developing a product, establishing product safety and shelf life, or when validating a production process or decontamination technology.

Important controls for products in this category include raw materials of good microbiological quality, validated lethality processes designed to eliminate the organism of concern, and rapid and controlled chilling to prevent the recontamination and growth of microorganisms surviving the cook process. In addition, there must be complete physical separation of raw and RTE products and ingredients and proper hygienic control of facilities, equipment, and employees in order to reduce cross-contamination and maintain product shelf life and safety. The application of postlethality processes such as postpasteurization or high-pressure processing (HPP), and the use of additives such as sodium or potassium lactate and sodium diacetate, provide an additional level of protection against the growth of foodborne pathogens throughout the shelf life of the product.

Although numerous interventions and additives are available that preclude microbial growth, thereby enhancing the safety and extending the shelf life of processed meat products; not all are appropriate for all types of products. This may be due to the negative effects various antimicrobial processes and ingredients have on the organoleptic properties of the product and/or the prohibitive costs involved in the investment and implementation of the intervention which may be particularly challenging for small processors. The number and types of commercially available thermal, nonthermal, antimicrobial chemical, biological, and other novel treatments that may be, and are, applied to foods and which are designed to inhibit, reduce, or inactivate microorganisms has increased considerably over the past several years. The driving force behind this proliferation and development of new and novel interventions for RTE meat and poultry products has primarily been the result of both proactive and reactive responses to outbreaks of foodborne illness, recalls, and regulatory actions associated with *Listeria monocytogenes*. Food safety concerns and public health implications related to *Listeria* stem primarily from contamination of RTE meat and poultry products that receive no additional cooking by the food preparer or the consumer.

There are a number of factors that may contribute to the contamination of processed meat and poultry products with pathogens such as *Listeria*. Pathogens may be present in raw materials and product ingredients and survive the lethality process. Pathogenic microorganisms may also be present in ingredients applied postlethality, such as glazes and spices. However, contamination of RTE meat and poultry products with pathogens, such as *L. monocytogenes*, occurs primarily during postprocessing steps such as slicing, dicing, packaging, and repackaging. Therefore, in order to maintain the safety of these products during distribution and storage, and in addition to adequate temperature control and effective cleaning and sanitation, any of a number of antimicrobial treatments can be applied during the postprocessing stage. Although it is almost impossible to completely eliminate *L. monocytogenes* from raw meat, most measures to control *L. monocytogenes* in RTE meat products involve continual management and a multiple hurdle approach with measures targeting several

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areas in the process. This includes, for the product, reducing levels of the organism in raw meat and nonmeat ingredients, avoiding cross-contamination of raw products with RTE-processed products, and preventing growth of the organism in the product itself. In the environment, limiting product contamination and proper sanitation including employee hygiene; preventing establishment of the organism in coolers, cold rooms, air-handling units, and equipment; and conveying systems of postlethality areas (RTE areas) of a processing plant or in a food preparation kitchen may reduce the risk of product contamination with this organism.

Some countries, such as the USA, have a zero tolerance policy in place for *L. monocytogenes* in RTE meat products. In 2003, the US Department of Agriculture's Food Safety and Inspection Service (FSIS) published requirements for the control of *L. monocytogenes* in postlethality-exposed RTE products. Although the intent of this article is to provide options (Alternative 1, Alternative 2, and Alternative 3) to meat processors for controlling *L. monocytogenes* in their meat and poultry products, it also provides incentives for processors of RTE meat and poultry products to use postlethality treatments such as antimicrobial ingredients in formulation and other intervention technologies in order to reduce or eliminate the presence of and/or suppress or limit the growth of *L. monocytogenes* on these products. These control measures can include a myriad of postlethality treatments (e.g., radiant heating, steam pasteurization, and high hydrostatic pressure), and antimicrobial agents (e.g., organic acids and/or their salts) used singly or in combination.

Thermal Technologies

In-Package and During Packaging Thermal Pasteurization

As previously discussed, contamination of processed meat and poultry products such as frankfurters, hams, deli meats, and sausages often occurs following a lethality process such as cooking, drying, or fermentation, and before packaging. This contamination is typically limited to surface contamination and is often due to exposure with the environment and associated handling or contaminated equipment and surfaces. Postpackaging heat treatments have long been used for whole muscle products that are unavoidably handled after initial heat processing in order to increase shelf life and safety of these products. Pasteurization of the product surface is achieved by exposing the surface of the product to hot water or steam for a specified amount of time necessary to eliminate the target organism. The original postpasteurization systems designed for whole muscle products were applied to products packaged in their final packaging at temperatures of 70–96 °C (~160–205 °F) for dwell times of up to 20 min and continue to be used today for larger products such as turkey breasts, hams, and roast beef. Although the technology has success for larger diameter products and singly packaged small diameter meats, this approach has limited value for smaller diameter products such as sausages or frankfurters that are often packaged with multiple pieces per package in a single or double layer. For the inactivating heat to reach all surfaces for all pieces in a package, these products required extended dwell times of exposure to elevated temperatures that often resulted in cooking the product in the package. This led to the development of an application method that involves applying a burst of steam or hot water to product just before packaging in order to eliminate pathogens such as *L. monocytogenes* from the surface. The integration of surface pasteurization into a commercial vacuum packaging system often minimizes any negative effect of heat on food quality by reducing treatment (exposure) time from minutes to seconds and also reduces the risk of product recontamination with pathogens by applying the intervention immediately before sealing the package at the very last step in packaging before the end user. There are currently no regulations or special labeling requirements for surface pasteurization of cooked meat products. The process needs to be established and validated for each product, based on the desired level of destruction of the target organisms at the product surface and based on maintaining desirable quality characteristics, particularly color, purge (yield), and flavor. Product shape is an important consideration as the smoother the surface, the more efficient the pasteurization process. Crevices or cuts in product, net marks, and/or wrinkles may be more impervious to heat treatments. Spacing of product, and avoiding product overlap, during the process, whether hanging in a batch house or continuous process or loaded on a carrier designed to move product through a pasteurization system, must allow for all sides (surfaces) of the product to be treated evenly. Additional factors which complicate this process include the package configuration and packaging materials that will need to withstand the extended dwell times at higher temperatures during pasteurization and the rechilling required for the longer postpasteurization treatments.

Infrared Heating

The infrared pasteurization process is an effective means of reducing contamination from meat surfaces immediately before packaging. Products are exposed to high temperatures as they pass through a tunnel of heated coils on a stainless steel conveyor. The process is currently used in commercial applications for processed RTE meat products such as hams, corned beef, and roast beef. The process may be applied, for instance, to products that are removed from their packaging following a lethality step for portioning, sectioning, and repackaging in order to pasteurize product surfaces that have been exposed to incidental contamination from equipment and handling. As with previously mentioned heat treatments, product characteristics such as the size and shape of the product and uniformity of the surface need to be taken into consideration as the heat generated by this process does not penetrate into wrinkles in product ends or cuts or crevices on product surfaces.

Nonthermal Technologies

Irradiation

Nonthermal technologies such as irradiation may inactivate pathogens by destroying or damaging their cell membranes

without producing a significant negative effect on product quality. Commercially available irradiation technologies, such as electron beam, gamma, and X-ray technologies, are well established as effective microbial interventions and a substantial amount of research has been performed on meat and poultry products in particular to demonstrate the reduction of pathogens on these products. Additional research has shown that certain common food additives, such as a combination of acetic acid salts and lactic acid used in processed meat formulations, make pathogens such as *Listeria* more sensitive to radiation, thus allowing a significant reduction in the dose of radiation needed to inactivate the pathogen and hence maintaining product quality attributes. Several limiting factors in the use and application of irradiation processing technologies have included widespread consumer acceptance of the technology and regulatory approval for the use of irradiation on RTE products. Irradiation was approved by the U.S. Food and Drug Administration (FDA) in July 1985. Since then, the USDA's FSIS has issued rules to allow the use of irradiation to only treat refrigerated or frozen raw meat and meat products to control parasites and pathogens. In 1999, the FDA was petitioned by an industry coalition to allow the use of irradiation to treat RTE meats, including hot dogs, deli ham, and turkey and bologna, in order to control *L. monocytogenes*. However, and well over a decade later, the FDA is still reviewing the petition.

Chemical Antimicrobials

The list of chemical interventions that have been developed to be applied to meat and poultry products or included as ingredients in product formulations is rather extensive and continues to grow with increasing demand. Traditional and commonly used antimicrobials applied to RTE meat and poultry products include: organic acids (lactic, acetic, and citric) and chemicals such as ozone, electrolyzed water, acidified calcium sulfate, potassium lactate, sodium diacetate (actually a mixture of acetic acid and sodium diacetate), sodium citrate, acetate, propionic acid, trisodium phosphate, and various salt compounds; naturally occurring spices and oils; and a variety of antioxidant compounds that exhibit antimicrobial properties. Most often these chemical interventions are used in combination or as a blend such as a blend of sodium or potassium lactate and sodium diacetate, which has been used extensively in product formulations such as sausages, hams, deli meats, and frankfurters in order to limit the growth of *Listeria* throughout product shelf life. The inclusion of lactate and diacetate as ingredients in a product formulation may prevent the outgrowth of the pathogen during extended refrigerated storage, and the addition of a surface treatment with an antimicrobial such as lauric arginate will provide an initial kill of the pathogen. However, not all of these antimicrobials are appropriate for all processed meat products. Processors using a chemical antimicrobial in their product must consider the impact these different antimicrobials have on the quality attributes (color, flavor, yield, etc.) of their products. The application method for the antimicrobial must also be taken into consideration in order to achieve the maximum lethality from the technology. Some chemical antimicrobials are used in product formulation, whereas others are applied as a spray or a dip to the surface of the product just before packaging or as a surface treatment sprayed into the package during packaging.

Clean Label Alternatives

Consumers are becoming more aware of product labels and are demanding products that are preservative free, have minimal processing, and are avoiding products containing ingredients that the average consumer does not recognize. Therefore, many food manufacturers are revisiting product formulations and cleaning up product labels by replacing ingredients that have a chemical-sounding name or any terms that implies that the product contains any artificial ingredients including preservatives. Any chemical-sounding name or any ingredient or process that does not sound natural and wholesome is generally not considered a clean ingredient. This presents a challenge to meat processors and product developers to use the latest available technology in order to ensure the safety of their products while maintaining a clean label at a reasonable cost. The newer, label-friendly antimicrobials formulated to meet these demands consist mainly of a blend of organic acid salts (primarily lactate) and sugars. The sugar source, which may be sucrose originating from cane or beet sugar or dextrose originating from corn, is fermented to organic acids, primarily lactic acid, by microorganisms commonly used in the food industry for the production of cheese or probiotics. The technology provides clean label solutions and may carry a simple ingredient statement including cultured (cane, beet, or corn) sugar and vinegar.

Probiotic Bacteria and Bacteriocins

Probiotic bacteria are bacteria, generally lactic acid bacteria, thought to be beneficial to the host. Bacteriocins are proteinaceous substances produced by one kind of bacteria to inhibit the growth of similar or closely related bacterial strain(s). The addition of bacteriocins, such as nisin and pediocin, and probiotic bacteria, such as lactic acid bacteria, has been evaluated as an alternative to chemical preservatives in order to inhibit the growth of *L. monocytogenes*. Although there are many natural antimicrobials, most do not have sufficient antimicrobial activity to be considered for commercial development or for inclusion in product formulations. Typical applications of bacteriocins for use in meat processes and products include the direct addition of purified bacteriocins to food products, the inoculation of a meat product with lactic acid bacteria that will produce bacteriocin in the product itself, such as a starter culture during fermentation, and the use of an ingredient in food processing that has been previously fermented with a bacteriocin-producing bacteria. The consideration and use of certain bacteriocins in commercial meat processing applications is in part due to the relative heat resistance of these substances as thermal processing is used extensively in meat processing. Both bacteriocins and probiotic bacteria are also evaluated on the basis of their scope of activity against a target organism such as *L. monocytogenes*, their regulatory acceptance, status as a natural ingredient, ease of

use, and cost. Antimicrobial bacteriocins and probiotic bacteria are among the several hurdle technologies and methods that have proven to be effective when used either separately or in combination with other technologies such as active packaging.

Bacteriophage

Bacteriophages, also known as phages, are viruses that infect bacteria. They are ubiquitous organisms and can be found in every living ecosystem including soil, water, and in the intestines of animals. Phages are highly specific for bacteria and therefore cannot infect eukaryotic cells such as those of humans/animals and plants. In addition, phages are very precise in their target such that one phage can only infect a subgroup of strains within the same bacterial species, without affecting strains of other bacterial species. Bacteriophages destroy their host cell by disrupting host cell's metabolic activity, leading to disruption of the cell membrane and eventual cell death. In 2006, the US FDA approved the use of a bacteriophage-based preparation, composed of six different bacteriophages specific against *L. monocytogenes*, as a food additive for use in RTE meat and poultry products. However, due to an unfavorable reception by consumers willing to have 'viruses' put onto their food, phage technology has yet to gain widespread acceptance or use.

Plant Extracts

Plant extracts have received a considerable amount of attention as they represent a natural alternative to many synthetic and chemical antimicrobials in use today. In addition to antimicrobial, specifically listericidal, properties, many extracts also have additional antioxidant properties that are attractive to consumers. Some of the extracts include those found in plums, prunes, grape seed, and in the essential oils of herbs and spices such as rosemary, basil, anise, and oregano. Although plant extracts have had some success when incorporated into packaging films, they are not in widespread use in the meat industry; however, they do offer an additional step as part of the company's multihurdle approach to maintaining product safety.

Active Packaging

A significant amount of research and development has been performed by the food industry, government, and academia in order to discover new and novel methods to control and/or eliminate microbial pathogens, specifically *L. monocytogenes*, from processed meat and poultry products. One of these novel technologies involves the incorporation of antimicrobials into packaging materials, also known as 'active packaging.' The concept is based on the slow release of the antimicrobial from the packaging onto the product to inhibit microorganisms on the surface of food during storage. For the technology, and the antimicrobial, to be effective, there must be substantial and complete contact between the packaging material and food product; therefore, the potential application is more appropriate for vacuum or skin-packaged processed meat products. Additionally, there must be a continual slow release of the antimicrobial throughout the shelf life of the product. Some of the antimicrobial compounds used in this 'active packaging' technology include organic acids, plant oils, and bacteriocins such as pediocin and nisin.

High-Pressure Processing

As the food industry strives to meet consumer demand for more natural and minimally processed RTE foods, the demand increases for innovative nonthermal intervention technologies. Additionally, food manufacturers want to maintain product quality attributes and extend the shelf life of their products while ensuring product safety. The application of heat to many products such as processed meat and poultry products not only reduces or eliminates bacteria but also often adversely affects many of the organoleptic characteristics of the food. Several alternative processing technologies are available to processors, including HPP, pulsed electric fields, radio frequency electric fields, ultraviolet light, and irradiation, which preserve the quality attributes of product while reducing levels of foodborne pathogens. Several of these technologies may be combined, or used in concert with minimal heat treatments, to achieve a greater reduction in levels of microorganisms. Although most of these technologies have undergone extensive research, they have not had widespread application to meat products and may be better suited for decontamination of surfaces or water used in processed meat processes. One nonthermal process that has gained widespread use, as irradiation of processed meats has not yet been approved in the USA, is HPP. Exposing RTE meat products, such as cold cuts and hams, to very high pressures such as 5500–9000 bar (~80 000–130 000 lb per square inch (psi)), for a few minutes or less, inactivates foodborne pathogens by interrupting their cellular function without the need for heat irradiation or chemicals. HPP will kill most vegetative microorganisms that grow in foods under normal storage conditions; however, it is not effective in killing microbial spores. The process also extends product shelf life by inactivating spoilage organisms and when appropriately used, HPP does not alter the texture, appearance, or flavor of foods. HPP is of particular interest for foods where thermal pasteurization is not an option due to flavor, excessive purge, texture, or color changes. Because products are processed in the final consumer package, the potential for recontamination is eliminated. When HPP first emerged in the 1990s, as a food pasteurization alternative to heat or irradiation, it was not as available to many processors as were other intervention methods such as chemical antimicrobials, due to the prohibitive costs of investing in the equipment and the lack of availability to the technology. However, as the process becomes more widely available through commercial HPP processors, it has also become more affordable for food processors. In addition, HPP has been a very promising technology for RTE meats as there are few barriers to approval by regulatory authorities, limited trade barriers, and no special labeling requirements. A major

challenge for implementation of HPP remains in keeping up with production throughput.

Validation

As with all validations, the objective of the process must be kept in mind during validation of an antimicrobial treatment. For example, is the postlethality treatment sufficient to eliminate the levels of *L. monocytogenes* contamination more likely to occur on the product? Another and equally important consideration for a company intending to use an antimicrobial ingredient or process is to determine the current regulatory approvals as written for their particular product for the country they are producing in or exporting to. In the USA, antimicrobials and approval for use in certain meat and poultry products and associated labeling requirements are listed in the USDA FSIS Directive 7120.1. These approvals vary from one product to another and a particular antimicrobial approved for application to beef bologna as a spray at a certain level (ppm) and/or for a specified amount of time may not be approved for use as a dip for the same product and may not be approved for any other processed meat products. These approvals are based on the data and documentation submitted by a company intending the use the product and/or by the manufacturer of the particular ingredient or process. Therefore, it is recommended that one of the first steps taken in the evaluation of an antimicrobial is a search of the current regulatory approvals related to that particular ingredient and/or process. During the validation and implementation of an antimicrobial intervention, ingredient, system, or process, all pertinent equipment and product characteristics should be considered. Thermal processes such as hot water or steam pasteurization will require equipment qualification and the development of a temperature profile by mapping the process in order to better understand the process and the impact of the product on the process and equipment performance. Chemical and biological antimicrobials may require a challenge study to determine their effectiveness in different products with varying product characteristics (i.e., pH, water activity, salt, meat block, etc.).

A combination of more than one intervention treatment may be used in a hurdle approach to microbial control where the synergistic effects of the combined treatments is more effective than either treatment used alone. Some of these interventions are applied directly to the product itself, some during packaging or to in-package product depending on the regulatory approvals, labeling requirements, and the overall desired effect on product and the target microorganism(s).

The primary objectives of any antimicrobial product or process is to maintain safety of the food supply and to determine the efficacy and suitability of the intervention process whether it be a thermal technology (i.e., postpasteurization or radiant heat), a nonthermal technology (i.e., ionizing irradiation or HPP), biological, chemical, or a combination of these. Processors must determine the best fit for any and all of these processes as each intervention has associated costs and may impact product quality. Additional considerations for systems that require equipment installation include space requirements, effluent output, and maintenance and upkeep of equipment. The result in using any or all of these interventions will likely outweigh the costs and inconvenience as most of these technologies result in an added improvement in product shelf life in addition to the larger goal of protecting human health and ensuring product safety.

See also: Biopreservation. Irradiation. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat. Microbiological Safety of Meat: Hurdle Technology; *Listeria monocytogenes*. Modeling in Meat Science: Microbiology

Further Reading

Doyle, M.E., 1999. Literature Survey of the Various Techniques Used in *Listeria* Intervention. FRI Briefings. Madison: Food Research Institute, University of Wisconsin.

Tompkin, R.B., 2002. Control of *Listeria monocytogenes* in the Food-Processing Environment. Journal of Food Protection 65, 709–725.

US Department of Agriculture, 2003. Control of *Listeria monocytogenes* in postlethality exposed ready-to-eat products. Federal Register 68, 34224–34254.

US Department of Agriculture. 2006. Food additives permitted for direct addition to food for human consumption; bacteriophage preparation. Federal Register 71, 47729–47732.

Zhu, M., Du, M., Cordray, J., Dong, U.A., 2005. Control of *Listeria monocytogenes* Contamination in Ready-to-Eat Meat Products. Comprehensive Reviews in Food Science and Food Safety 4, 34–42.

Relevant Website

http://www.fsis.usda.gov/oppde/rdad/FSISDirectives/7120.1.pdf
USDA FSIS.

Microbial Contamination of Fresh Meat

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Glossary

Acid–adaptation responses The stress response that confers microorganisms with resistance to organic acid food preservatives.

Enteric pathogens Rod-shaped Gram-negative bacteria in intestines of humans and animals.

Enterobacteriaceae Family of rod-shaped Gram-negative bacteria, most of which occur normally or pathogenically in intestines of humans and other animals.

Immunoassay A biochemical test that measures the presence or concentration of a macromolecule in a solution through the use of an antibody or immunoglobulin.

Mesophiles Organisms that grow best in moderate temperature, neither too hot nor too cold, typically between 20 and 45 °C (68 and 113 °F).

Nucleic acid probe Nucleic acid that complements a specific RNA or DNA molecule or fragment; used for hybridization.

Polymerase chain reaction (PCR) A biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA.

Psychotrophes The cold-tolerant bacteria that have the ability to grow at low temperatures, but have optimal and maximal growth temperatures above 15 and 20 °C, respectively.

Psychrophiles The microorganisms that are capable of growth and reproduction in cold temperatures, ranging from −15 °C to +10 °C.

Total viable counts A quantitative idea about the presence of microorganisms such as bacteria, yeast, and mold in a sample.

Introduction

Meat commences as sterile muscle in a living animal. During transport, lairage, stunning, slaughter, and dressing, the now exposed muscle is showered by bacteria and other contaminants while it undergoes anaerobic glycolysis and becomes meat. The ever-changing surface of the meat presents a range of suitable environments for growth of various microorganisms.

Microorganisms on fresh meat may be pathogens, i.e., microorganisms causing food poisoning that are a high risk to human health. They include *Salmonella*, *Escherichia coli*, *Campylobacter jejuni-coli*, *Yersinia enterocolitica* (pork), *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus*, etc. Other microorganisms on fresh meat could be spoilage bacteria – these are microorganisms that cause off-odors and slime on meat surfaces; this group include Gram-negative rods (*Acinetobacter–Moraxella*, *Aeromonas*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, and Enterobacteriaceae), Gram-positive rods (*Corynebacterium*, Lactic acid bacteria, *Brochothrix thermosphacta*, *Bacillus*), Gram-positive cocci (micrococci, staphylococci, fecal streptococci), yeasts, and molds.

Sources of Microbial Contaminants in Fresh Meat

Microbial contaminants are more common than any other form of meat contaminants as meat-producing animals naturally harbor them, either externally or internally (intestinal or respiratory tracts). Microbial status of fresh meat is affected by animal rearing, transportation, slaughtering, cutting, and packaging, besides hygiene and processing conditions of the slaughter plant. The natural surface flora of meat animals usually are not important as the contaminating microorganisms from their intestinal or respiratory tracts. However, hides, hooves, and hair contain not only large numbers of microorganisms from soil, manure, feed, and water but also important kinds of spoilage organisms. Meat animals may contain *Micrococci*, *Staphylococci*, and *Streptococci* on the skin or from the respiratory tract; these organisms may find their way onto the carcass (such as by an aerosol or by handling) and consequently contaminate the meat. The feces and fecal-contaminated products of animals can contain many enteric organisms including *Salmonella*; people working in meat-processing plants also can act as vector of many food-borne pathogenic bacteria. Findings suggest that processing procedures may generally be of far greater importance for determining the microbiological condition of dressed carcasses than is the condition of the hide of the animals. Plant design and operations as well as speed of slaughter and dressing may also be major factors in transfer of contamination from external animal and other surfaces to carcasses and cuts.

Slaughtering

As a rule, carcass meat is sterile immediately after slaughtering. However, the preslaughter environment can alter the rate of contamination in subtle ways. The animal origin and feed can affect the amount of excreta on the hides during transport and this can be exacerbated during lairage. If there is an excessively stressful preslaughter environment, the meat's ultimate pH can be adversely affected and this alters the way various microorganisms grow resulting in unacceptable odors and flavors

at relatively low bacterial numbers. This is particularly unacceptable in vacuum packaging. In the case of pork, the various scalding and dehairing and flaming procedures can result in variable numbers of bacteria on the surface. Poultry are defeathered and washed using various decontamination procedures so the exterior of whole birds may be affected. Bacterial contamination of the muscle from visceral bacteria does not usually occur through migration, but in exceptional cases, bacteria may end up in the muscle through contamination of intestinal contents. If good manufacturing practice (GMP) is applied during slaughtering, there is 1 bacterium/10–100 g of meat present.

Dressing

During dressing (removal of the hide, appendages, and viscera from animal carcass), contamination takes place via skin or hide, knives, hands, clothing, water, and equipment. If GMP is applied, the total number of bacteria for beef, mutton, and pork range between 10^2 and $10^6/cm^2$ and the total number of Enterobacteriaceae range between 10 and $10^3/cm^2$.

Chilling

Quick chilling at low temperatures with high air speed reduces the total viable count (TVC) on the surface of the carcass. Under less rigorous conditions, the TVC increases, but the number of mesophiles increase faster than the number of psychrophiles and psychotrophes, and because pathogens have a mesophilic character, the risk of food poisoning increases. GMP for chilling requires fast chilling to below 3 °C.

Cutting

Cutting and boning enlarge the relative surface that causes an increase in the TVC/g, depending on the initial contamination of the carcass, the conditions of hygiene, and the temperature/time in which those actions occur. Good results are achieved at 10 °C if efficiently cleaned and disinfected equipment is used.

Detection of Microbial Contaminants

Techniques explained by American Public Health Association (APHA) and International Commission on Microbiological Specification for enumeration and isolation of bacteria are widely acceptable although there are several other techniques available. Methods such as immunological, chemical, biochemical, biophysical, nucleic acid probe, polymerase chain reaction (PCR), and biosensor-based techniques have been developed to monitor the incidence of pathogenic bacteria in meat. However, problems associated with such methods include the following:

- Difficulties in the recovery of bacterial species from meat, as some coextractive materials come at the time of enrichment in selective broth medium.
- Excessive time taken to get samples that can significantly extend duration of the isolation and detection procedure.
- Inefficiencies in extraction of the target pathogens from the food matrix.
- Poor separation from elements of the competitive micro flora.

All of these can lead to subsequent problems in the accurate detection and/or differentiation of target organisms. Thus, coextractive materials can interfere with DNA hybridization test in PCR assay and immunoassay. Furthermore, these methods require approval by any governmental organization or other agencies such as Codex Alimentarius Commission (CAC), Association of Analytical Communities, APHA, International Organization for Standardization (ISO), etc. Traditional cultural and serological methods are used, though time-consuming and labor-intensive.

Traditional and standardized analysis of food for presence of bacteria relies on the enrichment and isolation of presumptive colonies on solid media, using approved diagnostic artificial media. The ISO has elaborated several standards for the detection of important pathogenical bacteria by a traditional method. For example, *Salmonella* (ISO 6579), *L. monocytogenes* (ISO 10560), thermo-tolerant *Campylobacter* (ISO 10272), *E. coli* O157 (ISO 16654), and *Staphylococcus* spp. (ISO 6888).

Indicator Tests for Pathogen Contamination in Meat

Meat can be contaminated with a variety of pathogens and spoilage bacteria and it would be difficult to monitor all of these organisms in a scientific manner. Indicator organisms are therefore used and are groups of bacteria that indicate the possible presence of organisms of concern, and may point to the origins of microbial contamination. Generally, it can be assumed that the numbers of a pathogen are less than the numbers of the corresponding indicator organism. Also, a reduction in the number of indicator organism will produce a similar reduction in the number of any pathogen associated with it.

Total Viable Counts

Most of the bacteria on raw meat originate from the skin and the intestine of the animals. Some of the contamination is of fecal origin but it is supplemented by the normal flora of the skin (staphylococci, micrococci, pseudomonads, yeasts, and molds) as well as a variety of organisms from soil and water. Only a small proportion of bacteria present is able to grow once the meat has been chilled and factors such as temperature, surface dryness, and gaseous atmosphere influence how quickly these bacteria can multiply.

Under moist and aerobic conditions, the bacterial population increases quickly and is probably dominated by pseudomonads. Off-odors and slime on the meat surface are evident when pseudomonads reach 100–500 million per cm^2. Imminent spoilage of the meat can be anticipated if a total bacterial count approaches these numbers. However, in vacuum-packed meat, the packaging brings about changes in the bacterial flora, and the storage life depends more on the nature

of the flora that develops during storage rather than on the total number of bacteria present after processing.

Generic *E. coli*

Escherichia coli forms part of the rumen or the lower intestinal tract contents, therefore, *E. coli* presence on fresh meat is considered to be a specific indicator of fecal contamination during the slaughtering and dressing processes.

The growth and survival characteristics of *E. coli* are broadly comparable to many pathogenical Enterobacteriaceae species such as *Salmonella* and pathogenical *E. coli*. Therefore, increases in *E. coli* during chilling, storage, and distribution suggest that the meat has been subjected to conditions that would also allow growth of other pathogens.

Coliforms

Part of the Enterobacteriaceae family are coliforms, which include *E. coli*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Many of them are capable of surviving below 5 °C; this attribute makes the use of coliforms as indicators of pathogen contamination in chilled meat rather suspect, resulting in erroneous conclusions regarding pathogens. *Escherichia coli* cannot grow below 7 °C, so a high coliform count does not imply growth of fecal pathogens. Processing or unsatisfactory postprocess contamination might cause an increase in the number of coliforms in meat, but the history of the product must be examined closely before the exact source of contamination can be ascertained.

Microbiological Criteria

Microbiological criteria may be used to define the acceptability of a process, product, or food batch. The criteria could be the absence, presence, or number of microorganisms, and/or the quantity of their toxins/metabolites in samples.

Microbiological criteria may be used either

- by an individual establishment, to verify that their process control systems are working as intended to prevent contamination; or
- to set national baselines to allow benchmarking against the overall performance of all meat processors and to satisfy market access issues.

The CAC of the United Nations World Health Organization has established internationally accepted guidelines for the development of microbiological criteria. The guidelines state that microbiological criteria should only be established if it is practical and necessary to do so. Codex states that the following factors are relevant for assessing need and practicality:

- Evidence of actual or potential hazards to health;
- Effect of further processing on the likely microbiological status of the food and intended use of the product;
- Likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage, and use;
- The underlying health of the consumers concerned.

The American Meat Science Association convened a panel of leading microbiologists, statisticians, and other food safety experts from the USA, Canada, Australia, New Zealand, and UK to examine the role of microbiological testing in a beef food safety program. The panel was asked to document the science behind the sampling process and to present clear recommendations for the evaluation of sampling programs. The panel found that

- at no stage during a process will pathogen testing assure food safety;
- pathogens or other microorganisms at a low incidence cannot be used to assess process control;
- food-borne pathogens will not be detected consistently when they are not randomly distributed and/or occur at low incidence;
- testing for appropriate nonpathogenical organisms will allow validation and verification of process control systems designed to improve food safety;
- effective microbiological testing programs are based on sound food-safety objectives with definable microbiological performance criteria; and
- the main purpose of microbiological testing of foods is to validate and verify process control measures in the context of a properly implemented hazard analysis and critical control points system.

Processes to Reduce Microbial Contamination on Meat Carcasses and Cuts

- For the meat industry to implement interventions with the objective of improving the microbiological condition of carcasses at slaughter and consequently of resulting meat cuts, decontamination processes must be applied – these include animal washing and chemical dehairing, carcass knife-trimming, steam vacuuming and immersion, flooding, cascading, deluging, rinsing or spray-washing with water or chemical solutions, before and after evisceration (removal of viscera – internal organs, especially those in the abdominal cavity). The effectiveness of these treatments in reducing microbial contamination is affected by a number of factors including water pressure, temperature, chemicals used and their concentration, time of exposure (which depends on speed of slaughter and length of the application chamber), method of application, and time or stage of application during slaughter or carcass dressing. There are a number of concerns associated with the application of decontamination interventions.
- Depending on the spraying pressure used, application of spraying/rinsing treatments to carcasses may cause penetration of bacteria into the meat or their spreading and redistribution over the carcass. The problem may be solved by using decontamination interventions that may inactivate (hot water, steam, and chemical solutions) rather than remove contamination.
- The period of time before decontamination has an important effect on bacterial attachment; biofilm formation and potential protection of the microorganisms from

exposure to the decontamination treatment is another concern. Spray washing of carcasses before evisceration may owe its usefulness to the fact that it removes contamination very quickly after removal of the hide, while bacterial attachment is still minimal.

- The physiological stage of the bacterial cells or the development of resistance in microorganisms exposed to sublethal decontamination treatments (e.g., acids, hot water, or steam) may be of major concern.

The use of two, three, or more decontamination processes in sequence may yield synergistic or additive decontaminating effects. The higher the initial contamination level, the greater the decontamination effect of single or multiple sequential decontamination technologies. In multiple decontamination interventions, the sequence of processes is of great importance. In addition, increased water temperatures (50–55 °C) enhance the effect of acid solutions.

Carcass decontamination interventions are very useful because they may contribute to the production of carcasses with lower levels or inactive microbial contamination and to a reduction in the prevalence of enteric pathogens. However, further studies are required on possible additional consequences of decontamination treatments related to food safety. Studies have shown that exposure of pathogens to conditions similar to those occurring during decontamination may result in the development of acid–adaptation responses, increased resistance to the conditions of the gastrointestinal environment, and possibly increased pathogenesis. In addition, further investigation is required of the effect of decontamination treatments on the background microflora of meat that may act antagonistically to the growth of pathogenic bacteria during product storage.

See also: Biofilm Formation. Carcass Composition, Muscle Structure, and Contraction. Conversion of Muscle to Meat: Glycogen; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Cutting and Boning: Hot Boning of Meat. Equipment Cleaning. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat. Microbiological Analysis: DNA Methods; Indicator Organisms in Meat; Standard Methods. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium perfringens*; Emerging Pathogens; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; Prions; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Viruses; Yeasts and Molds; *Yersinia enterocolitica*. Modeling in Meat Science: Microbiology. Parasites Present in Meat and Viscera of Land Farmed Animals. Preslaughter Handling: Preslaughter Handling; Welfare of Animals. Risk Analysis and Quantitative Risk Management. Slaughter-Line Operation: Other Species; Poultry

Further Reading

Bachhil, V.N., Jaiswal, T.N., 1988. Occurrence of *Salmonella* in meats. Journal of Food Science and Technology 25, 310–312.

Bacon, R.T., Belk, K.E., Sofos, J.N., *et al*., 2000. Microbial populations of animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. Journal of Food Protection 63, 1080–1086.

Banerjee, R., Kapoor, K.N., Agarwal, R.K., Ghatak, S., 2001. Verotoxin producing *Escherichia coli* (VETO) in foods of animal origin. Journal of Food Science and Technology 38, 82–84.

Biswas, A.K., Kondaiah, N., Anjaneyulu, A.S.R., Mandal, P.K., 2011. Causes, concerns, consequences and control of microbial contaminants in meat – a review. International Journal of Meat Science 1, 27–35.

Brown, M.H., 2000. HACCP in primary processing: Red meat. In: Brown, M.H. (Ed.), HACCP in the Meat Industry. Cambridge: Woodhead Publishing, pp. 81–117.

Food Safety and Inspection Service, Microbiology Division, Microbiology Division, 1994. Nationwide Beef Microbiological Data Collection Program: Steers and Heifers. Washington, DC: USDA.

Food Safety and Inspection Service, 1996. Pathogen Reduction: Hazard Analysis and Critical Control Point System; Final Rule. Federal Register 61, 38805–38989.

Gill, C.O., 1998. Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: Davies, A., Board, R. (Eds.), The Microbiology of Meat and Poultry. London: Blackie Academic and Professional, pp. 118–157.

Grau, F.H., 1986. Microbial ecology of meat and poultry. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research: Vol. 2, Meat and Poultry Microbiology. Westport, CT: AVI, pp. 136.

Jay, J.M., 2000. Fresh meats and poultry. In: Modern Food Microbiology, fifth ed. Gaithersburg, MD: Aspen Publishers Inc, pp. 59–85.

Koutsoumanis, K., Sofos, J.N., 2004. Microbial contamination of carcasses and cuts. In: Jensen, W.K. (Ed.), Encyclopedia of Meat Sciences, 1st ed Amsterdam, The Netherlands: Elsevier Academic Press, pp. 727–737.

Nottingham, P.M., 1982. Microbiology of carcass meats. In: Brown, M.H. (Ed.), Meat Microbiology. Harlow: Applied Science, pp. 13–65.

Nychas, G.-J.E., Drosinos, E.H., 1999. Spoilage of meat. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology. London, UK: Academic Press Ltd, pp. 1253–1260.

Sofos, J.N., Cabedo, L., Zerby, H., Belk, K.E., Smith, G.C., 2000. Potential interactions between antioxidants and microbial quality. In: Faustman, C., Decker, E. (Eds.), Dietary Strategies for Improving Muscle-Based Food Products. New York: Wiley, pp. 427–453.

Sofos, J.N., Smith, G.C., 1998. Nonacid meat decontamination technologies: Model studies and commercial applications. International Journal of Food Microbiology 44, 171–189.

Sofos, J.N., 1994. Microbial growth and its control in meat, poultry and fish. In: Pearson, A.M., Dutson, T.R. (Eds.), Quality Attributes and Their Measurements in Meat, Poultry and Fish Products. Glasgow, UK: Blackie Academic and Professional, pp. 359–403.

Stanbridge, L.H., Davies, A.R., 1998. The microbiology of chill-stored meat. In: Davies, A., Board, R. (Eds.), The Microbiology of Meat and Poultry. London: Blackie Academic and Professional, pp. 174–212.

Microbial Contamination of Processed Meat

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Glossary

Aerobic It means requiring air (oxygen).
Anaerobic It means without air (oxygen).
Bacilli Taxonomic class of Gram-positive, rod-shape bacteria; can be obligate aerobes or facultative.
Bacteriocins Proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s).
Biocidal Chemical agent, such as a pesticide, that is capable of destroying living organisms.
Cocci Any bacterium that has a spherical shape.
Gram-positive Bacteria that do not retain crystal violet dye in the Gram staining protocol.
Microbes A microscopic organism.
Pathogenic microorganisms Microorganisms that can cause disease in other organisms or in humans, animals, and plants.
Virulence factors Molecules expressed and secreted by pathogens (bacteria, viruses, fungi, and protozoa) that enable them to start an infection.

Introduction

Processed meat could be defined as any meat preserved by smoking, curing, or salting, or with the addition of chemical preservatives; examples include bacon, salami, sausages, hot dogs or processed deli, or luncheon meats. These meat products maintain at least one-half of their original meat integrity after processing (i.e., the original meat qualities have reduced to one-half when a meat is processed into meat products); meat products are the most popular and one of the most consumed and highly savored food products worldwide.

Safe food supply is a challenge worldwide due to the presence of spoilage and pathogenic microorganisms, which has bewitched food processing and supply industries. 'Microbial contamination of food accounts for approximately 2500 cases of illness and approximately US$200 million in monetary loss in the United States annually.' In spite of modern innovations in harvest hygiene and food production techniques, the safety of commercially processed meat products is a major area of concern.

The absence of centralized harvest facilities and the small volume of retail business (i.e., the retail venture is usually a small market), prohibitive capital costs on mechanized infrastructure, and recurring expenditures have been the hurdles for hygienic processing of meat.

Reduction of initial bacterial load in meat is of prime importance in an attempt to improve the shelf life of products. Shelf life of meat products depends on several factors – the most important of which is the microbiological quality. Several studies have indicated that consumption of meat and meat products has been associated with incidence of outbreaks of foodborne illnesses. Foodborne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medicare and social costs. Foodborne disease and microbial spoilage of food results from the failure or inability to control microorganisms at one or more stages of the food chain from raw material to consumption of the final product. The implications of situations that result in food poisoning outbreaks or food spoilage can be severe for food producers, retailers, consumers, and regulatory authorities.

Microbial growth during storage is one of the main factors affecting the quality of meat products leading to contamination and spoilage and, hence, economic loss. Microbiological criteria should take into account any organisms that are likely to be present in the meat product. The levels of tolerance applied at the time of manufacture should be such that, even after allowing for predictable growth of these organisms, products should remain safe and wholesome till the end of shelf life, provided they are stored under the appropriate conditions.

The safety of foods of animal origin for human consumption has become an essential component of public health debates. Thus, microbiological ecology of meat products will mainly depend on the environment, kind of meat and raw material, equipment, handling practices, processing, packaging, and storage temperature.

Traditionally, control of microorganisms in food has been demonstrated by microbiological testing of samples at various stages of production, including the final product. The potential for growth and/or toxin production of residual microbial population in finished products depends on the type of organisms present and their ability to grow to a level of concern under the storage conditions applied during the product shelf life.

Sources of Microbial Contaminants in Meat Products

Meat products can be contaminated with microorganisms from meat handlers who carry pathogenic microorganisms during the processes of manufacturing, packing, and marketing. One of the major risks of food contamination originates from the working practices of food handlers and disease-causing microorganisms present in or on the food handler's body that are subsequently transported from the food handler to the food during the handling process. Personal hygiene is very important in food processing because a major source of contamination leading to food poisoning caused by *Staphylococcus aureus* is human. Poor personal hygiene practices, such as negligence to wash hands after visiting the bathroom may result in

up to 10^7 CFU (colony-forming units) ml^{-1} pathogens under the fingernails of food handlers. Spice, which has an important role in meat products, can be contaminated with bacteria, molds, and yeasts. Microbial load of spice depends on its variety, processing method, granule size, and moisture content. Minced meat and spice mix have been identified as the primary contamination sources in sausage manufacturing. The most probable reason for minced meat having high microbial load is the poor hygienic quality of raw meat. Microbial quality of raw meat and nonmeat ingredients affects the quality of final products. The hygienic condition and level of sophistication of the harvesting and processing equipment are also contributory sources of contamination in processed meat products. Studies have revealed that contamination of meat with microbes decreased with increased sophistication of harvest facilities. Also, the incidence of various foodborne pathogens in processed meat may be different in different parts of carcasses from which the processed meat is obtained; for instance, studies have shown that thigh muscles were highly prone to contamination compared to the breast muscle, irrespective of the processing conditions.

Some Microbial Contaminants of Processed Meat

Many groups of microorganisms potentially contribute to meat spoilage under appropriate conditions. This makes the microbial ecology of spoiled meat products very complex, and thus the spoilage is very difficult to prevent. Many studies have determined the presence of foodborne pathogens in meat products, such as *Listeria monocytogenes*, *S. aureus*, *Escherichia coli*, *Clostridium perfringens*, and *Samonella* spp.

Listeria monocytogenes is a Gram-positive, nonspore forming, highly mobile, rod-type, facultative anaerobic bacterium. It can grow in a wide range of temperature conditions and pH range. The organism can tolerate salt and nitrite, and is widely spread in the environment. The Centers for Disease Control and Prevention reported that a multistate outbreak between 1998 and 1999, which caused 101 cases and 21 deaths, was linked to the contamination by *L. monocytogenes* in frankfurters and deli meats. In 2000, a multistate outbreak involving deli turkey meat resulted in 29 cases, 4 deaths, and 3 miscarriages or stillbirths. The recall of 26 million pounds of meat products in 2002 indicates the economic consequences of contamination with *L. monocytogenes*. The U.S. Department of Agriculture (USDA) has thus established a 'zero tolerance' policy for *L. monocytogenes* in meat products. Therefore, it is important to prevent contamination in meat products.

Staphylococcus aureus is one of the most common agents in bacterial food poisoning outbreaks; it is also a major causative pathogen of clinical or subclinical mastitis of dairy domestic ruminants. Poultry, meat and egg products as well as milk and milk products have been reported as common foods that may cause staphylococcal food poisoning. *Staphylococcus aureus* is one of the commonest etiological agents of bacterial diseases worldwide due to its ability to produce a broad range of exotoxins and other virulence factors. Among them, the staphylococcal enterotoxins produced by some *S. aureus* strains are the main cause of most widespread foodborne intoxications. Staphylococcal food poisoning, together with toxic shock syndrome toxin-1 are responsible for toxic shock syndrome and staphylococcal scarlet fever. Studies have shown that augmentation of the additives, onion garlic meal, pepper, and E vitamin can decrease the microbial agents in meat products.

Other contamination bacteria in processed meat products include *Salmonella*, *Bacillus*, *Clostridium*, *Escherichia*, *Campylobacter*, *Acinetobacter*, *Aeromonas*, *Enterococcus*, *Moraxella*, and *Psychrobacter*. Some of the pathogenic bacteria, such as *Salmonella*, can be found in fermented meat products because this organism can tolerate or adapt to a wide variety of environmental stresses during meat fermentation. Food poisoning outbreaks caused by the consumption of fermented meat products contaminated with *Salmonella*, such as *Salmonella typhimurium* DT 124 and *S. typhimurium* PT 193, in salami have been reported. Although these pathogenic bacteria can be destroyed during cooking by heat treatment, some consumers prefer to consume raw or medium-cooked products.

Intervention against Microbial Contaminants of Processed Meat

Most microbial vegetative cells and toxic products are sensitive to heat treatment and can easily be inactivated by cooking. Therefore, postcooking recontamination during packaging is the main concern. Postpackage decontamination methods such as in-package thermal pasteurization and irradiation, and formulating meat products with antimicrobial additives are common approaches to control microbial contaminants in processed meat. Therefore, it is to the manufacturer's advantage to take measures for reducing contamination in food.

Irradiation

Ionizing radiation is a process in which products are exposed to radiant energy. Ionizing radiation includes gamma rays, electron beams, and X-rays. E-beam irradiation was reported to be more effective than gamma-ray irradiation in decreasing *Bacillus cereus* and *E. coli* O157:H7, but not for *L. monocytogenes*. In cooked pork chops and hams inoculated with *L. monocytogenes*, low-dose (0.75–0.90 kGy) irradiation reduced *L. monocytogenes* by >2 log. Irradiation treatments were significantly more lethal under aerobic packaging than in either vacuum or modified atmosphere packaging conditions. One concern about using modified atmosphere packaging in irradiated meat or poultry is that pathogens may grow and/or produce toxins because of low competing organisms. This is of even greater concern if spoilage is suppressed and does not provide the usual warning signals. Temperature effects must be carefully considered because reduced irradiation temperatures result in fewer adverse changes in the sensorial properties of meat and poultry products. However, low temperature conditions require greater radiation doses to inactivate the foodborne pathogens. The irradiation dose rate is another factor because, at low dose rates, microbial enzymes may have more time to repair damage to cells,

resulting in higher resistance. Generalization of the effects of irradiation can be misleading because the effectiveness of irradiation is affected by irradiation conditions and product compositions.

Food Preservatives

Chemical antimicrobials: The salt of lactate (SL) is frequently used as an antimicrobial in meat products due to its beneficial properties to meat quality when applied at appropriate concentrations. The addition of lactate to food products with neutral pH offers good prospects for prolongation of shelf life. In general, Gram-positive bacteria were more sensitive toward lactate than Gram-negative bacteria under optimum growth conditions (pH 6.5, 20 °C). The combined application of lactate and diacetate resulting in a synergistic inhibitory effect on the growth of pathogenic organisms in meat products has been reported to be very effective. A chemically synthesized short-chain peptide composed of 6 leucine and 8 lysine residues was shown to be biocidal against several foodborne organisms, including *L. monocytogenes* suspended in phosphate buffer at concentrations 5–50 μg ml^{-1}. Peptide concentrations of 100 μg ml^{-1} inhibited aerobic and anaerobic microorganisms present in meat exudate. Sodium hypochlorite, quaternary ammonium compound, and peroxyacetic acid used as sanitizers in meat processing plants were effective in eliminating *L. monocytogenes*.

Lactobacilli, probiotic bacteria, and bacteriocins: Biopreservation with various strains of lactic acid bacteria is a suitable alternative to chemical preservatives. The antimicrobial activity of a bacteriocin-producing *Lactobacillus plantarum* MCS strain against *L. monocytogenes* was observed in naturally and artificially contaminated salami, all showing strong antimicrobial effects. Thus, the application of *Lactobacillus*, *Pediococcus*, or *enterococci* bacteria in starter cultures may provide an additional hurdle against pathogens in fermented meat products. Lactic acid bacteria can also be used to inhibit the growth of bacterial contaminants in nonfermented products. Addition of *Lactobacillus sakei* Lb 706 prevented the growth of *L. monocytogenes* pasteurized minced meat and comminuted cured raw pork during the first few days after production. The *L. sakei* strain applied to cooked products at a concentration of 10^5–10^6 CFU g^{-1} immediately before slicing and vacuum packaging inhibited the growth of a cocktail of 3 rifampicin-resistant mutant *L. monocytogenes* strains both at 8 °C and 4 °C. Bacteriocins are ribosomally synthesized polypeptides produced by bacteria with an ability to kill or inhibit the growth of similar bacterial strain(s). Nisin is the most commercially important bacteriocin due to its relatively long history of safe use. It is currently recognized as a safe food preservative in approximately 50 countries.

Plant extracts: Plant extracts, due to their antioxidant and antimicrobial activities, have a broad spectrum of antimicrobial activity against many genera of bacteria and fungi. It has been reported that eugenol (clove extract) and pimento extract significantly inhibit the growth of *Aeromonas hydrophila* and *L. monocytogenes* inoculated in cooked beef slices. The numbers of *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* in treated raw ground beef declined when 1% pine bark extract was used as an antimicrobial on the ground beef after 9 days of refrigerated storage. These results suggest that natural plant extracts have a potential to be used with other preservation methods to reduce pathogens in processed meat.

In-Package Thermal Pasteurization

Effects of surface pasteurization temperatures on the survival of *L. monocytogenes* in low-fat turkey bologna showed that all the *L. monocytogenes* cells were destroyed after exposure to an 85 °C water bath for 10 s (> 6-log reduction), but viable cells were detected at up to 10 min of heating at 61 °C (< 6-log reduction). The effectiveness of in-package pasteurization in inactivating pathogenic organisms depended upon package size and the roughness of the product surface. The strains of bacteria also influence the effectiveness of thermal pasteurization. Cells grown at 42.8 °C before heat treatment were more thermotolerant than those grown at 37 °C. Heating at slowly increasing temperatures (0.7 °C min^{-1}) enhanced the thermotolerance of *L. monocytogenes*, and starvation in phosphate-buffered saline pH 7 for 6 h at 30 °C increased the heat resistance of the organism in broth.

High-Pressure Processing

High-pressure processing (HPP) is a novel, nonthermal method of food processing where food is subjected to elevated pressures with or without addition of heat. HPP can inactivate microorganisms without significant changes in texture, color, or nutritional value of food. HPP is not only a powerful tool to control pathogenic organisms but also is effective on spores and viruses. The decontamination efficacy of HPP also depends on many other factors, such as level of pressure, treatment temperature, exposure time, pH, water activity, and food composition. Many studies have shown that the effectiveness of HPP was slightly reduced at room temperature compared with refrigerated temperature. Also, the presence of oil reduced the effectiveness of high pressure in killing *L. monocytogenes* and cell morphology also had an effect on HPP, with bacilli being more sensitive to pressurization than cocci. When HPP was combined with antimicrobials, like bacteriocins, death rate increased because of sublethal injuries to living cells.

Combination Therapy Technology

The concept of combination therapy technology is based on the application of combined preservative factors to achieve microbiological safety and stability of foods. The most important hurdles used in food preservation are temperature, water activity, acidity, redox potential, antimicrobials, and competitive microorganisms. A synergistic effect could be achieved if the hurdles hit at the same time at different targets that disturb the homeostasis of the microorganisms present in foods. For meat products, the most frequently applied hurdles include thermal processing, vacuum packaging, refrigerated storage, and nitrite. However, these hurdles seem insufficient for *L. monocytogenes* due to its ubiquitous nature, ability to grow at refrigerated temperature and anaerobic condition, and resistance to salt and nitrite.

Microbial Contamination Intervention and Processed Meat Quality

Although interventions are very effective in controlling foodborne pathogens in meat, it generates free radicals that can cause lipid peroxidation and other chemical changes, which influence the quality of processed meat. Irradiated meat products can develop a characteristic odor described as 'bloody sweet' or 'barbecued corn-like.' Sensory analysis indicated that sulfur odor increased as irradiation dose increased. In addition to sulfur compounds, irradiation dramatically increased other volatiles in the meat products. Irradiation can induce a variety of color changes depending on irradiation dose, animal species, muscle type, pH, and reducing potential of meat and packaging type. The color change induced by irradiation is associated with carbon monoxide production during irradiation, which is correlated with increased redness of irradiated meat. They characterized the pigment that causes pinkness in irradiated turkey meat as carbon monoxide-myoglobin (CO-Mb). Irradiation is also reported to cause the oxidation of amino acids by generating high yields of side-chain hydroperoxides that relates to the oxidation of proteins and lipids.

Injection of SL to cooked, vacuum-packaged beef top rounds resulted in higher cooking yields and darker, redder color with less gray surface area. Flavor notes associated with fresh beef were also enhanced by the addition of SL, and flavor deterioration during storage was minimized. In Chinese-style sausage, the addition of 3% SL resulted in better quality regarding physicochemical characteristics. Research reports have shown that SL added to fresh pork sausage did not affect the internal lean color but resulted in more rapid surface discoloration, and that 2% potassium lactate had no effect on quality and sensory properties of low-fat pork sausage or lean color during refrigerated aerobic storage. Adding 2% SL to turkey breast rolls resulted in lower color values, but increased hardness, springiness, cohesiveness, chewiness, and resilience of turkey breast rolls. Including 3.3% commercial SL in frankfurter formulation did not affect textural profile of sausage. Addition of potassium sorbate up to 0.1% or sodium benzoate up to 0.1% in product formulation had no effects on the texture of products. These results suggest that the effect of SL on the quality of products depends on SL level and product types. A high concentration of sodium diacetate (SDA) has a negative effect on flavor of ham products. However, at lower levels (0.1%), SDA does not influence the quality of meat products. The addition of potassium benzoate greatly increased the content of benzene in the volatiles of irradiated RTE turkey ham and breast rolls, suggesting that benzoate salt is not a good antimicrobial to be used in products for irradiation. HPP causes minimal changes in 'fresh' characteristics of foods because it can be conducted at ambient or refrigerated temperatures. However, there is no doubt that HPP causes quality changes of meat. Some of the changes such as color and lipid oxidation are detrimental, whereas other changes such as pressure tenderization and pressure-assisted gelation are beneficial.

Conclusion

Microbial contamination can occur and can be reduced at different stages of processing of meat. Experiments have confirmed that coliform bacteria, fecal coliform bacteria, *E. coli*, total *Enterococcus* spp. and aerobic plate count are the most important indicator organisms that are most commonly used to ensure food safety. In some instances, enumeration of yeast performed on samples taken during processing in small businesses to verify good manufacturing practices is also good. This verification through monitoring was found to be an attractive alternative to the examination of end products.

To ensure the microbiological quality of final products, raw meat and ingredients must be inspected before entering the processing plant. Certified suppliers must be selected. Strong criteria for hygienic quality of raw meat must be set for suppliers. After receiving raw meat and ingredients, they must be stored in appropriate conditions until use. Effective cleaning and sanitation programs must be performed in processing plants. Personnel should follow the standard hygienic procedures and health conditions of personnel must be monitored regularly, and finally, appropriate time and temperature settings for meat processing should be selected.

See also: Biofilm Formation. Conversion of Muscle to Meat: Rigor Mortis, Cold, and Rigor Shortening. Environmental Contaminants. Foreign Bodies. Growth of Meat Animals: Muscle. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Disease Control and Specific Pathogen Free Pig Production; Exotic and other Species; Meat Production in Organic Farming; Poultry; Red Meat Animals. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat. Microbiological Analysis: DNA Methods; Indicator Organisms in Meat; Standard Methods. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium perfringens*; Emerging Pathogens; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; Prions; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter;* Viruses; Yeasts and Molds; *Yersinia enterocolitica*. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Modeling in Meat Science: Meat Quality; Microbiology; Refrigeration. Nutrition of Meat Animals: Pigs; Poultry; Ruminants. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production. Species of Meat Animals: Cattle; Finfish; Game and Exotic Animals; Pigs; Poultry; Sheep and Goats; Shellfish

Further Reading

Ahn, D.U., Lee, E.J., 2004. Mechanisms and prevention of off-odor production and color changes in irradiated meat. American Chemical Society Symposium Series 875, 43–76.

Ahn, J., Grun, I.U., Mustapha, A., 2004. Antimicrobial and antioxidant activities of natural extracts *in vitro* and in ground beef. Journal of Food Protection 67, 148–155.

Cegielska-Radziejewska, R., Pikul, J., 2004. Sodium lactate addition on the quality and shelf life of refrigerated sliced poultry sausage packaged in air or nitrogen atmosphere. Journal of Food Protection 67, 601–606.

Centers for Disease Control and Prevention (CDC), 2002. Multistate outbreak of listeriosis – United States. Morbidity and Mortality Weekly Report 51, 950–951.

Chen, H., Hoover, D.G., 2003. Bacteriocins and their food applications. Comprehensive Reviews in Food Science and Food Safety 2, 82–100.
European Food Safety Authority (EFSA), 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to 2-isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials. European Food Safety Authority Journal 293, 1–15.
Jacobsen, T., Budde, B.B., Koch, A.G., 2003. Application of *Leuconostoc carnosum* for bio-preservation of cooked meat products. Journal of Applied Microbiology 95, 242–249.
Jay, J.M., Loessner, M.J., Golden, D.A., 2005. Modern Food Microbiology, fifth ed. New York, NY: Springer Science and Business Media Inc., p. 790.
Lunden, J.M., Autio, T.J., Sjoberg, A.M., Korkeala, H.J., 2003. Persistent and non-persistent *Listeria monocytogenes* contamination in meat and poultry processing plants. Journal of Food Protection 66, 2062–2069.
Murphy, R.Y., Duncan, L.K., Driscoll, K.H., Marcy, J.A., Beard, B.L., 2003b. Thermal inactivation of *Listeria monocytogenes* on ready-to-eat turkey breast meat products during post-cook in-package pasteurization with hot water. Journal of Food Protection 66, 1618–1622.
Nam, K.C., Ahn, D.U., 2003b. Effects of irradiation on meat color. Food Science and Biotechnology 12, 198–205.
Ross, A.I., Griffiths, M.W., Mittal, G.S., Deeth, H.C., 2003. Combining non-thermal technologies to control foodborne microorganisms. International Journal of Food Microbiology 89, 125–138.
Stekelenburg, F.K., 2003. Enhanced inhibition of *Listeria monocytogenes* in Frankfurter sausage by the addition of potassium lactate and sodium diacetate mixtures. Food Microbiology 20, 133–137.
Zhu, M., Du, M., Cordray, J., Ahn, D.U., 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. Comprehensive Reviews in Food Science and Food Safety 4, 34–42.
Zhu, M.J., Mendonca, A., Ismail, H.A., *et al.*, 2005. Impact of antimicrobial ingredients and irradiation on the survival of *Listeria monocytogenes* and quality of ready-to-eat turkey ham. Poultry Science 84, 613–620.

Relevant Website

ftp://ftp.cordis.europa.eu/pub/fp7/kbbe/docs/traditional-foods.pdf
European Commission.

MICROBIOLOGICAL ANALYSIS

Contents

DNA Methods

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Glossary

Amplicon Target amplified deoxyribonucleic acid (DNA) sequence.
Amplification The process of making multiple copies of DNA.
Endonucleases Enzymes that cleave DNA at specific sequences.
Horizontal gene transfer The transfer of genes between microorganisms.
Indels Mutation where insertion or deletion of DNA bases occurs.
Melt curve The analysis of disassociation of DNA from double stranded to single stranded with the addition of heat.
Nucleotide substitutions A single base pair change in DNA between two populations.
Primer A small piece of DNA that acts as a starting point for DNA synthesis and extension.
Template The DNA from organisms to which primers bind in order that amplification can occur.
Thermal cycling The cycling of temperatures to allow DNA denaturation, primer annealing, and DNA extension.

Molecular Detection of Foodborne Pathogens

Traditional methods of detection of pathogenic bacteria on meat products constitute a challenge, as the bacteria are often present in low numbers, camouflaged by the food matrix and background microflora, and are frequently injured or stressed. Therefore, conventional culture methods include long preenrichment/enrichment steps, along with isolation, and are often followed by biochemical/serological/immunological confirmation. This multistep process is time and labor extensive, creating a high demand for development of rapid identification methods. Molecular detection plays a key role in decreasing the amount of time needed to obtain critical results by reducing enrichment time required due to corresponding increase in sensitivity. Importantly, the decision to accept or reject fresh meat products or to release ready-to-eat meat products can be made by screening enrichment for the presence or absence of virulence gene(s) carried by the pathogen of interest. Molecular methods provide increased sensitivity/specificity, higher throughput, minimal technician training, and less subjective test results. Development of novel molecular detection platforms helped to increase speed of detection; however, recent advances in full genome sequencing have really revolutionized molecular detection of pathogens in meat products by revealing target deoxyribonucleic acid (DNA) sequences unique to pathogens of interest.

Polymerase Chain Reaction-Based Detection

Conventional polymerase chain reaction-based detection methods

Conventional polymerase chain reaction (PCR) is a technique used to amplify millions of copies of a predetermined sequence of DNA by replicating the DNA replication process *in vitro*. The method uses short pieces of synthetic DNA (primers) that flank the DNA sequence of interest along with enzyme, cofactor, substrate, and buffers in order to amplify template DNA. To generate millions of copies of the targeted DNA, PCR relies on thermal cycling – cycles of repeated heating and cooling to allow double-stranded DNA to denature, primers to anneal to the template, and for enzymatic extension or 'replication' of the DNA to occur (Figure 1).

Agarose gel electrophoresis is most commonly used to resolve the DNA products. This process separates nucleic acids by an electrical current, which allows the movement of negatively charged particles through the agarose. Migration of DNA

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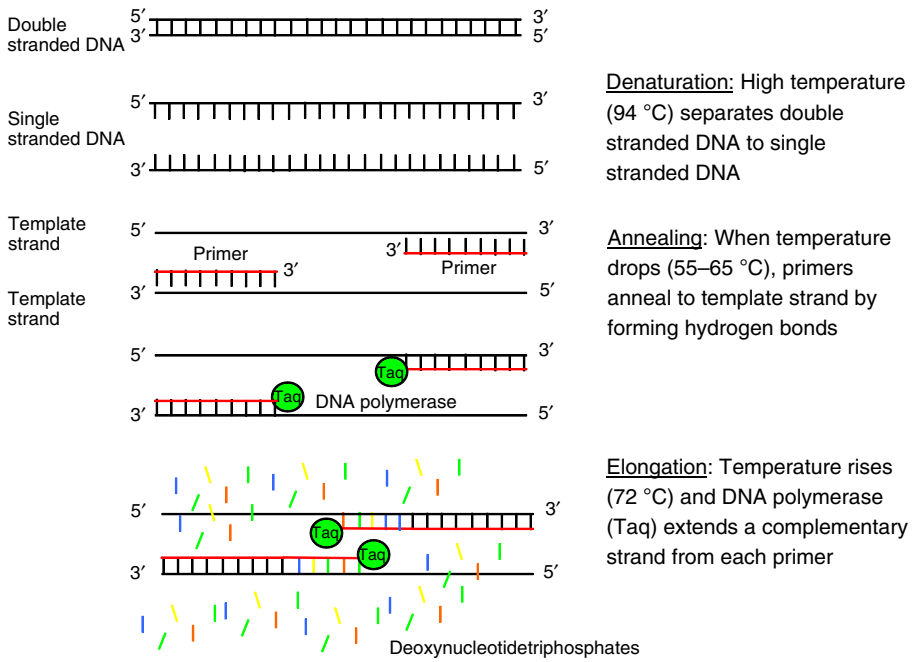

Figure 1 Polymerase chain reaction (PCR).

fragments through the agarose matrix is largely determined by their molecular mass, so that comparison of the distance of migration of a DNA fragment with that of size standards can verify that the amplified DNA fragment is of identical size as that of desired target amplicon. The DNA fragments can be visualized by preparing or staining the gel with fluorescent DNA intercalators, such as Ethidium Bromide or products such as SYBR® safe.

Multiplex polymerase chain reaction

Multiplex PCR is a variant of conventional PCR, permitting simultaneous amplification of more than one target of interest in a single PCR reaction by incorporating more than one pair of primers. Multiplex PCR is a rapid, convenient, and repeatable assay that may include analyses of mutations (e.g., nucleotide substitutions, indels, or horizontal gene transfer events) that have been accumulated in the genomes of different bacterial isolates. Typically, multiplex PCR is used for identification applications where simultaneous analysis of multiple markers is required. Conventional PCR may be used to simultaneously screen for the presence or absence of multiple pathogens (e.g., *Escherichia coli* O157:H7, non-O157 shiga toxin-producing *E. coli* (STEC) of regulatory significance, and *Salmonella*) from the same enrichment. Additionally, incorporation of multiple targets is a simple way to increase specificity. Multiplexing requires optimization of primer concentrations and annealing temperature to deter formation of primer dimers and to facilitate amplification of all target sequences. An internal amplification control should be included in conventional PCR assays for testing purposes to insure that the food matrix and enrichment media have not inhibited the PCR reaction. Although, universal primers that amplify 16 s rDNA (present in all bacteria) may seem like an obvious choice for an amplification control, the potential for the amplification control to outcompete targets in the presence of high levels of background microflora should be carefully considered.

Real-time polymerase chain reaction-based detection methods

Real-time PCR follows the same procedure as conventional PCR; however, the amplified DNA is quantified in real time as fluorescence accumulates after each amplification cycle, rather than an amplicon being determined at the end of the reaction as in conventional PCR. The amount of DNA is quantified either by the use of fluorescent dyes, such as SYBR® green, that intercalate with double-stranded DNA, or by using fluorescently labeled probes that hybridize the DNA fragment flanked by a set of PCR primers. The point at which the fluorescent signal is measured in order to calculate the initial template quantity can be either at the end of the reaction (endpoint semiquantitative PCR) or at the time when the amplification is still in progress (real-time quantitative PCR). For probe-based chemistries, eventually, enough accumulated fluorescence will be produced to drive the amplification signal above baseline. The intensity of fluorescence detected is proportional to the concentration of double-stranded DNA in the reaction and thus provides a measure of the quantity of newly synthesized product. If a standard curve is created from samples of known concentration, the initial number of target cells/DNA can be determined. Although SYBR® green-based assays are performed on real-time equipment, results cannot technically be obtained in real time and thus the assay should be referred to as endpoint semiquantitative PCR; this is because accumulation of SYBR® green is not quantified until the melt-curve analysis is run. A melt or dissociation curve is the gradual melting of the PCR products at the end of the PCR run. In short, it charts the change in fluorescence observed when double-stranded DNA with the intercalated dye dissociates or

'melts' into single-stranded DNA as the temperature of the reaction is raised, ultimately preventing results from being interpreted in real time as fluorescence accumulates.

Commercial systems that use real-time PCR-based chemistries are available, such as the DuPont Qualicon BAX® detection system, PALL GeneDisc® system, Applied Biosystems MicroSeq® detection kit, BIOFIRE R.A.P.I.D.® LT Food Security System, AES Chemunex ADIAFOOD PCR kits, Bio-Rad iQ-Check real-time PCR test kit, and the BIOTECON foodproof® kit, to name but a few. These systems are available for detection of meatborne pathogens such as *Campylobacter* (spp. *jejuni*, *coli*, and *lari*), *Listeria* (*monocytogenes* and spp.), *Salmonella* spp., and *E. coli* (*E. coli* O157:H7, the top six serogroups of non-O157 STEC, and Enterohemorrhagic *E. coli* (EHEC)). These systems consist of preparation on enriched samples, followed by automated amplification and detection utilizing fluorescent dyes, such as SYBR® green or fluorescently labeled probes (i.e., Scorpion probe or Taqman© probe) developed for targeted organisms.

Isothermal Detection

Novel discoveries in molecular biology of DNA synthesis *in vivo* revealed that DNA amplification can occur in isothermal conditions *in vitro*, negating the need for expensive thermocycling equipment and time required to perform two- or three-step cycling to amplify DNA. The use of DNA polymerase with high-strand displacement activity and other various accessory proteins allows replication at a constant temperature of 60–65 °C. Owing to the use of multiple primer sets to identify distinct regions on the target gene, there is also a direct increase in assay specificity.

The Neogen amplified nucleic single temperature reaction (ANSR)™ Isothermal Pathogen Detection System is a commercially available kit for *Salmonella* and *Listeria* spp testing that uses ANSR technology for *in vitro* DNA amplification. Nicks created in double-stranded DNA by specific endonucleases allows polymerization of target nucleic acid and thus amplification and detection of target sequences in real time using fluorescent molecular beacon probes. ANSR removes many of the limitations that antibody- and PCR-based technologies are associated with in terms of food matrix effects on detection. Additionally, results are available within as little as 10 min after a minimally enriched sample is loaded into the system.

The commercially available 3 M™ Isothermal Molecular Detection System© also utilizes isothermal DNA amplification but does so in combination with bioluminescence detection for detection of *E. coli* O157, *Salmonella* spp, and *L. monocytogenes* and other *Listeria* spp. Briefly, the assay works by producing pyrophosphate ions during targeted DNA amplification. These pyrophosphate ions and adenosine 5′ phosphosulfate are enzymatically converted into adenosine triphosphate (ATP) by ATP Sulfurylase. This ATP drives the luciferase-mediated conversion of luciferin to oxyluciferin, which generates visible light in amounts that are proportional to the amount of ATP. The light produced is subsequently quantified, indicating the presence of DNA from the target organism. This rapid system allows the simultaneous amplification and detection process to complete in 75 min postenrichment.

Ribonucleic Acid Detection

Roka Bioscience© pathogen detection assays uniquely target ribosomal ribonucleic acid (rRNA); however, the system is able to target mRNA or DNA as well. rRNA is an optimal target for molecular technology in meat- and foodborne pathogen detection applications mainly because targeting rRNA permits increased sensitivity due to the high cellular copy levels of rRNA versus low levels of DNA, ultimately reducing the time needed for an enrichment step. Currently, Roka Bioscience© has developed the Atlas™ System for both *Listeria* and *Salmonella*. A system for *E. coli* is estimated to be Association of Analytical Communities approved this year. Unlike other commercial kits, these assays combine the use of target capture, transcription-mediated amplification, and hybridization protection.

Target capture concentrates and purifies the nucleic acids before the amplification step, much like other immunomagnetic bead separation techniques. In short, oligonucleotides, which are bound to magnetic beads, capture the targeted nucleic acid. These magnetic beads, now containing bound target nucleic acids, are then drawn to the side of the tube using magnetism. Keeping the nucleic acids of interest bound and stable in the tube allows inhibitory particles to be washed away before amplification begins. Transcription-mediated amplification is an RNA transcription-mediated isothermal amplification system. Briefly, it uses two enzymes to drive the reaction: RNA polymerase and reverse transcriptase, enabling amplification of either DNA or RNA. Finally, the Atlas™ System also uses hybridization protection, where a specific oligonucleotide probe is used for final detection. The probe is labeled with an acridinium ester detector molecule that emits a chemiluminescent signal and hybridizes with target rRNA.

Microarray/Gene Chip

A microarray or gene chip is an assembly of DNA spots attached to a solid surface. DNA microarrays are often used to simultaneously measure the expression levels of large numbers of genes. Each DNA spot contains a specific DNA sequence (probes/oligos) that is a short section of a gene/DNA segment which is used to hyrbridize cDNA. cDNA is DNA that has been made from conversion of mRNA to DNA using reverse transcriptase. Probe–target hybridization is most often detected and quantified by chemiluminescence-, fluorophore-, or silver-labeled targets used to quantify target nucleic acid sequences.

VereFoodborne™ is a nucleic acid-based device that combines two molecular biological technologies: multiplex PCR and microarray hybridization. This allows the system to detect, differentiate, and identify multiple foodborne pathogens simultaneously in a single test. Pathogens that the system is able to simultaneously detect includes: *Vibrio* spp., *Staphylococcus aureus*, *Listeria* spp., *Bacillus* spp., *Clostridium perfringens*, *Campylobacter* spp., STECs (*E. coli* O157 and O104), *E. coli* spp., *Shigella* spp., *Salmonella* spp., *Cronobacter sakazakii*, and Norovirus genogroups I and II. The process of the

VereFoodborne™ chip includes DNA extraction directly from enrichment, culture, an isolated colony, or surface swab. PCR amplification is then carried out to amplify copies of target DNA that is unique to the specific foodborne pathogens of interest. This step is followed by hybridization of amplified product onto the microarray chip. After washing the chip, the array can then be analyzed. Analysis of the chip allows you to visualize what unique DNA segments from your unknown sample hybridized to the DNA spot on the chip.

Beacon's BrightSPOT™ is another system that uses microarray and DNA chip technology. It uniquely combines an extremely sensitive light sensor with very bright light-producing molecules from the deep oceans. As stated above in this section, most chip technologies that are based on light detection use chemiluminescence, such as firefly luciferase. This system utilizes *Gaussia* luciferase (originates from a deep-sea copepod) and produces a bright blue light approximately 10 000 times brighter than other luciferases. The chip is able to perform up to a hundred different diagnostic tests on a 10 µl biological sample. The unique design of the BrightSPOT™ System allows it to test samples without the need for time-consuming sample preparation or enrichment, giving you results in approximately 35 min.

DNA Subtyping of Foodborne Pathogens

Subtyping can be defined as the ability to characterize a group of isolates beyond the species or subspecies level. It has two broad categories: (1) phenotypic methods that differentiate isolates on the basis of enzymes, proteins, or toxins and other cellular metabolites that are expressed and (2) molecular methods that differentiate isolates on the basis of interrogation of nucleic acid sequences, which can be accomplished using a variety of approaches. PCR-, restriction band-, and DNA sequence-based methods are widely and routinely employed to molecularly detect or confirm the presence of a foodborne pathogen in a given sample and to further characterize isolates representing a pathogen beyond the species or subspecies level. Along with pathogen detection, the implementation of DNA-based subtyping methods has provided important insight into genetic diversity, evolution, and population structure of clinically important foodborne bacterial pathogens, including *Campylobacter*, STEC, *L. monocytogenes*, and *Salmonella*.

DNA sequence-based subtyping methods continue to be developed and have become increasingly more popular for improving the discriminatory power and epidemiological concordance to characterize bacterial foodborne pathogens beyond the species or subspecies level. Although macrorestriction band-based molecular methods continue to serve as the 'gold standard' for subtyping of foodborne pathogens, the enhanced availability of full genome sequence data in recent years has served as the impetus for the development of novel DNA sequence-based subtyping methods. Deciphering results of sequence data is much less subjective than interpreting patterns from band-based subtyping methods, and unlike band-based methods, results are easily transferable and comparable between laboratories.

Polymerase Chain Reaction-Based Subtyping

Molecular serotyping

The cell wall of pathogenic bacteria contains numerous components (e.g., proteins and lipopolysaccharides) and each of these different structures can be termed an antigen. The three types of antigens, which are of importance in prokaryotes, are the flagellar (H), somatic or polysaccharide (O), and capsular (K) antigens. Conventional serotyping, which is a phenotypic subtyping method, probes a pathogen's reaction with many different antisera to determine which antigens are present on the cell surface. Based on the combination of antigens that are detected, isolates can be further differentiated beyond the species level into serotypes.

Classifying a foodborne bacterial pathogen isolate into a serotype provides clinically relevant information and can facilitate surveillance of foodborne diseases and epidemiological investigations during an outbreak of foodborne disease. For example, serotyping is potentially useful for defining subtypes and clonal groups of *L. monocytogenes*. More than 95% of human listeriosis cases are caused by a few specific *L. monocytogenes* serotypes (i.e., 1/2a, 1/2b, 1/2c, and 4b), where serotype 4b is responsible for a majority of listeriosis epidemics. The development of a simple multiplex PCR-based serotyping method has provided one with a molecular assay for the identification and grouping of *L. monocytogenes* into four groups, each containing one of the most common human disease-associated serotypes (1/2a, 1/2b, 1/2c, and 4b). Molecular serotyping protocols have also been developed for *E. coli* and *Salmonella* that targets, genetically, the same antigens used in conventional serotyping. Serotyping is the basis of pathogenic *Salmonella* diagnostics. The flagellar, polysaccharide, and capsular antigens determine the strain's pathogenicity. The Luminex© xMAP® *Salmonella* Serotyping Assay is a Centers for Disease Control (CDC)-developed, molecular *Salmonella* serotyping assay that drastically improves the speed of determining serogroup, a very important tool in an epidemiological setting. The xMAP® assay is a suspension analysis system and consists of three separate tests (PCR, bead technology, and flow cytometry) that determine O and H antigens simultaneously. The assay tests for 7 O Groups and 35 H antigens and their results are based on combinations of O and H antigen PCR products. This novel molecular technology can differentiate the top 100 most commonly occurring human pathogen serotypes of *Salmonella enterica*.

DNA Macrorestriction Band-Based Subtyping

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) is a band-based molecular subtyping method that does not include PCR but rather involves macrorestriction of total bacterial DNA (chromosomal and plasmid) using an infrequent cutting restriction enzyme. The large DNA fragments produced from restriction digest are susceptible to mechanical shearing; therefore, in order to prevent DNA breakage, whole bacterial cells are embedded into an agarose plug. Once whole cells are embedded, they are subjected to lysis detergents/enzymes and extensively washed to remove remaining cellular or chemical contaminants. The agarose plug containing the genomic DNA is

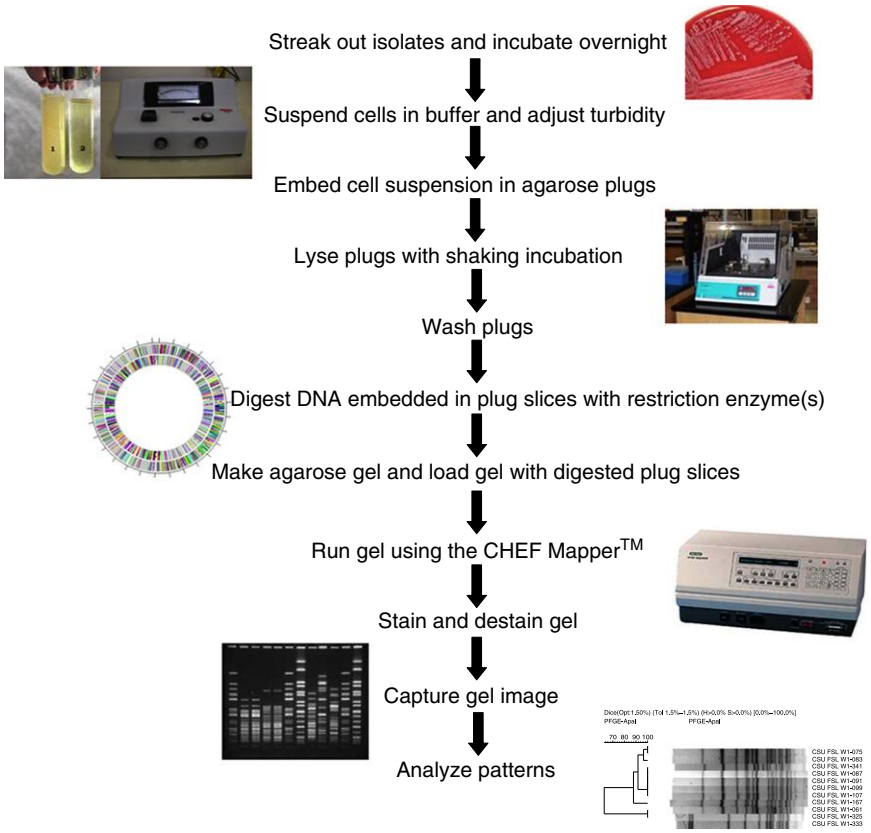

Figure 2 Pulsed field gel electrophoresis (PFGE).

then digested with a restriction enzyme known as a 'rare cutter.' Following digestion, a slice of the digested plug is loaded into an agarose gel as in PCR; however, it is then separated using a unique electrophoresis apparatus called the contour-clamped homogeneous electric field electrophoresis system. This system, unlike post-PCR gel electrophoresis, uses 24 electrodes evenly spaced in a hexagonal arrangement. This electrophoresis system produces uniform and homogeneous electric fields across the gel by constantly alternating current at 120° angles, moving the large DNA segments evenly through the gel and eliminating distorted bands at the bottom of the lanes (Figure 2).

PFGE is the current 'gold standard' subtyping method used to deposit molecular subtyping data into the PulseNet system. PulseNet is a national surveillance program, which was created by the CDC in collaboration with the Association of Public Health Laboratories (APHL), to provide scientists at public health laboratories with access to a searchable electronic database of PFGE patterns in order to facilitate real-time comparison of bacterial PFGE patterns isolated from cases of foodborne illness throughout the country. The CDC, along with state public health laboratories, PulseNet, US Food and Drug Administration, and the United States Department of Agriculture-Food Safety and Inspection Services employ PFGE to identify epidemiologically and genetically related isolates associated with clinical cases during outbreaks of foodborne illness. Since 1996, PulseNet has been imperative in detection, investigation, and control of numerous outbreaks caused by STEC O157:H7, *L. monocytogenes*, and *Salmonella*.

Ribotyping

Ribotyping is a molecular subtyping method that analyzes genetic components that code for rRNA (i.e., 5 S, 16 S, and 23 S rRNA). rRNA genes or rDNA are located throughout the chromosome, making ribotyping an effective approach to probe genetic diversity throughout the genome. rRNA genes are housekeeping genes that are needed for proteins synthesis and are thus pertinent for cell survival. As rRNA, along with other housekeeping genes, diversifies slowly, analysis of rRNA genes, therefore, provides a stable target for assigning molecular subtypes. Briefly, ribotyping is performed by lysing the bacterial cell and releasing total bacterial DNA. This is subsequently followed by digestion with a 'frequent cutter' restriction enzyme. The digested DNA is then electrophoresed to separate DNA fragments by size, and DNA fragments are then captured and immobilized on a nylon membrane. Once on the membrane, hybridization of the DNA with a chemically labeled rRNA operon probe occurs. After the DNA fragments and the rRNA operon probes hybridize, the membrane is washed and a chemiluminescent substrate is added to allow the bands containing rRNA to be visualized. Banding pattern data are then normalized against a molecular marker and similarity analysis is used to classify isolates into a ribotype on the basis of an existing database.

With the exception of cell lysis, ribotyping may be fully automated if performed using the Riboprinter® System (DuPont Qualicon), and thus requires minimal labor. The development of fully automated ribotyping systems improved the repeatability and reproducibility of interlaboratory

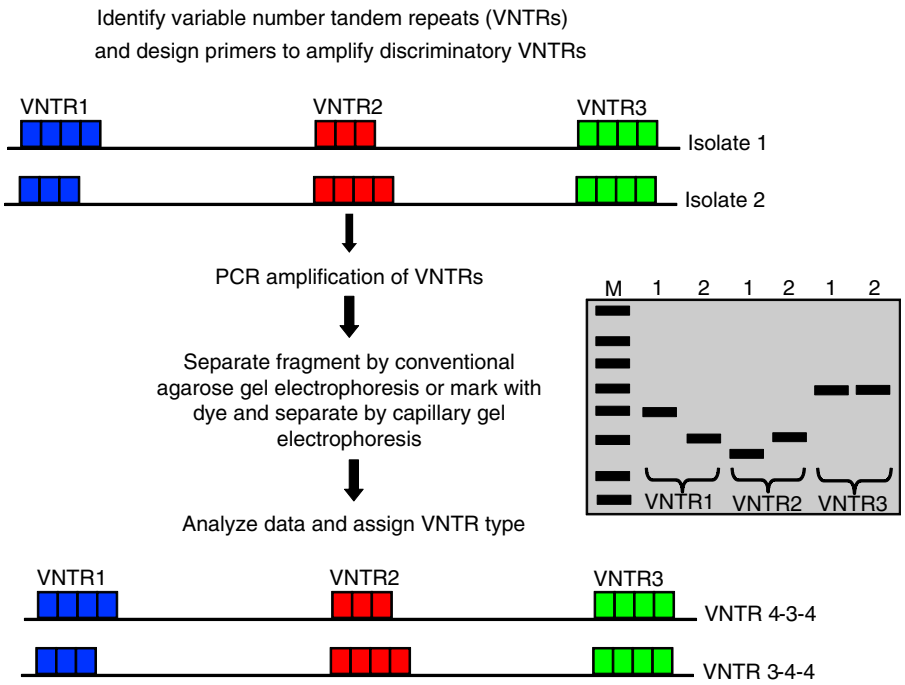

Figure 3 Multiple-locus variable-number tandem repeat analysis (MLVA).

ribotyping. The RiboPrinter® includes a database of bacterial ribotype patterns that includes more than 5700 patterns encompassing more than 180 genera and 1200 species. Ribotyping has most commonly been used to trace the spread of particular bacterial pathogens, especially *L. monocytogenes*, from farm to fork. Most importantly, it has been used for pathogen tracking during meat processing to help to identify harborage sites and routes of transmission.

DNA Sequence-Based Subtyping

Multilocus sequence typing

Multilocus sequence typing (MLST) is a nucleotide-based approach to subtyping bacterial foodborne pathogens. The MLST method is based on sequence variability within a set of five to seven genes or gene fragments, which typically focuses on housekeeping genes. In the MLST method, internal fragments ranging from approximately 400–600 bp in size (from particular genes such as virulence or housekeeping genes) are amplified and subsequently sequenced to determine the allelic and MLST types for each isolate. Once sequence data for all genes are ascertained, each gene is assigned a numerical allelic profile and is compared with other strains to determine genetic relatedness by nucleotide base changes rather than DNA fragment size.

Housekeeping genes, which are most commonly chosen genes to type, are not as subject to selective forces. Diversification occurs slowly within housekeeping genes by the accumulation of neutral variations and consequently highly conserving these genes within a species. If accumulation of genetic variation occurs rapidly (i.e., as in other genes, such as virulence genes), there is difficulty in distinguishing whether its descendants came from a common ancestor. Therefore, studying evolutionary questions using MLST data from housekeeping gene data provides more reliable information about the relationship between strains.

Multiple-locus variable-number tandem repeat analysis

Multiple-locus variable-number tandem repeat analysis (MLVA) methods are novel subtyping methods that have been designed by data gathered from genomic sequencing projects. The most recent analysis of multiple prokaryotic genomes revealed a high percentage of DNA that consists of multiple copies of repeat sequences, which are termed variable number of tandem repeats (VNTRs). The repeat regions can either be clustered in one specific area in the genome or be dispersed throughout the genome and are ideal for genomic events, such as DNA polymerase slippage and recombination. Recently, a number of studies have looked into the use of these VTNRs as a way to subtype bacterial strains. For very clonal organisms, such as *E. coli* O157, MLVA has been proven to be an appropriate way to address the minimal genetic diversity of such highly homogeneous species. The MLVA method uses these DNA elements repeated in tandem within bacterial chromosomes to base the observation that individual strains often carry the same elements with different copy numbers. Multiplex PCR primers are utilized to amplify regions of the genome that contain the VNTRs, and the repeat unit is subsequently assessed on agarose gels, or more frequently by separation of labeled amplicons using capillary electrophoresis. The resulting repeat size defines the repeat copy number; therefore, the allele type for each locus, ultimately designating an MLVA type (Figure 3).

Single-nucleotide polymorphism genotyping

With the increasing number of prokaryotic genomes that have been fully or partially sequenced, single-nucleotide polymorphism (SNP) genotyping is a recent development in

subtyping of bacterial pathogens. An SNP is a change in a single nucleotide (A, T, C, or G) in one sequence relative to another. As bacteria evolve, genetic divergence occurs through nucleotide substitutions, insertion, or deletion of genetic sequences, horizontal gene transfer, and recombination. The past decade has experienced an increased demand for high-throughput DNA analysis and SNP genotyping to detect genetic markers correlated with an important phenotype. SNPs can be detected using a multitude of methods and chemistries that enable allelic discrimination. The majority of SNP genotyping assays can be classified into one of four groups based on molecular mechanisms: (1) allele-specific hybridization, (2) primer extension, (3) oligonucleotide ligation, and (4) invasive cleavage.

See also: Microbial Contamination: Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: Standard Methods. Microbiological Safety of Meat: *Bacillus cereus*; *Clostridium Perfringens*; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*

Further Reading

Carlson, B.A., Nightingale, K.K., 2010. Molecular subtyping and tracking of foodborne bacterial pathogens. In: Juneja, V.K., Sofos, J.N. (Eds.), Pathogens and Toxins in Foods. Washington, DC: ASM Press, pp. 460–477.

De Boer, E., Beumer, R.R., 1999. Methodology for detection and typing of foodborne microorganisms. International Journal of Food Microbiology 50, 119–130.

Dunbar, S.A., 2006. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clinica Chimica Acta 363, 71–82.

Foley, S., Lynne, A.M., Nayak, R., 2009. Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. Infection, Genetics, and Evolution 9, 430–440.

Hyytia-Trees, E., Cooper, K., Ribot, E.M., Gerner-Smidt, P., 2007. Recent developments and future prospects in subtyping of foodborne bacterial pathogens. Future Microbiology 2, 175–185.

McPherson, M., Moller, S., 2006. PCR, second ed. New York, NY: Taylor and Francis Group.

Pagotto, F., Corneau, N., Scherf, C., *et al*., 2005. Molecular typing and differentiation of foodborne bacterial pathogens. In: Fratamico, P.M., Bhunia, A. K., Smith, J.L. (Eds.), Foodborne Pathogens: Microbiology and Molecular Biology. Norwich, England: Caister Academic Press, pp. 51–75.

Swaminathan, B., Barrett, T.J., Hunter, S.B., Tauxe, R.V., 2001. Pulsenet: The molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerging Infectious Diseases 7, 382–389.

Wiedmann, M., 2002. Subtyping of bacterial foodborne pathogens. Nutrition Research Reviews 60, 201–208.

Relevant Websites

http://en.wikipedia.org/wiki/Amplicon
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Amplification_of_DNA
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/DNA_microarray
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Endonuclease
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Horizontal_gene_transfer
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Indel
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Melting_curve_analysis
Wikimedia Foundation Inc.

http://en.wikipedia.org/wiki/Primer_(molecular_biology)
Wikimedia Foundation Inc.

Indicator Organisms in Meat

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Glossary

Hazard analysis and critical control point (HACCP) A comprehensive system designed to manage and insure food safety.

Indicator organism An organism to be considered of optimal use in identifying potential hazards.

Most probable number (MPN) A microbiological sampling technique designed to convert a series of yes/no results from test tubes containing samples at different dilutions into a quantitative assessment of the number of organisms present.

National Shellfish Sanitation Program (NSSP) A program administered by the US Food and Drug Administration that works together with the US state government officials and the regulated industry to assure the safety of clams, oysters, and mussels.

Salmonella A foodborne pathogen. Indicator organisms are used to indicate the presence of pathogens like *Salmonella*.

Introduction

This article will provide a brief overview of the historical development of indicator organisms, reasons for using indicators, and the qualities of an acceptable indicator. The benefits and limitations of indicators to detect hazardous contamination of raw materials, assess the adequacy of processing treatments to reduce or eliminate dangerous pathogens, and evaluate the recontamination of meat products will be discussed. Finally, this article will conclude with a brief discussion of the use of indicator organisms within hazard analysis and critical control (HACCP) and regulatory systems.

Historical Perspective

To understand the current application of indicators in meat systems it is desirable to understand their earliest use in food safety. The concept of indicator organisms had its beginnings in the early history and development of shellfish sanitation. As early as 1603, oysters and other shellfish were being implicated as the vehicle for typhoid fever and other enteric diseases, but it was not until the late nineteenth century that many European scientists began routinely collecting bacteriological and epidemiological data to confirm the correlation. By 1879, it was widely accepted by European health officials that 'typhoid bacillus' (now known as *Salmonella typhi*) was present in raw sewage. If this sewage was dumped into shellfish beds, *Salmonella* could subsequently contaminate those oysters and thus cause typhoid fever in individuals consuming shellfish from those beds. Between 1904 and 1909, 124 of 855 typhoid cases in Birmingham, England implicated mussels as the source of infection. Bacterial examination of mussels in the Birmingham Markets over the course of a year found a correlation between the quantity of microorganisms and the purity of the mussel harvest sites. This, in turn, led to the development of a classification system that was based on (1) the total number of microorganisms, (2) the number of *Bacillus* spores, (3) the number of glucose-fermenting organisms, and (4) the number of *Streptococci* detected in mussels and harvest beds.

Concurrent to European activities, consumption of shellfish in the US was also high, interest in sanitation was low, and the number of typhoid fever outbreaks was rising steadily. A well-documented typhoid fever outbreak on a Connecticut college campus involving oysters led investigators to begin microbiological testing of shellfish in the US.

In the early 1900s, it was very difficult to compare results between laboratories as no standard microbiological methodologies had yet been developed. The American Public Health Association charged a committee to provide the industry with a system for scoring bacteriological results from the newly developed most probable number (MPN) technique on *Bacillus coli* (now *Escherichia coli*) in 1909; however, this committee was not prepared to give any sanitary significance to these MPN scores.

Investigations into controlling shellfish contamination continued periodically and the issue was finally given priority after outbreaks in Chicago, New York, and Washington, DC in 1924. One year later a committee appointed by the National Shellfish Sanitation Program (NSSP) recognized that *B. coli* was often being used as an indicator of pathogens from fecal origin, yet it was only 'roughly related to the presence of disease-causing germs.' The committee did agree, however, that the level of *B. coli* in fresh shellfish was a fair index of the cleanliness or contamination of the waters from which they originated. Subsequently, various guidelines based on the presence and levels of *B. coli* in water were developed and implemented at the state level.

The use of *B. coli* as an appropriate indicator of contamination was by no means unanimous. Total coliforms were judged by some to be the indicator of choice based on data collected on the fate of coliform bacteria within oysters. The New York State Conservation Department, for example, set acceptable limits using total coliform counts in clams. This standard was derived using an average ratio of pathogens to coliform density in drinking water supplies, the number of typhoid cases, and several other assumptions. The suitability of either standard is still an active topic of debate and the current NSSP guidelines allow a water quality standard based on either total or fecal coliform count for shellfish waters.

Objectives of Indicators

The main objective for any use of microbial indicator organisms is to assess the microbial quality of a food product. Indicators may also reveal flaws in process controls that could allow food to be contaminated or a pathogen to multiply to dangerous levels. A specific pathogen may not be present in the sample being tested, but the presence of indicator organisms is meant to suggest that pathogens have a reasonable likelihood of occurring in other samples of the same product. There are many types of organisms that may be used as indicators, and Figure 1 shows the relationships between such organisms.

Qualities of a Good Indicator Organism

For an organism to be considered of optimal use in identifying potential hazards it must fulfill many requirements. The source of the indicator organism should be known. If an organism is being used to indicate pathogens of fecal origin, it must be from a fecal source. Ideally, the indicator will always be present when the hazard is present.

The indicator organism should also behave in a physiologically similar manner to the pathogen of interest; inactivation and growth rates should be similar, as should their resistance to environmental conditions. If the indicator organism multiplies rapidly at the normal storage temperature of a meat product but the pathogen does not, indicator results will be skewed. Additionally, if the indicator organism is inactivated more quickly during processing than a pathogen, the utility of the indicator has been lowered. Ideally, a change in pathogen concentration should result in a concomitant increase or decrease in the corresponding indicator organism.

An indicator organism should be easy to culture and differentiate from any other microorganisms normally present in the meat product. There should be standard methods of detection for this organism and they should be simple, reliable, accurate, rapid, and widely accepted. If the tests are either more difficult or less accurate than those that test for the pathogen of concern, then little is gained. An optimal indicator organism will be present in the food at levels that can be correlated to the pathogen, yet will be absent from foods not contaminated with the hazard. Even though such a wish list is nice in principle, there is no single indicator organism capable of meeting all of these requirements. Although no single indicator has been shown to be a perfect surrogate, it has been suggested that if measurements are taken with sufficient frequency to detect changes in the distribution and level of these indicators, performance could be improved.

Common Uses of Indicator Organisms

Identification of Contaminated Raw Materials

The quality and safety of a finished meat product is highly dependent on the raw materials used. Indicator organisms are often used to assess quality and identify hazardous contamination of carcasses and raw meat products. Aerobic plate count (APC) can help to evaluate the impact of time–temperature history or slaughter sanitation conditions if these factors are unknown. If counts are very high or vary widely among samples from different lots or within the same lot,

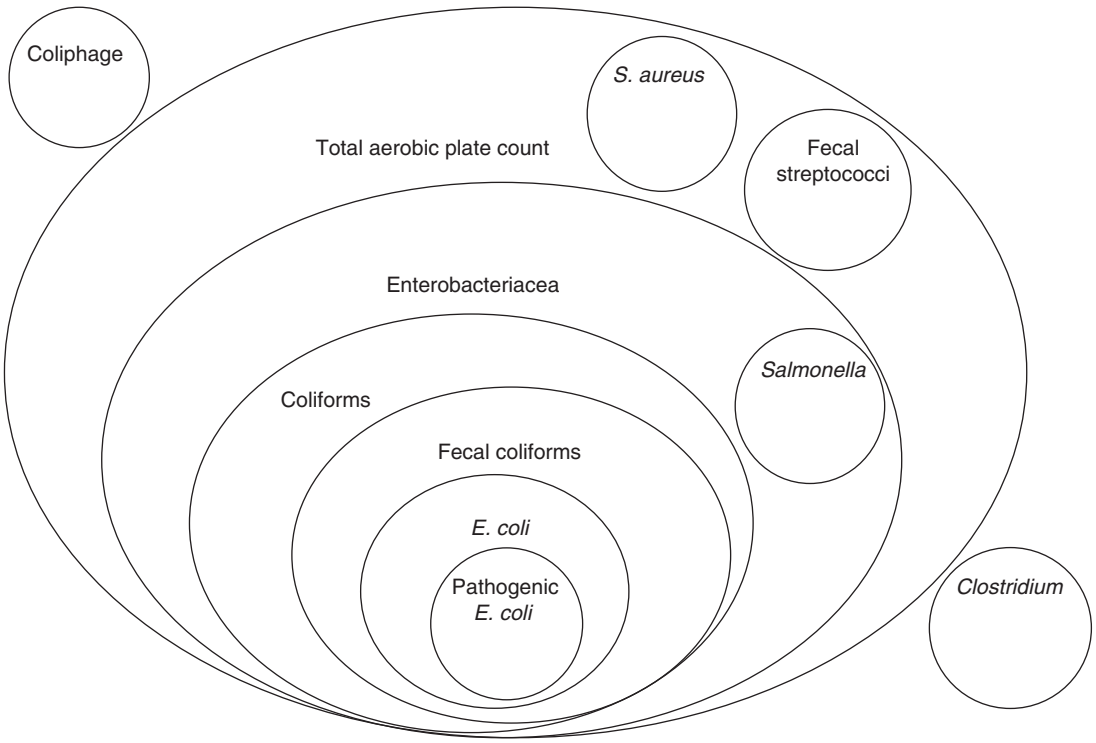

Figure 1 A Venn diagram showing the relationships between different groups of organisms used as indicator organisms.

inadequate microbiological control during processing or transport may be one explanation. Although APC has some utility, it is generally less accurate than other indictors in indicating the presence of pathogens in meats. Normal and spoilage microflora are typically included in the APC, and thus APC values are skewed when these counts are high.

Cross-contamination of meat with coliforms originating on hair, hooves, or hide during slaughter can be very common and has thus been responsible for their suggested use as an indicator of enteric pathogen contamination. Total coliforms include all aerobic and facultative anaerobic Gram-negative, nonspore-forming bacilli that have the ability to ferment lactose to acid and gas within 48 h at 35 °C. This group is classified according to biochemical reactions, not genetic relationships, and thus coliforms do not represent any specific taxonomic group. Organisms included in this group are *E. coli*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*. Coliforms are relatively easy to detect yet classical methods are targeted at 'typical' organisms and sacrifice accuracy for speed. Coliforms inhabit both human and animal intestinal tracts and have been associated with both fecal and nonfecal matter. The overall levels of Enterobacteriaceae have been shown to be an accurate predictor of the absence of *Salmonella* in a beef carcass after slaughter, yet with many species of this organism found naturally in the environment, including meat processing plants, this indicator may not be as reliable as *E. coli*.

It is very difficult to avoid *E. coli* contamination of carcasses during slaughter due to the organism's close association with animals. *Escherichia coli* is considered the most prominent fecal coliform, and some believe it is the only indicator that should be used to suggest the presence of pathogens from fecal origin. A limitation to utilizing *E. coli* as an indicator of pathogens of fecal origin is the cost and complexity of *E. coli* testing compared with those of other methods. As with other indicators, the presence of *E. coli* does not guarantee that pathogens of fecal origin are present, nor does its absence ensure that contamination has been avoided. Additionally, seasonality effects should be considered when using *E. coli* as an indicator as significantly higher counts have been observed during summer months, impacting correlations to pathogenic targets. Although numerous studies have reported positive results measuring *E. coli* as an indicator of process controls, its use in isolation to measure cross-contamination events during slaughter is not advised.

A correlation between fecal streptococci and enteric pathogens in unprocessed, raw meats has also been suggested. The correlation has been shown to be much better than with meats that are heated, cured, frozen, or dried. Fecal streptococci (or Enterococci) are Gram-positive, catalase-negative cocci from selective media that grow on bile aesculin agar at 45 °C and are loosely referred to as fecal streptococci. These organisms are able to survive outside the intestines better than coliforms. Fecal streptococci include *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus equinus*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus avium*, and numerous *Enterococcus* spp. The correlation of fecal streptococci with enteric pathogens in meat products is still controversial. Fecal streptococci may be found in the intestinal tracts of humans and warm-blooded animals, on the hide, hair, hooves, and feathers of animals, and are also widespread in nature. Their universal presence may restrict their use as an indicator of pathogens of fecal origin. The presence of *Streptococcus bovis* and *S. equinus* indicates animal pollution possibly during slaughter, as neither is associated with humans.

Aeromonas has been investigated for use as an indicator of hygiene in a processing plant. Increasing numbers of *Aeromonas caviae* or *Aeromonas hydrophila* on processing equipment, such as dehairing machines, mesh gloves, and belts, could indicate inadequate sanitation. *Aeromonas* was also identified as a possible indicator for assessing carcass dressing procedures during swine slaughter.

The presence of spore-forming bacteria suggests the possible presence of *Clostridium perfringens*, *C. botulinum*, or *Bacillus cereus*, all of which have been associated with meat and meat-containing products. Spores present in raw meat have the ability to survive thermal processing and proliferate in finished meat products.

A high degree of correlation between fecal coliforms and F^{+}RNA coliphage counts has been seen while monitoring the microbiological quality of raw meat and poultry. Coliphages are viruses that infect *E. coli* bacteria. Because methodologies for concentrating and detecting coliphages are relatively simple and rapid, this new technology has shown promise as a future indicator of pathogens of fecal origin. Additional emerging technologies, including nucleic acids, biosensors, and nanotechnology, have been shown to provide rapid results with a low investment of resources by detecting multiple organisms or molecular markers concurrently.

Adequacy of Processes to Destroy Pathogens

APC is often used to test sanitation on a processing line during production. Ingredients may be sampled before or after addition to the product, or a complete product may be sampled before and after processing or during or after a period of delay. In addition, APC may be used to identify key steps involved in contamination during processing; APC will generally be low or at baseline levels before the processing step causing the problem, but will be significantly higher after contamination has occurred. Additionally, thermal processing will inactivate a high number of vegetative organisms present in raw materials, but some spore-forming pathogens are highly heat resistant and will probably survive most cooking operations. Most microorganisms found on meat are also very sensitive to drying and freezing and are thus less useful as indicator organisms for frozen or dried products. It may not be advisable to use APC as a microbial indicator in fermented meats because these products may have high APC from the starter cultures used in their manufacture. That being said, starter organism colonies do have a different appearance on agar plates, and thus experienced technicians may still be able to differentiate high counts due to contamination versus those due to the starter culture itself.

Another group utilized to indicate inadequate processing is the Enterobacterieae family. The Enterobacteriaceae have been classified as Gram-negative, glucose-fermenting, oxidase-negative, usually catalase-positive, and nitrate-reducing organisms. This includes not only many bacteria commonly associated with fecal

matter but also many nonfecal organisms. Common genera of this family include *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia*, *Hafnia*, *Klebsiella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, and *Yersinia*. The detection of any member of the Enterobacteriaceae family present in a meat product has been used to imply the presence of enteric pathogens, but a direct correlation between Enterobacteriaceae presence and concentration and enteric pathogen presence and concentration is not assured. Enterobacterieae levels have been shown to be very similar to coliform concentrations, as there is a great deal of species overlap between these two groups. Enterobacterieae may also be used to evaluate hygiene and sanitation in processing plants, as they are readily inactivated by sanitizers and capable of colonizing a variety of niches when sanitation is inadequate. However, the presence or absence of high concentrations of these organisms cannot confirm the presence or absence of enteric pathogens.

Fecal coliforms, a subset of the total coliforms group, have the ability to multiply in hospitable environments, are destroyed by pasteurization and normal cooking, and are typically inactivated by freezing conditions, thus making them an indicator candidate for analysis of process controls. Fecal coliforms are defined as Gram-negative bacilli that ferment lactose within 48 h at 44.5–45.5 °C. Multiplication at higher temperatures often results in this group being referred to as 'thermo tolerant coliforms.' This group includes *E. coli*, *Enterobacter* spp., *K. pneumoniae*, and *C. freundii*. Testing for fecal coliforms arose from attempts to find rapid, dependable methods to detect *E. coli* without the need to isolate a pure culture. Fecal coliforms are generally specific to the intestinal environment of warm-blooded animals and thus high levels are found in fecal matter, but some organisms of nonfecal origin will occasionally test positive as fecal coliforms, and therefore care must always be taken in interpreting test results. Fecal coliforms are relatively easy and reliable to detect.

Escherichia coli have been shown to have similar rates of inactivation during thermal processing to enteric pathogens, including *Salmonella* spp., and thus have been suggested as indicators of process control. As new meat processing technologies (e.g., irradiation) are developed, care will need to be taken to insure that any difference in sensitivities is considered before determining the final suitability of *E. coli* as an indicator.

Fecal streptococci have been used as evaluators of frozen food plant sanitation due to their ability to survive freezing. They have also been used as indicators of adequate sanitation for their ability to resist inactivation by thermal processes, drying, detergents, and disinfectants. Yet use of organisms with increased resistance can lead to an indicator with undesirable stringency, as they outlast less durable pathogens, such as *Salmonella* and *Shigella*.

Identification of Recontamination of Processed Products

High numbers of coliforms may be representative of improper handling or storage of meat products, which allows for the multiplication of any coliforms present. High counts of coliforms on cooked meat products would indicate postprocessing contamination. High levels of Enterobacterieae can suggest postprocessing contamination or microbial proliferation due to inadequate storage conditions.

Staphylococcus aureus' association with human skin and oral–nasal cavities has prompted its use as an indication of inadequate employee sanitation and contamination due to handling. *Staphylococcus aureus* has also been suggested as an indicator to detect multiplication of bacteria during prolonged storage of processed meats without refrigeration.

Microbial Indicators and Hazard Analysis and Critical Control

HACCP plans are currently required in all meat and poultry plants within the US. The use of indicator organisms can offer assistance for establishing, monitoring, and verifying critical control points in HACCP operations. Indicators can best be used within an HACCP system to control processes that have the greatest influence on the level of microorganisms rather than to determine whether to accept or reject a given lot of product. When used for critical control point evaluation, a large number of samples can be progressively collected throughout a process. HACCP can use index organisms to assess the integrity of an evisceration procedure or thermal process by determining levels of indicator organisms before and after each process is completed. Indicator organisms can be used to establish upper limits for pathogen numbers, and thus actions should be implemented that strive to reduce indicator organism numbers to the lowest level possible. It is widely accepted that the regular monitoring of process controls in the meat industry must replace end-product testing.

Regulatory Issues

Current regulations require that meat and poultry processing plants test for generic *E. coli* within their HACCP plans to verify process controls. Testing for *Salmonella* spp. has been mandated to confirm that plants are controlling pathogens within meat plants. Slaughter plants are also required to sample carcasses for generic *E. coli* to verify the prevention and removal of fecal contamination from raw meat. Generic *E. coli* was chosen due to its suggested association with pathogens of fecal origin and the relative ease and low cost of enumeration. Although most global regulatory authorities do not require microbiological testing of raw meat products, for import into the US, processors must meet the United States Department of Agriculture regulations. Australia and New Zealand, which do require testing, were able to establish regulations for food quality and safety through government and industry collaboration.

Conclusions

None of the indicators mentioned above are obligate inhabitants of the intestinal tract of humans or warm-blooded animals. Environmental reservoirs for each have been identified. Many of the organisms mentioned here are commonly present in meat manufacturing facilities and may become part of the natural microflora. Because no one indicator can be

applicable for all pathogens or all situations, it has been suggested that several indicator organisms can be used for several different pathogens. Whatever decision is made regarding the use of indicator organisms in the meat processing plant, it should be made with the full knowledge of both the benefits and the limitations of the microbial indicators. No microbiological specification, no matter how perfect, should ever be used blindly or without common sense. Finally, a trained food microbiologist should be consulted when implementing or changing the use of microbial indicators in a food processing environment.

See also: Hazard Analysis Critical Control Point and Self-Regulation. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: Standard Methods. Microbiological Safety of Meat: Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*

Further Reading

Altekruse, S.F., Berrang, M.E., Marks, H., *et al*., 2009. Enumeration of *Escherichia coli* cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the US. Applied and Environmental Microbiology 75, 3522–3527.

Brown, M.H., Gill, C.O., Hollingsworth, J., *et al*., 2000. The role of microbiological testing in systems for assuring the safety of beef. International Journal of Food Microbiology 62, 7–16.

Clem, J.D., 1994. Historical overview. In: Hackney, C.R., Pierson, M.D. (Eds.), Environmental Indicators and Shellfish Safety. New York: Chapman & Hall, pp. 1–29.

Dewaele, I., Ducatelle, R., Herman, L., Heyndrickx, M., De Reu, K., 2011. Sensitivity to disinfection of bacterial indicator organisms for monitoring the *Salmonella* Enteritidis status of layer farms after cleaning and disinfection. Poultry Science 90, 1185–1190.

Downes, F.P., Ito, K., 2001. Compendium of Methods for the Microbiological Examination of Foods. Washington, DC: American Public Health Association.

Ghafir, Y., China, B., Dierick, K., De Zutter, L., Daube, G., 2008. Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. Journal of Food Protection 71, 35–45.

Hsu, F.C., Shieh, Y.S.C., Sobsey, M.D., 2002. Enteric bacteriophages as potential faecal indicators in ground beef and poultry meat. Journal of Food Protection 65, 93–99.

ICMSF, 1978. Microorganisms in Foods 1: Their Significance and Methods of Enumeration. Toronto, ON: University of Toronto Press.

Kennedy, J.E., Williams, S.K., Brown, T., Minerich, P., 2006. Prevalence of *Escherichia coli* O157:H7 and indicator organisms on the surface of intact subprimal beef cuts prior to further processing. Journal of Food Protection 69, 1514–1517.

Niebuhr, S.E., Laury, A., Acuff, G.R., Dickson, J.S., 2008. Evaluation of nonpathogenic surrogate bacteria as process validation indicators for *Salmonella enterica* for selected antimicrobial treatments, cold storage, and fermentation in meat. Journal of Food Protection 71, 714–718.

Ruby, J.R., Zhu, J., Ingham, S.C., 2007. Using indicator bacteria and *Salmonella* test results from three large-scale beef abattoirs over an 18-month period to evaluate intervention system efficacy and plan carcass testing for *Salmonella*. Journal of Food Protection 70, 2732–2740.

Saini, P.K., Marks, H.M., Dreyfuss, M.S., *et al*., 2011. Indicator organisms in meat and poultry slaughter operations: Their potential use in process control and the role of emerging technologies. Journal of Food Protection 74, 1387–1394.

Sheridan, J.J., 1995. The role of indicator systems in HACCP operations. Journal of Food Safety 15, 157–180.

Smoot, L.M., Pierson, M.D., 1997. Indicator microorganisms and microbiological criteria. In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology, Fundamentals and Frontiers. Washington, DC: ASM Press, pp. 66–80.

Relevant Website

http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/93-016F.pdf
USDA.

Standard Methods

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Glossary

CAMP A diagnostic test for *Listeria* based on production of factors that interact synergistically with *Staphylococcus aureus* β-lysin. It is named after researchers Christie, Atkins, and Munch–Petersen.
Enumeration Methods which determine the concentration of cells in a sample.
Pathogens Bacteria which produce toxins during growth on food that can cause foodborne illness if ingested.
Qualitative methods Methods of analysis which determine the presence or absence of the target organism in a sample.
Spoilage bacteria Bacteria which do not cause illness. Its metabolic activity during replication induces unacceptable sensory changes in a food.
Standard methods Defined as recognized methodologies that have been collaboratively studied and validated before acceptance.
Verotoxigenic *Escherichia coli* (VTEC) *Escherichia coli* which carry the genes for and produce the virulence factor verotoxin. The terms shiga toxin producing *E. coli* and enterohemorrhagic *E. coli* are synonymous. *Escherichia coli* O157:H7 is the most common serotype of VTEC in North America.

Introduction

Standard methods can be defined as recognized methodologies that have been collaboratively studied and validated before acceptance. In some instances they form the basis for official, regulatory control actions, whereas in others they may be used for quality control purposes. Some international agencies concerned with the development and assessment of microbiological sampling plans and methodologies for foods include the AOAC International (formerly known as the Association of Official Analytical Chemists), the International Commission on Microbiological Specifications for Food (ICMSF), the International Organization for Standardization (ISO), the Comité Européen du Normalization (CEN), and the Nordic Committee on Food Analysis (NMKL). Regulatory authorities may publish their own official methods (US Department of Agriculture Food Safety Inspection Service, *Microbiology Laboratory Guidebook*; US Food and Drug Administration, *Bacteriological Analytical Manual*; and Health Canada, *The Compendium of Analytical Methods*) or make use of methods published by international agencies; for example, ISO methods are required by the European Union for regulatory testing for pathogens on fresh meats.

Although standard methods for determining pathogens and indicator bacteria in meats are based on the same general principles, they are highly variable and are continually being modified to suit the needs of the agency and meat product in question. There are no standard methods for the recovery and enumeration of meat-borne spoilage bacteria. Thus, there are no truly international standard methods that are universally applied to characterize and enumerate bacteria contaminating meats.

In view of this, the objective of this chapter is to provide a concise reference of verified cultural and biochemical procedures that will enable the selection of reliable methods for the recovery, detection, and enumeration of the most potent meat spoilage bacteria and relevant human pathogens.

Types of Culture Media

Media can be formulated to be nutritionally complete (general purpose) or can be selective, differential, or elective. Nutritionally complete media would favor the unrestricted growth of most meat-borne genera and enable the recovery of the largest population of bacteria. Unfortunately, bacterial types cannot be distinguished on these media and additional cultural and biochemical tests must be undertaken to confirm the identity of selected colonies. The random selection and characterization of 30 colonies from countable plates gives a good estimate of bacterial composition.

Selective media contain restrictive antimicrobial substances that, in combination with incubation conditions, allow the growth of a specific group of microorganisms while suppressing others (Tables 2 and 4). Unfortunately, these selective agents can reduce the recovery of targeted strains (particularly sublethally injured cells), their selectivity is not absolute, and further characterization of the isolates may be warranted.

Differential media contain substrates or reagents that allow the differentiation of colonies on the plate. This can be due to a change in the appearance of the colonies themselves or to a change in the medium surrounding the colonies.

Media of value in improving the recovery of lactic acid bacteria (LAB), for example, from meats have been termed elective. These media satisfy the complex nutritional requirements of these bacteria, but they are not selective or differential.

Spoilage Bacteria

Characteristics

The biochemical and cultural characteristics of bacteria most commonly associated with meat spoilage, including the

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Table 1 Standard cultural and biochemical criteria for differentiating meat spoilage bacteria

Organism	*Gram reaction*	*Morphology*	*Motility*	*Growth at pH 5.7*		*Catalase*	*Oxidase*	*Glucose metabolism*[b]	*Spoilage characteristics*
				O_2	No O_2				
Pseudomonas spp.	−[a]	Bacilli	+	+	−	+	+	O/−	Putrid odor
Brochothrix thermosphacta	+	Cocco-bacilli/ chains	−	+	−	+	−	F	Pungent cheesy odor
Enterobacteriaceae	−	Bacilli	+	+	−	+	−	F	Putrid odor and gas
Lactobacillus spp.	+	Bacilli	−	+	+	−	−	F	Sulfide odors, sour/ dairy odors, gas, and discoloration
Leuconostoc spp.	+	Bacilli	−	+	+	−	−	F	Sour/dairy odors and flavors
Carnobacterium spp.	+	Bacilli	−	±[c]	±[c]	−	−	F	Sour/dairy odors, flavors, and discoloration
Shewanella putrefaciens	−	Bacilli	+	−	−	+	+	−/O	Green discoloration and sulfide odors

[a] − Negative reaction; + positive reaction.
[b] O, oxidative; F, fermentative.
[c] Carnobacteria are sensitive to acetate at low pH.

characteristic spoilage defects, are depicted in Table 1. It is evident that a few simple tests can be utilized to differentiate the major spoilage flora; the most important of those include the Gram reaction, motility, oxygen requirements, catalase activity, oxidase activity, and glucose metabolism. Bacterial metabolism of arginine to produce alkaline by-products has also been suggested as a means to distinguish bacteria, but test results are highly variable and may not be a reliable tool.

Sample Preparation

Processing of meat samples is generally conducted by homogenization and dilution using 0.1% (w/v) peptone water. Diluted samples can be stored in this diluent for up to 2 h at room temperature or 6 h at 2 °C without appreciable change in bacterial numbers.

Common plating methods to recover and enumerate spoilage bacteria include pour plate, spread plate, spiral plate, and hydrophobic grid membrane filtration (HGMF). The pour plate method is usually performed by pipetting 1.0 ml of a sample dilution into a sterile Petri dish, followed by the addition of molten agar tempered to 45 °C and gentle mixing to distribute the cells. The spread plate technique utilizes Petri plates of solidified, predried agar media, which are inoculated by pipetting 0.1 ml of sample dilution onto the surface and then spreading using a sterile L-shaped glass or plastic rod to ensure an even distribution. Spiral plating is a semiautomated variation of spread plating in which a machine uses a stylus to deposit a volume of sample dilution on the surface of agar media. The sample dilution is deposited at a constant rate as the stylus moves from the center of the agar plate to the rim, resulting in a decreasing concentration of deposited sample; this permits a 2–3 log range of cell concentration to be enumerated on a single plate. The HGMF method usually involves the filtration of up to 10 ml of sample dilution through a 0.45 μm membrane filter (ISO-GRID, QA Life Sciences Inc., San Diego, CA, USA), after which the filter is placed on the surface of predried agar plates containing a suitable indicator dye. Treatment with enzymes and surfactants are often necessary to clarify meat suspensions before filtration. The HGMF method gives most probable number (MPN) results.

Sampling, diluting, and plating methods (pour and spread plate) that have a lower limit of sensitivity of 10–100 bacteria per unit volume, area, or weight are usually more than adequate for enumerating the prevailing flora in a spoiled meat product. If one is more concerned with the precise composition of the spoilage bacteria initially contaminating meats when numbers are expected to be low, it may be necessary to employ HGMF, which will facilitate detection of one bacterium per unit volume, area, or weight.

Media for Enumeration of Spoilage Bacteria

With knowledge of the type and history of the meat product and the spoilage symptoms, it is possible to select appropriate media to recover the targeted organism. Table 2 summarizes the performance of some of the more useful media for the recovery and enumeration of the dominant spoilage bacteria from meats.

An alternative to these direct plating procedures is HGMF methods. The ISO-GRID counting range is such that less sample dilutions are required and the lower limit of detection is less than that of conventional plating procedures. HGMF methods have been used to determine total psychrotrophs, *Pseudomonas* spp., *Brochothrix thermisphacta*, *Enterobacteriaceae*, and LAB from raw, chilled beef (Table 3) and counts were not different from those determined by conventional agar plating methods.

Aerobic and psychrotrophic plate count

An acceptable procedure for total plate counts is the spread plating of appropriate dilutions on plate count agar (PCA)

Table 2 Performance of selective media for enumerating meat spoilage bacteria

Media and incubation	*PCA aerobic 25 °C, 2 d*	*TSA aerobic 25 °C, 2 d*	*CFC aerobic 25 °C, 2 d*	*PI agar aerobic 25 °C, 2 d*	*STAA aerobic 25 °C, 3 d*	*VRBGA aerobic, 35 °C, 24 h*	*MRS anaerobic 25 °C, 2 d*	*Rogosa agar anaerobic 25 °C, 4 d*	*PIA aerobic 25 °C, 3 d*
Pseudomonas spp.	+[a]	+	+	±	−	−	−	−	−
Brochothrix thermosphacta	+	+	−	−	+	−	+	−	±
Enterobacteriaceae									
Escherichia coli	+	+	−	−	−	+	+	−	+
Hafnia alvei	+	+	−	−	−	+	+	−	+
Serratia liquefaciens	+	+	+	+	−	+	+	−	+
Lactic acid bacteria									
Leuconostoc spp.	+	+	−	−	−	−	+	−	−
Lactobacillus spp.	+	+	−	−	−	−	+	+	−
Carnobacterium spp.	+	+	−	−	−	−	+	−	+
Shewanella putrefaciens	+	+(O)[b]	∓	∓	−	−	−	−	+(B)[c]

[a] + indicates growth and typical colonial appearance with the exception of peptone-iron agar (PIA) for which blackening of the culture medium was found only with *S. putrefaciens*.

[b] O indicates a light orange pigmentation of the colonies.

[c] B indicates blackening of the colony and surrounding medium.

Abbreviations: CFC, cephaloridine–fucidin–cetrimide agar; d, day; h, hour; MRS, deMan, Rogosa, and Sharpe agar; PCA, plate count agar; PI, Pseudomonas isolation agar; STAA, streptomycin-thallous acetate-actidione agar; TSA, tryptic soy agar; VRBGA, violet red bile glucose agar. All inoculations made from broth cultures by surface plating except that pour plate was used for VRBGA and PIA.

Table 3 Media for the recovery and enumeration of meat spoilage bacteria using HGMF

Bacteria	*Medium*[a]	*Appearance of colonies*
Total psychrotrophs	Plate count agar (PCA) + 0.25 g l^{-1} fast green (7 °C, 10 d)	Yellow/green
	PCA (7 °C, 10 d) + 0.1% triphenyltetrazolium chloride postincubation	Dark red
Pseudomonas spp.	Cephaloridine–fucidin–cetrimide agar + 0.25 g l^{-1} fast green (25 °C, 2 d)	Dark green/blue
Brochothrix thermosphacta	Streptomycin–inositol–neutral red agar (25 °C, 3 d)	Pink/red
Enterobacteriaceae	Violet red bile glucose agar (no overlay) 35 °C, 18–24 h, anaerobic)	Red
Lactic acid bacteria	Tryptone–phytone–yeast extract agar + 0.2 g l^{-1} erioglaucine (25 °C, 4 d, anaerobic)	Light green/pale blue

[a] The performance of these media was not different from that of traditional plate count methods using naturally contaminated or inoculated beef steaks. d, day; h, hour.

followed by incubation at 25 °C for 48 h. Tryptic soy agar (TSA) is a suitable alternative and produces counts of a similar magnitude.

Recommended methods for enumeration of total psychrotrophs are PCA with incubation at 5–7 °C for 7–10 days. If a total anaerobic plate count is required, PCA can be incubated in the absence of oxygen and in an environment containing 4–10% CO_2 (v/v). This can readily be achieved by utilizing anaerobic jars and commercially available disposable gas generators that remove oxygen and generate an atmosphere of 4–10% CO_2.

HGMF can be used for total counts by incorporating 0.25 g l^{-1} fast green in the PCA or TSA (Table 3). Colonies developing on this medium are yellow/green. A more tedious approach is to place the membrane filter on TSA and flood the plate with 1% triphenyltetrazolium chloride after incubation by introducing 2 ml of it under the filter to visualize and enumerate the red colonies.

Indicator bacteria

Both *Escherichia coli* and total Enterobacteriaceae counts have been proposed as indicators of hygiene and safety; they can be enumerated by direct plating procedures or using HGMF (Tables 2 and 3) and there are proposed standard methods (ISO and AOAC).

Pseudomonads

The most common meat spoilage pseudomonads are *Pseudomonas fragi* and *Pseudomonas fluorescens*. Cephaloridine–fucidin–cetrimide agar (CFC) is a selective medium routinely used to recover and enumerate spoilage pseudomonads from meats. The sample dilutions are spread plated and the medium is incubated aerobically at 25 °C for 2 days. Colonies developing on this medium are cream colored and are 2–5 mm in diameter. Although *Serratia liquefaciens* grows on CFC, it can be differentiated from the oxidase-positive pseudomonads using the oxidase reagent (1% w/v tetramethyl-*p*-phenylene-diamine hydrochloride). Also, CFC can be modified to differentiate pseudomonads from enterics by the addition of 1% (w/v) arginine and 0.002% (w/v) phenol red. The metabolism of arginine by the pseudomonads produces pink colonies.

HGMF methods can readily be applied by incorporating 0.25 g l^{-1} fast green into the CFC medium. Pseudomonad colonies are dull green/blue on the membrane filter (Table 3).

Brochothrix thermosphacta

The medium formulated for the selective enumeration of *Brochothrix thermosphacta*, streptomycin-thallous acetate-actidione agar (STAA), is highly selective and usually excludes all other members of the spoilage flora in meats, with the rare exception of some pseudomonads. Although *Xanthomonas campestris* grows on STAA, it would not be expected to contaminate meats.

Sample dilutions are spread on STAA and after aerobic incubation at 25 °C for 3 days, *B. thermosphacta* colonies are typically pale straw colored and approximately 1–2 mm in diameter.

To enable the use of HGMF for enumeration of *B. thermosphacta*, streptomycin–inositol–neutral red agar has been used. *B. thermosphacta* ferments inositol, giving rise to pink/red colonies on the membrane filter (Table 3).

Enterobacteriaceae

Members of the Enterobacteriaceae of importance to meat spoilage include *Hafnia alvei*, *Enterobacter agglomerans*, and *S. liquefaciens*. The traditional procedure for total Enterobacteriaceae counts for meats utilizes violet red bile glucose agar (VRBGA) and the pour plate technique. Solidified plates are then overlaid with approximately 5.0 ml VRBGA and incubated at 35 °C for 18–24 h. Purple/red colonies, 1–2 mm in diameter and surrounded by a reddish halo of precipitated bile, are presumptive enterics.

The short incubation time, the overlay, and the mesophilic incubation conditions enhance the selectivity of the procedure and limit the growth of other Gram-negative organisms (aeromonads and pseudomonads) that might give false positive results on this medium. The oxidase test can be used to assist in differentiating the oxidase-negative Enterobacteriaceae.

Total psychrotrophic Enterobacteriaceae are estimated following incubation of VRBGA at 4 °C for 10 days, but there is an increased risk of the proliferation of nonenterics under these conditions and some have suggested that enumeration of all visible colonies provides an estimate of the total Gram-negative counts.

The VRBGA medium can also be utilized with the HGMF method. After filtration of the diluted meat homogenate, the membrane filter is placed on a plate of VRBGA and incubated anaerobically in an atmosphere of CO_2 at 35 °C for 18–24 h. Enterobacteriaceae produce red colonies on the membrane filter.

Lactic acid bacteria

Members of the genera *Lactobacillus*, *Leuconostoc*, and *Carnobacterium* are the important spoilage LAB in meats.

The medium of choice for estimating LAB bacterial numbers in meats is the medium of deMan, Rogosa, and Sharpe (MRS). This medium contains acetate, which may restrict the growth of carnobacteria, but these bacteria can be selectively recovered from meats using cresol red–thallium acetate–inulin agar.

An acceptable method for recovering the largest population of LAB from meats is the anaerobic incubation of MRS agar at 25 °C for 4 days (spread plate) in an atmosphere containing CO_2. LAB colonies are small (1–2 mm) and characteristically white or cream colored. As evident from Table 2, most other members of the spoilage flora proliferate on this nonselective medium and a simple catalase test readily differentiates the catalase-negative LAB from other bacteria on the plates.

Rogosa agar and acetate agar have also been utilized to recover LAB from meats. These media have the selective pressure of both high acetate content and a lower pH (5.5), which favors the growth of meat-borne lactobacilli.

There is an HGMF procedure that utilizes tryptone–phytone–yeast extract agar containing 0.2 g l^{-1} erioglaucine. After anaerobic incubation in CO_2 at 25 °C for 4 days, presumptive LAB are light green to pale blue on the membrane filter.

Shewanella putrefaciens

This bacterium produces large amounts of hydrogen sulfide and can cause green discoloration of meat with a pH of 6.0 or greater. When this type of spoilage is evident, diluted samples are plated using peptone-iron agar (PIA) by the pour plate technique and the solidified medium is overlaid with approximately 5 ml of PIA. Incubation is done at 25 °C for 3 days. Colonies producing hydrogen sulfide are 1–2 mm in diameter and gray-black in color; in some instances the surrounding medium is blackened.

Because this medium is a nonselective, differential medium and other bacteria can produce hydrogen sulfide, it is necessary to select colonies for confirmation using cultural and biochemical criteria.

Meat-Borne Pathogens

In contrast to spoilage bacteria, pathogens are usually present in low numbers (i.e., less than one per gram or per 100 cm^2). Most pathogens associated with meat and meat products are mesophiles whose growth is restricted at refrigeration temperatures but are capable of surviving throughout the product's shelf life. Even with growth inhibited it is not possible to establish safe levels of exposure for some pathogens (e.g., verotoxin-producing *E. coli* and *Salmonella)* as the estimated infectious dose may be as low as 10 cells. Consequently, pathogen detection commonly uses qualitative methods, which include an enrichment step and determine the prevalence of the pathogen.

Although several pathogenic bacteria are found on meats, only six types are commonly associated with illness. *Salmonella* spp., pathogenic *E. coli*, and *Campylobacter jejuni/coli* are of most relevance to raw, chilled meats, whereas *Clostridium perfringens*, *Listeria monocytogenes*, and *Staphylococcus aureus* are of more importance to the safety of processed and ready-to-eat meats.

Pigs are an important reservoir for *Yersinia enterocolitica*. *Yersinia* can replicate on chilled meat at temperatures as low as −1 °C and is frequently recovered from pork-processing environments and pork tissues. Although outbreaks of meat-borne illness attributable to *Y. enterocolitica* are rare, some consideration is given to it, as it has the potential to emerge as an important pathogen.

Conventional procedures for determining both the prevalence and numbers of these bacteria are reviewed.

Bacterial Prevalence

Qualitative methods may be used to determine the prevalence of pathogens and have four stages: enrichment, screening, isolation, and confirmation (Table 5).

Enrichment

Enrichment is an initial culture step, with the aim of amplifying the concentration of the pathogen. The food sample is enriched by suspension in a liquid medium and incubation at a temperature permissive for growth. Enrichment simultaneously resolves a number of challenges in the bacteriological analysis of foods in that it converts solid samples to an aqueous suspension, raises the concentration of the target organism, and homogenizes distribution. This ensures that the success of screening and isolation is not dependent on the probability that aliquots of the sample suspension contain the target pathogen.

If pathogens have been exposed to environmental stress (heating, freezing, and pH) the cells may be injured and demonstrate an extended lag phase or be unable to replicate in the presence of selective agents or conditions. To improve the recovery of injured cells, enrichment may begin with a period of resuscitation, under relatively unrestrictive growth conditions (Table 5). Enrichment in nonselective media amplifies microflora indiscriminately, which may interfere with screening for the target or its isolation. To reduce nontarget microflora the enrichment can continue with increasing selectivity by the addition of selective agents or the transfer of an aliquot to a more selective media. In some protocols, to increase the probability of successful detection, the enrichment may be conducted in parallel in media with differing selective characteristics.

Screening

Following enrichment, analysis may proceed directly to isolation, or the enrichment broth may be screened for the presence of the pathogen. Screening tests commonly use serological methods or polymerase chain reaction (PCR) to determine the presence of molecules or gene sequences associated with the pathogen. The purpose for screening enrichment broths is not detection of the target organism, as the enrichment broth contains a mixed population of organisms and there is a potential for false positive results, which need to be eliminated by isolation and confirmation. Instead, the purpose of screening tests is to reduce the number of samples that need to proceed to isolation, by identifying which samples are negative for the pathogen. Screening tests for the major pathogens associated with meat are commonly available commercially in a kit format.

Isolation

The purpose of isolation is to establish a pure colony of the target pathogen, separating it from other microflora that can interfere with analysis or the interpretation of results. Isolation is conducted by plating an aliquot of the enrichment broth onto agar media. The agar media ideally have selective and differential characteristics which aid the analyst in identification of target colonies. As with enrichment, multiple media may be used for isolation to increase the probability of success. Isolation is completed by the establishment of pure subculture on nonselective media.

To reduce the amount of nontarget cells transferred to the agar media from the enrichment broth, immunomagnetic separation (IMS) may be used. In IMS, cells bearing a target surface antigen are bound to magnetic beads conjugated with an antibody to the anitgen. The magnetic beads are then recovered from the enrichment broth with a magnet and repeatedly washed to remove cells that are not bound to the antibody. The washed IMS beads are then plated onto agar media. IMS beads are commercially available for *Listeria* spp., *Salmonella* spp., and a variety of *E. coli* O types associated with verotoxin production (O157, O26, O45, O103, O111, O121, and O145).

Confirmation

Once multiple presumptive isolates of the pathogen have been subcultured, a series of tests are conducted to verify the identity of the pathogen on the basis of species and the presence of virulence or virulence-associated characteristics. Individual confirmatory tests may be biochemical, serological, or molecular tests for species and virulence-associated traits. As with screening tests, some confirmatory tests for major pathogens are available in kit format.

Enumeration

Direct plating

For some pathogenic bacteria, direct plating may be a suitable alternative to enrichment procedures and provides an estimate of numbers. Direct plating is applicable only if the numbers of the pathogen are high enough (>100 CFU g^{-1}).

If cells are injured, a resuscitation step may be required. This can be achieved by agar overlay or membrane filter plating. In agar overlay the sample diluent is spread plated onto a nonselective agar media, which is then incubated for a period sufficient for growth to occur, but before visible colonies form (4–6 h), the agar plate is then overlain with a layer of molten selective agar at 45 °C. The second agar layer is allowed to gel and then incubation is continued. Alternately, if samples are spread onto the surface of a membrane filter or the sample is filtered through membrane filters, the filters may be first incubated on nonselective media and then transferred to the surface of a selective agar medium for continued incubation.

Most probable number determination

The determination of the MPN of pathogens in a meat sample utilizes the advantages of both enrichment and enumeration and allows very low concentrations of cells to be enumerated. Known weights of sample are introduced into replicate tubes (commonly 3–10) containing enrichment broth and additional 10-fold dilutions of the sample and replicate tubes are prepared. For example, if 1 g of meat is introduced into the first set of five tubes, 0.1 g would be put in the second set of five tubes and 0.01 g in the third set of five tubes. Tubes are incubated and observed for growth or a particular metabolic characteristic and scored as positive or negative. The specificity of an MPN analysis can be increased

by plating broth from tubes onto an appropriate agar medium and confirming the identity of the resulting colonies. The concentration of cells in the original sample is estimated statistically with the assistance of MPN tables or computer applications, a variety of which are available from online resources.

Hydrophobic grid membrane filtration

As with direct plating onto membrane filters, HGMF can be used to both resuscitate and enumerate pathogens. Media have been designed to have the potential to detect and enumerate *Salmonella* spp., *S. aureus*, *Listeria* spp., and *E. coli* O157:H7 (Table 6).

Considerations for Specific Pathogens

Campylobacter spp.

In most meat samples, except possibly for raw poultry, numbers of *Campylobacter* spp. can be expected to be low. *Campylobacter* spp. are microaerophilic and capnophilic. They can lose viability under atmospheric oxygen concentrations and so must be cultured in an environment with 5% oxygen, 10% carbon dioxide, and 85% nitrogen. The optimum growth temperature for *Campylobacter* spp. is 42–43 °C. Enrichment and plating media are composed of a nutritious medium containing laked or lysed horse or sheep blood or charcoal with selective agents (Table 4). If cells of young colonies are observed microscopically, they will appear as small, thin, and curved rods with corkscrew motility. Latex agglutination tests and enzyme-linked fluorescent assays can be used to confirm the identity of *Campylobacter* spp.

Clostridium perfringens

Clostridium perfringens can frequently be encountered in raw and cooked meats. It must be present in high numbers to cause illness and in the event of a food-borne illness outbreak attributable to *C. perfringens*, direct plating is usually appropriate. Sulfite-cycloserine agar without egg yolk, prepared by the pour plate method, is the preferred isolation medium (Table 6) and *C. perfringens* metabolizes sodium metabisulfite and ferric ammonium sulfate to form black colonies. For examination of lecithinase activity, some isolation media include egg yolk.

Alternative plating media include horse blood agar with neomycin, but neomycin is likely to inhibit some strains.

If cells are expected to be stressed, *Bacillus cereus–C. perfringens* broth (BCP) containing catalase, hydrogen peroxide degraders, and fresh egg yolk is effective for cell recovery before plating. If an MPN analysis is needed, iron milk medium incubated for up to 18 h at 45 °C is recommended. *Clostridium perfringens* causes a 'stormy fermentation' from the production of acid from lactose and fractionation of the curd. Motility and the ability to reduce nitrate, to ferment lactose, and to liquefy gelatin are characteristics used to confirm *C. perfringens*.

Escherichia coli O157:H7 and Nonmotile O157 Strains

Escherichia coli O157:H7 and nonmotile O157 strains can be isolated with relative ease in the presence of other *E. coli*, as they normally do not ferment sorbitol and lack β-glucuronidase activity. A variety of differential agar media based on these traits are commercially available. Selectivity can be imparted to enrichment and isolation media by the use of the antimicrobials novobiocin, tellurite, and cefixime, to which *E. coli* O157 strains commonly display superior resistance.

Large sample sizes have become established for the detection of *E. coli* O157 in some beef products. The USDA-FSIS requires a sample size of 325 g (or 5 × 65 g) for ground beef or ground beef precursor material. The sample is enriched and then screened for potential *E. coli* O157 by PCR or lateral flow device (Table 5). Isolation is performed using IMS beads conjugated with anti-O157 antibodies and plating onto selective/differential agar. Confirmation of presumptive positive samples would include latex agglutination, hemolysis on blood agar plates, and biochemical, serological, and molecular confirmation, including expression of verotoxin and the O157 antigen.

Verotoxin-Producing *Escherichia coli*

Escherichia coli O157:H7 is one serotype of a group of *E. coli* pathogens known as verotoxin-producing *E. coli* (VTEC) or shiga toxin-producing *E. coli* (STEC). These other VTEC serotypes are increasingly recognized as an important cause of food-borne disease and standard methods for their detection have been established. Analysis for VTEC in the presence of other *E. coli* is challenging because, unlike *E. coli* O157, they have no shared physiological characteristics on which selective or differential culture media can be based.

Standard methods for a limited range O-serogroups, associated with higher public health risk (US, O26, O45, O103, O111, O121, O145; EU, O26, O103, O111, and O145), are published. These methods use enrichment and isolation media that are permissive for *E. coli* growth. Enrichment broths are screened by PCR for verotoxin (*stx*) and O-group genes; some protocols test for additional virulence factors, such as the intimin (*eae*). In an attempt to increase selectivity in isolation, IMS for target serogroups is used, but large numbers of colonies of diverse morphology must still be tested.

Other Pathogenic *Escherichia coli*

No cultural methods provide enrichment specific for pathogenic types of *E. coli*, nor are there any bacteriological media that allow their differentiation from nonpathogenic strains. Individual colonies are examined for virulence factors using molecular approaches. In meat samples, numbers of pathogenic strains are usually low and numerous colonies must be examined, with little chance of recovering a pathogenic strain. An example of a pre-enrichment and enrichment protocol is shown in Table 5. Typical colonies are selected for further characterization. If large numbers of *E. coli* are expected, surface plating may be appropriate (Table 6).

Table 4 Examples of selective and diagnostics agents used for the recovery and differentiation of pathogenic bacteria

Enrichment		*Plating*			*Appearance of colonies*
Medium	*Selective agents*	*Medium*	*Selective agents*	*Differential agents*	
Campylobacter spp.					
Bolton broth	Cefoperazone, vancomycin, trimethoprim, and cycloheximide	*Campylobacter* medium with charcoal	Cefoperazone and amphotericin B	NA[a]	NA[a]
Listeria spp.					
Listeria enrichment broth (UVM-1)	Acriflavin and nalidixic acid	Modified oxford agar	Colicin and moxalactam	Ferric ammonium citrate and aesculin	Hydrolysis of aesculin produces black halo around colonies. No change in color
Fraser broth	Acriflavin and nalidixic acid	Palcam agar	Polymyxin, ceftazidime, acriflavine, and lithium chloride	Mannitol, phenol red, ferric ammonium citrate, and aesculin	Hydrolysis of aesculin produces black halo around colonies. No change in color of medium and colorless colonies owing to lack of mannitol fermentation
Pathogenic *E. coli*					
Tryptone phosphate broth		Levine's eosin–methylene blue agar		Lactose, eosin Y, and methylene blue	Dark-centered colonies with or without a metallic sheen
		MacConkey agar	Bile salts	Lactose and neutral red	Fermentation of lactose results in formation of brick red colonies with or without a surrounding precipitate
Escherichia coli O157:H7/NM					
EHEC enrichment broth	Cefixime, cefsoludin, and vancomycin	Tellurite-cefixime sorbitol MacConkey agar	Bile salts and crystal violet	Sorbitol and neutral red	No change in color of the medium and colorless colonies owing to lack of sorbitol ferrmentation
Salmonella spp.					
Tetrathionate broth	Bile salts, brilliant green, and iodine	Bismuth sulfite agar	Brilliant green	Ferrous sulfite, bismuth sulfite indicator Lactose, sucrose, salicin, acid fuchsin, bromothymol blue, sodium thiosulfate, and ferric ammonium citrate	Brown, gray, or black colonies with or without a metallic sheen. Surrounding medium may be discolored
Rappaport–Vassiliadis medium	Magnesium chloride, sodium chloride, and malachite green	Hektoen enteric agar	Bile salts	Lactose, sucrose, salicin, acid fuchsin, bromothymol blue, sodium thiosulfate, and ferric ammonium citrate	Blue or blue green colonies with or without black centers. Colony may appear to be completely black
Yersinia spp.					
Irgasan–ticarcillin–chlorate broth	Ticarcillin, irgasan, potassium chlorate, and malachite green	Cefsulodin–irgasan–novobiocin agar	Cefsulodin, irgasan, novobiocin, crystal violet, and sodium deoxycholate	Mannitol and neutral red	Transparent colonies with deep red centers with entire or irregular edges

[a]Not applicable. Only *Campylobacter* will grow.

Abbreviation: EHEC, enterohaemorrhagic *E. coli*.

Table 5 Examples of pre-enrichment, enrichment, and plating procedures used for the recovery of meat-borne pathogens

Primary enrichment	*Secondary enrichment*	*Plating*	*Agency*[a]
Campylobacter spp.[b]			
Bolton broth a. Sample taken within 10 days of production, incubate for 4 h at 37 °C b. Sample taken 10 days or older from production, incubate at 30 °C for 3 h and at 37 °C for 2 h	Transfer primary enrichment broth to microaerobic atmosphere (42 °C for 24–48 h)	Abeyta–Hunt–Bark or modified Campylobacter blood-free agar (37–42 °C, 24–48 h)	USDA-FDA
Park and Sanders Enrichment Broth, incubated under microaerobic atmosphere (37 °C for 3–4 h)	Transfer primary enrichment broth to microaerobic atmosphere (42 °C for 24–48 h)	Campylobacter medium with charcoal and deoxycholate with cefoperazone, amphotericin, and Preston agar (37 °C, $\leq$72 h)	HC
None	Blood-Free Bolton enrichment broth incubated for 48 $\pm$ 2 h at 42 °C, under microaerobic atmosphere	Campy–Cefex agar (42 °C, 42–72 h) under microaerobic atmosphere	USDA-FSIS
Listeria spp.			
Buffered Listeria Enrichment Broth with sodium pyruvate, without selective agents (30 °C, 4 h)	Add selective agents to primary enrichment broth (30 °C, 20 and 44 h)	Oxford agar or Palcam agar or Modified Oxford agar (35 °C, 24–48 h) or lithium chloride-phenylethanol-moxalactam medium (LPM) fortified with esculin and Fe^{3+} (30 °C for 24–48 h)	USDA-FDA
Listeria Enrichment Broth (UVM-1 formulation) (30 °C, 48 h)	Inoculate Modified Fraser broth with primary enrichment broth after 24–48 h incubation (35 °C, 24–26 and 48 h)	Oxford agar and one of ALOA formulation agar, A.L. Agar, BBL CHROM agar Listeria, Chromogeneic Listeria Agar Plate, modified Oxford agar, PALCAM agar, RAPID'L.Mono (35 °C, 48), or LPM (30 °C, 48 h)	HC
UVM (Incubate at 30 °C, 23–26 h)	MOPS-BLEB (35 °C, 18–24 h)	Horse Blood Overlay Medium agar (35 °C, 18–26 h) and Modified Oxford agar (35 °C, 24–53 h)	USDA-FSIS
Pathogenic *E. coli* (non-O157)			
Brain-heart Infusion (35 °C, 3 h)	Transfer primary enrichment to double strength Tryptone-phosphate broth (44 °C, 20 h)	Levine's eosin–methylene blue and MacConkey agars (35 °C, 20 h)	USDA-FDA
Escherichia coli O157:H7 and O157:NM			
Modified Buffered Peptone water with pyruvate (37 °C, 5 h)	Add selective agents (Acriflavin–Cefsulodin–Vancomycin) to pre-enrichment (42 °C, 18–24 h)	TC-SMAC and Rainbow® Agar O157 or R&F® *E. coli* O157:H7 agar (37 °C, 18–24 h)	USDA-FDA
None	Modified Tryptone Soy broth (mTSB) with (42 °C, 15–24 h)	IMS with anti-O157 beads, acid treated or directly plated onto Rainbow agar with 5 mg l^{-1} novobiocin, 0.05 mg l^{-1} cefixime, and 0.15 mg l^{-1} potassium tellurite (35 °C, 20–24 h)	USDA-FSIS
mTSB with 20 mg l^{-1} novobiocin or for samples with high bacterial load Enterohemorrhagic *E. coli* Enrichment Broth (42 °C, 22–24 h)	Optional: Enterohemorrhagic *E. coli* Enrichment Broth (35 °C, 18–24 h)	IMS with anti-O157 beads, plated onto two agar media, either two group 1 or one group 1 and one group 2 Group 1: Modified Hemorrhagic Coli Agar with Tellurite and Cefsulodin (42 °C, 18–24 h) mHC Agar with CT supplement (35 °C, 18–24 h) BBL CHROMagar for *E. coli* O157 (BD) Group 2: Modified Sorbitol MacConkey agar with Tellurite, Cefixime, and Cefsulodin (42 °C, 18–24 h)	HC

(*Continued*)

Table 5 Continued

Primary enrichment	*Secondary enrichment*	*Plating*	*Agency*[a]
		CT-SMAC or CR-SMAC (35 °C, 18–24 h)	
VTEC serogroups O26, O45, O103, O111, O121, and O145			
None	mTSB with (42 °C, 15–24 h)	IMS beads acid treated or directly plated onto Rainbow agar with 5 mg l^{-1} novobiocin, 0.05 mg l^{-1} cefixime, and 0.15 mg l^{-1} potassium tellurite (35 °C, 20–24 h)	USDA-FSIS
Salmonella spp.			
Nutrient broth or buffered peptone water (35 ± 0.5 °C, 18–24 h)	Rappaport–Vassiliadis medium and tetrathionate brilliant green broth (42.5 °C, 24 ± 2 h)	Two of bismuth sulfite (BS) agar, brilliant green sulfa agars, or Brilliance Salmonella agar (35 °C, 24 h; BS can be incubated up to 48 h)	HC
Lactose broth (35 °C, 24 ± 2 h)	Rappaport–Vassiliadis medium (42 °C, 24 ± 2 h) and tetrathionate broth (43 °C if microbial load is high and at 35 °C if microbial load is low, 24 ± 2 h)	Bismuth sulfite, xylose lysine deoxycholate, and hektoen enteric agars (35 °C, 24 ± 2 h)	USDA-FDA
Buffered peptone water (35 °C, 20–24 h) or mTSB (42 °C, 15–24 h)	Rappaport–Vassiliadis R10 broth and TT broth (Hajna) (42 ± 0.5 °C, 22–24 h)	Double-modified lysine-iron agar (DMLIA), and brilliant green sulfa agars (35 °C, 18–24 h)	USDA-FSIS
Yersinia spp.[c]			
None	Irgasan–ticarcillin–cholate broth (25 °C, 2 and 3 day)	KOH treatment before plating or direct plating onto Cefsulodin–irgasan–novobiocin (CIN) agar (32 °C, 18 h)	USDA-FSIS
Tryptic soy broth (25 °C, 24 h)	Bile–oxalate–sorbose broth (25 °C, 3 and 5 day)	Salmonella-Shigella deoxycholate calcium agar (SSDC; 30 ± 1 °C, 24 h) and KOH treatment before plating or direct plating onto CIN agar (32 °C, 18 h)	USDA-FSIS
None	Phosphate buffered saline (4 °C, 14 day)	KOH treatment before plating or direct plating onto CIN agar (32 °C, 18 h)	USDA-FSIS

[a]USDA-FDA, United States Department of Agriculture, Food and Drug Administration; USDA-FSIS, United States Department of Agriculture, Food Safety Inspection Services; HC, Health Canada.
[b]Incubation conditions for *Campylobacter* spp. are microaerobic, established by flushing with a gas mixture (10% CO_2, 5% O_2, and 85% N_2) or using gas-generating kits.
[c]All three enrichment procedures are recommended followed by plating.

Listeria spp.

Listeriae are psychrotrophic and can grow under microaerobic conditions so that their numbers can increase during refrigerated storage of meats, both anoxically and aerobically packaged. *Listeria* spp. can be difficult to recover and two enrichment broths are usually used (Table 5). After enrichment, PCR screening test may be performed and enrichment media is streaked onto at least two selective media to ensure isolation of as many *Listeriae* as possible. Typical colonies are selected for biochemical and serological characterization. Recovery of injured cells is problematic with *Listeria* and the use of Listeria Repair Broth has been suggested. *Listeria* can be confirmed and differentiated using a kit such as the API Listeria kit, observation of hemolysis, and the CAMP test for β-lysin factor.

Salmonella spp.

Salmonella spp. are usually present in low numbers and form only a small part of the total microflora. Usually two enrichment broths and two plating media are recommended to allow recovery of most strains (Table 5). Enrichment broths may be screened by PCR for gene markers. Kits such as the AP1 20E (bio Merieux, Marcy-l'Etoile, France), MICRO-ID (bio Merieux), VITEK (bio Merieux), or Enterotube II (Becton Dickinson, Franklin Lanes, NJ) can be used to characterize putative salmonellae, but confirmation of *Salmonella* requires somatic (O) antigen and flagellar (H) antigen agglutination reaction using polyvalent and single grouping antisera.

Staphylococcus aureus

Staphylococcus aureus can be isolated from both raw and cooked meats; however, it is a poor competitor and its growth in raw meats is limited. It does grow well in cooked meats. Illness from *S. aureus* infection is the result of ingestion of a heat-resistant toxin, so that large numbers of *S. aureus* must have been present. Foods are usually examined for *S. aureus* using direct plating (Table 6) and there is almost universal acceptance that Baird–Parker agar is the most appropriate medium. It

Table 6 Examples of direct plating procedures for isolation of pathogens

Organism	*Filtration*	*Plating medium and incubation conditions*	*Additional steps*
Clostridium perfringens			
	Not used	Sulfite-cycloserine agar (pour plate, 35 °C, 24 h)	Count black colonies as presumptive *C. perfringens*
Escherichia coli			
	Membrane filter and surface plating	Nutrient agar (35–37 °C, 4 h), followed by tryptone-bile agar (44.5 °C, 20 h)	Soak filter in p-dimethylaminobenzaldehyde dissolved in 1 mol l^{-1} HCl and count pink (indole positive) colonies as *E. coli*
	Not used	Tryptone bile *X*-glucuronide agar (30 °C, 4 h followed by 44 °C, 18 h)	Count blue colonies as *E. coli*
	Hydrophobic grid membrane filter	Lactose–monensin–glucuronate agar (LMG, 35–37 °C, 24 ± 2 h), followed by buffered 4-methylumbelliferyl-β-D-glucuronide agar (BMA, 35–37 °C, 2 h)	Illuminate BMA agar plates with long-wavelength UV light and count blue-while fluorescent colonies as presumptive *E. coli*
Escherichia coli and *E. coli* O157:H7			
	Hydrophobic grid membrane filter	SD-39 agar (44.5 °C, 24 h)	Count green colonies as presumptive *E. coli*, pink as presumptive *E. coli* O157: H7
Staphylococcus aureus	Spread plating or hydrophobic grid membrane filter	Baird–Parker agar (35 °C, 48 ± 2 h)	Count black colonies[a] with a clear zone with or without a white precipitate in the medium as presumptive *S. aureus*
Listeria spp. and *Listeria monocytogenes*			
	Hydrophobic grid membrane filter	LM-137 agar with 50% egg yolk emulsion (35–37 °C, 22–24 h)	Count pink colonies as presumptive *Listeria* spp. and *Listeria monocytogenes.* Confirm *Listeria monocytogenes* using further testing

[a]When using HGMF, only the color of the colonies is considered.

contains both selective agents (lithium chloride, glycine, and potassium tellurite), which inhibit most other bacteria, and differential agents (egg yolk and potassium tellurite). Further biochemical tests include the coagulase test using human or rabbit blood plasma and identification using commercial systems such as API Staph or VITEK Gram-positive identification card.

Yersinia spp.

Yersinia spp. are able to grow at refrigeration temperatures and one approach to their isolation is cold enrichment (Table 5). Cultural methods for the isolation and identification of yersiniae require a great deal of effort and are thought to underestimate its presence. Molecular methods, either by deoxyribonucleic acid (DNA) colony hybridization or by nested PCR, have been shown to increase sensitivity significantly (25% using nested PCR). *Yersinia* spp. are resistant to alkaline treatment, so exposure to potassium hydroxide is used as an additional tool to separate *Yersinia* spp. from background flora. Identification of isolates as *Yersinia* spp. includes no utilization of citrate, only carbohydrate fermentation, no production of hydrogen sulfide, and evidence of urease activity.

Conclusion

In relation to meat-borne pathogens, standard methods are promulgated by regulatory agencies throughout the world. Such methods are necessary to support microbiological standards with legal status. In contrast, methods to recover, enumerate, and characterize meat-borne spoilage bacteria are considerably more variable and can best be described as 'recommended procedures' that have been widely practiced and are generally accepted. Harmonization of methodologies will be essential if it is required to compare meat-borne bacterial numbers reported by different laboratories.

See also: Microbiological Analysis: DNA Methods; Indicator Organisms in Meat. Microbiological Safety of Meat: *Clostridium Perfringens*; *Listeria monocytogenes*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*, *Yersinia enterocolitica*. Spoilage, Factors Affecting: Microbiological

Further Reading

AOAC International, 2014. Official Methods of Analysis. Available at: http://www.eoma.aoac.org/ (accessed 29.03.14).

Corry, J.E.L., Curtis, G.D.W., Baird, R.M. (Eds.), 2012. Handbook Culture Media for Food and Water Microbiology, third ed. Cambridge, UK: The Royal Society for Chemistry.

Downes, F.P., Ito, K. (Eds.), 2001. Compendium of Methods for the Microbiological Examination of Foods, fourth ed. Washington, DC: American Public Health Association.

Gharst, G., Oyarzabal, O.A., Hussain, S.K., 2013. Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. Journal of Microbiological Methods 95, 84–92.

Gill, C.O., Greer, G.G., 1993. Enumeration and Identification of Meat Spoilage Bacteria. Technical bulletin 19938E, Lacombe Technical Bulletin No. 6. Lacombe, AB: Agriculture and Agri-Food Canada. Revised 1999.

Greer, G.G., Dilts, B.D., 1997. Enumeration of meatborne spoilage bacteria with hydrophobic grid membrane filtration. Journal of Food Protection 60, 1388–1390.

Health Canada, 2014. The Compendium of Analytical Methods: Official Methods for the Microbiological Analysis of Foods. Available at: http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php (accessed 29.03.14).

Holzapfel, W.H., 1992. Culture media for non-sporulating gram-positive food spoilage bacteria. International Journal of Food Microbiology 17, 113–133.

International Organization for Standardization, 2014. Available at: http://www.iso.org/iso/home/standards.htm (accessed 29.03.14).

Jay, J.M., 2002. A review of aerobic and psychrotrophic plate count procedures for fresh meat and poultry products. Journal of Food Protection 65, 1200–1206.

Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), 2000. The Microbiological Safety and Quality of Food, vols. 1–2. Gaithersburg, MD: Aspen Publishers.

Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), 2000. Encyclopedia of Food Microbiology, vols. 1–3. London: Academic Press, UK.

Sharpe, A.N., Peterkin, P.I., 1988. Membrane Filter Food Microbiology. Letchworth: Research Studies Press.

Sidari, R., Caridi, A., 2011. Methods for detecting enterohaemorrhagic *Escherichia coli* in food. Food Reviews International 27, 134–153.

Taskila, S., Tuomola, M., Ojamo, H., 2012. Enrichment cultivation in detection of food-borne. Salmonella Food Control 26, 369–377.

U.S. Department of Agriculture, Food Safety and Inspection Service, 2014. Microbiology Laboratory Guidebook (MLG). Available at: http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook (accessed 29.03.14).

U.S. Department of Health and Human Services, Food And Drug Administration, 2014. Bacteriological Analytical Manual Online (BAM). Available at: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm (accessed 29.03.14).

Wasney, M.A., Holley, R.A., Jayas, D.S., 2001. Cresol red thallium acetate sucrose inulin (CTSI) agar for the selective recovery of *Carnobacterium* spp. International Journal of Food Microbiology 64, 167–174.

Relevant Websites

http://www.eoma.aoac.org/
AOAC International.

https://www.cen.eu
Comité Européen du Normalization (CEN).

http://www.food.gov.uk/
Food Standards Agency, UK.

http://www.foodstandards.gov.au
Food Standards Australia, New Zealand.

http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index-eng.php
Health Canada.

http://www.icmsf.org/
International Commission on Microbiological Specifications for Foods.

http://www.iso.org/iso/home/standards.htm
International Organization for Standardization.

http://www.nmkl.org/Engelsk/
Nordic Committee on Food Analysis.

http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
U.S. Department of Agriculture, Food Safety and Inspection Service.

http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
U.S. Department of Health and Human Services, Food And Drug Administration.

MICROBIOLOGICAL SAFETY OF MEAT

Contents

Aeromonas spp.

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Glossary

Cytotoxin A chemical substance (usually a protein) having specific toxicity to certain organs or tissues.
Endotoxin Refers to a bacterial toxin retained by the cell and not released extracellularly. Usually synonymous with the lipopolysaccharide component of the outer cell membrane of Gram-negative bacteria.
Enterotoxin A bacterial toxin causing vomiting, diarrhea, or abdominal pain.
Gastroenteritis Infection of the intestine usually resulting in diarrhea.
Hemolysin A biologically active protein molecule capable of rupturing red blood cells.
ß-Hemolysis A zone of clearing surrounding a bacterial colony on a blood agar plate.
Hemolytic uremic syndrome Bacterial infection resulting in lysis of red blood cells and severe damage to the kidneys.
Hepatobilliary Refers to the liver, gall bladder, and bile ducts.
Meningitis Infection of the meningeal membrane surrounding the brain.
Septicemia Occurrence of bacteria in the blood.

Introduction

The genus designation *Aeromonas* was established for bacterial cells with a single polar flagellum producing gas from glucose to distinguish such isolates from members of the genus *Vibrio* that characteristically also produce a single polar flagellum but do not produce gas from glucose. Members of the genus *Aeromonas* are Gram-negative, facultatively anaerobic, catalase positive, cytochrome oxidase positive, and rod-shaped bacteria. The genus *Aeromonas* has undergone a number of taxonomic changes over the past two decades. It was originally allocated to the family *Vibrionaceae*; however, subsequent phylogenetic studies indicated that the genus *Aeromonas* is not closely related to vibrios. This resulted in the establishment of a new family, the *Aeromonadaceae* under which the genus *Aeromonas* is housed.

The aeromonads are composed of two major subgroups. The mesophilic group, typified by *Aeromonas hydrophila*, consists of motile isolates that grow well at 35–37 °C and are associated with a variety of human infections. The second group consists of psychrophilic strains allocated to the species *Aeromonas salmonicida* that cause diseases in fish, are nonmotile,

and have optimal growth temperatures of 22–25 °C. In the last edition (2005) of Bergy's Manual of Systematic Bacteriology, deoxyribonucleic acid (DNA)–DNA hybridization studies led to the expansion of the original 5 *Aeromonas* species to recognition of 17 hybridization groups (HGs) or genospecies and 14 phenospecies including the 6 human pathogenic species, *A. hydrophila*, *Aeromonas caviae*, *Aeromonas veronii*, biotype sobria, *A. veronii* biotype veronii, *Aeromonas schubertii*, and *Aeromonas jandaei*. The term 'phenospecies' was coined to refer to a single heterogeneous species containing multiple HG groups within it.

There are presently 24 species names in the genus *Aeromonas* in the published literature derived from recent descriptions of new species, some of which are considered synonymous with previously described species. A minimum of five well-characterized strains has been recommended for each valid species in addition to almost complete 16S ribosomal ribonucleic acid (rRNA) sequences, phenotypic properties, and molar G+C content of the genomic DNA. The phylogenetic depth of the 16S rRNA gene tree for the genus *Aeromonas* is considered notably shallow, with all species exhibiting interspecies sequence similarity values of approximately 98% or higher and in most cases >99%.

Habitats

Members of the genus *Aeromonas* are found in a wide variety of habitats including freshwater rivers, lakes and streams, and marine water estuaries, and have been isolated from soil, the intestines of healthy cattle and humans, meat and vegetable products, and are frequently present in dairy products.

Occurrence of *Aeromonas* in Raw Meat and Poultry

A 1993 study indicated that *Aeromonas* spp. were commonly isolated from ground beef and poultry. The dominant species in ground meat were *A. hydrophila* and *A. caviae*. In chicken, *A. sobria* was common. *Aeromonas hydrophila* was isolated from 75% of ground meat samples and from 62% of chicken samples.

A later study indicated that a relatively high percentage 16/23 (70%) of *Aeromonas* containing samples from poultry (5/6), red meat (9/14), and other meat products (2/3) was reported by Neyts *et al*. in 2000 in a Belgium study. *Aeromonas* counts from one sample were as high as 10^5 cells per gram.

In a more extensive study, in a total of 563 samples of various foodstuffs purchased, 287 were found to contain mesophilic *Aeromonas* spp. The most frequently contaminated samples were poultry (79.3%) and offal (84.3%). The researchers concluded that both raw and cooked foods are potential sources of *Aeromonas* species such as *A. hydrophila* and *A. sobria*. This conclusion implies postprocessing contamination of cooked ready-to-eat foods.

A more recent study indicated that among 80% of raw turkey meat samples, 43 (53.8%) were positive for the presence of motile *Aeromonas* species. *Aeromonas hydrophila* was the most prevalent *Aeromonas* species followed by *A. sobria* and *A. caviae*. A separate study resulted in *Aeromonas* species isolated from 53/92 (57.6%) raw poultry samples and 27/158 (17%) minced meat samples. *Aeromonas hydrophila* and *A. caviae* were the most frequently isolated *Aeromonas* species followed by *A. sobria*, *A. jandaei*, and *A. veronii* in that order of frequency. A somewhat earlier study involving cuts of raw beef, goat, and lamb found 53/60 (88%) of samples to be positive for *Aeromonas*. The most frequent species isolated was *A. sobria* (67.3%) and *A. hydrophila* (21.2%). Three additional species occurred in minor proportions, with all the *Aeromonas* isolates exhibiting β-hemolysis. Initial *Aeromonas* counts for a variety of refrigerated meats have been found to range from $<10^2$ to $>10^5$ CFU g^{-1} and that after 7 days, numbers can increase from 1 to 3 logs in most raw meat products.

Phenotypic Characteristics

All members of the genus *Aeromonas* have been found to exhibit a number of common phenotypic features, which are listed in Table 1 along with characteristics of 98–99% of all isolates. The production of extracellular DNase, elastase, hemoysin, and H_2S has been found to be variable.

Virulence Factors

Although pathogenicity of *Aeromonas* was earlier open to some question, mounting evidence in recent years involving aeromonads solely present in septicemic, meningitis, and other infections and the recognition of the production of various toxins by the implicated isolates have contributed to the recognition that aeromonads are primary as well as opportunistic pathogens.

Pathogenicity among *Aeromonas* spp. is associated with the ability to produce exotoxins (hemagglutinins and hemolysins), cytotoxins, endotoxins, enterotoxins, siderophores, invasins, adhesins (pili), S-layer (surface array protein layer), flagella, several hydrolytic enzymes, and to invade tissue cultured cells. The hemolysin (also known as aerolysin) is encoded by the *aero* gene and has been shown to have hemolytic, enterotoxic, and cytolytic activity. Aerolysin is a hydrophilic protein that binds to eukaryotic cells and forms pores in the membrane resulting in the destruction of membrane permeability and cell lysis. Among these various virulence factors presumably contributing to the pathogenicity of *Aeromonas*, β-hemolysis seems to be related to enterotoxigenicity. There is evidence that adherence to tissue culture cells and hemolysis are plasmid mediated.

Human and Veterinary Clinical Infections

The large majority of *Aeromonas* clinical isolates consist of *A. hydrophila*, *A. caviae*, and *A. veronii* biovar sobria (Table 2). The genus *Aeromonas* comprises species that exhibit opportunistic and primary human pathogenicity causing septicemia among the immunocompromised, wound infections in otherwise healthy individuals, and various other illnesses including peritonitis, meningitis, and infections of the eyes, joints, and bones, in addition to gastroenteritis. Aeromonads can cause invasive infections, especially in patients with hepatobiliary diseases, malignancy, and diabetes mellitus.

Table 1 Phenotypic characteristics common to all species of *Aeromonas*

Characteristic	*Phenotype*[a]	*Characteristic*	*Phenotype*[b]
Cytochrome oxidase	+	Motility	+
Catalase	+	β-Galactosidase activity	+
Nitrate utilization	+	Resistance to O/129	+
Acid from glucose	+	Urea hydrolysis	–
Gas from glucose	+	Pectinolysis	–
Acid from D-trehalose	+	Acid from arabitol	–
Malonate utilization	–	Acid from D-raffinose	–
Mucate utilization	–	Acid from D-amygdalin	–
Fermentation of adonitol	–		
Fermentation of dulcitol	–		
Fermentation of erythritol	–		
Fermentation of D-xylose	–		
Growth in 0% NaCl	+		
Growth in 3% NaCl	+		
Growth in 6% NaCl	–		

[a]100% of all isolates.
[b]98–99% of all isolates.

Table 2 Identification tests for distinguishing motile *Aeromonas* species of frequent human clinical interest

Biochemical test	Aeromonas hydrophila	Aeromonas caviae	Aeromonas veronii bv. sobria
Esculin hydrolysis	+	+	–
Growth in potassium cyanide broth	+	+	±
H_2S from cysteine	+	–	±
L-arabinose utilization	+	+	–
Fermentation of salicin	+	+	–
Fermentation of mannitol	+	+	+
Gas from D-glucose	+	–	+
Methyl red test	+	+	–
Voges–Proskauer test	+	–	+
Indole production	+	+	+
Urocanic acid utilization	±	+	–
Stapholysin production	+	–	–
Lysine decarboxylase	+	–	–

Source: Reproduced from Koca, C., Sarimehmetoglu, B., 2009. Isolation and identification of motile *Aeromonas* spp. in turkey meat. Ankara Universitesi Veteriner Fakultesi Dergisi 56, 95–98; Abbot, S., Cheung, W., Kroske-Bystrom, S., Malekzadeh T., Janda, J., 1992. Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. Journal of Clinical Microbiology 30, 1262–1266; Abbott, S., Cheung, W., Janda, J., 2003. The genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. Journal of Clinical Microbiology 41, 2348–2357; and Janda, J., Abbott, S., 2010. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews 32, 35–73.

Diarrhea due to *Aeromonas* presents with varied clinical symptoms. Watery and self-limited diarrhea is common. Patients with cholera-like diarrhea and rice water stools have been reported. However, some patients may develop fever, abdominal pain, and bloody diarrhea. Approximately 35% of patients exhibit fever and vomiting. It has been difficult to prove in a definitive manner that aeromonads are causative organisms of gastroenteritis. No single clonally related outbreak of diarrhea has ever been reported to be associated with an aeromonad. In addition, no suitable animal model exists to allow Koch's postulates to be fulfilled, by inducing similar diarrhetic symptoms in a laboratory animal. However, there are a small number of cases in the literature where *Aeromonas* is unquestionably the cause of gastroenteritis, with the diagnosis based not only on isolation of the organism from feces but also on an immune response. One recent study of travelers to Africa, Asia, and Latin America, found that 50% of the individuals returning with *Aeromonas*-associated diarrhea had symptoms lasting 14 days or longer. The vast majority (>80%) of cases of *Aeromonas* septicemia occur in severely immunocompromised individuals. Predisposing factors for systemic infection are hepatic necrosis (54%) and malignancy (21%).

On an annual basis there are large numbers of individuals who are stricken each year with acute gastroenteritis, resulting in only members of the genus *Aeromonas* being isolated from feces, and the absence of other pathogens. In 1995, a food poisoning outbreak occurred involving *A. hydrophila*. A group of 27 people consumed a typical Swedish 'landgång' containing shrimps with mayonnaise, liver paté, ham, sausage, and legume salad purchased from a food store. Twenty-two of the 27 individuals became ill within 20–34 h after consumption. Symptoms included severe acute diarrhea, abdominal pain, headache, fever,

and vomiting. Among the remaining five healthy persons who consumed the leftover food the next day, two became similarly ill. Symptoms lasted for several days. Bacteriological examination of the leftover food samples resulted in the isolation of *A. hydrophila* from the shrimp with mayonnaise, smoked sausage, liver paté, and boiled ham. The number of *A. hydrophila* per gram of these foods was 10^6–10^7 per gram of food sample. *Aeromonas hydrophila* was not isolated from the legume/mayonnaise salad samples.

Among 1485 patients with acute gastritis reporting to a hospital in India in 2002, a total of 67 patients yielded *Aeromonas* strains as the sole bacterial pathogen. *Aeromonas hydrophila* (64.2%) was the dominant isolate followed by *A. sobria* (28.4%) and *A. caviae* (7.4%). The sources of the *Aeromonas* isolates were not identified.

An outbreak of acute diarrhea due to *A. sobria* in Benghazi occurred during a 1-month period in 1977. Among 69 patients admitted with acute gastroenteritis, 28 were positive for *A. sobria*. The source of the infections was not determined, nor was molecular typing of the isolates undertaken to determine if a single monoclonal strain was responsible.

Soft-tissue infections following water-related injuries are frequently due to *Aeromonas*. A number of pneumonia cases have been reported due to near drowning, resulting from aspiration of freshwater into the lungs, with symptoms occurring in less than 24 h with a death rate of 63%. Snake bites have been reported to result in infections by aeromonads. Infections in burn patients by aeromonads are notably rare, with less than two-dozen cases reported to date. Some of these cases yielded *Aeromonas* isolates from infected burn tissues and others from primary septicemic infections. *Aeromonas* appears to be a major nosocomial pathogen in patients with hepatic cirrhosis, resulting in septicemia with a rapidly fatal outcome. *Aeromonas hydrophila* and *A. veronii* biotype sobria have been reported to be causative agents in spontaneous empyema (puss in the pleural cavity) among hepatic cirrhotic patients exhibiting pleurisy. At least six cases of hemolytic uremic syndrome due to *Aeromonas* have been reported involving *A. sobria*, *A. hydrophila*, and *A. veronii* biotype sobria involving a cytotoxin with homology to Shiga toxin 1. These three species account for approximately 85% of all clinical isolates of *Aeromonas*. *Aeromonas hydrophila* has been reported to be the cause of abortion in sheep and cattle, whereas *A. sobria* has been associated with abortions in buffaloes. There are more than 96 distinct serogroups of *Aeromonas* on the basis of unique somatic antigens and they are not species specific.

In an interesting study in 2012, poultry farmers were found to be a high-risk group for *Aeromonas* infections. The study was initiated from recognition that there is a high incidence of *Aeromonas* in poultry stool samples, carcasses, and processing plant wastewater. Among a total of 63 stool samples from asymptomatic poultry workers employed by 23 poultry farms in Brazil, 12 (18.5%) were positive for *Aeromonas*. In contrast, stool samples from a control group of 72 from an urban community were all negative for *Aeromonas*. Poultry stools allowed the identification of *Aeromonas* in only 6 of the 23 farms (26.1%). In four of these farms, 50–70% of the workers were contaminated. Although asymptomatic at the time of analysis, 78% of the *Aeromonas*-positive workers complained of frequent diarrhea during the previous 6 months. Interestingly, all of the workers (those testing positive and negative) reported frequent gastrointestinal tract disorders during the first month of employment in the poultry farms.

Quorum-sensing molecules have been detected in *A. hydrophila* and *A. salmonicida* that result in the activation of one or more genes at a critical cell density. Little is known regarding the role of quorum sensing among these species, which potentially may influence biofilm formation and control of virulence genes.

The association of aquatic environments and human infections is exemplified by the tsunami that struck Thailand in December 2004. One study indicated that among the 305 tsunami survivors with skin or soft-tissue infections, *Aeromonas* was the single most frequently isolated pathogen, accounting for more than 20% of the 641 isolates identified.

Controlling the Numbers of *Aeromonas* in Foods

Considering that the ultimate source of *Aeromonas* spp. associated with meat and poultry is the intestines of warm-blooded animals, one approach to reducing the numbers of Gram-negative, nonspore-forming pathogens, such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Campylobacter*, and *Aeromonas*, is to scald the internal and external surface of carcasses with water at 176 °F (80 °C) and to guard against recontamination during further handling and processing. Giblets from poultry represent a subset of tissues to which this is not readily applicable. It is, therefore, essential that giblets be well cooked. A precautionary defense against the presence of Gram-negative pathogens, including *Aeromonas*, recommended by the World Health Organization, is to heat all meat to an internal temperature of at least 158 °F (70 °C).

An additional mechanism for the destruction of aeromonads is the application of gamma irradiation. In a recent study, chicken tissue was seeded with a mixture of *A. salmonicida*, *A. caviae*, *A. jandaei*, *A. hydrophila*, and *A. veronii*. The decimal reduction dose (D_{10}) value was found to be 0.089 ±0.003 kGy. This translates to the fact that a low dose of 1 kGy will destroy 10 log cycles of cells, which is far more than would be encountered on a raw meat product.

Cross-contamination from raw meat to raw vegetables should always to be avoided. This is most readily achieved in homes by vigorous washing of hands with hot water and soap or detergent after handling raw meat, and ideally applying a 70% alcohol-based handwash before handling raw vegetables. Some preservation methods, such as refrigeration, vacuum packaging, and modified atmosphere packaging appear ineffective in inhibiting the growth of *A. hydrophila*.

Antibiotic Sensitivity and Resistance

Aeromonas species have been reported to be largely resistant to penicillin, ampicillin, first-generation cephalosporins, carbenicillin, clindamycin, ticarcillin, and vancomycin. Susceptibility to chloramphenicol, trimethoprim-sulfamethoxazole, and fluoroquinolones has been consistently reported. A high level of *in-vitro* sensitivity to aztreonam, imipenem, and fluoroquinolones against *Aeromonas* species has been observed. Third-generation cephalosporins or fluoroquinolones,

especially ceftriaxone and cefotaxime are considered the agents of choice.

Isolation of Aeromonads

Aeromonads grow well at an alkaline pH range of up to pH 9.0, which is used in alkaline-peptone enrichments (pH 8.5–9.0) from stool, environmental, and food samples. Some of the mesophilic mobile aeromonads grow from 0 °C to 45 °C with an optimum growth temperature of 22–35 °C.

Ampicillin blood agar containing 20 mg ml^{-1} of ampicillin has been found to yield a higher recovery of *Aeromonas* directly from stool samples than Cefsulodin-Irgasan-Novobiocin (CIN) agar. It also has the advantage over CIN agar in that hemolytic colonies can be directly tested for cytochrome oxidase, which greatly reduces screening. It is to be noted that approximately 10% of all *Aeromonas* isolates are non-hemolytic. An alternative medium is *Aeromonas* agar (AA) which contains Irgasan, and uses D-xylose (which areomonads do not ferment) for differentiation. Pseudomonads which are indistinguishable from aeromonads on AA can be distinguished from aeromonads on the basis of their obligately aerobic metabolism using the Hugh-Leifson medium.

Commercial identification systems have proven to be unreliable for the identification of *Aeromonas* species, tending to erroneously identify most clinical *Aeromonas* isolates as *A. hydrophila*. It has been suggested that the cytochrome oxidase test be performed on all stool isolates to distinguish *Aeromonas* from enterobacteria.

Molecular Characterization of *Aeromonas* Isolates

A number of evolutionary markers have been applied to members of the genus *Aeromonas* for determining phylogenetic relationships among species. These include 16S rRNA, *gyrB* (B-subunit of DNA gyrase), *rpoD* (σ^{70}, RNA polymerase subunit), *rpoB* (β-subunit of DNA-dependent RNA polymerase), and *dnaJ* (heat shock protein 40 genes). Recent results indicate that there is less divergence in 16S rRNA gene sequences than there is within these four housekeeping genes, making them more valuable for species discrimination.

The use of the polymerase chain reaction (PCR) targeting the cytotoxin, enterotoxin, and hemolysin virulence genes present in mobile aeromonads from a variety of raw foods revealed 59%, 26%, and 33.3% of samples from fish, poultry, and shrimp to possess aeromonads carrying these genes. PCR assays of samples from hams and raw cured beef yielded 10.5% of *Aeromonas* isolates from both sources possess these virulence genes.

Fatty acid methyl ester analysis and amplified fragment length polymorphism have been used to identify *Aeromonas* isolates from foods to the DNA hybridization (HG) level.

The hemolysin and aerolysin genes have been used for genotyping *Aeromonas* isolates. A statistically significant correlation was found between cytotoxin levels (from Vero cell culture cytotoxicity assays) and the hemolysin genotype. Genotype 4 isolates (possessing both *ahh1* and *aerA* genes) expressed higher cytotoxin titers than isolates of other genotypes suggesting that genotype 4 isolates may have a greater clinical significance.

Polymerase Chain Reaction Detection of *Aeromonas*

Two fundamental approaches to the PCR detection of aeromonads have been utilized. One involves a pair of primers that amplify a conserved 16S rDNA sequence uniformly unique to the genus *Aeromonas*. The second involves a pair of primers unique and common to a specific species of *Aeromonas* such as the β-hemolysin or the cytolytic autolysin *aero* gene for the detection of *A. hydrophila*. Table 3 lists a number of PCR primers and the *Aeromonas* genes that have been targeted for amplification.

Distribution of *Aeromonas* Virulence Genes

The distribution of three toxin genes among 115 *Aeromonas* isolates from 1735 children with diarrhea has been studied. Alt is a heat-labile cytotonic enterotoxin; Ast is a heat-stable cytotonic enterotoxin; and Act is a cytotoxic enterotoxin. In addition, 27 aeromonads isolated from 830 control children, and 120 randomly selected aeromonads from different components of surface water in Bangladesh were also examined for the distribution of these three toxin genes. The number of isolates positive only for the presence of the *ast* gene was significantly higher for the environmental samples than for samples from diarrheal children. Isolates positive only for the presence of the *act* gene were not found in any of the three sources. Importantly, the number of isolates positive for both the *alt* and *ast* genes was significantly higher for diarrheal children than control children and the environment. Among the 11 *A. hydrophila* isolates from diarrheal children, none harbored the *alt* or *ast* genes individually, but 6 (54.6%) harbored both the genes. In contrast, none of the five isolates of *A. hydrophila* from diarrheal children harbored both the *alt* and *ast* genes whereas among the *A. hydrophila* isolates isolated from the environment, only two (11.1%) harbored both the *alt* and *ast* genes. The products of both the *alt* and *ast* genes may therefore synergistically act to induce severe diarrhea.

The presence of a type III secretion system (T3SS) has been reported for *A. hydrophila* and its contribution to virulence established. A cytotoxin-designated *Aex*T associated with a T3SS having ADP-ribosyltransferase activity has been described. The toxin was derived from a diarrheal isolate of *A. hydrophila*. A new *Aeromonas* T3SS effectors (*aexU*) has been described and characterized. The *aexU* gene was found in various isolates among 250 aeromonads from clinical and water sources.

Restriction Fragment Length Polymorphism Studies

The characterization of the PCR products from *Aeromonas* isolates from clinical, environmental, and food sources by PCR-restriction fragment length polymorphism (PCR-RFLP) using the endonuclease *Hpa*II and PCR-amplicon sequence analysis revealed three types of amplicons indicating that the virulence genes are classified into three main groups: (1)

Table 3 *Aeromonas* polymerase chain reaction primers and deoxyribonucleic acid (DNA) probes[a]

Primer or probe	*Sequence (5′→3′)*[a]	*Size of sequence (bp's)*	*Amplified Gene or DNA target sequence*	*References*
AP1	CAA-GGA-GGT-CTG-TGG-TGG-CGA-CA	208	*β*-Hemolysin	Xia *et al.* (2004)
AP2	TTT-CAC-CGG-CGG-TAG-CAG-GAT-TG			Xia *et al.* (2004)
EUB f933	GCA-CAA-GCG-GTG-GAG-CAT-GTG-G	500	16S rDNA	Ji *et al.* (2004)
EUB r1387	GCC-CGG-GAA-CGT-ATT-CAC-CG			Ji *et al.* (2004)
16S rDNA-F	AGG-TTG-ATG-CCT-AAT-ACG-TA	–	16S rDNA	Bi *et al.* (2007)
16S rDNA-R	CGT-GCT-GGC-AAC-AAA-GGA-CAG			Bi *et al.* (2007)
Aero-F	TGT-CGG-SGA-TGA-CAT-GGA-YGT-G	720	*aero*	Kong *et al.* (2002)
Aero-R	CCA-GTT-CCA-GTC-CCA-CCA-CTT-CA			Kong *et al.* (2002)
aexUCF	TTG-CCA-GCT-GTC-ACC-AGT-GC	–	*aexU*	Sha *et al.* (2007)
aexUCR	TTA-CAG-ATA-GTC-AGC-CCC-GAC			Sha *et al.* (2007)
AERO1	CCA-AGG-GGT-CTG-TGG-CGA-CA	–	*aero*	Tombelli *et al.* (2000)
AERO2	TTC-CAC-CGG-TAA-CAG-GAT-TG			Tombelli *et al.* (2000)
AERO probe	CAC-CAG-GTA-TTG-GAC-GCT-GTC-CC	–	*aero*	Tombelli *et al.* (2000)
1a	CCA-AGG-GGT-CTG-TGG-CGA-CA	209	*aero*	Özbas *et al.* (2000)
1b	TTT-CAC-CGG-TAA-CAG-GAT-TC			Özbas *et al.* (2000)
Aero 2A	AAG-CAA-TAT-TGT-CGG-CAT-GA	150	*aero*	Özbas *et al.* (2000)
Aero 1b	TTT-CAC-CGG-TAA-CAG-GAT-TC			Özbas *et al.* (2000)
Aerola	CCA-AGG-GGT-CTG-TGG-CGA-CA	209	*aero*	Pollard *et al.* (1990)
Aerolb	TTT-CAC-CGG-TAA-CAG-GAT-TG			Pollard *et al.* (1990)
UPF	AAA-CTC-AAA-GGA-ATT-GAC	500	16S rDNA	Peng *et al.* (2002)
UPR	GAC-GGG-CGG-TGT-GTA-CAA			Peng *et al.* (2002)
AHCF1	GAG-AAG-GTG-ACC-ACC-AAG-AAC-A	232	AHCYTOEN	Kingombe *et al.* (1999)
AHCR1	AAC-TGA-CAT-CGG-CCT-TGA-ACT-C			Kingombe *et al.* (1999)
AHH1F	GCC-GAG-CGC-CCA-GAA-GGT-GAG-TT	130	*Ahh*1	Wang *et al.* (2003)
AHH1R	GAG-CGG-CTG-GAT-GCG-GTT-GT			Wang *et al.* (2003)
AH-aerAF	CAA-GAA-CAA-GTT-CAA-GTG-GCC-A	309	*aer*A	Wang *et al.* (2003)
AH-aerAR	ACG-AAG-GTG-TGG-TTC-CAG-T			Wang *et al.* (2003)
ASA1F	TAA-AGG-GAA-ATA-ATG-ACG-GCG	249	*asa*1	Wang *et al.* (2003)
ASA1R	GGC-TGT-AGG-TAT-CGG-TTT-TCG			Wang *et al.* (2003)
A16SF	GGG-AGT-GCC-TTC-GGG-AAT-CAG-A	356	16S rDNA	Wang *et al.* (2003)
A26SR	TCA-CCG-CAA-CAT-TCT-GAT-TTG			Wang *et al.* (2003)
AHCF1	GAG-AAG-GTG-ACC-ACC-AAG-AAC-A	232	*ahc*	Fukushima *et al.* (2003)
AHCR1	AAC-TGA-CAT-CGG-CCT-TGA-ACT-C			Fukushima *et al.* (2003)
Aer-F	AGA-GTT-TGA-TCA-TGG-CTC-AG	1502	16S rDNA	Borrel *et al.* (1997)
Aer-R	GGT-TAC-CTT-GTT-ACG-ACT-T			Borrel *et al.* (1997)
FP	TCA-TGG-CTC-AGA-TTG-AAC-GCT	599	16S rDNA	Arora *et al.* (2006)
RP	CGG-GGC-TTT-CAC-ATC-TAA-CTT-ATC			Arora *et al.* (2006)
FP	GCA-GAA-CCC-ATC-TAT-CCA	252	*aero*	Arora *et al.* (2006)
RP	TTT-CTC-CGG-TAA-CAG-GAT-TG			Arora *et al.* (2006)

[a]S = G or C; Y = C or T.

aerolysins-hemolysins, (2) cytolytic enterotoxins, and (3) cytotonic enterotoxins.

RFLP has been applied to reference strains of all species of *Aeromonas* and 76 clinical isolates of diverse origin. PCR primers were used to amplify a 1502-pb sequence of the 16S rDNA of all *Aeromonas* species. The resulting amplicons were restricted with *Alu*I and *Mbo*I and the resulting DNA fragments (33–346-bp) resolved as banding patterns by agarose gel electrophoresis. The resulting banding patterns were used to identify isolates of *Aeromonas* at the species level. Most RFLP results were in agreement with biochemical identification.

See also: Biofilm Formation. Environmental Contaminants. Foodborne Zoonoses. Irradiation. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat. **Microbiological Analysis:** DNA Methods. **Microbiological Safety of Meat:** Hurdle Technology. **Microorganisms and Resistance to Antibiotics, the Ubiquity of:** Antibiotic Resistance by Microorganisms; Potential Environmental and Wildlife Sources of Microorganisms in Meat. **Minced Meats. Modeling in Meat Science:** Microbiology. **Nutrition of Meat Animals:** Poultry. **Physical Measurements:** Temperature Measurement. **Quality Management:** Abattoirs and Processing Plants. **Species of Meat Animals:** Cattle; Pigs; Poultry; Sheep and Goats; Shellfish

Further Reading

Abbot, S., Cheung, W., Kroske-Bystrom, S., Malekzadeh, T., Janda, J., 1992. Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. Journal of Clinical Microbiology 30, 1262–1266.

Abbott, S., Cheung, W., Janda, J., 2003. The genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. Journal of Clinical Microbiology 41, 2348–2357.
Fricker, C., Ampsett, S., 1989. *Aeromonas* spp. in foods: A significant cause of food poisoning? International Journal of Food Microbiology 9, 17–23.
Garcia-Gimeno, R., Sanchez-Pozo, M., Amaro-Lopez, M., Zurera-Cosano, G., 1996. Behavior of *Aeromonas hydrophila* in vegetable salads stored under modified atmosphere at 4 and 15 °C. Food Microbiology 13, 369–374.
Hänninen, M., 1993. Occurrence of *Aeromonas* spp. in samples of ground meat and chicken. International Journal of Food Microbiology 18, 339–342.
Ingham, S., 1990. Growth of *Aeromonas hydrophila* and *Plesiomonas shigelloides* on cooked crayfish tails during cold storage under air, vacuum and modified atmosphere. Journal of Food Protection 53, 665–667.
Janda, J., Abbott, S., 2010. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews 32, 35–73.
Koca, C., Sarimehmetoglu, B., 2009. Isolation and identification of motile *Aeromonas* spp. in turkey meat. Ankara Universitesi Veteriner Fakultesi Dergisi 56, 95–98.
Krovacek, K., Dumontet, S., Eriksson, E., Baloda, S., 1995. Isolation, and virulence profiles, of *Aeromonas hydrophila* implicated in an outbreak of food poisoning in Sweden. Microbiology and Immunology 39, 655–661.
Levin, R.E., 2009. Molecular methods for detecting and discriminating virulent aeromonads associated with foods and human clinical infections – A mini review. Food Biotechnology 23, 1–7.
Martin-Carnaham, A., Joseph, S., 2005. Genus I. *Aeromonas* Stanier 1943., 213[AL]. In: Brenner, D.J., Kried, N.R., Staley, J.T., Garrity, G.M. (Eds.), Bergey's Manual of Systematic Bacteriology, second ed., vol. 2, part B. New York: Springer-Verlag, pp. 557–578.
Nagar, V., Bandekar, J., 2011. Effectiveness of radiation processing in elimination of *Aeromonas* from food. Radiation Physics and Chemistry 80, 911–916.
Neyts, K., Huys, G., Uyttendaele, M., Swings, J., Debevere, J., 2000. Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. Letters in Applied Microbiology 31, 359–363.
Taher, A., Rao, B., Alganay, L., El-arabi, M., 2000. An outbreak of acute gastroenteritis due to *Aeromonas sobria* in Benghazi, Libyan Arab Jamahiriya. Eastern Mediterranean Health Journal 6, 497–499.
Vasaikar, S., Saraaswathki, K., De, A., Varaiya, A., Gogate, A., 2002. *Aeromonas* species isolated from cases of acute gastroenteritis. Indian Journal of Medical Microbiology 20, 107–109.
Zanella, J., deLuz, R., Fadanelli, R., *et al*., 2012. High prevalence of *Aeromonas* spp. in poultry farmers from a rural community of South Brazil. Asia-Pacific Journal of Molecular Biology and Biotechnology 20, 93–98.

Relevant Websites

www.sciencedirect.com/science/article/pii/016816059390156B
Elsevier Science Direct.
www.sciencedirect.com/science/article/pii/S0740002099903052
Elsevier Science Direct.
www.ncbi.nlm.nih.gov/pubmed/1904764
National Center for Biotechnology Information.
www.ncbi.nlm.nih.gov/pubmed/8484917
National Center for Biotechnology Information.
www.ncbi.nlm.nih.gov/pubmed/8643030
National Center for Biotechnology Information.
foodsafety.govt.nz/elibrary/industry/Aeromonas-Science_Research.pdf
New Zealand Food Safety Authority.
dergiler.ankara.edu.tr/dergiler/11/872/11066.pdf

Bacillus cereus

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Glossary

Chromogenic Media Culture media incorporating at least one chromogen, which is a substance that can be converted into a detectable pigment or dye. The ability to utilize the chromogen substrate is diagnostic of the target organism/s.
Dodecadepsipeptide Dodeca (*Gk*. 12) and depsipeptide is a peptide in which one or more of the amide (-CONHR-) bonds are replaced by ester (COOR) bonds. Cereulide is a twelve-chain depsipeptide.
Emetic syndrome Vomiting caused by a toxin that survives high temperatures and survives in cooked foods.
Enterotoxin Toxin results in a diarrheal illness – it is heat labile and the most common cause of *B. cereus* food poisoning.
Enzyme-linked immunosorbent assay (ELISA) An immunological assay technique making use of an enzyme bonded to a particular antibody or antigen.
Liquid chromatography–mass spectrometry (LC–MS) LC–MS is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (HPLC) with the mass analysis capabilities of mass spectrometry.

Introduction

Bacillus cereus is ubiquitous in the environment and can be found in soil, dust, air, water, decaying matter, and plants. Consequently, raw foods of plant origin are the major source of *B. cereus*. Dried herbs, spices, and dehydrated foods have all been shown to contain *B. cereus*. The widespread distribution of this organism, ability of its spores to survive dried storage, and the thermal resistance of spores mean that processed foods can contain *B. cereus*. Thus, control measures to prevent growth of *B. cereus* in those foods are required, especially if cooking has eliminated the competing microflora.

Bacillus cereus is an aerobic endospore former next in importance to *Bacillus anthracis* as a pathogen of humans and other animals. *Bacillus cereus* was first associated with food poisonings in the 1950s, when outbreaks involving vanilla sauce were reported. It causes two forms of food poisoning: an emetic syndrome and a diarrheal illness. The emetic syndrome is caused by a toxin that survives high temperatures and exposure to trypsin, pepsin, and pH extremes. This syndrome is mostly associated with cooked rice and other cereal-based foods. For example, in an incident involving seven people, the heat stable toxin was indirectly ingested and caused illness via contaminated hands following the use of contaminated rice in a children's kindergarten craft activity. The diarrheal illness is caused by a heat-and acid-labile enterotoxin and is mostly associated with proteinaceous foods. Reported outbreaks of *B. cereus* food poisoning due to meat products have been of the latter type.

Although *B. cereus* can be isolated from raw meat, it is mostly the ingredients added to processed meat products such as sausages, or seasonings added to spiced meat dishes, that is the cause of *B. cereus* food poisoning involving meat products. When processed meats containing *B. cereus* are improperly stored after cooking, surviving endospores may germinate and grow to high numbers.

Characteristics of *Bacillus cereus*

Bacillus cereus is a Gram-positive or Gram-variable spore-forming rod (Figure 1). It grows best aerobically but grows slowly under anaerobic conditions. The rods, which are typically 1.0–1.2 μm in diameter and 3.0–5.0 μm in length, tend to grow in long chains. The optimum temperature for growth is between 28 and 35 °C, although the growth temperature range extends from 5 to 50 °C; it has been reported that some strains can grow even at 55 °C. It can grow over a pH range from 4.3 to approximately 9.3 and at salt concentrations up to 7.5% w/v. The minimum water activity (a_w) for vegetative growth is 0.912. The generation time is in the range 18–27 min, under optimum conditions.

Although vegetative cells of *B. cereus* are readily killed by heat, the spores are moderately heat resistant. Heat resistance varies with differences in food matrices. Heat resistance of spores is increased in high-fat and oily foods. For example, in soybean oil, the time for *B. cereus* numbers to be reduced by

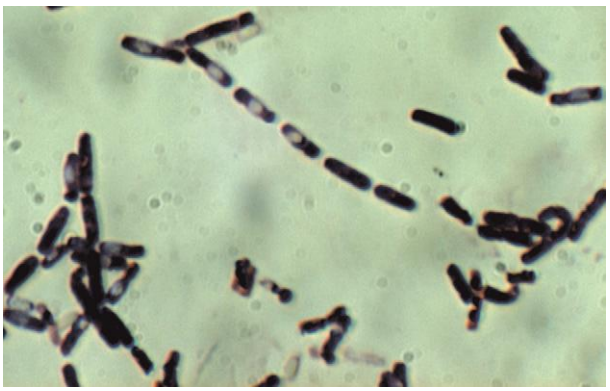

Figure 1 Gram stain of *Bacillus cereus* cultured on sheep blood agar showing typical growth in chains and endospore formation.

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90% (the *D* value) is 30 min at 121 °C. Other examples of heat resistance that have been recorded are D_{85} °C = 33.8–106 min in phosphate buffer and D_{95} °C = 1.5–36.2 min and 1.8–19.1 min in distilled water and milk, respectively. Heat resistance is also enhanced in foods of low water activity.

Spores of different strains vary considerably in heat resistance. Some have *D*-values at a given temperature up to 20 times greater than those of more sensitive strains. Spores are more resistant to dry heat than to moist heat and can remain dormant for long periods in dried foods stored at ambient temperatures.

Once it is formed, boiling, frying, roasting, and microwave heating will not destroy or biologically inactivate the extremely heat-stable emetic toxin. The toxin can survive 90 min at 126 °C and is not affected by extremes of pH (2–11). In contrast, the diarrheal toxins are inactivated at 56 °C in 5 min.

Isolation and Identification

Most procedures for isolation of *B. cereus* from meat involve direct plating onto a selective agar. The selective agars commonly used are polymyxin egg yolk mannitol bromothymol blue agar (PEMBA), mannitol egg yolk polymyxin (MYP) agar, and Kim and Goepfert agar. The antibiotic polymyxin B is added to the media as the preferred selective agent, because it inhibits growth of most of the Gram-negative bacteria that usually predominate in meat microflora.

Most media incorporate egg yolk for ready recognition of the *B. cereus* colonies. A turbid zone forms around a colony of *B. cereus* in the presence of egg yolk, owing to the organisms' secretion of an enzyme complex that includes lecithinase and phospholipase, which react with the egg yolk. However, some strains of *B. cereus* have lost the ability to form a turbid zone, probably because the genes for these enzymes are not expressed. Most media include mannitol and an indicator to allow discrimination between fermenters and nonfermenters of mannitol. *Bacillus cereus* does not ferment mannitol, but it hydrolyzes casein in order to change the pH of the medium to alkaline under and around the colony. On MYP agar, the agar changes to pink color, whereas on PEMBA, the color of the colonies is bluish. Chromogenic media are being used more frequently in food laboratories, and the media properties that allow selection of *B. cereus*, *Bacillus thuringiensis*, and *Bacillus weihenstephanensis* are improving. Each chromogenic medium has its own advantages and limitations. Some limitations seem to be resolved by incubation at 30 °C instead of 37 °C, as is recommended.

Bacillus cereus may occur in foods as vegetative cells, as sporulating sporangia or, as free spores. However, in organic liquid media such as milk, vegetative cells do not sporulate readily at refrigeration temperatures. The recovery of *B. cereus* from most foods does not usually require a resuscitation step, as most spores germinate readily. Therefore, isolation by spread plating on the media mentioned above is adequate. There are certain foods such as dried meat meals or protein powders that may have been subjected to heating and drying processes that leave *B. cereus* present as only dormant spores. In this case, it may be necessary to use a method that includes a resuscitation step, such as a three-tube most probable number (MPN) method using tryptic soy broth supplemented with polymyxin B. Positive growth in each tube is confirmed by streaking a loopful of the contents onto MYP agar plates. The MPN indicated by the pattern of positive tubes is a good estimate of the concentration of bacteria in the tested food.

Bacillus cereus and organisms closely related to it, including *B. anthracis*, *B. thuringiensis*, *B. mycoides*, and *B. weihenstephanensis* are included in the *B. cereus* complex. *Bacillus* sp. strain Ba813 carries a chromosomal marker, Ba813, that is otherwise found only in *B. anthracis*. *Bacillus thuringiensis* is an insect pathogen that has become very important in food microbiology. Its use as a commercial insecticide has increased its presence in food commodities where it can be mistakenly identified as *B. cereus*. Although there have been no outbreaks linked to *B. thuringiensis*, toxigenic strains of the organisms have been identified. *Bacillus thuringiensis* may then have some role in foodborne illness. *Bacillus mycoides*, (previously, *B. cereus* var. *mycoides*) is used as a promoter of plant growth. It has recently been separated into two species, *B. mycoides* and *B. pseudomycoides*. *Bacillus weihenstephanensis* now encompasses psychrotolerant strains of *B. cereus*. This new species grows at 7 °C but not at 43 °C and can be identified rapidly using rDNA or PCR targeted to a gene for the shock protein *cspA*. The taxonomic position of *B. weihenstephanensis* may still be in doubt, because strains intermediate between it and *B. cereus* exist and there are psychrotrophic strains of *B. cereus* that cannot be classified as *B. weihenstephanensis* as they do not carry the *cspA* gene. In at least one study, cytotoxicity and the growth temperature range *of B. cereus* strains were found to be independent. Some strains of *B. cereus* may be able to adapt to cold storage, and strains of *B. weihenstephanensis* have been shown to tolerate exposure to 47 °C after exposure to the nonlethal temperature of 38 °C. Exposure to other stresses such as high salt concentration, low pH, a high ethanol concentration, or low temperature also result in development of increased heat tolerance in *B. weihenstephanensis*. The hemolytic enterotoxin HBL seems to be widely distributed among strains of the *B. cereus* group. The enterotoxin is not associated with a particular species or a specific environment. In one study of 50 *B. weihenstephanensis* strains, 72% were not cytotoxic, although all strains had part of at least one of the *B. cereus* enterotoxins Hbl, Nhe, or CytK. *Bacillus weihenstephanensis* was found to produce the emetic toxin cereulide at temperatures as low as 8 °C.

It has been suggested that the *B. cereus* group may constitute a single species; difficulties remain with conventional laboratory differentiation of the current species. Evaluation of *Bacillus* growth characteristics on Anthrax Blood Agar and Cereus Ident Agar showed that some strains of *B. cereus*, *B. mycoides*, *B. thuringiensis*, and 30% of *B. weihenstephanensis* strains could be misidentified as *B. anthracis*. Indeed, the Australian Standard Method used in NATA-accredited laboratories for isolating *B. cereus* from foods draws attention to the fact that although *B. cereus* can be reported on the certificate of analysis, the methodology does not differentiate *B. cereus* and *B. thuringiensis*.

Identification of *B. cereus* isolates in most laboratories is based on a traditional morphological and biochemical classification scheme. If this approach is complemented with molecular techniques, classification of isolates can be more complicated. However, identification based on methods such

as gas chromatography–mass spectrometry, whole-cell fatty acid analysis, 16 s RNA gene sequencing, or other methods of genetic analysis is not commonly performed in food laboratories.

The best approach is to confirm that the isolate belongs to the *B. cereus* complex (Table 1) and then to differentiate *B. cereus* from the other species included in the complex (Table 2). When access to a fully equipped microbiology laboratory is limited, the miniaturized API 50 CHB kit (bioMérieux, Marcy-l'Étoile, France) can be used.

Characteristics of Foodborne/Meatborne Disease

The *B. cereus* emetic type of illness occurs 1–6 h after consuming contaminated food, whereas the diarrheal illness occurs 10–12 h after consumption. The symptoms of the emetic syndrome, which result from ingestion of preformed toxin in foods, are nausea and vomiting, occasionally followed by diarrhea. The illness can be confused with *Staphylococcus aureus* intoxication.

Diarrheal symptoms result from ingestion of vegetative cells or spores and their subsequent multiplication and toxin production within the intestinal tract. Although diarrheal toxins may be produced during exponential growth in foods, their sensitivity to low pH and proteolytic enzymes precludes survival of the toxin in the environment of the stomach. The symptoms of abdominal pain, watery diarrhea, and occasional nausea are indistinguishable from those arising from *Clostridium perfringens* infection.

Recovery is rapid for both syndromes, usually within 12–24 h. Very few fatalities have been reported. However, in a large outbreak associated with the consumption of vegetable puree that affected 44 elderly persons, 6 patients developed bloody diarrhea and 3 died of necrotic enteritis.

All people are believed to be susceptible to intoxication and infection, but the intensity of symptoms may vary between individuals. There are no known long-term effects of *B. cereus* intoxication or infection. Unusual forms of *B. cereus* illness, such as invasive infection in immunocompromised children following consumption of tea, have been reported. Also, toxigenic *B. cereus* complex organisms have been isolated from commercial ground roasted coffee, which suggests that coffee may be a vehicle for *B. cereus* food poisoning.

Generally, large numbers of *B. cereus* ($>10^5$ g^{-1} of food) are required to produce enough toxin to cause illness or infection. However, some strains may cause food poisoning at concentration as low as 10^3–10^4 bacteria per gram. Any food containing more than 10^4*B. cereus* per gram can be considered unsafe for consumption.

Most *B. cereus* food poisoning incidents result from eating cooked foods that have been cooled slowly and stored incorrectly. Outbreaks of emetic-type illness generally result from eating rice dishes or starchy foods such as potato and pasta. Outbreaks of diarrheal type illness result from eating a variety of foods, from vegetables and salads to meat and

Table 1 Confirmation of an isolate as a member of the *Bacillus cereus* complex

Characteristics	*Description*
Width of rod	1.0–1.2 μm, medium-length broad rods
Voges–Proskauer reaction	Positive
Catalase	Positive
Mannitol	Negative
Egg yolk turbidity factor	Positive (some strains could have delayed reaction)
Anaerobic growth	Positive
Spore[a]	Presence of oval spores stained green. Do not swell cell
Lipid granules[a]	Presence of lipid granules within cells, stained dark blue. Other parts of vegetative cells stained red

[a]Rapid confirmatory test includes staining with 5% malachite green for spores, with 0.3% Sudan black for presence of lipid granules, and with 0.5% safranin as a counterstain (Bennett and Belay, 2001).

Source: Adapted from Jenson, I., Moir, C.J., 1997. *Bacillus cereus* and other *Bacillus* species. In: Hocking, A.D., Arnold, G., Jenson, I., Newton, K., Sutherland, P. (Eds.), Foodborne Microorganisms of Public Health Importance, fifth ed. Sydney, Australia: AIFST (NSW Branch), pp. 379–406.

Table 2 Characteristics for differentiating *Bacillus cereus* from other members of the *B. cereus* complex

Characteristics	B. cereus[a]	B. thuringiensis	B. mycoides	B. anthracis
Hemolysis on sheep blood agar	Positive	Positive	Positive (weak)	Negative
Rhizoid growth	No	No	Yes	No
Motility	Yes	Yes	No	No
Parasporalcrystal[b]	No	Yes	No	No
Lysis by gamma phage[c]	No	No	No	Yes

[a]Includes *B. weihenstephanensis*, a psychrotolerant species proposed by Lechner *et al.* (1998). The only difference from *B. cereus* is that this species can grow at 4–7 °C and expresses the cold shock protein homolog *cspA* gene.

[b]Best viewed when vegetative cells are in the sporangia stage.

[c]Claus and Berkeley (1986).

Source: Adapted from Jenson, I., Moir, C.J., 1997. *Bacillus cereus* and other *Bacillus* species. In: Hocking, A.D., Arnold, G., Jenson, I., Newton, K., Sutherland, P. (Eds.), Foodborne Microorganisms of Public Health Importance, fifth ed. Sydney, Australia: AIFST (NSW Branch), pp. 379–406.

casseroles, in which *B. cereus* organisms have grown to large numbers. Meat products that have been responsible for *B. cereus* diarrheal food poisoning outbreaks include sausages, meat loaf, barbecued chicken, forcemeat, tongue, beef stew, veal broth, and cooked turkey. In Hungary, there has been a clear association between the large number of *B. cereus* food poisoning cases and consumption of highly spiced meat dishes.

Bacillus thuringiensis has been isolated from an institutional food poisoning outbreak, which also involved Norwalk-like virus, but *B. mycoides* has not yet been linked to any outbreak, although some strains carry enterotoxin genes. Species of *Bacillus* that are not members of the *B. cereus* complex, namely *B. subtilus* and *B. licheniformis*, have been associated with foodborne illness. Cooked turkey, peppered steak, meat pasties, pies and rolls, meat curry, stuffed poultry meat, meat paté, and Greek meat dishes have been vehicles of *B. subtilis* food poisonings. Meat dishes incriminated in *B. licheniformis* food poisonings include meat and poultry pies and pasties, meat stews, curried meat and poultry, chicken with sauce, boiled sausages, meat paté, and cold cooked meats.

Mechanism of Pathogenicity

The toxins that induce the two syndromes are very different from one another. The emetic toxin is a 1.2-kDa dodecadepsipeptide and is preformed in the food. Also known as cereulide, this emetic toxin is believed to be enzymatically synthesized rather than being a gene product. Cereulide exhibits fungistatic action and so may give *B. cereus* a competitive advantage in food ecosystems. The toxin does not appear to be associated with sporulation. Nonspecific toxin activity assays, such as formation of vacuoles in Hep-2 or Vero cells in tissue culture, are used for detection of cereulide. When incubated with boar spermatozoa, cereulide disrupts the outer membrane of mitochondria, causing them to swell. The toxin does not cause fluid accumulation in rabbit and mouse ileal loops. It has been shown to stimulate the vagus afferent nerves by recognition of the 5-HT_3-receptor site. However, the mechanism of action of cereulide is still not defined.

The diarrheal syndrome is caused by two types of enterotoxin. These related toxins are the hemolytic BL enterotoxin (HBL) and the nonhemolytic enterotoxin (NHE). Both enterotoxins are composed of three proteins. The HBL enterotoxin can be detected by the *B. cereus* Enterotoxin Reverse Passive Latex Agglutination (BCET-RPLA) test kit from Oxoid (Unipath, Basingstoke, UK). The L2 component is the antigen detected by this kit. This protein is not the enterotoxigenic component; the B component is. These two components form a complex that produces the biological effect.

The NHE enterotoxin, which is also known as the *Bacillus* diarrheal enterotoxin or BDE, can be detected by the TECRA *Bacillus* Diarrheal Enterotoxin Visual Immunoassay (VIA) kit (Tecra Diagnostics, Roseville, Australia). The TECRA VIA kit detects the 40-kDa and 41-kDa nontoxic components of the NHE enterotoxin. A third type of toxin, enterotoxin T, has been cloned and sequenced. It has limited homology with the nucleotide sequence of the enterotoxin B component of the HBL toxin, but its biological significance remains to be determined.

Strains of *B. cereus* that produce emetic toxin may also produce one or both of the diarrheal enterotoxins. This explains the occasional manifestation of diarrhea in emetic intoxications. Because cereulide is very stable to heat, low pH, and proteolytic enzymes, preformed toxin in foods is unlikely to be inactivated during passage through the stomach. Cereulide is not antigenically active and no ELISA-style commercial test kit is available for easy detection of the emetic toxin. Only very specialized laboratories with tissue culture capability and specialized skills have been able to detect and quantify this toxin. However, recently it has been shown that emetic toxin can be accurately quantified by high performance liquid chromatography (HPLC)–mass spectrometry. LC–MS/MS techniques have now been developed to detect cereulide.

Epidemiology

The true burden of illness acquired from food is unknown, because episodes involving less commonly identified pathogens such as *B. cereus* are less likely to have their etiologies confirmed; these organisms are not always considered in clinical, epidemiological, and laboratory investigations of food-associated illness; and they commonly cause sporadic cases rather than major outbreaks. Illness associated with food products may also be underreported, because few of those affected seek medical attention owing to the mild nature and short duration of symptoms.

Food poisoning caused by *B. cereus* is recognized by symptoms such as vomiting, cramps, abdominal pain, and diarrhea. The order in which symptoms are experienced and the incubation period between consumption of suspected food and onset of symptoms are important for initial diagnosis of food poisoning. Other information that can assist with epidemiological investigations are details of recent food consumption before the episode, attack rates relating to types of food consumed, and isolation of *Bacillus* organisms from feces or vomit during the acute stage of the illness. Demonstration of enterotoxin in fecal samples further assists the diagnosis.

Bacillus cereus is not part of the normal gut flora. The presence of these organisms in feces could represent ingestion of vegetative cells or spores in normal food intake. In healthy individuals, counts are usually low or below the level of detection. However, after a bout of illness, up to 10^9 CFU g^{-1} can be found in feces. One or both types of enterotoxin can be demonstrated from fecal homogenates if both the Oxoid and TECRA kits are used for detection.

Typing of *B. cereus* isolated from food poisoning episodes can be useful in understanding the epidemiology of outbreaks. So far, serotyping of the flagellar (H) antigens has been used. It has been shown that the H1 flagellar type is more common (approximately 70%) in emetic than in diarrheal types of *Bacillus* food poisonings. In one outbreak, H26 was found to be the common type. However, overall the diarrheagenic types were too inconsistent for H typing to be of value. The H antisera are not commercially available and the typing scheme is used routinely only in the UK.

Plasmid profiling, phage typing, and biotyping based on biochemical reactions have also been used with differing degrees of success. Information published before 1984 showed

that certain serotypes of emetic toxin producers are incapable of hydrolyzing starch or fermenting glycogen, whereas diarrheagenic strains tend to ferment salicin. Recently, molecular fingerprinting methods based on randomly amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) and multiplex RAPD–PCR have been found to be useful in typing isolates commonly involved in outbreaks.

Control and Preventive Measures

Although *B. cereus* is found in many foods, the ingestion of small numbers in ready-to-eat foods will not cause illness. The vegetative form is inactivated by most cooking procedures, but problems remain with the survival and subsequent outgrowth of spores during inadequate cooling and storage, particularly of moist protein-based foods and rice. For the occurrence of food poisoning, large numbers of enterotoxigenic *B. cereus* cells must be present in the food ($>10^5$ CFU g^{-1}); hence, it is important that control measures should be directed toward effective temperature management. Cooked foods should, therefore, be stored at >60 °C or cooled rapidly and stored under refrigeration (<5 °C). The Australia New Zealand Food Standards Code, Standard 3.2.2, for example, defines a two-step process of cooling foods: cool from 60 to 21 °C within 2 h, followed by cooling from 21 to 5 °C in a further 4-h time period. There is potential for *B. cereus* to be found in reheated foods if this cooling requirement is not followed, as reheating temperatures are usually not high enough to inactivate bacterial spores. The time taken for cooling and reheating thus provides an opportunity for bacteria to grow. Composite food products may be at a higher risk of *B. cereus* contamination due to the numerous ingredients that may carry spores. Spore-forming bacteria are problematic for the food industry as it is not always possible to apply enough heat during food processing to destroy spores without detrimental effect on desired qualities of the food. *Bacillus* species can represent up to 95% of the total bacteria in herbs and spices. A study of 45 isolates of *B. cereus* from spices found that all were capable of producing enterotoxins. Thus, the presence of *B. cereus* in spices could well result in food poisoning incidents when they are added to foods that are subjected to no or minimal heat treatment. Irradiation or fumigation of spices can be used to avoid such situations. Surveys of the distribution, prevalence, and concentration of *B. cereus* in a broad range of retail foods have been performed. In one such survey, *B. cereus* was not detected in chilled raw beef; however, chilled raw diced chicken had the highest prevalence and mean count of *B. cereus*, at 5.5% and 4.3 log cfu g^{-1}, respectively. In other surveys, *B. cereus* has been detected in poultry feed and in raw poultry meat. If *B. cereus* spores are present in raw foods, they will only pose a food safety risk if they survive the cooking process and the food is subsequently temperature abused for a sufficient time. Holding foods at refrigeration temperatures is the recommended means of controlling bacterial growth. However, it has been shown that psychrotolerant strains of *B. cereus* are able to grow in foods stored at 4–6 °C. A study of enterotoxigenic strains of *B. cereus* isolated from spices found that 50% and 87% were able to grow at 5 °C and 7 °C, respectively. Another study showed 53% *B. cereus* isolates from pasteurized milk were able to grow at 7 °C. These could compromise the safety of foods, even when they are stored at refrigeration temperatures. Cells of *B. cereus* can also be adapted to grow at low temperatures by prior storage at cold temperatures. Refrigeration temperatures less than 4 °C may be needed to avoid growth of *B. cereus* spores in pasteurized or cooked ready-to-eat foods.

Preservatives can be used to inhibit the growth of *B. cereus*. When preservatives are used in combination with reduced pH, increased salt concentrations, modified atmospheres, and/or heat treatment, the levels of preservatives can be reduced. Growth of *B. cereus* has been shown to be inhibited by 0.26% sorbic acid at pH 5.5 and by 0.39% potassium sorbate at pH 6.6. Nisin is commonly used to inhibit spore germination and growth in processed cheese, dairy desserts, canned foods, cured meats, and high-moisture baked products, such as crumpets and pikelets. Other antimicrobials that have an effect on *B. cereus* include benzoate, sorbate, ethylenediaminetetraacetic acid, and polyphosphates.

Most chemical sanitizers used routinely in the food industry will destroy vegetative *B. cereus* on food preparation surfaces. Sanitizers such as chlorine, iodophors, and peroxyacetic acid compounds are sporicidal. The commonly used quaternary ammonium compounds (QAC) are not sporicidal but will inhibit growth of both spores and vegetative cells. QAC are especially effective against Gram-positive bacteria. As with most chemicals, correct usage of sanitizers in the right concentrations and appropriate situations is important for their optimum effectiveness.

Spores are more resistant to radiation than vegetative cells are; however, *B. cereus* spores become sensitive to heating at 90 °C after irradiation at 4 kGy. It has been observed that *B. cereus* spores in vegetables and broth were more susceptible to gamma-irradiation than those in cereals. Gamma-irradiation of ready-to-eat foods with up to 10 kGy did not eliminate *B. cereus* spores, nor did such treatments damage their enterotoxin-coding genes.

See also: Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: DNA Methods; Standard Methods. Microbiological Safety of Meat: *Staphylococcus aureus*

Further Reading

Bennett, R.W., Belay, N., 2001. Bacillus cereus. In: Downes, F.P., Ito, K. (Eds.), Compendium of Methods for the Microbiological Examination of Foods, fourth ed. Washington, DC: American Public Health Association, pp. 311–316.

Claus, D., Berkeley, R.C.W., 1986. Genus *Bacillus*. In: Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (Eds.), Bergey's Manual of Systemic Bacteriology, vol. 2. Baltimore, USA: Williams and Wilkins, pp. 1105–1139.

Eglezos, S., Huang, B., Dykes, G.A., Fegan, N., 2010. The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. Journal of Food Protection 7, 867–870.

El Saleeby, C.M., Howard, S.C., Hayden, R.T., McCullers, J.A., 2004. Association between tea ingestion and invasive *Bacillus cereus* infection among children with cancer. Clinical Infectious Disease 39, 1536–1539.

Fermanian, C., Fremy, J.-M., Lahellee, C., 1993. *Bacillus cereus* pathogenicity: A review. Journal of Rapid Methods and Automation in Microbiology 2, 83–134.
Granum, P.E., 1997. Bacillus cereus. In: Doyle, M.P., Beuchat, L.R., Montville, T.D. (Eds.), Food Microbiology: Fundamentals and Frontiers. Washington, DC: ASM Press, pp. 327–336.
Hendriksen, N.B., Hansen, B.M., 2011. Diagnostic properties of three conventional selective plating media for selection of *Bacillus cereus, B. thuringiensis* and *B. weihenstephanensis*. Folia Microbiologica 56, 535–539.
International Commission on Microbiological Specifications for Foods, 1996. Bacillus cereus Micro-Organisms in Foods 5. Microbiological Specifications of Food Pathogens. London: Blackie Academic and Professional, pp. 20–35.
Jenson, I., Moir, C.J., 1997. *Bacillus cereus* and other *Bacillus* species. In: Hocking, A.D., Arnold, G., Jenson, I., Newton, K., Sutherland, P. (Eds.), Foodborne Microorganisms of Public Health Importance, fifth ed. Sydney, Australia: AIFST (NSW Branch), pp. 379–406.
Kramer, J.M., Gilbert, R.J., 1989. *Bacillus cereus* and other *Bacillus* species. In: Doyle, M.P. (Ed.), Foodborne Bacterial Pathogens. New York: Marcel Dekker, pp. 21–70.
Lechner, S., Mayr, R., Francis, K.P., *et al*., 1998. *Bacillus weihenstephanensis* sp. nov.is a new psychrotolerant species of *Bacillus cereus* group. International Journal of Systematic Bacteriology 48, 1373–1382.
Logan, N.A., 2011. Bacillus and relatives in foodborne illness: A review. Journal of Applied Microbiology 112, 417–429.
Logan, N.A., Halket, G., 2011. Developments in the taxonomy of the aerobic, endospore-forming bacteria. In: Logan, N.A., De Vos, P. (Eds.), Aerobic, Endospore-forming Soil Bacteria. Berlin: Springer-Verlag, pp. 1–29.
Notermans, S., Batt, C.A., 1998. A risk assessment approach for food-borne *Bacillus cereus* and its toxins. Journal of Applied Microbiology, Symposium (Supplement 84), 51S–61S.
Oliwa-Stasiak, K., Kolaj-Robin, O., Adley, C.A., 2011. Development of real-time PCR assays for detection and quantification of *Bacillus cereus* group species: Differentiation of *B. weihenstephanensis* and rhizoid *B. pseudomycoides* isolates from milk. Applied and Environmental Microbiology 77, 80–88.

Relevant Website

http://www.fda.gov
United States Food and Drug Administration, United States Department of Health and Human Services.

Clostridium botulinum and Botulism

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Botulism

Foodborne botulism is an intoxication resulting from consumption of foods in which *Clostridium botulinum* has grown and produced toxin. Depending on the amount of toxin ingested, the onset of botulism usually begins 18–36 h after ingesting toxin-containing food. As with other bacterial foodborne illnesses, the initial symptoms may include diarrhea and vomiting. Botulinum intoxication results in a descending symmetrical flaccid paralysis. Cranial nerves are affected initially, resulting in one or more of blurred and double vision, fixed and dilated pupils, ptosis (drooping eyelids), dysphonia (difficulty swallowing), dysphagia (difficulty speaking), and dry mouth. Paralysis may descend causing muscle weakness and paralysis affecting the diaphragm. Fatal cases are normally the result of respiratory failure. Botulism is frequently confused with other illnesses, especially in geographical areas where the disease has a low incidence. The symptoms of botulism are commonly confused with Guillain–Barré syndrome, stroke, and myasthenia gravis.

A diagnosis of botulism can be made by demonstration of botulinum neurotoxin in the patient's serum or the toxin and/or the organism in feces. As *C. botulinum* is not normally part of the human intestinal microflora, its isolation from feces or gastric contents, in association with classic symptoms of the disease, is diagnostic. Owing to improved medical care, such as the use of respiratory support systems and timely administration of antitoxin, the fatality rate due to botulism has fallen significantly in recent years.

In Europe, more than 2500 cases of foodborne botulism were reported in 1999–2000. Countries with a high reported incidence include Armenia, Azerbaijan, Belarus, Georgia, Poland, Russia, Turkey, and Uzbekistan. A smaller but significant number of cases are reported annually in France, Germany, Italy, China, and the USA. It should be noted that the true incidence of foodborne botulism is likely to be much higher, with underreporting being an issue. Foodborne botulism is not reportable in all countries and the efficiency with which potential outbreaks are investigated varies from country to country.

Being described first in 1976, infant botulism has become the most commonly reported form of botulism in the US, with approximately 80–100 cases occurring annually. Unlike foodborne botulism, infant botulism is caused by ingestion of spores, followed by colonization of the intestinal tract by *C. botulinum*, with *in situ* production of neurotoxin. A third form is wound botulism, wherein the organism infects tissue and produces toxin at a wound site. With no cases being reported in the UK before 2000, wound botulism has now become the most common form of botulism in the UK as a result of injection of illicit drugs. As only foodborne botulism relates to the microbiological safety of meat, further discussion will be limited to that type of illness.

Foodborne botulism is likely as old as food preservation. 'Sausage poisoning' was first seriously studied following an outbreak in Wildbad, Germany, in 1793, which resulted in 13 cases with 6 deaths. Although originally attributed to belladonna poisoning (caused by consumption of leaves or berries of the Nightshade plant), it was more widely accepted that the cause was consumption of a locally produced blood sausage 'Blunzen,' which was a pig stomach filled with blood and other ingredients, briefly boiled, and preserved by smoking. Following the Wildbad outbreak, the number of reported cases of sausage poisoning rapidly increased, prompting an 1829 study of the disease by the local health officer, Justinius Kerner, who described 230 cases of sausage poisoning over a period of 25 years. The illness became known as 'botulism' after '*botulus*,' the Latin word for sausage. Kerner's report was the first published complete description of the clinical symptoms of foodborne botulism.

An outbreak of botulism involving 34 cases caused by salted ham served at a gathering of amateur musicians in Ellezelles, Belgium, occurred in December 1895. The outbreak was investigated by Prof. E. Van Ermengem of the University of Ghent. Van Ermengem reported that portions of ham fed to mice, guinea pigs, and monkeys caused flaccid paralysis and death. Filtered extracts of ham had the same effects as macerated ham. Van Ermengem found an anaerobic sporulating bacillus, which was named *Bacillus botulinus*, in cultures of the ham as well as a culture of spleen from a deceased victim. Cultures and culture filtrates had the same effects as the macerated ham and ham filtrates, indicating involvement of a filterable toxin that was produced by the bacterium. In the investigation of this outbreak, Van Ermengem established that botulism was an intoxication, not an infection, and the toxin was produced by a spore-forming obligately anaerobic bacterium.

C. botulinum

Clostridium botulinum is the 'species' name assigned to an assemblage of anaerobic, spore-forming, rod-shaped bacteria, all of which produce botulinum neurotoxin that causes the severe neuroparalytic condition known as botulism. Morphologically, members of the *C. botulinum* assemblage are Gram-positive, anaerobic, motile rods, 4–6 μm by 0.9–1.2 μm in size with oval, subterminal spores (**Figure 1**).

Originally, all organisms known to produce botulinum neurotoxin were included in this species. There are seven types of *C. botulinum*, A through G, based on the serological specificity of the neurotoxin produced. Human botulism, including foodborne, wound, and infant botulism, is associated with

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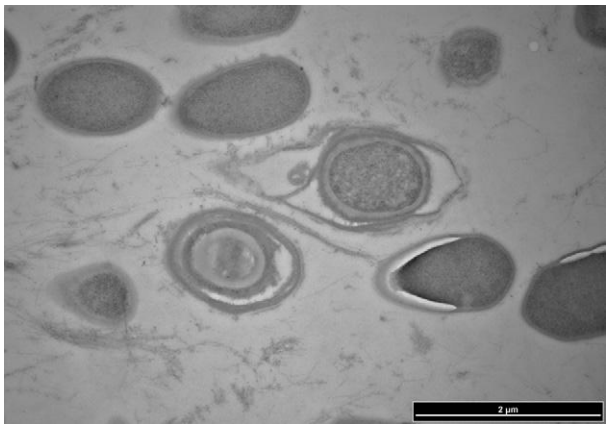

Figure 1 A transmission electron micrograph of a thin section through several vegetative cells and spores of *Clostridium botulinum*. Bar represents 2 µm.

types A, B, E, and F. Types C and D cause botulism in animals. To date, there is no direct evidence linking type G to the disease.

The species is also divided into four groups on the basis of physiological and genomic differences. Strains belonging to group I include all type A strains and proteolytic strains of types B and F. *Clostridium botulinum* Group II includes all type E strains and nonproteolytic strains of types B and F. *Clostridium botulinum* Group III includes all type C and D strains. *Clostridium botulinum* group IV, or *C. botulinum* type G, has been renamed *Clostridium argentinense*. A high degree of relatedness exists among strains within each group, but there is little relatedness between groups.

Group I strains are proteolytic and are typified by strains that produce neurotoxin type A. The optimal temperature for growth is 37 °C, with growth occurring between 10 °C and 48 °C. High levels of neurotoxin are typically produced in cultures. Spores of proteolytic strains are resistant to heat, with D_{100} °C values of approximately 25 min (the D value is the time required to inactivate 90% of the population at a given temperature). To inhibit growth, the pH must be less than 4.6, salt concentration more than 10%, or the water activity (a_w) less than 0.94.

Group II strains are nonproteolytic, have a lower optimum growth temperature (30 °C), and are psychrotolerant, that is to say, being capable of growth at temperatures as low as 3.0 °C. The spores have a much lower heat resistance than those of group I strains, with D_{100} °C values less than 0.1 min. Group II strains are inhibited at pH less than 5.0, salt concentrations more than 5%, or water activities (a_w) less than 0.97. The original strain isolated by van Ermengem in 1895 was probably nonproteolytic *C. botulinum* type B, and many type B cases in Europe are still due to strains of nonproteolytic *C. botulinum*. Recorded outbreaks of botulism involving nonproteolytic *C. botulinum* have most frequently been associated with meat, fish (e.g., salted, dried, vacuum, or smoked), and homemade foods prepared by the peoples of Alaska and northern Canada, such as aged beaver tail and paw, aged salmon roe and 'muktuk' (i.e., whale skin and fat).

Group III includes strains producing types C and D neurotoxins, which are not involved in human botulism but cause animal botulism. These strains are nonproteolytic, grow optimally at 40 °C, and do not grow at temperatures less than 15 °C. Group IV strains, which produce type G neurotoxin, grow optimally at 37 °C and have a minimal growth temperature of 10 °C.

These four groups, plus neurotoxin-producing strains of *Clostridium butyricum* type E and *Clostridium baratii* type F, make a total of six distinct genomic groups that produce botulinum neurotoxin. With a few exceptions, infant botulism is caused by proteolytic strains of *C. botulinum*. In the few exceptional cases, other clostridia (*C. baratii* producing type F toxin and *C. butyricum* producing type E toxin) have been implicated.

Isolation and Characterization of *C. botulinum*

The diagnosis of foodborne botulism is generally confirmed if, in addition to the clinical syndrome, botulinum toxin and/or viable *C. botulinum* are detected in a suspect food or a clinical specimen. Suitable specimens for toxin analysis are serum, feces, enema fluid, and stomach contents. The same specimens, except serum, are also suitable for the detection of *C. botulinum*. Botulinum toxin is detected by injecting serum or extracts from foods and clinical specimens into mice, and then observing them for characteristic symptoms including ruffled fur, labored breathing, and a pinched waist. These symptoms, combined with the absence of these symptoms in specimens neutralized with specific antisera, can be considered an earlier endpoint for the mouse assay negating the requirement for death as an endpoint.

The detection of *C. botulinum* involves anaerobic incubation of foods and clinical specimens in liquid media, and then subsequent toxin analysis. In major outbreaks or in the isolation of uncommon serotypes, the analyses are followed by the isolation of the incriminated microorganism. The customary procedure for isolation of *C. botulinum* from food is based on an enrichment procedure followed by detection and serotyping of toxin in culture supernatant fluid. Commonly used enrichment procedures include preliminary heat or ethanol treatments to destroy competing nonsporing bacteria, followed by anaerobic culture in media such as cooked meat medium or trypticase-peptone-glucose-yeast extract broth. Enrichment incubation is normally at temperatures between 26 °C and 35 °C for 5–10 days. A selective and differential agar medium containing antibiotics and egg yolk may be used for isolation of the organism from enrichment broth.

Botulinum Neurotoxin

The specificity of botulinum toxin for neurons makes it exquisitely toxic. The lethal dose of botulinum toxin for a 70 kg human is estimated to be approximately 0.09–0.15 µg intravenously or intramuscularly and 70 µg orally. Following ingestion, the toxin is absorbed through the gastric and upper intestinal mucosa from where it enters the bloodstream. Botulinum neurotoxin then gains access to the nervous system, where it blocks the release of the neurotransmitter acetylcholine,

preventing muscle contraction and thereby causing flaccid paralysis.

The active toxins are first synthesized as single polypeptides with low activity that require posttranslational proteolytic cleavage to form the active dichain molecule consisting of heavy and light chains joined together by a disulfide bridge. Strains of Group I *C. botulinum* are proteolytic and self-activate the toxin with endogenous proteases. However, Group II strains, all of which are considered nonproteolytic, need exogenous proteolytic enzymes or trypsin treatment to activate the toxin. The toxin is stable in acidic conditions (pH 3.5) but is easily inactivated in slightly alkaline conditions. The toxin is heat labile; consequently, normal cooking procedures should ensure a food's safety with respect to botulism. However, freezing does not destroy the toxin.

Incidence of *C. botulinum* in Meats

Spores of proteolytic and nonproteolytic *C. botulinum* are widespread in nature and are found in soils, aquatic sediments, and the gastrointestinal tracts of animals. Given the ubiquitous nature of *C. botulinum* spores, their presence in meats must be assumed and appropriate controls to prevent growth and toxin production must be applied. Information regarding the prevalence rates for *C. botulinum* on meat is not readily available, mainly because of the cost and difficulties of detecting *C. botulinum* in foods. However, the studies that have been done indicate that the incidence and levels of *C. botulinum* in meats are low, compared with fish and fishery products. Several studies indicate that the incidence of *C. botulinum* in meat samples is often less than 10%, with levels at approximately 1 spore per kg. The more frequent occurrence of botulism acquired from pork than from other meat products suggests more frequent contamination of pork than that of beef, lamb, and other meats. Toxin type B is typically implicated in cases of meat-borne botulism. Survey results reveal that nearly all toxin types identified from raw and semi-preserved meats were either A or B. The finding of low numbers of spores of *C. botulinum* in pig and cattle's feces indicates that clinically healthy animals occasionally carry *C. botulinum* spores. Thus, the contamination of carcasses with *C. botulinum* spores during carcass dressing seems likely.

Botulism Incidents Involving Meat Products

Home processing of meat is more common in continental Europe than in the UK or North America and an increased incidence of foodborne botulism, most frequently associated with meat products, has been observed in eastern European countries. Nonproteolytic type B strains are the etiological agents in most outbreaks of botulism in Germany, France, and Portugal that have been caused by home-prepared salted or cured hams. Homemade bottled pork meat is the main vehicle of botulism in Poland. Three incidents occurring in the UK and Ireland involved Polish nationals and were associated with the consumption of home-prepared meat products originating from Poland. Of 106 cases of botulism in Romania diagnosed during 2007–09, 86 (81.1%) were associated with the consumption of home-prepared pork products (ham, bacon, blood pudding, mosaic salami, raw sausages, and smoked-dried meat), whereas commercially canned products were involved in 20 cases (18.9%).

Most cases of botulism due to meat products in North America result from consumption of traditional northern foods by Inuit or Eskimos. Peccant foods include meat and fat from seal, whale, walrus, and beaver. Other than these northern foods, botulism from meat products is rare in Canada and the US. Commercial pate was the cause of two cases of botulism in Quebec in 1995, whereas commercial jarred pork caused a single case in Quebec in 2001. In July of 2007, an outbreak from commercially canned hotdog chili sauce caused 10 cases of botulism in Texas and Indiana. A 15-case botulism outbreak, also caused by chili, occurred in Texas in 2001. As both these cases involved chili, a food with several ingredients, it was not determined whether meat, or another ingredient, contributed the spores. Occasional single cases were reported in the US from home-prepared foods including stew and home-canned beef and peas; however, in these cases, it is also impossible to determine whether meat was the responsible ingredient.

Although home and artisanal production remain the principal causes of botulism outbreaks, the proportion of cases attributable to commercial products is increasing, especially in Europe, where recent outbreaks have been linked to widely distributed, commercially produced foods. Methods for preserving food products are changing, and fresh products that are vacuum-packed and refrigerated with extended shelf-lives, or products that are heat treated at temperatures and times too mild to inactivate *C. botulinum* spores, are increasing the risk of botulism from commercial foods.

Control of *C. botulinum* in Meats

Safe food production is based on either destroying spores of *C. botulinum* using a thermal process or inhibiting growth of the organism in foods, in combination with low storage temperatures and limited storage times. Incorporation of a combination of inhibitory factors such as reduced pH or water activity, added preservatives, and competing microflora provide multiple barriers and help processors to achieve a safe product. In addition to inhibition of growth of *C. botulinum*, the microbiological safety of foods also relies on good control and monitoring of processes throughout manufacture and distribution.

Refrigeration

The psychrotolerant nature of nonproteolytic strains of *C. botulinum* makes these strains of particular concern in refrigerated products. Psychrotrophic *C. botulinum* has the ability to survive mild heat treatment of minimally processed foods and may grow during cold storage. Growth of psychrotrophic *C. botulinum* occurs in response to extrinsic parameters such as storage at temperatures and redox potentials permissive for growth and parameters intrinsic to the food, such as pH and water activity.

Toxin formation by *C. botulinum* is both time and temperature dependent. At its lowest growth temperatures, it takes a *C. botulinum* strain many weeks to produce toxin. However, a slight rise in incubation temperature is accompanied by a rapid increase in growth rate of *C. botulinum* and a reduction in time to toxin detection. Storage of foods for several weeks at refrigeration temperatures is needed for toxigenesis by nonproteolytic strains. Thus, refrigerated products with a short shelf-life normally would not present significant risk. However, products with extended shelf-lives stored at refrigeration temperatures for extended periods may produce toxin by nonproteolytic strains of *C. botulinum*.

As strict temperature control of refrigerated products is often not maintained during their storage, distribution, display, and subsequent handling in domestic, food service, or institutional facilities, the likelihood of toxin production by nonproteolytic strains of *C. botulinum* in extended shelf-life products is increased. However, low-temperature storage cannot be reliably used as the sole means of controlling growth and toxin production by *C. botulinum*.

Consumer demands for ready-to-eat convenience foods, combined with an emphasis on little or no preservatives and less processing, have placed a new focus on *C. botulinum*. The lack of a heat treatment sufficient to destroy spores of *C. botulinum*, use of packaging that mostly or totally exclude oxygen, prolonged storage at chill temperatures, and the lack of heating before consumption combine to increase the risk of botulism from these foods. These products often do not use additional preservative systems such as reduced pH or water activity. In those foods where such treatment would adversely affect product quality, other controls, such as reduced water activity or acidification, should be considered. Subinhibitory levels of several factors can be combined to control growth of *C. botulinum* by application of what is known as the hurdle concept.

Thermal Processing

A great number of botulism outbreaks during the early twentieth century in Europe and North America were associated with the widening use of canning and bottling processes to extend shelf-life. This led to the development and enforcement of the 'botulinum cook' for commercial processing. The 'botulinum cook' was developed as a heat process for low acid foods (>pH 4.6) in order to destroy the spores of *C. botulinum*, and its widespread use led to substantial reductions in the number of botulism outbreaks. Spores are a dormant form of the organism that are resistant to high temperatures, high pressure, UV light, and desiccation. The 'botulinum cook' process is designed to reduce the population of the most resistant *C. botulinum* spores to 10^{-12} of their original numbers. The decimal reduction time (D value) is the time in minutes at a given temperature in order to produce a one log reduction in *C. botulinum* spore number. Much higher temperatures are required to inactivate spores of Group I strains, compared with those of Group II. Group I strains typically have D values in the range of 0.21 min at 121 °C, whereas Group II strains have D values of 2.4 min at 82.2 °C. Botulism in canned foods results from underprocessing or postprocess contamination. In the case of underprocessing, it is the heat-resistant proteolytic strains that are of concern with regard to product safety. *C. botulinum* is controlled in shelf-stable canned meats using thermal processing.

Redox Potential and Atmosphere

C. botulinum is a strict anaerobe. However, even within foods exposed to oxygen, the redox potential is often low enough to support the growth of this organism. Vacuum packaging and modified atmosphere packaging were developed for the extension of product shelf-life, but concerns have been raised about risks from these products with respect to botulism. In modified atmosphere packaging of certain meat products, the air in the headspace of the package is replaced by CO_2 and N_2, resulting in a low O_2 environment. There is concern about the safety of these products with respect to the potential for growth and toxin production by nonproteolytic *C. botulinum*. Vacuum or modified atmosphere packaging in atmospheres without O_2 restricts the growth of aerobic spoilage bacteria but not of clostridia or other anaerobic bacteria. There have been a number of reports describing low temperature spoilage of vacuum-packaged meats caused by psychrotolerant *Clostridium* spp. These incidents demonstrate that conditions within a chilled vacuum pack may select for the growth of psychrotolerant clostridia. Outbreaks of type E botulism in the 1960s were linked to vacuum-packed smoked fish. Studies have shown no difference in the rate of toxin production in high- and low-oxygen barrier films. However, it is of concern that the use of high-barrier oxygen film may increase the organoleptic acceptability of a toxic product. Inhibition of competing organisms may permit toxin production by *C. botulinum* in refrigerated modified atmosphere packaged foods without obvious sensory evidence of spoilage.

pH

C. botulinum will not grow in highly acidic foods that have a pH value less than 4.6. Consequently, acidification is widely used to control the growth of *C. botulinum* in food products. Cases of botulism have been linked to the consumption of high-acid foods in which the growth of yeasts or molds has raised the pH sufficiently to allow the growth of *C. botulinum*. In general, proteolytic strains of *C. botulinum* are more tolerant of acidic conditions than the nonproteolytic strains.

Water Activity

The generally accepted minimum a_w for growth of *C. botulinum* in foods, under otherwise optimal conditions, is 0.94 and 0.97 for proteolytic and nonproteolytic strains, respectively. The values 0.94 and 0.97 correspond to salt concentrations of approximately 10% and 5%, respectively. Today, high salt concentrations in foods are unacceptable to most consumers. Lower salt concentrations can, however, be effective when used in combination with other inhibitory factors such as pH and nitrite.

Preservatives

Nitrite

Sodium nitrite is a multifunctional food additive, responsible for the characteristic color and flavor associated with cured meats and at the same time providing protection against growth and toxin formation by *C. botulinum* in cured meats subjected to temperature abuse. The exact mechanism of botulinum inhibition by nitrite is not known. Its efficacy depends on interactions involving pH, salt, heat treatment, storage temperature and time, and the composition of the food matrix. It has been shown that nitrite interacts with nitrogenous compounds to form carcinogenic nitrosamines and intestinal bacteria mediate the formation of nitrosamines in the body. As a result, in recent years, consumer and regulatory pressure has mounted to reduce the levels of nitrite in processed meats. The maximum concentration of sodium nitrite ($NaNO_2$) in meat products typically is 150 mg kg^{-1}. *C. botulinum* in cured meat products is primarily controlled by refrigeration and the use of nitrite. The control measure to apply is the addition of a sufficient quantity of nitrite salt (defined by the sodium nitrite ($NaNO_2$) concentration).

Nisin

Bacteriocins, antimicrobial peptides that inhibit the growth of a broad spectrum of Gram-positive microorganisms, offer a natural alternative as biopreservatives for safeguarding minimally processed foods. Nisin is a peptide antibiotic produced by some strains of *Lactococcus lactis*. It has been used as a food preservative in many countries for more than 50 years and has been granted generally regarded as safe status. Nisin is not only active against closely related lactic strains but is also effective for inhibiting the growth of the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, and *C. botulinum* under certain conditions. Nisin sensitivity varies among *C. botulinum* strains. Growth conditions and food components also affect nisin's effectiveness. Factors decreasing nisin's ability to inhibit *C. botulinum* growth include a low-acid environment and high protein and phospholipid concentrations in foods.

Competing Microorganisms

In most foods, competing organisms, when present, often grow more rapidly than *C. botulinum*, as this microorganism is a poor competitor. The growth of organisms such as the lactic acid bacteria lowers the pH and, consequently, inhibits the growth of *C. botulinum*. Strains of lactic acid bacteria have been found to produce bacteriocins (low molecular weight proteins) that are inhibitory to *C. botulinum*. These strains hold promise for use in the food industry, where they may provide 'natural' protection against *C. botulinum* hazards.

Hurdle Technology

Although hurdles to growth of *C. botulinum* have been reviewed separately, they are typically used in combination. Thermal processing resulting in a 12 log reduction of *C. botulinum* spores, although acceptable for canned foods, would render many meat products inedible. Reliance on refrigerated storage alone results in the risk that a food may be temperature abused.

Perishable meat products rely on chilling, in combination with intrinsic factors, in order to control growth of *C. botulinum*. Products such as luncheon meats in hermetically sealed containers receive a mild heat treatment but remain shelf stable due to refrigerated storage, and nitrites and salt are added to inhibit growth of *C. botulinum*. Bacon and some fermented, undried sausages are preserved by chilling, in combination with nitrite, and reduced water activity and pH.

Shelf-stable meat products rely on intrinsic factors to prevent growth of *C. botulinum*. For example, Italian mortadella relies on a combination of a thermal process, reduced water activity, low pH, and the addition of nitrite and smoke. Other meat products rely more on a limited number of hurdles. For example, canned unsalted meat relies only on the thermal process, whereas Parma-type ham relies primarily on reduction of water activity with salt, with a lesser reliance on reduced pH.

See also: Biopreservation. Canning. Chemical Analysis for Specific Components: Curing Agents. Microbiological Safety of Meat: Hurdle Technology

Further Reading

Austin, J.W., Leclair, D., 2011. Botulism in the North: a disease without borders. Clinical Infectious Diseases 52, 593–594.

Emanuelle, E., Vaillant, V., de Valk, H., Popoff, M.R., 2003. France recalls internationally distributed halal meat products from the plant implicated as the source of a type B botulism outbreak. Eurosurveillance 7 (38), 2295.

Fagan, R.P., McLaughlin, J.B., Castrodale, L.J., *et al.*, 2011. Endemic foodborne botulism among Alaska Native persons – Alaska, 1947–2007. Clinical Infectious Diseases 52, 585–592.

Lucke, F.-K., Roberts, T.A., 1993. Control in meat and meat products. In: Hauschild, A.H.W., Dodds, K.L. (Eds.), *Clostridium botulinum*. Ecology and Control in Foods. New York: Marcel Dekker, pp. 177–207.

Neghina, A.M., Marincu, I., Moldovan, R., Iacobiciu, I., Neghina, R., 2010. Foodborne botulism in southwest Romania during the post-communism period 1990–2007. International Journal ofInfectious Diseases 14, 96–101.

Neghina, A.M., Neghina, R., 2011. Epidemiology of foodborne botulism in Romania 1980–2009. Foodborne Pathogens and Diseases 8, 907–911.

Peck, M.W., Stringer, S.C., 2005. The safety of pasteurised in-pack chilled meat products with respect to the foodborne botulism hazard. Meat Science 70 (3), 461–475.

Peck, M.W., Stringer, S.C., Carter, A.T., 2011. *Clostridium botulinum* in the post-genomic era. Food Microbiology 28, 183–191.

Clostridium perfringens

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Glossary

Enterotoxin A toxin produced in the intestine.
Gastroenteritis The inflammation of stomach or intestine.
Plasmid A genetic element separate from the nucleus and which is not required for growth. The genes carried in plasmids provide antibiotic resistance or mediate toxin formation.
Polymerase chain reaction (PCR) A laboratory method of amplifying specific gene(s) that may be involved in virulence by certain bacteria.
Spore A dormant structure produced by bacteria which is usually resistant to physical agents such as heat.

Introduction

Clostridium perfringens is an opportunistic pathogen capable of causing a broad spectrum of human and veterinary disease conditions, including gas gangrene, meningitis, appendicitis, respiratory and urinary tract infections, septicemia, sudden infant death syndrome, and a variety of gastrointestinal disorders. The association of this microorganism with outbreaks of food poisoning has been known since the turn of the nineteenth century, but its etiological role as a causative agent of human foodborne disease was not established until the 1940s. Today, *C. perfringens* is recognized as a principal causative agent of two forms of foodborne disease: *C. perfringens* type A gastroenteritis and *C. perfringens* type C necrotic enteritis. The symptoms of the gastroenteritis are usually mild and rarely fatal; however, owing to its high incidence this condition is a substantial public health concern. The necrotic enteritis, also known as pigbel or darmbrand, is a severe disease with a mortality rate of up to 25%, but it is exceptionally rare in Western countries. Consequently, the emphasis in this article will be on *C. perfringens* type A gastroenteritis and its causative agent(s).

Characteristics of *C. Perfringens*

Clostridium perfringens is a Gram-positive, spore-forming, encapsulated microorganism.

Vegetative cells of *C. perfringens* are straight rods with blunt ends, occurring singly or in pairs. Spores are large and oval, distending the cell at either the central or subterminal position. Spores are rarely observed in foods and/or in laboratory cultures.

Clostridium perfringens is proteolytic and saccharolytic. The majority of *C. perfringens* strains reduce sulfite to sulfide, hydrolyze gelatin, reduce nitrate to nitrite, produce acid from lactose, are lecithinase-positive and ferment sucrose. In contrast to other sulfite-reducing anaerobes, *C. perfringens* is nonmotile.

Clostridium perfringens grows optimally at temperatures between 43 and 47 °C, and at these temperatures it has a generation time of less than 10 min. Typically, the growth range is from 15 to 50 °C. Vegetative cells of *C. perfringens* are sensitive to low temperatures, such as those employed for refrigerated storage of foods. Generally, *C. perfringens* cells are sensitive to heat and in laboratory media are easily destroyed by exposure to temperatures above 55 °C. However, the sensitivity of vegetative cells to heat is thought to be strain specific. Spores of *C. perfringens* are relatively cold resistant and can survive refrigeration or freezing at −18 °C. *C. perfringens* spores are renowned for their high resistance to heat, with D_{95} °C values (the time in minutes at 95 °C required for a 90% reduction in a population of viable spores) exceeding 200 min in meat-based media. Heat resistance of *C. perfringens* spores varies depending on both genetic and environmental factors. Reports indicate that strains implicated in *C. perfringens* type A gastroenteritis outbreaks may show D_{95} °C values 60 times higher than those obtained for nonoutbreak strains. This reflects the location of the *cpe* gene on the chromosome or on a plasmid (see section Molecular Methods).

Clostridium perfringens grows optimally at pH values between 6.0 and 7.0, and its growth is inhibited at pH values below 5 and above 8.3. The minimum water activity (a_w) that will permit growth of *C. perfringens* is between 0.93 and 0.97. The organism is relatively tolerant to oxygen but will not produce colonies on the surface of solid media exposed to air. Under carbon dioxide atmospheres, the growth of *C. perfringens* cells is somewhat inhibited but not completely prevented. Growth is completely inhibited by either 6–8% NaCl, 10 000 ppm $NaNO_3$, or 400 ppm $NaNO_2$. However, when these salts are used together, the inhibition of growth occurs with substantially lower concentration of each salt.

Clostridium perfringens produces a variety of toxins that are detrimental to humans and animals. Of the greatest significance to food-borne disease is *C. perfringens* enterotoxin (CPE), which belongs to a group of endotoxin that are produced inside the bacterial cell. CPE is a polypeptide of 319 amino acids and has a molecular weight of approximately 35 kDa. It is a unique protein that has only limited amino acid sequence homology with other bacterial proteins. CPE is sensitive to heat, and it is readily inactivated by 5 min exposure to a

temperature of 60 °C. In contrast to vegetative cells of *C. perfringens*, CPE retains its biological activity during refrigerated storage. Intracellular synthesis of CPE occurs throughout the various growth stages of the bacterium but increases exponentially (at least 1500-fold) during sporulation. CPE accumulates in the cytoplasm of the sporulating cell and is released only when *C. perfringens* cells lyse to free mature spores.

In addition to CPE, the organism produces at least 15 extracellular toxins or exotoxins, with novel *C. perfringens* toxins continuing to be discovered. Exotoxins are thought not to be responsible for the symptoms of gastroenteritis, but β1 and β2 toxins induce hemorrhagic necrosis of the intestinal mucosa, which is characteristic of food-borne necrotic enteritis. On the basis of mouse lethality tests and according to the various combinations of four major exotoxins (alpha, beta, epsilon, and iota), *C. perfringens* is divided into five toxinotypes, A–E. Each toxinotype causes distinct disease symptoms, with *C. perfringens* type A being associated with either gas gangrene or gastroenteritis symptoms, and *C. perfringens* type C being responsible for necrotic enteritis symptoms.

Clostridium perfringens toxinotypes correspond to a diverse range of genotypes. The full genome sequence of *C. perfringens* identified a total of 25 genes that contribute to the organism's virulence. Genotypes of the majority of strains include the *plc* gene that encodes alpha toxin and, at the same time, none, one or two genes encoding other type-specific exotoxins. Each genotype may or may not carry the *cpe* gene. *C. perfringens* exotoxin genes reside either on the chromosome or on large plasmids that occur singly or in low copy numbers. Similarly, the genome location of the gene encoding CPE may vary, with the *cpe* gene residing on the chromosome in *C. perfringens* strains associated with foodborne disease but positioned on a plasmid in strains associated with nonfoodborne gastrointestinal disorders (e.g., antibiotic-associated or sporadic gastroenteritis) or in strains of veterinary origin. When located chromosomally, the *cpe* gene can be found on mobile genetic elements, such as a lysogenized phage or a transposon.

Isolation and Identification

Quantitative recovery of viable *C. perfringens* cells from, and detection of CPE in, food and/or patient specimens are key steps for the diagnosis of *C. perfringens* gastroenteritis. In addition, molecular methods are increasingly being used for the detection of *C. perfringens* toxin genes and epidemiological typing of *C. perfringens* isolates.

Conventional Methods

The procedure for enumeration of *C. perfringens* in meat is based on initial recovery of microorganism on solid media for sulfite-reducing anaerobes, followed by confirmation of colonies as *C. perfringens* with further biochemical tests.

Tryptose sulfite cycloserine (TSC) agar is the medium presently recommended by both Association of Official Analytical Chemists (AOAC) International and the International Organization for Standardization (ISO) for use in quantitative recovery of *C. perfringens*. The initial selection is on TSC agar with or without egg yolk. Egg yolk has traditionally been added to media to enable detection of lecithinase activity previously thought to be characteristic of *C. perfringens*. However, it is now recognized that not all strains of *C. perfringens* are lecithinase-positive. Consequently, the lecithinase reaction is not required as a differential property of the TSC agar, and the egg yolk can be omitted. It should be noted, however, that adding lysozyme or egg yolk to the medium may enhance the recovery of thermally injured *C. perfringens* spores. TSC agar contains the antibiotic cycloserine, which suppresses the growth of facultative anaerobes. Ferric ammonium citrate incorporated into the medium reacts with the sulfide that is produced during sulfite reduction, forming a black iron sulfide precipitate. Black colonies that develop during incubation are reported as sulfite-reducing anaerobes. ISO recommends that TSC agar be inoculated using the pour plate method. Alternatively, the medium can be inoculated using the spread plate method and overlayed with a small volume of TSC agar. Incubation is at 37 °C under anaerobic conditions.

Owing to the sensitivity of *C. perfringens* to low temperatures, food samples to be tested for the presence of this microorganism must not be refrigerated or frozen before analysis. Instead, samples should be tested as soon as possible after collection. However, activation of the heat-resistant spores of *C. perfringens* is heat dependent and only approximately 4% of spores of food-poisoning strains will germinate without prior heat shock. Patient specimens should be heat treated before plating on TSC agar to maximize the recovery of spores.

Isolates recovered from TSC agar are subsequently screened with biochemical tests for motility, nitrate reduction, lactose fermentation, and gelatin hydrolysis. Isolates that are nonmotile, reduce nitrate, ferment lactose, and hydrolyze gelatin are identified as *C. perfringens*. Species-level identification of *C. perfringens* can be achieved using commercially available miniaturized test kits, for example, the RAPID ID 32A (bioMérieux, Marcy-l'Etoile, France) or BBL Crystal Anaerobe kits.

Detection of *C. Perfringens* Enterotoxin

The detection of CPE in the stools of patients with food poisoning enables a definitive diagnosis of *C. perfringens* gastroenteritis to be made. CPE can be routinely detected in stool specimens by enzyme-linked immunosorbent assay (ELISA) and reverse passive latex agglutination (RPLA) assay. These assays are capable of detecting as little as 2–4 ng g^{-1} CPE in fecal material, well below the levels of 1 μg g^{-1} that are expected to occur in stool specimens from *C. perfringens* gastroenteritis patients. Even higher levels of assay sensitivity for CPE detection can be achieved with Western immunoblotting.

Molecular Methods

A number of DNA-based methods exist for the detection and identification of *C. perfringens*. These methods include polymerase chain reaction (PCR) assays and gene probes for the specific detection of the *cpe* gene to establish the

isolates 'or foods' potential for toxigenicity. Multiplex PCR has been developed for simultaneous detection of several toxins, including exotoxins and CPE, and this assay can be used to replace conventional toxin typing. Recently, a duplex PCR assay was described for differentiation of *C. perfringens* type A isolates carrying chromosomal *cpe* genes from isolates carrying this gene on a plasmid. DNA-based methods also include real-time PCR and a PCR assay for the detection and/or identification of *C. perfringens* using 16S rDNA-based specific primers.

Plasmid profiling, phage typing, ribotyping, pulsed-field gel electrophoresis (PFGE), and amplification of fragment length polymorphism (AFLP) analysis have been used for tracing *C. perfringens* isolates from patients back to the outbreak food sources. While ribotyping, PFGE and AFLP each demonstrate significant discriminatory power for strain-specific differentiation of *C. perfringens* isolates, the genetic diversity of *C. perfringens* strains and necessary expertise may prove inhibitory for successful use of these methods in routine epidemiological investigations. As both CPE-producing and CPE-negative *C. perfringens* strains may possess indistinguishable fingerprints, it is recommended that before traceback studies, the potential for CPE production by outbreak isolates is established using a *cpe* gene detection assay.

Characteristics of *C. Perfringens* Foodborne Disease

In contrast to foodborne botulism, the symptoms of *C. perfringens* foodborne disease develop after ingestion of food containing viable *C. perfringens* cells rather than preformed toxins. *C. perfringens* type A gastroenteritis is usually characterized by profuse diarrhea and cramping in the lower abdomen. The onset of the disease usually begins 6–12 h after the ingestion of foods containing at least 4×10^9–6×10^9 *C. perfringens* vegetative cells (equivalent to 8–10 mg of CPE). Vomiting and fever are uncommon. The symptoms lessen after a further 24 h and usually resolve spontaneously. The mortality rates associated with *C. perfringens* type A gastroenteritis are low, but death may occur in immunocompromised patients, for example, the elderly. Gastroenteritis symptoms caused by *C. perfringens* type A and *Bacillus cereus* are virtually indistinguishable. *Clostridium perfringens* type C necrotic enteritis takes the form of severe gastroenteritis characterized by bloody diarrhea, cramps, nausea, vomiting, and necrotic inflammation of the small intestine. The disease has a high mortality rate of up to 25%.

Traditionally, a diagnosis of *C. perfringens* type A gastroenteritis was made on the basis of clinical signs and lesions as well as quantitative recovery of *C. perfringens* from patient specimens and food specimens. *Clostridium perfringens* was typically verified as a causative agent of the disease when (1) *C. perfringens* was present in implicated food at a level of 10^5 colony-forming units (CFU) g^{-1} or higher; (2) *C. perfringens* was present in patients' stool samples at level of 10^6 CFU g^{-1} or higher; and (3) serotyping indicated similarity of serotypes of *C. perfringens* strains isolated from food and stools of patients. However, the traditional diagnostic scheme has significant drawbacks, such as occasional carriage of *C. perfringens* in stools of asymptomatic individuals at levels approaching those present in clinical specimens, or untypeability with commercially available antisera of many outbreak strains owing to the antigenic heterogeneity of *C. perfringens*. Consequently, the criteria for diagnosis of *C. perfringens* type A gastroenteritis were expanded to include evidence for the presence of CPE in stool specimens of affected patients. More recently, it was proposed that diagnosis may also be helped by the detection of the *cpe* gene in food and fecal material, and by establishing the chromosomal location of this gene in outbreak strains.

Mechanism of Pathogenicity

The events leading to pathological changes within the gastrointestinal tract that later manifest themselves as symptoms of gastroenteritis are typically initiated with the ingestion of vegetative cells of *C. perfringens*. It is thought that the majority of these cells survive exposure to the acidic environment of the stomach and start to multiply rapidly once the alkaline environment of the small intestine is reached. Sporulation of *C. perfringens* occurs readily in the presence of human intestinal contents. CPE accumulates inside the cell during the sporulation and is subsequently released into the lumen.

In the small intestine, CPE interacts with several proteins of the epithelial cells. The toxin binds initially to a claudin protein receptor of the intestinal cell brush border and forms part of a small, and then large, protein complex that becomes associated with the cell membrane. On formation of the large complex, cell membrane permeability is affected, so that leakage of small molecules occurs and, subsequently, synthesis of DNA, RNA, and protein is inhibited. These changes result directly in the death of the affected epithelial cells.

CPE induces a number of morphological changes in the small intestine. The histopathological effects include formation of blebs; shortening, desquamation, and necrosis of the tips of the intestinal villi; and loss of the folded configuration of the brush border. It is thought that the histopathological damage is responsible for the disturbances to fluid and electrolyte transport in the intestinal loops that eventually manifest as acute diarrhea.

Epidemiology

Reservoirs of *C. Perfringens*

Clostridium perfringens is one of the most widely distributed pathogenic bacteria and can be found in soil, dust, water, and air of natural environments. *Clostridium perfringens* type A occurs in soil at levels of 10^3–10^4 CFU g^{-1}. Strains of *C. perfringens* known to cause gastroenteritis in humans are normal inhabitants of the intestinal tract of humans and animals, occurring in feces of healthy or asymptomatic individuals at levels of up to 10^5 CFU g^{-1}.

Food reservoirs of *C. perfringens* include raw beef, chicken and pork, fresh and vacuum-packed fish and shellfish, raw fruit and vegetables, soups, sauces and gravy mixes, cheese, and spices. It is thought that approximately 50% of consumer-ready items of raw meat and poultry contain *C. perfringens*. The organism may be found on the surface of beef, pork, and lamb carcasses and is also recognized as an intrinsic organism

present in the deep tissues or internal organs (spleen, liver, kidneys or lymph nodes) of slaughter animals.

Traditionally, the widespread presence of *C. perfringens* in the environment and foods was thought to explain the high prevalence of *C. perfringens* type A food poisoning. However, early surveys rarely identified the potential for toxigenicity of clostridial isolates. Retrospective screening of *C. perfringens* isolates showed that nearly half of the strains recovered during outbreaks did not carry the *cpe* gene and were thus genetically incapable of causing the disease. At present, it is estimated that less than 5% of the total population of culturable *C. perfringens* strains possess the *cpe* gene. Recently, none of the *C. perfringens* isolates that were obtained in a comprehensive survey of retail foods in the US was found to carry the *cpe* gene. Consequently, high incidence rates obtained for *C. perfringens* in foods and the environment may be of little relevance to food safety. At present, the reservoirs for *C. perfringens* strains that cause human gastroenteritis remain unknown. A novel proposal is that humans are a source of enterotoxigenic foodborne isolates.

Studies have indicated that despite the widespread presence of *C. perfringens* in domestic animals, only a small proportion of veterinary strains are CPE positive, carry the chromosomal *cpe* gene, or produce heat-resistant spores. Depending on the species, the incidence of *C. perfringens* strains capable of CPE production in domestic animals in the US ranges from 0% to 22%, with prevalence in steers/heifers reaching 1.0% and that in cows/bulls reaching 2.7%. Only a few of these CPE-positive strains have a chromosomally located *cpe* gene. Consequently, food animals have a lower potential for causing *C. perfringens* gastroenteritis than was initially thought.

Characteristics of Outbreaks

Outbreaks of *C. perfringens* type A gastroenteritis typically occur when meat or poultry containing *C. perfringens* spores are cooked in advance, left to cool slowly with inadequate refrigeration, and not reheated adequately before serving. The dishes are usually prepared in a manner that favors the development of anaerobic conditions, for example, as rolled roasts or in large vats. The outbreaks are large, with a median size of 25 or more cases, and frequently result from consumption of food cooked by commercial catering companies. Commercial meat processors are rarely implicated in outbreaks of *C. perfringens* type A gastroenteritis.

It is thought that, under the conditions just described, heat-resistant *C. perfringens* spores will survive and become activated during the cooking process. These spores will germinate, outgrow, and multiply rapidly after the temperature of the prepared dish falls below 50 °C and before it reaches ~15 °C. The resulting vegetative cells, now present in the dish at 10^6–10^7 cells per gram, will not be destroyed in the absence of adequate refrigeration and/or during inadequate reheating.

The usual vehicles for *C. perfringens* gastroenteritis outbreaks are meat and poultry dishes. Between 1973 and 1987, approximately 30% of *C. perfringens* outbreaks in the US were associated with consumption of beef, whereas chicken and turkey dishes accounted for approximately 15% of outbreaks. More recently, meat and poultry dishes were confirmed as vehicles in approximately one-third of all reported cases of foodborne *C. perfringens* gastroenteritis in the US.

Incidence of the Disease

Of the three spore-forming bacteria most commonly associated with foodborne disease, that is, *Bacillus cereus*, *Clostridium botulinum*, and *C. perfringens*, *C. perfringens* causes the most outbreaks and cases. *C. perfringens* gastroenteritis is the second most common cause of cases of bacterial foodborne disease in the US after *Salmonella*.

In Europe also, *C. perfringens* gastroenteritis is the second most common cause of foodborne disease after *Salmonella*. In 2000, 22% of deaths due to foodborne disease in England and Wales were caused by *C. perfringens*. In the UK, the fall in the numbers of cases and deaths due to *C. perfringens* gastroenteritis observed during the past decade is thought to be due to a decline in the consumption of red meats.

In many countries, *C. perfringens* type A gastroenteritis is not a notifiable disease. Because of lack of adequate reporting, the incidence of this disease and its economic impact are likely to be underestimated.

Control and Preventive Measures

A number of factors contribute to the ability of *C. perfringens* to cause outbreaks of foodborne disease. Of these factors, its widespread presence in foods and the environment, its formation of heat-resistant spores and its short generation times significantly influence the approach to control of this microorganism, and the disease it causes.

Because *C. perfringens* is prevalent in the farm environment, the organism is frequently detected in slaughter animals, on dressed carcasses and in abattoir environments. Traditionally, it was thought that during slaughter and dressing, carcasses inevitably became contaminated with *C. perfringens* spores because of their transfer to the carcass as opening cuts are made in the skin. Consequently, the possibility of raw meat being free from *C. perfringens* was thought to be unrealistic and its presence in meat was regarded as unavoidable. Now it is recognized that only a small proportion of *C. perfringens* strains from food animals and abattoir environments are capable of causing foodborne disease, control of carcass/cut contamination with *C. perfringens* spores may become feasible, by identification of farm and/or abattoir reservoirs of contamination with outbreak strains. Once these reservoirs are identified, specific control measures can be developed to reduce the initial contamination of raw meat and eliminate transfer of clostridial spores to processed meats. Similarly, following the identification of reservoirs of foodborne disease-causing *C. perfringens* strains, control measures could be directed at specific operations within the retail sector, commercial catering facilities, and/or food industries.

In the past, removal or inactivation of *C. perfringens* spores present on carcasses or cuts proved unsuccessful. Unless extreme measures are employed, various carcass decontamination treatments frequently result in only approximately 1 log reductions in the levels of *C. perfringens* spores. With the high

D_{95} °C values of some strains, the use of thermal treatments to destroy *C. perfringens* spores without impairing the desired organoleptic attributes and palatability of prepared foods is generally not possible.

Traditionally, the approach to control of *C. perfringens* type A gastroenteritis centered on inhibiting the multiplication of the organism in food. The most practical way of preventing *C. perfringens* gastroenteritis appears to be by preparing meat and poultry dishes shortly before they are served, and by adequate cooling, refrigeration, and reheating of cooked products. The USDA guidelines recommend that cooked meat dishes are cooled from an internal temperature of approximately 54 °C to approximately 27 °C within less than 1.5 h, and from approximately 27 °C to approximately 4 °C within less than 5 h in order to avoid multiplication of *C. perfringens*. Following cooling, food should be stored refrigerated, and should be reheated immediately before serving to reach an internal temperature of 70 °C or above.

Interestingly, regardless of initial contamination of the raw material, times and temperatures employed during cooking of food and cooling rates achieved for the cooked product, *C. perfringens* type A gastroenteritis could be avoided if food was reheated to 70 °C before serving. It seems that regardless of the other, various possibilities for control of *C. perfringens* during food manufacturing, education of food service workers remains paramount for effective prevention of *C. perfringens* foodborne disease.

See also: Microbiological Analysis: DNA Methods; Standard Methods

Further Reading

De Jong, A., Baumer, R., Rombouts, F., 2002. Optimizing sporulation of *Clostridium perfringens*. Journal of Food Protection 65, 1457–1462.

Food and Drug Administration. 2011. *Clostridium perfringens*. Bacteriological Analytical Manual (Chapter 16). Available from http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm (accessed 11.10.13).

Gao, Z., McClane, B., 2012. *Use of Clostridium perfringens* enterotoxin and the enterotoxin receptor-binding domain (C-CPE) for cancer treatment: Opportunities and challenges. Journal of Toxicology. doi:10.1155/2012/981626.

Grant, K., Kenyon, S., Nwafor, I., *et al*., 2008. The identification and characterization of Clostridium perfringens by real-time PCR, location of enterotoxin gene and heat resistance. Foodborne Pathogens and Disease 5, 629–639.

Heikinheimo, A., Lindstrom, M., Liu, D., Korkeala, H., 2010. Clostridium. In: Di, L. (Ed.), Molecular Detection of Foodborne Pathogens. Boca Raton, FL: CRC Press, pp. 145–156.

International Organization for Standardization, 2004. Microbiology of Food and Animal Feeding Stuffs-Horizontal Methods for the Enumeration of Clostridium perfringens – Colony Count Technique. Geneva: International Organization for Standardization ISO 7937:2004.

Labbe, R., Grant, K., 2011. *Clostridium perfringens* in food service. In: Hoorfar, J. (Ed.), Rapid Detection, Characterization, and Enumeration of Foodborne Pathogens. Washington, DC: ASM Press, pp. 381–392.

Labbe, R., Heredia, N., 2013. *Clostridium perfringens*. In: Labbe, R., Garcia, S. (Eds.), Guide to Foodborne Pathogens, second ed. London: Wiley and Sons.

Li, J., McClane, B., 2006. Further comparisons of temperature effects on growth and survival of *Clostridium perfringens* type A isolates carrying a chromosomal or plasmid-borne enterotoxin gene. Applied and Environmental Microbiology 72, 4561–4568.

Lin, Y.-T., Labbe, R., 2003. Enterotoxigenicity and genetic relatedness of *Clostridium perfringens* isolates from retail foods in the United States. Applied and Environmental Microbiology 69, 1642–1646.

Lindstrom, M., Heikinheimo, A., Lahti, P., Korkeala, H., 2011. Novel insights into the epidemiology of *Clostridium perfringens* type A food Poisoning. Food Microbiology 28, 192–198.

McClane, B., 2007. Clostridium perfringens. In: Doyle, M., Beuchat, L. (Eds.), Food Microbiology, Fundamentals and Frontiers, third ed., pp. 423–444. Washington, DC: ASM Press, pp. 423–444.

Miki, Y., Miyamoto, K., Kaneko-Hirano, I., Fujiuchi, K., Akimoto, S., 2008. Prevalence and characterization of enterotoxin gene-carrying *Clostridium perfringens* isolates from retail meat products in Japan. Applied and Environmental Microbiology 74, 5366–5372.

Scallan, E., Hoekstra, R., Angulo, F., *et al*., 2011. Foodborne illness in the United States – major pathogens. Emerging Infectious Diseases 17, 7–15.

USDA/FSIS, 2001. U.S. Department of Agriculture, Food Safety and Inspection Service. Performance standards for the production of certain meat and poultry products; final rule. Federal Register 64, 732–749.

Relevant Websites

http://www.fda.gov/Food/FoodborneIllnessContamination/default.htm
Foodborne Illness and Contaminants.

http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm
U.S. Food and Drug Administration.

Emerging Pathogens

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Glossary

Alpha (α)-haemolysis Haemolytic activity that results in the formation of green zones around bacterial colonies on blood agar media.
Bacteriocin A protein toxin produced by some strains of bacteria which inhibits the growth of, or kills of similar or closely related bacterial strains.
Binary toxin Toxins consisting of two independent polypeptides which are produced by some *Clostridium* spp.
Cytotoxin A substance that has a toxic effect on certain cells.
Endocarditis An inflammation of the endocardium (the membrane that lines inside of the heart), particularly of the heart valves.
Enterotoxin A type of harmful protein produced by bacteria that is specific for the mucous membrane of the intestine and causes the vomiting and diarrhea associated with food poisoning.
Fimbriae A fringe like structure of certain bacteria that is thinner and shorter than a flagellum, and is associated with antigenic properties of the cell surface.
Natural casings Edible casing for sausages made from the submucosa layer of animal intestines which consists mainly of collagen.
Plasmid An extrachromosomal self-replicating structure of bacterial cells that carries genes for a variety of functions not essential for cell growth and that can be transferred to other cells by conjugation or transduction.
Polyarthritis An inflammation that involves more than one joint. The inflammation may migrate from one joint to another, or there may be simultaneous involvement of two or more joints.

Introduction

Emerging pathogens have been defined as organisms that cause illnesses that have appeared or been recognized only recently, or which are rapidly increasing or spreading. For foodborne pathogens, the term has been extended to include organisms long established as human pathogens but for which food has only recently been recognized as a vehicle for their transmission. Those definitions obviously encompass a wide and diverse range of organisms, including organisms that are well recognized as pathogens as well as organisms that have only recently been associated with human disease. Among the former group are a number of pathogens that can be acquired from meat such as verotoxigenic *Escherichia coli*, *Campylobacter jejuni*, and *Yersinia enterocolitica*. These are the subjects of individual articles in this Encyclopedia. Among the latter group are many organisms for which the modes and vehicles of their transmission have not been established. Clearly, proper consideration in a short article of all the possibly meatborne organisms to which the label 'emerging pathogen' has been attached would not be possible. Therefore, for the purposes of this article, emerging meatborne pathogens are defined as organisms that have been found on meat, have been recognized recently as causes, or are suspected of being causes of human illness that are increasing in incidence, and are known or suspected of being acquired from meat. Four species of bacteria that comply with this definition and about which concerns have been increasing are discussed as representative emerging meatborne pathogens.

Acrobacter butzleri

Arcobacter spp. are members of the family Campylobacteraceae, which includes the genera *Campylobacter* and *Sulfurospirillum*. *Campylobacter* spp. are generally nutritionally demanding microaerophilic organisms that do not grow at temperatures much below 30 °C, and colonize mucosal surfaces of the digestive or urogenital tracts of animals and birds. Many campylobacters are established or suspected pathogens of animals, birds, and/or humans. In contrast, *Sulfurospirillum* are free-living organisms, various species of which can reduce sulfur, arsenate, or tetrachloroethane. Arcobacters are superficially similar to campylobacters in that they are Gram-negative, curved or s-shaped organisms that are motile by means of polar flagella, and grow optimally under microaerobic conditions. As with *Campylobacter*, species of *Arcobacter* are difficult to distinguish by phenotypic characteristics because of limited and weak biochemical activities. However, arcobacters are tolerant of aerobic conditions; at least some species can grow anaerobically using nitrate or other electron acceptors for anaerobic respiration; and they grow at temperatures of 15 °C or less.

Five species of *Arcobacter* have been isolated from animals and birds. Three of those species, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii*, have been isolated from both healthy livestock and animals with disease conditions of the urinogenital and gastrointestinal tracts. The involvement in human illness of *A. skirrowii is* uncertain, possibly because of difficulty with its isolation from stool samples; but *A. butzleri* and *A. cryaerophilus* are associated with gastroenteritis in humans. Of the latter two organisms, *A. butzleri* is the species most often isolated from diarrheic stools. Diarrhea attributed to *A. butzleri* is reported to be more persistent and watery than diarrhea caused by *Campylobacter* spp. and, unlike with *Campylobacter* spp., stools are not bloody.

Arcobacter spp. have been isolated directly and, generally with greater frequency, by enrichment from feces of pigs, cattle, sheep, horses, and cloacal contents of poultry. Carriage of

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Arcobacter is apparently frequent in pigs, but relatively uncommon in broiler chickens. Even so, the prevalence of *Arcobacter* spp. on chicken carcasses and portions is often high and generally greater than the prevalence on pork. *Arcobacter butzleri* is greatly predominant among arcobacters recovered from meats on retail sale, even though *A. cryaerophilus* may be recovered more frequently from animals or newly dressed carcasses. These various findings suggest that *A. butzleri* found on meat are mostly not fecal contaminants but instead originate largely from meat processing environments. Other findings that support this suggestion are that *A. butzleri* has been recovered from fresh and sea waters, shellfish, and meat processing environments; the organism can survive in cold waters for long times; and it has been shown to grow at 5 °C and form biofilms on surfaces when cultivated in a poultry meat exudate medium. Because its minimum temperature for growth has not been determined, it might be less than 5 °C. In addition, sequencing of the *A. butzleri* genome has shown that it contains a large number of genes associated with survival and growth under a broad range of environmental conditions, which is indicative of *A. butzleri* being a free-living organism.

The basis for *A. butzleri* pathogenicity is uncertain. Genes in *A. butzleri* that correspond with those for some putative virulence factors in *Ca. jejuni* have been identified; but those do not include genes coding for the *Campylobacter* cytolethal distending toxin. Whether or not *A. butzleri* produces toxins of any sort remains to be determined. Resistance to multiple antibiotics is apparently common among *A. butzleri* strains, with resistance genes being chromosomal rather than being borne on plasmids. Plasmids may not be of common occurrence in *A. butzleri*. Evidently, much remains to be learned about the role of *A. butzleri* in enteric illness.

Clostridium difficile

Clostridium difficile is a Gram-positive, strictly anaerobic, spore-forming bacterium that was originally isolated from the feces of newborn infants. It was found to be a usual component of the fecal flora of infants, and subsequently to be commonly present in neonates of a wide range of wild and domestic animals. The organism was not regarded as a pathogen until 1978, when it was identified as the cause of pseudomembranous colitis, i.e., inflammation of the gut wall, with the formation of pseudomembranes composed of fibrin, mucin, neutrophils, and cell debris. Since then, the incidence of *Cl. difficile* infection (CDI) has steadily increased in most developed countries to become the most common cause of nosocomial, i.e., hospital-acquired diarrhea. Consequently, *Cl. difficile* and CDI have been extensively studied in recent years.

Pathogenicity is associated with toxin production by some strains of the organism. A chromosomal pathogenicity locus encodes two toxins and factors that control transcription of the toxin genes. Both toxins were thought to be necessary for the development of CDI, with damage to epithelial cells by the enterotoxin TcdA being required for entry and inactivation of cells by the cytotoxin TcdB. However, TcdA-negative strains that cause CDI have been identified, and laboratory-created mutants that express only one or other of the toxins have been shown to cause disease in laboratory animals. In addition, *Cl. difficile* can produce a binary toxin, but what role it may have in CDI is not known.

CDI is associated with the treatment of patients with broad-spectrum antibiotics, which suppresses the normal gut microflora therby facilitating extensive colonization of the gut by *Cl. difficile*. The disease, with symptoms that range from mild diarrhea to often fatal pseudomembranous or fulminant colitis, occurs predominantly in patients aged over 65 years who receive antibiotic therapy during prolonged hospitalization. The increasingly frequent outbreaks of the disease in hospitals can be difficult to contain, because the highly resistant spores produced by the organism readily survive outside the host to be spread during normal hospital procedures.

Since the early years of the current century there has been a continuous rise in the incidence of CDI in North America and Europe. Recent estimates for CDI in the USA are approximately 500 000 cases per year with up to 20 000 fatalities. Although a large majority of cases still occur among elderly hospital patients that receive antibiotic therapy, CDI in individuals who have not been treated with antibiotics and have no association with hospitals is becoming increasingly common. Such community-associated CDI has been found to occur in groups, such as the young and pregnant women, for which the risk of CDI was thought to be low.

Food has obviously been considered as a possible source of the *Cl. difficile* responsible for community-associated CDI, with meat being a possible vehicle for the organism. *Clostridium difficile* was known to be the cause of sometimes fatal enteric disease in horses, but its occurrence in food animals has been investigated only in recent years. Findings are that large numbers, possibly majorities, of piglets and chickens in at least some herd or flocks are infected with *Cl. difficile*. Substantial fractions of calf populations also carry the organism. *Clostridium difficile* causes diarrhea in piglets, but the illness is rarely fatal. It apparently does not cause illness in chickens; and any role it may have in illness in calves remains unclear. Certainly, many young of all three species can carry *Cl. difficile* without any symptoms of illness. How infants and young animals are protected, more or less, from CDI has not been established. Healthy adults and mature animals can also carry *Cl. difficile*, but apparently at frequencies of only a few percent. Strains of *Cl. difficile* involved in human CDI have been isolated from food and companion animals.

Clostridium difficile has been recovered from beef, pork, chicken, and ready-to-eat meats offered for retail sale. The isolates recovered from meats have commonly been found to be toxigenic, and have included strains that could not be distinguished from strains recovered from CDI patients. The prevalence of *Cl. difficile* in various meats have mostly been reported to be between 1% and 10%. However, in some studies, no *Cl. difficile* was recovered from some meats, whereas in others high prevalence up to 40% were found.

Although spores of *Cl. difficile* are less resistant to inactivation by heating than spores of some other Clostridia, their numbers were reduced by little more than 1 log unit by heating to 71 °C for 30 min. Cooking of ground beef and other meats to 71 °C is generally considered sufficient to ensure the meat is free of enteric pathogens; yet spores of *Cl. difficile* have been recovered from ground beef at numbers approximately 3 log cfu g^{-1}. It therefore seems that the *Cl. difficile*

spores in meat might well survive cooking relatively often, with consumers being exposed to spores with corresponding frequency. The available information could, therefore, be taken as strongly suggesting that community-associated CDI might be acquired by consumption of meat contaminated with *Cl. difficile* spores. However, there is as yet no direct evidence for such an occurrence.

Mycobacterium avium subsp. *paratuberculosis*

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the cause of Johne's disease in cattle, sheep, and other ruminants. Apparently, infection with MAP usually occurs when animals are young, by ingestion of the organism; but symptoms of diarrhea and chronic loss of weight generally appear only in animals that are 2 years or older, and many infected older animals might have no symptoms.

Infection with MAP is initiated by invasion of the ileum and associated lymph nodes. The infection can spread to much of the small intestine, and to the large intestine in some species. When the disease is advanced, MAP can be disseminated in organ and muscle tissues, and lymph nodes throughout the body. Dissemination may also occur episodically at early stages of infection. Both young and old animals infected with MAP shed the organism sporadically in feces. The MAP-infected gut wall is infiltrated by white blood cells, becomes granulomatous and thickened, and develops a characteristic, corrugated appearance. In advanced cases of the disease, mycobacteria in the tissues can be numerous and easily detected as acid-fast bacteria in tissue sections prepared for microscopy. However, infected tissues of characteristic appearance may contain very few acid-fast organisms.

In Crohn's disease, a chronic inflammatory disorder of the human gut, the appearance of the gut wall is similar to that seen in Johne's disease. Consequently, it has long been considered that MAP might be the cause of Crohn's disease; or at least be involved in the condition. However, this has proved difficult to confirm or disprove. MAP has been found to be present in gut tissues from patients with Crohn's disease, but not consistently and never in high numbers. Also, in some studies, MAP has been detected in the blood of patients with Crohn's disease; but in other studies it was apparently present in similar prevalence in blood of both Crohn's patients and healthy individuals, or it was not detected in blood from either group.

The uncertainty as to whether or not MAP is involved in human disease is in large part due to difficulties with establishing its presence unambiguously. Mycobacteria mostly grow only slowly, and MAP is the slowest growing of mycobacteria that can be cultivated on microbiological media. With a generation time of several days, incubation for a minimum of 6 weeks and usually much longer is necessary if colonies are to be obtained. As the organism is nutritionally demanding, media that will support its growth will allow the much more rapid growth of other microorganisms. Therefore, media for MAP are supplemented with antibiotics to which the organism is relatively resistant. Even so, overgrowth of MAP by other organisms is likely; so samples are usually subjected to a decontaminating treatment before they are used to inoculate MAP-selective media. Like all mycobacteria, MAP has a thick and waxy cell wall that renders the cells resistant to various, harsh, decontaminating treatments that are effective against other organisms. However, the extent to which MAP is affected by any decontaminating treatment is uncertain and very likely variable. Thus, reliable recovery of MAP when it is present in samples in small numbers cannot be expected.

Because of the difficulties with, and the effort required to recover MAP from samples, many studies have been conducted using indirect methods such as enzyme-linked immunosorbent assays for detection of MAP antibodies in blood or detection of MAP deoxyribose nucleic acid (DNA) sequences by polymerase chain reaction (PCR) tests. However, such tests can give both false-positive and false-negative results, and generally cannot establish the presence of viable MAP in samples.

The same problems arise with establishing the presence of viable MAP in foods. Thus, in the only two studies of MAP in meat on retail sale, no viable MAP were recovered; but in one no MAP DNA was detected in ground beef by PCR, whereas in the other MAP DNA corresponding to MAP at numbers up to 4 log cfu g^{-1} was detected in beef, pork, chicken, and fermented sausages by quantitative PCR. The far more numerous studies of MAP in milk and milk products have yielded similarly ambiguous results. Nonetheless, the presence of viable MAP in at least some raw meat products is likely. Animals with symptoms of Johne's disease are culled; and their meat is considered suitable for human consumption because MAP is currently not regarded as a human pathogen. Most meat from culled cattle is ground, so some ground beef must inevitably include MAP-infected muscle and lymphatic tissue from animals in which the organism was disseminated. In addition, all beef and lamb will be contaminated to some extent with fecal organism that can include MAP. Moreover, fresh natural casings are likely to be contaminated with MAP, and some may survive the preservation of casings with salt.

Whether or not MAP is involved in Crohn's disease or in other diseases, such as type I diabetes with which it has been linked, it is frequently suggested that the exposure of humans to MAP should be minimized, as a precautionary measure. In view of the widespread occurrence of the disease among farmed animals and the difficulties with identification of infected animals, exclusion of MAP-infected animals from milk and meat production is hardly a practicable proposition at present. Instead, reduction in the exposure of humans to MAP may well result from improving the control over contamination of meat with fecal organisms, and ongoing efforts in many countries to reduce the prevalence of MAP among farm animals.

Streptococcus suis

Streptococcus suis is a Gram-positive, facultatively anaerobic coccus that possesses cell wall antigens related to those of enterococci, such as *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus durans*, and other organisms that are members of Group D in the Lancefield classification of streptococci. However, it is not closely related genetically to the other member of this group. All strains of this species are α-hemolytic on sheep blood agar plates, but unlike enterococci

they are not able to grow in broth containing 6.5% NaCl. *Streptococcus suis* has been recovered from many species of mammals and birds. It is a usual component of the microflora of upper respiratory, urinogenital, and gastrointestinal tracts of healthy pigs. However, it is a cause of disease in pigs of all age groups, but with *S. suis* disease being most common among neonatal and young animals. Disease conditions caused by the organism include septicemia, meningitis, pneumonia, endocarditis, and polyarthritis. Infected animals can die suddenly, or may have various symptoms such as dyspnea (labored breathing), cyanosis, and/or wasting. The disease is endemic among pigs in most countries with sizable pig populations, and is the cause of substantial economic losses.

Thirty-five serotypes of *S. suis* have been identified based on capsular polysaccharide antigens. There is a wide variation in virulence within as well as between serotypes. Only some serotypes have been found to be responsible for infections in pigs, with serotype 2 being the most common. Knowledge concerning the virulence factors of *S. suis* serotype 2 is limited. There are ongoing problems with identification of virulence factors for the organism, although there is agreement that there are virulent and nonvirulent strains of *S. suis* serotype 2. Molecules that have been suggested to be associated with virulence include the capsule polysaccharide (CPS), which confers resistance to phagocytosis, muramidase-released protein (MRP), extracellular protein factor (EF), and suilysin. Despite the fact that CPS is the only proven critical virulence factor, nonvirulent encapsulated strains have been reported; and whereas MRP, EF, and suilysin protein are produced by the most virulent European strains, they are absent in most North American strains. Recently, other virulence factors, including adhesins, proteolytic enzymes, bacteriocins, and fimbriae have also been suggested.

The mechanisms of *S. suis* pathogenesis are not fully understood. Pigs may acquire infection via either vertical transmission, during farrowing, or horizontal transmission due to close contact with carrier or infected animals, their feces, and/or housing structures. There is no clear explanation of why *S. suis* successfully colonize some piglets and not others. Carrier animals typically harbor the bacteria in their tonsils and might never develop disease, whereas others will eventually develop bacteremia, septicemia, and meningitis. In these latter cases, *S. suis* must breach the mucosal epithelium in the upper respiratory tract to reach the bloodstream and invade various organs to cause inflammation.

In 1968, *S. suis* was first reported as the cause of human disease in Denmark. Since then, human cases have been found in many countries, but with few occurring in North America and Europe in comparison with the number of cases in South East Asia. As with pigs, *S. suis* disease in humans is caused mainly by serotype 2. Infections usually present as meningitis, but present as septicemia in approximately 20% of cases. Mortality is higher with the latter than with the former type of illness, but loss of hearing is a common sequel to meningitis.

Streptococcus suis can be transmitted to humans in close contact with sick or carrier pigs, such as pig farmers, abattoirs workers, meat processing workers, and veterinarians, via exposed cuts and abrasion in their skin. Consequently, most cases of human *S. suis* disease have occurred sporadically among such groups of people. However, in recent years there have been large outbreaks of human *S. suis* disease that were contemporaneous with extensive occurrence of disease caused by the organism among pigs in the outbreak regions. Patients in these outbreaks included people who had no contact with pigs or pig products other than the consumption of pork. It, therefore, appears that humans might be infected by contact with or consumption of contaminated pork.

Streptococcus suis serotype 2 can survive in water at 60 °C for 10 min. Consequently, cross contamination of carcasses with the organism might occur during carcass scalding as well as during dressing operations on carcass heads and throats. Survival of the organism on carcasses for up to 6 weeks has been reported. Pork may then commonly be contaminated with the organism; particularly when the meat is from carcasses dressed without being suspended in traditional Asian abattoirs, and when there is an outbreak of *S. suis* disease in local herds of pigs. Fortunately, *S. suis* is inactivated by most common disinfectants and cooking of meat to 70 °C. Thus, acquisition of the disease by consumption of pork should be readily contained if basic hygienic precautions are observed during handling of raw pork and the meat is cooked to a medium–well done condition, i.e., to at least 71 °C. However, hygienic handling of the meat during traditional marketing of pork in developing countries is not practicable. Therefore, further outbreaks of human *S. suis* disease in some East Asian countries are likely to occur.

Conclusion

The continuing emphasis on food safety in developed countries is likely to at least contain, and probably reduce infections with established human pathogens acquired from meat by consumers. Even so, the incidences of some infections may apparently increase, because of improved detection of some organisms, and/or increased reporting of the diseases they cause. Moreover, increasing production of meat in developed countries where the infrastructure needed for hygienic handling of meat is as yet limited might result in increased incidences of some diseases caused by meatborne pathogens. The broad definition of emerging pathogens would allow all organisms found to be involved in human disease with increasing frequency to be classified as such.

In addition, ongoing advances in medical and food microbiology are likely to result in the identification of disease conditions caused by meatborne microorganisms that currently are poorly characterized or are regarded as benign. Such organisms will, of course, be categorized as emerging pathogens. However, some meatborne organisms currently viewed as probably pathogenic for humans, such as MAP, might prove to be uninvolved in human disease. Others, such as *Cl. difficile*, might be carried by, but not be acquired from meat. Evidently, any organism characterized as being of those sorts cannot properly be referred to as emerging meatborne pathogens.

See also: Microbiological Safety of Meat: *Aeromonas* spp.; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; Thermotolerant *Campylobacter*, *Yersinia enterocolitica*

Further Reading

Gill, C.O., Saucier, L., Meadus, W.J., 2011. *Mycobacterium avium* subsp. *paratuberculosis* in dairy products, meat, and drinking water. Journal of Food Protection 74, 480–499.

Gottschalk, M., Xu, J., Calzas, C., Segura, M., 2010. *Streptococcus suis*: A new emerging or an old neglected zoonotic pathogen? Future Microbiology 5, 371–391.

Ho, H.T.K., Lipman, L.J.A., Gasstra, W., 2006. *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent. Veterinary Microbiology 115, 1–13.

Keessen, E.C., Gaastra, W., Lipman, L.J.A., 2011. *Clostridium difficile* infection in humans and animals, differences and similarities. Veterinary Microbiology 153, 205–217.

Miller, W.G., Parker, C.T., Rubenfield, M., *et al*., 2007. The complete genome sequence and analysis of the Epsilonproteobacterium *Arcobacter butzleri*. PLoS ONE 2 (12), e1358. doi:10.1371/journal.pone.0001358.

Motarjemi, M., Adams, M., 2006. Emerging Foodborne Pathogens. Cambridge, UK: Woodhead Publishing

Rupnik, M., Wilcox, M.H., Gerding, D.M., 2009. *Clostridium difficile* infection: New developments in epidemiology and pathogenesis. Nature Reviews Microbiology 7, 526–536.

Smith, T.C., Harper, A.L., Nair, R., *et al*., 2011. Emerging swine zoonoses. Vector Borne Zoonotic Diseases 11, 1225–1234.

U.S. National Advisory Committee on Microbiological Criteria for Foods, 2010. Assessment of food as a source of exposure to *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Journal of Food Protection 73, 1357–1397.

Weese, J.S., 2010. *Clostridium difficile* in food – Innocent bystander or serious threat. Clinical Microbiology Infection 16, 3–10.

Relevant Websites

http://www.uoguelph.ca/foodsafetynetwork/arcobacter-spp
Food Science Network Acrobacter spp.

http://www.gutpathogens.com/content/1/1/15
Gut Pathogens.

http://www.ift.org/Knowledge-Center/Read-IFT-Publications/Science-Reports/Expert-Reports/~/media/Knowledge%20Center/Science%20Reports/Expert%20Reports/Emerging%20Microbiological/Emerging%20Micro.pdf
Institute of Food Technologists.

http://www.extension.iastate.edu/Pages/ansci/swinereports/asl-1505.pdf
Iowa State University.

http://www.ohioline.osu.edu/vme-fact/0003.html
Ohio State University Fact Sheet.

http://www.health.gov.on.ca/en/ccom/cdi/
Ontario Ministry of Health and Long-Term Care.

Hurdle Technology

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Glossary

Extrinsic factors Factors associated with the environment external from the food product.
Homeostasis Maintenance of cellular equilibrium.
Intrinsic factors Inherent properties associated with a food product.
Redox potential The tendency of the food matrix to either donate (reduce) or accept (oxidize) electrons, which is most commonly influenced by the amount of oxygen in a food product.
Water activity The amount of water that is not bound by the food matrix and available for microorganisms to utilize for growth.

Introduction

Food preservation has been practiced for centuries, with salting generally recognized as the earliest form of preservation. Several intrinsic and extrinsic factors associated with foods serve to promote preservation, the most important of which include: water activity (a_w), temperature (low or high), preservatives (i.e., nitrite), acidity (pH), competitive microorganisms (i.e., lactic acid bacteria), and redox potential (Eh). These extrinsic and intrinsic factors are limited when applied singly. However, when combined with one another in a sequence or applied simultaneously, the activity of each is considerably enhanced. This effect is likened to a series of hurdles that become increasingly harder to overcome the more hurdles that are utilized. The result is synergistic food preservation referred to as a hurdle effect. To fully understand the effects of these hurdles on microbial populations, extensive research has focused on defining the critical limits for growth, survival, and death of the most significant microorganisms associated with the food supply. This information has provided a foundation for designing effective food preservation strategies. Thus, the term hurdle technology represents the intentional combination of hurdles, without necessarily listing them, at independently sublethal levels to preserve novel and traditional foods. Hurdles can be strategically combined such that it is possible for a food to become increasingly economical, have improved microbial safety and stability, as well as enhanced nutritional and sensory characteristics. The overall goal is for the hurdles to control the naturally occurring microbial population by either inhibiting growth or inactivating the microorganisms.

Aspects of Hurdle Technology

Creating a hostile environment for microorganisms is an important aspect of food preservation. Microbial death or growth is dependent on their response to the hostile conditions imposed on them by hurdle technology, which is implemented by altering intrinsic and extrinsic factors. To fully understand their response, an understanding of microbial homeostasis, stress reactions, and how the effects of multiple stressful conditions can lead to metabolic exhaustion is necessary.

Organisms require a certain amount of internal stability and uniformity for survival and growth. For example, the osmotic pressure of a living cell must be well maintained to achieve homeostasis. Thus, hurdles can be employed to disrupt the homeostasis of microbial cells, thereby restricting growth or causing cell death. Food preservation is accomplished when hurdles effectively disturb the homeostasis of microorganisms in a food either permanently or temporarily.

Maintaining homeostasis can rapidly deplete microbial cellular energy and lead to metabolic exhaustion. Hurdle technology subjects microorganisms to multiple hostile factors, some of which may result in sublethal injury to the cell. Thus, microorganisms will employ every possible repair mechanism to overcome the antagonistic environment and return to a state of cellular homeostasis. In doing so, they completely deplete their energy, which leads to an inability to maintain cellular functionality (i.e., metabolic exhaustion) and death. This process will eventually result in 'autosterilization' of hurdle technology foods. Therefore, the safety of such foods improves throughout storage, particularly when they are stored at ambient temperatures, as hurdles can have a lasting residual impact on the death of the cell.

The efficacy of food preservation hurdles can be limited by the various stress reactions induced by microorganisms. For example, stress shock proteins are synthesized by bacteria as they become more resistant, and in some cases more virulent, under stressful conditions (i.e., ethanol, heat, pH, a_w, and starvation). Fortunately, this does not result in a complete loss of hurdle efficacy, as microorganisms typically have more difficulty with turning on stress shock protein genes when a variety of stresses are experienced simultaneously. This requires energy consumption by the cell to synthesize multiple stress shock proteins concurrently. Bacteria are unable to meet this need and succumb to metabolic exhaustion.

For hurdle technology to be efficacious, a strategic combination of hurdles must be applied simultaneously with the intent of attacking various targets within the cell (i.e., cell membrane, pH, DNA, etc.), an approach known as multitarget preservation. By applying multitarget preservation, the

hurdles in a food begin to act synergistically with one another to disrupt homeostasis within microbial cells. As a cell is simultaneously exposed to hurdles aimed at multiple cellular targets, the cell is forced to initiate a variety of repair mechanisms, including stress shock protein synthesis. This overwhelms the cell, ultimately leading to metabolic exhaustion and cell death. Thus, applying different preservation hurdles in small amounts could be more effective than using large amounts of a single preservative, as multiple preservatives will target a variety of cellular components and act synergistically.

Hurdle Technology and Food Quality

Food preservation hurdles present within a food might influence stability and safety of a food, but may also affect the nutritive, sensory, economic, or technological properties of a product. Hurdles have the potential to either negatively or positively impact the overall quality of a food and a single hurdle may have a positive or negative impact depending on hurdle intensity. For example, water activity must be sufficiently low in fermented sausages to restrict pathogenic bacteria; however, the water activity cannot be so low that taste and texture are compromised. Some hurdles, like the addition of nitrite in the process of curing meat, provide antimicrobial activity while also creating the characteristic 'cured meat taste' and a color that is desirable. Thus, hurdle technology is also applicable to food quality, and motivation for implementing food preservation hurdles should not be driven solely by the microbial stability and safety of a food product. However, for maximal food safety and quality to be achieved, effective application of hurdle technology requires a specific understanding of the efficacy of each hurdle within a particular product and each hurdle must remain within their respective optimal range.

Application of Hurdle Technology in the Meat Industry

The conversion of live animals into raw meat and meat products allows for the introduction of spoilage and pathogenic microorganisms to be introduced onto the carcass via cross-contamination from animal hides, viscera, employees, equipment, and food-contact surfaces. Because meat processors in most countries of the world strive to produce products with low amounts of contamination, particularly pathogenic microorganisms, hurdle technology has been implemented extensively in the meat industry worldwide.

Fresh meat commonly has a water activity above 0.90 and is classified as a high-moisture food. Because many microorganisms are capable of survival or growth in this range, water activity at this level is not a sufficient preservative. Thus, other food preservation hurdles are necessary to control the microbial safety and stability of fresh meat products.

During animal harvest, hurdle technology is implemented as a simultaneous or sequential systems approach consisting of two or more chemical and/or physical procedures designed to decrease carcass contamination in an additive or synergistic fashion. Such processes commonly include hide cleaning before or after knocking, knife-trimming of visible contamination, a pre-evisceration carcass rinse (hot water or chemicals), steam-vacuuming, steam or hot water treatment, and organic acid rinses. Warm acid solutions and a combination of chemicals may also be applied to the carcass simultaneously to achieve synergism. These hurdles are strategically utilized throughout animal harvest to attain extensive reductions in the microbial population present on a carcass such that control of any remaining microorganisms can be more readily achieved by subsequent hurdles (i.e., refrigeration, freezing, and packaging). Some hurdles are also applied to chilled carcasses, subprimals and beef trim. Research has shown that implementation of multiple hurdles in a sequential fashion results in progressively reduced bacterial counts as carcasses advance through harvest and additional reductions can be achieved during fabrication.

Numerous nonthermal interventions may be combined with the sequential hurdle approach described above to enhance meat preservation. Extensive research has described the efficacy of refrigeration/freezing, irradiation, chemical preservatives, biopreservation/natural antimicrobials (i.e., nisin), physical intervention technologies (i.e., high hydrostatic pressure), and packaging (i.e., modified atmosphere packaging (MAP)) as additional fresh meat hurdles. For maximal effectiveness, these hurdles can be intelligently combined in a strategic sequence, and applied at their optimal intensity, in order to maximize antimicrobial capacity of the hurdle system without selecting for microbial stress adaptation or resistance.

The combination of natural antimicrobials with other nonthermal interventions has been investigated as a means to enhance antimicrobial capacity. Synergism is achieved when these nonthermal hurdles weaken bacterial cell membranes causing increased susceptibility to natural antimicrobial activity. For example, bacteriocins can be used as a hurdle before packaging of both raw and or cooked meat products as a means to prevent microbial growth. These antimicrobial peptides/proteins are manufactured by bacteria and do not have specific molecular sites that are targeted and the capability exists for some to rapidly destroy bacterial membranes. When applied in combination with other interventions such as chelating agents (i.e., ethylenediaminetetraacetic acid) or high hydrostatic pressure, bacteriocins have exhibited enhanced antimicrobial activity. For example, nisin, which is generally most effective against gram positive cells, has efficacy against gram negative microorganisms when used in conjunction with chelating agents and organic acids. When in the presence of organic acids and their conjugate salts, bacteriocins have increased solubility and a net charge that allows for diffusion of the bacteriocin through the bacterial cell membrane to exert their antimicrobial activity within the cell. Live lactic acid bacteria can also be added directly to whole muscle cuts or ground beef (trim or final product) to kill foodborne pathogens throughout the process. Certain strains of lactic acid bacteria have been identified that kill foodborne pathogens during refrigerated storage but do not grow during storage and, therefore, do not negatively impact the quality of the meat.

Combining hurdles with MAP or vacuum packaging have also been used to optimize antimicrobial activity.

Organic acids (i.e., lactic acid) have been utilized in conjunction with MAP (i.e., 80% $CO_2 + 20\%$ O_2) to control pathogenic microorganisms while MAP has also been combined with plant essential oils to decrease spoilage microbes and extend product shelf life. Irradiation can be an effective hurdle; however, it can also lead to the production of sulfur compounds associated with an off-odor of the product. For this reason, combining irradiation with vacuum packaging may be beneficial, as this environment can decrease oxidative activity thereby limiting the production of volatile compounds believed to be associated with off-odors. Combining irradiation with MAP has been effective at reducing microbial loads and extending the shelf life of meat and meat products.

A well-established product in the meat industry preserved by hurdle technology is shelf-stable sausages. Many hurdles are represented in this process, including additives (i.e., salt, nitrites/nitrates) and competitive microflora (i.e., lactic acid bacteria cultures) that lower the redox potential as well as the pH and decreased water activity from product drying. Some semidry sausages also undergo smoking, which adds an additional hurdle for microorganisms to overcome. Stability of a fermented meat product manufactured in this fashion is achieved when the pH ranges from 4.6 to 5.3 with a water activity of <0.95, whereas a pH of <4.5 or a water activity of <0.91 would be required if both hurdles were not in place. Furthermore, by implementing two mild hurdles with the same antimicrobial efficacy as one harsher hurdle, meat quality can also be considered.

Design of Effective Hurdle Technology for Meats

The design of many meat products is a multidisciplinary task that requires a team of experts to create a product that is both safe and stable and retains consumer acceptance properties. A microbiologist will assess the types and intensity of hurdles required, whereas a food technologist and meat scientist will identify the ingredients and/or processes necessary to institute these hurdles into the product. In doing so, the sensory, nutritive, technological, and legal restrictions must be accounted for, while also considering the marketing, economic, and engineering components of the product.

The use of computer-based predictive microbiology has shown promise for designing hurdle technology products. This approach allows for quantitative predictions of microbial activity (growth, survival, and death) in foods; however, models may be limited by the number of hurdles that can be evaluated simultaneously. Given the number of hurdles that could potentially be included in a meat product preserved by hurdle technology, it is not possible to include every model in the model. Thus, predictive microbiology essentially predicts the fate of microorganisms in a product using the most important hurdles. Because not all hurdles may be accounted for in a model, the predicted efficacy of the resultant safety and stability of the product will be on the conservative side. Although predictive microbiology does not replace the need for challenge testing and process validation, it does provide a useful tool for product design.

Conclusion

The use of hurdle technology in the meat industry is a simple concept but usually requires a strategic combination of hurdles, involving water activity (a_w), temperature (low or high), preservatives (i.e., nitrite), acidity (pH), competitive microorganisms (i.e., lactic acid bacteria), and redox potential (Eh). The various procedures commence during animal processing and progress throughout product packaging and storage and retail activities. Meat products preserved by hurdle technology result in improved shelf life and safety with little to no adverse effects on quality. Because quality is more readily maintained by implementing multiple hurdles at a lesser intensity than a single intervention, hurdle technology is a successful approach for producing mildly processed meat products that are both safe and desirable to consumers.

See also: Biopreservation. Foodborne Zoonoses. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Modeling in Meat Science: Meat Quality; Microbiology. Preservation Methods of Animal Products. Slaughter-Line Operation: Cattle; Other Species; Pigs; Poultry; Sheep and Goats

Further Reading

Aymerich, T., Picouet, P.A., Monfort, J.M., 2008. Decontamination technologies for meat products. Meat Science 78, 114–129.

Chen, J.H., Ren, Y., Seow, J., *et al*., 2012. Intervention technologies for ensuring microbiological safety of meat: Current and future trends. Comprehensive Reviews in Food Science and Food Safety 11, 119–132.

Geornaras, I., Sofos, J.N., 2005. Combining physical and chemical decontamination interventions for meat. In: Sofos, J.N. (Ed.), Improving the Safety of Fresh Meat, first ed. Boca Raton, FL: CRC Press, pp. 433–460.

Huffman, R.D., 2002. Current and future technologies for the decontamination of carcasses and fresh meat. Meat Science 62, 285–294.

Leistner, L., 2000. Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology 55, 181–186.

Leistner, L., 2007. Combined methods for food preservation. In: Rahman, M.S. (Ed.), Handbook of Food Preservation, second ed. Boca Raton, FL: CRC Press, pp. 867–894.

McIntyre, L., Hudson, J.A., 2009. Something old, something new: Hurdle technology – a marriage of preservation techniques. Food New Zealand 9, 15–20.

Sofos, J.N., 2008. Challenges to meat safety in the 21st century. Meat Science 78, 3–13.

Zhou, G.H., Xu, X.L., Liu, Y., 2010. Preservation technologies for fresh meat – A review. Meat Science 86, 119–128.

Relevant Websites

http://www.beefresearch.org/cmdocs/beefresearch/beefdecontaminationtechnologies.pdf
The Center for Research & Knowledge Management in the Department of Science & Product Solutions at the National Cattlemen's Beef Association.

http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094145.htm
U.S. Food and Drug Administration.

http://demonstrations.wolfram.com/FoodPreservationHurdleTechnology/
Wolfram Demonstrations Project.

Listeria monocytogenes

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Glossary

Biofilm A thin layer of microorganisms adhering to the surface of a structure by means of the material that they secrete.

Haptens A small molecule that can elicit an immune response only when attached to a large carrier such as a protein.

Chemotaxonomy The identification and classification of organisms by comparative analysis of their biochemical composition.

Lineage A group composed of species, taxa, or individuals related by descent from a common ancestor.

Phylogenetic Study of evolutionary relatedness among various groups of organisms.

Introduction

Listeria monocytogenes is a foodborne pathogen that causes a group of human illnesses collectively called listeriosis. Human listeriosis is mostly acquired by ingestion of ready-to-eat (RTE) foods contaminated with *L. monocytogenes*. Consumption of such foods and concerns about listeriosis have increased greatly during the past 30 years in developed countries. Factors contributing to concerns about listeriosis include the increased proportion of immune-compromised persons at risk for listeriosis; increased use of refrigeration to extend perishable food shelf life; increased consumer demand for RTE, refrigerated, or frozen foods; improved diagnostic methods; and enhanced public health surveillance. Foodborne listeriosis in less developed nations with less developed food supply systems is less frequent. *Listeria monocytogenes* was confirmed as a foodborne pathogen after the occurrence in the mid-1980s of large common-source outbreaks associated with various foods, including meats. Although there has been a large worldwide research effort into different aspects of foodborne *L. monocytogenes* infections during the past 25 years, many aspects remain unclear.

General Characteristics of *Listeria*

Taxonomy

Since the first description of *L. monocytogenes* in 1926, when it was called *Bacterium monocytogenes*, the pathogen has been reclassified repeatedly. Numerous studies over the past 30 years, based on numerical taxonomy, chemotaxonomy, and ribosomal ribonucleic acid (rRNA) sequencing, show that among the other prokaryotes, *Listeria* is most closely related to *Brochothrix*. *Listeria* belongs to the *Clostridium* subbranch together with *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Brochothrix*, and is characterized in this phylogenetic position by a low average guanine–cytosine content of 38%. Presently, on the basis of deoxyribonucleic acid (DNA) homology, 16S rRNA sequencing homology, chemotaxonomy, and multilocus enzyme electrophoresis (MEE) analysis, the genus *Listeria* comprises six species: *L. monocytogenes*, *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria grayi*. Recently, phylogenetic analyses have identified and described novel species of avirulent *Listeria* named *Listeria rocourtiae* sp. and *Listeria marthii*, which were isolated from precut lettuce and the natural environment, respectively. Among all the *Listeria* species cited, only *L. monocytogenes* and *L. ivanovii* are pathogenic. *Listeria monocytogenes* infects both man and animals, whereas infection by *L. ivanovii* is more frequent among animals than among humans.

Morphology, Culture, and Metabolism

Listeriae are Gram-positive, short rods (diameter 0.5 μm, length 0.5–2 μm) with rounded or sometimes coccoidal ends (Figure 1). They do not form spores or capsules. *Listeria* are motile with peritrichous flagella at 20–25 °C, but are not motile, or less noticeably motile at 37 °C. All listeriae show characteristic tumbling motility when viewed microscopically in hanging-drop fresh broth cultures and, owing to their microaerophilic nature, grow in 'umbrella' forms approximately 0.5-cm below the surface of stabbed semisolid media.

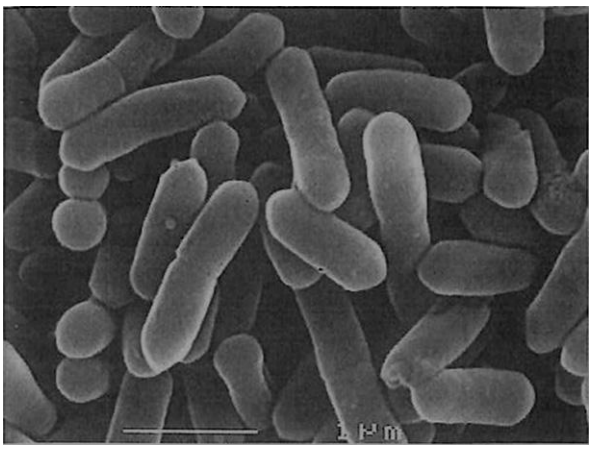

Figure 1 Photomicrograph of *Listeria monocytogenes*; bar = 1 μm. Courtesy of JA Vazquez-Boland. Copyright © JA Vazquez-Boland.

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Listeriae are fastidious, and grow on common bacteriological media. After overnight incubation, the colonies are smooth, bluish-gray, translucent, and 0.2–0.8 mm in diameter, but after prolonged incubation can be much larger. Rough colonies are sometimes observed. Colonies on a clear medium, which when viewed under a microscope with obliquely transmitted light, typically appear sparkling blue-green, a characteristic useful for distinguishing them from colonies of other microorganisms.

Listeria utilize glucose, forming lactate, acetate, and acetoin under aerobic conditions, but acetoin is not produced anaerobically. They are oxidase-negative and catalase-positive; hydrolyze aesculin but not urea, gelatin, or casein; and are methyl red- and Voges–Proskauer-positive. *Listeria* are facultatively anaerobic but prefer microaerophilic atmospheres when growing aerobically. *Listeria* require cystine, leucine, isoleucine, arginine, valine, cysteine, riboflavin, biotin, thiamin, and thioctic acid for growth. *Listeria* are able to grow at temperature, pH, and water activity (a_w) ranging from 0 to 45 °C, 5.6 to 9.6, and 0.90 to >0.97, respectively.

Isolation and Identification

Conventional Methods

Foods can simultaneously contain relatively low numbers of *L. monocytogenes* and large numbers of background microorganisms, which can make isolation of the pathogen difficult. Previously, a cold-enrichment procedure was attempted, with incubation of the enrichment broth at 4 °C for weeks or months to increase the numbers of listeriae before their isolation on solid media. Subsequently, modern *Listeria*-selective enrichment broths and solid media, which require shorter incubation times and contain selective agents to inhibit background microorganisms, were developed (**Table 1**). However, selective agents may not allow growth of all *Listeria* strains, and can inhibit injured *Listeria* cells that are often present in foods. This is problematic, especially for methods involving direct plating on selective agars without prior enrichment, as their effectiveness is determined by the fitness and levels of both *Listeria* and the background microorganisms.

Although a nonselective enrichment (buffered peptone water or a universal broth) can be used to recover injured listeriae before subculturing in a selective enrichment, there is a risk of listeriae being overgrown by competing organisms in the process. As an alternative, the thin agar layer (TAL) method has been used for recovering injured *L. monocytogenes* from foods. The TAL method requires the preparation of plates of a selective agar overlaid with a nonselective agar shortly before the plates are incubated. During the first hours of incubation, injured cells recover and start to grow on the nonselective agar. After diffusion of agents from the selective agar into the upper layer, colonies typical of those on the selective agar are formed whereas other microorganisms are inhibited. A list of official methods for the isolation of *L. monocytogenes* from food and the environment are presented in **Figure 2**. For the identification of *Listeria* species, tests including hemolysis and acid production from D-xylose, L-rhamnose, α-methyl-D-mannoside, and mannitol are recommended. Additional identification tests include a number of commercially available, multicomponent, miniaturized biochemical assays, as well as some DNA- or polymerase chain reaction-based methods.

Serotyping is a quick, traditional method for subspecies characterization of *L. monocytogenes* isolates, based on the detection of specific somatic (O) and flagellar (H) antigens. Currently, 13 serotypes are distinguished: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 5, 6a, and 6b. Three serotypes, 1/2a, 1/2b, and 4b, have been implicated in more than 90% of all human *L. monocytogenes* infections. Serotypes 1/2a and 1/2b are more commonly isolated from foods, but serotype 4b more frequently causes foodborne outbreaks of listeriosis. Molecular subtyping methods show that *L. monocytogenes* can be separated into three genetic groups. Serotypes 1/2b, 3b, 4b, 4d, and 4e form lineage I; serotypes 1/2a, 3a, 1/2c, and 3c form lineage II; and serotypes 4a, 4b, and 4c form lineage III. Lineage III strains appear frequently among isolates from animals with listeriosis. The distribution of serotypes is different in different geographic regions. The main causes of human listeriosis in the US are strains of lineage I serotype 4b, whereas lineage II serotype 1/2a strains cause the majority of human listeriosis in Europe and Canada. Lineage II strains were responsible for a large outbreak of listeriosis associated with RTE meat products in 2008 in Canada, and acid curd

Table 1 Examples of selective agents and related media for the isolation of *Listeria monocytogenes*

Selective agents	*Role*	*Broths with selective agents (numbered)*	*Agars with selective agents (numbered)*
1. Lithium chloride/ phenylethanol	Promotes *Listeria*, slowing growth of Gram-negatives	Fraser[a] (1+2+3)	Modified Oxford agar[a] – MOX (1+5)
2. Nalidixic acid	Inhibits Gram-negatives	UVM (2+3)	PALCAM agar[a] (1+3+4+5/6)
3. Acriflavin/trypaflavin	Inhibits Gram-positive cocci	FDA enrichment[a] (2+3)	ALPAMY agar (1+3)
4. Polymyxin B	Inhibits Gram-negatives and streptococci	L-PALCAMY (1+3+4+5/6)	RAPAMY agar (1+2+3)
5. Moxalactam	Broad spectrum: inhibits many Gram-positives and Gram-negatives	IDF enrichment[a] (2+3+6)	Modified McBride agar[a] (1)
6. Ceftazidime/ cycloheximide	Broad-spectrum antibiotic	LRB selective (2+3+6)	AC agar (3+6)

[a]Data from Curtis, G.D.W., Lee, W.H., 1995. Culture media and methods for the isolation of Listeria monocytogenes. International Journal of Food Microbiology 26, 1–13; Donnelly, C.W., 1999. Conventional methods to detect and isolate Listeria monocytogenes. In: Ryser, E.T., Marth, E.H. (Eds.), *Listeria*, Listeriosis, and Food Safety. New York: Marcel Dekker, pp. 225–260 (Chapter 7); and Farber, J.M., Peterkin, P.I., 2000. *Listeria monocytogenes*. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food, vol. II. Gaithersburg: Aspen Publishers, pp. 1178–1232.

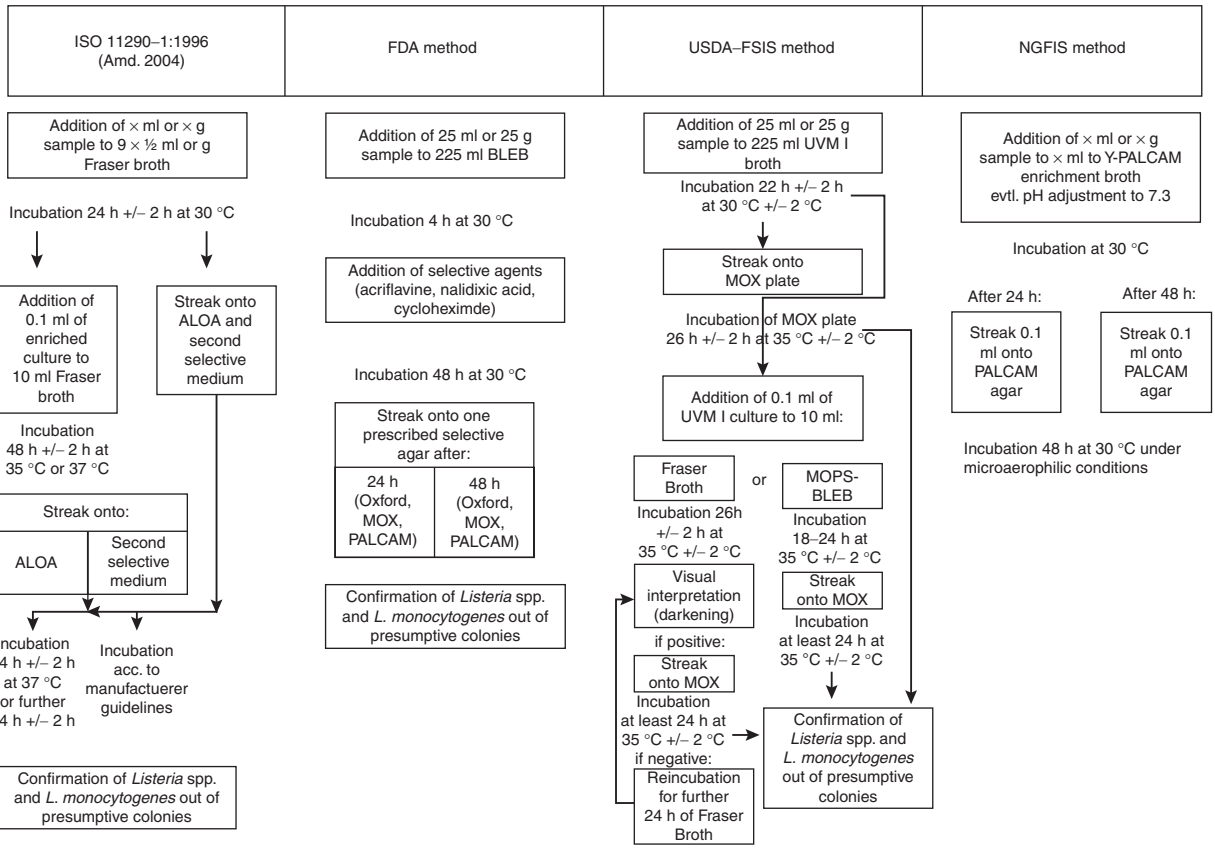

Figure 2 Official methods for the isolation of *L. monocytogenes* from food and the environment. Reproduced from Zunabovic, M., Domig, K.J., Kneifel, W., 2011. Practical relevance of methodologies for detecting and tracing of *Listeria monocytogenes* in ready-to-eat foods and manufacture environments – A review. Food Science and Technology 44, 351–362.

cheese contaminated with a serotype 1/2a strain caused outbreaks in Austria and Germany in 2009/2010.

Phage typing is another traditional method that is inexpensive, and easy to conduct. It can be more discriminatory than serotyping, but up to 50% of strains are not phage typeable.

Rapid Detection Methods

Conventional methods for the detection of *L. monocytogenes* are relatively slow, and unsatisfactory for purposes such as end product testing or various epidemiological investigations, for which results should ideally be obtained rapidly. Relatively rapid molecular methods have therefore been developed.

Nucleic acid amplification-based detection methods frequently target virulence genes of *L. monocytogenes*, particularly *hly*A (the gene producing *Listeria* listeriolysin O; LLO). These methods variously use the ligase chain reaction (LCR), PCR, or nucleic acid sequence-based amplification (NASBA). LCR methods use DNA targets such as the 16S rRNA gene as a template, and their particular strength is high specificity. PCR methods use thermostable DNA polymerase and flanking oligonucleotide primers to amplify targeted nucleic acid sequences, which include those of *hly*A, the genes for invasion-associated protein (*iap*), phospholipase B (*plc*B), or the delayed-type hypersensitivity factor. PCR methods are highly sensitive. NASBA amplifies nucleic acid targets with a series of enzymes including RNA polymerase and reverse transcriptase, and the templates used include 16S rRNA and *hly*A mRNA. Generally, nucleic acid amplification-based detection methods are rapid, but they can produce both false negative results due to inhibition of enzymatic reactions and false positives due to detection of nonviable cells or extracellular DNA.

Antibody-based methods detect *Listeria* antigens using enzyme-linked immunosorbent assay formats, where cells are either directly absorbed or immune-captured onto wells of microtiter plates and are then detected using antibodies carrying haptens or reporter enzymes. Alternatively, antibody-based assays can employ flow cytometry for the detection of immune-captured cells in solutions; or with immunomagnetic separation techniques, antibodies conjugated to magnetic beads can be used to capture and concentrate cells before plating onto *Listeria*-selective media.

Further improvements of current rapid methods will require the reduction or elimination of time-consuming enrichment steps, and the specific detection of only pathogenic *L. monocytogenes* rather than *L. monocytogenes* in general, or all *Listeria* spp.

Subtyping methods are important tools for confirming the identities of microorganisms associated with foodborne outbreaks, identification of new and emerging bacterial pathogens, tracking organisms as they colonize surfaces in food processing plants, and contribute to understanding the

genetics, epidemiology and ecology of foodborne pathogens such as *Listeria*. More modern, molecular subtyping methods include pulsed-field gel electrophoresis (PFGE), chromosomal DNA restriction endonuclease analysis (REA), MEE, restriction fragment length polymorphism (RFLP; ribotyping), random amplification of polymorphic DNA (RAPD), and arbitrarily primed PCR (AP-PCR). PFGE analysis of DNA macrorestriction patterns is presently considered the best subtyping method for *L. monocytogenes*, as it can distinguish closely related strains indistinguishable by other methods. Nevertheless, it is relatively slow (2–3 days) and expensive in terms of both reagents and equipment required. REA is universally applicable for *L. monocytogenes* and is easy and cost effective, but its limitations include the production of very complex and difficult to compare profiles. MEE, which distinguishes strains on the basis of the electrophoretic mobility of their enzymes, is a powerful tool in taxonomic studies of *L. monocytogenes*, but is moderately discriminatory in epidemiological investigations and is rather laborious. RFLP, which analyses polymorphisms associated with ribosomal operons, is widely used and can be conducted using a commercially available, automated system. As its discriminatory power for *L. monocytogenes* is not very high, it should be used in combination with other typing methods such as PFGE or PCR. RAPD and AP-PCR methods are both based on PCR. They are relatively simple and have good discriminatory power for *L. monocytogenes*, but results obtained with these methods are not very consistent. Other, promising subtyping methods that are yet to be fully developed include DNA sequence-based techniques.

Listeriosis

Listeriosis in Meat Animals

Listeriae are ubiquitous in animal environments. Listeriosis in meat animals is not notifiable in most countries; so it is difficult to compare the prevalences among countries. In all animal species, a proportion of clinically healthy animals can shed the pathogen in their feces, and *L. monocytogenes* can also be shed in milk from animals with or without symptoms of mastitis. The most common forms of animal listeriosis are encephalitis ('circling' disease), placentitis with abortion, and septicemia. Infection can occur via the gut from contaminated feeds, with poor-quality, i.e. insufficiently acidic, silage frequently being a vehicle for the pathogen. Infection through lesions in the mouth, nostrils, or conjunctiva can also occur. Factors contributing to infection include poor animal husbandry, stress, losing or cutting teeth, sudden changes in diet, concurrent disease, introduction of new animals to herds, and overcrowding.

In adult sheep and goats, meningoencephalitis is the most common form of the disease, with death often occurring in 2 or 3 days. Septicemia occurs primarily in neonates and lambs. Sheep seemingly have a relatively high natural resistance to *L. monocytogenes* because often only one-tenth of animals in an exposed flock exhibit clinical symptoms.

In cattle, listeriosis encephalitis is probably the most common form, but diseased cattle survive longer than diseased sheep. In pigs, listeriosis primarily manifests itself as septicemia, whereas encephalitis and abortion are rare. Diseased piglets usually die, but adult pigs generally survive. Listeriosis in horses is not common and is usually associated with feeding silage, or housing or grazing horses with cattle or sheep.

In domestic and wild birds, the proportion of healthy *L. monocytogenes* carriers is generally higher than the proportion in meat animals, probably owing to pecking of contaminated materials including soil. In contrast, clinical listeriosis is much less frequent in poultry than in meat animals. The most common form of listeriosis in poultry is septicemia, with few overt symptoms and death within 5–10 days. Encephalitis in poultry is rare but nearly always fatal.

Listeriosis in Humans

Listeria monocytogenes can be excreted in the feces by 1% to >50% of healthy people. Factors that tend to increase carriage of the organism include laboratory work with *L. monocytogenes*, household contacts with listeriosis patients, and working in cheese factories.

The incubation period for listeriosis is highly variable and can range from 1 day to more than 3 months. The reasons for this variability are not known, but they may include variations in the dose ingested or in the responsiveness of the host's immune system. Maternal listeriosis occurs during pregnancy, most commonly during the last 3 months and more frequently among women with multiple pregnancies. The infected mother can be asymptomatic or have relatively mild nonspecific (flu-like) symptoms, but the infection can be transmitted via the placenta to the fetus and may cause abortion or stillbirth. Neonatal listeriosis can occur as early- or late-onset disease, which is often fatal. The early-onset form is due to intrauterine infection of the infant with the development of listeriosis, usually presenting as sepsis or meningitis, either at birth or within a few days of birth. The late-onset form of the disease occurs between 1 week and several weeks after the birth of usually healthy infants and often presents as meningitis. The infection may have been acquired during the intrauterine phase, during passage through birth canal, or from other neonates in the hospital.

In other than expectant mothers, listeriosis occurs primarily in 'at risk' individuals who are immunocompromised owing to acquired immunodeficiency syndrome, immunosuppressive therapy or old age, or who have malignant diseases, diabetes, or hepatic conditions. The most common clinical symptoms are meningitis/meningoencephalitis, with mortality rates between 20% and >50% and sepsis. Other forms of disease caused by *L. monocytogenes* include endocarditis, cutaneous infections in farmers and veterinarians associated with handling infected animals, and variously localized infections.

Some reports indicate that noninvasive listerial infections can occur in healthy adults, primarily after ingestion of high numbers of the pathogen in food, with an incubation time of 24 h and febrile gastroenteritis symptoms, including vomiting and diarrhea. This condition is usually self-resolving but sometimes progresses to bacteremia.

The drug of choice for the treatment of listeriosis is a β-lactam antibiotic (e.g., penicillin) alone, or in the case of immunocompromised patients in combination with an aminoglycoside (e.g., gentamicin). As a second choice, especially

for patients allergic to β-lactams, trimethoprim and sulfonamide (e.g., sulfamethoxazole) are used in combination. The treatment may last as long as 6 weeks in immunocompromised patients, owing to the risk of relapse from *L. monocytogenes* surviving within the host cells. Although *L. monocytogenes* strains from food and food processing environments as well as human isolates are susceptible to the antibiotics commonly used for the treatment of listeriosis, some strains are resistant to one or more of the antibiotics nalidixic acid, oxacillin, clindamycin, tetracycline, and oxytetracycline. Usually, the antibiotic resistance of *Listeria* spp. is due to the acquisition of mobile genetic elements. Therefore, the potential for acquisition of resistance by *Listeria* spp. to a broader range of antibiotics is possible, with consequent difficulties for management of invasive infections.

Mechanism of Pathogenicity

The pathogenesis of listerial infection is still poorly understood. Although small numbers of *L. monocytogenes* may often be ingested with foods, the prevalence of listeriosis is low. The infective dose is variable, as is the incubation period. These variations are caused by interrelationships between the three main factors that determine the course of an infection. These are the number of *Listeria* cells ingested, the pathogenicity/virulence of the strain involved, and the immunological status of the host.

Virulence heterogeneity among *L. monocytogenes* isolates has been commonly observed. It is of interest that most isolates from foods belong to serogroup 1/2, but more than 50% of listeriosis cases worldwide are caused by serotype 4b. It might be that serotype 4b is particularly well adapted to host tissues. Further, some species-related tropisms exist among strains of *L. monocytogenes*. For example, some ribotypes of *L. monocytogenes* serotype 4b associated with human foodborne outbreaks are only infrequent causes of ruminant listeriosis.

Colonization of the Host

Ingested *L. monocytogenes* cells apparently invade the host tissue via the M cells that overlay Peyer's patches in the intestine; but the mechanism by which they enter these cells is still unclear. Subsequently, the pathogen is carried in the blood and lymph to the mesenteric lymph nodes, the liver, and the spleen. Resident macrophages in the liver and spleen rapidly clear the pathogen from the blood with most of the invading cells being captured and killed by liver Kupffer cells. However, surviving *L. monocytogenes* multiply within the hepatocytes and spread directly between them. Hepatocytes respond by releasing neutrophil chemoattractants, initiating apoptosis, and producing a local immune response. After mononuclear cells and lymphocytes from the blood become involved, characteristic granulomas are formed. Several days after infection, *L. monocytogenes* starts to disappear gradually from the organs as a result of a complex immune reaction involving macrophage activation and the action of cytotoxic T lymphocytes that finally destroy *Listeria*-infected cells. In the immune host previously exposed to the pathogen from foods or the environment, this chain of events results in rapid elimination of the pathogen from the liver. In immunocompromised hosts, the immune response in the liver is inadequate and, consequently, *L. monocytogenes* can proliferate, recontaminate the blood, and subsequently infect a range of tissues, but with significant tropism for the pregnant uterus and central nervous system.

Intracellular Cycle of Infection

Listeria monocytogenes can be internalized within various types of eukaryotic cells. The process starts with the pathogen's adhesion to the cell surface, on which it recognizes a number of receptors. It then is gradually engulfed by the host cell and becomes internalized within a phagocytic vacuole.

Listeria monocytogenes can remain viable in the vacuole because it prevents phagosome maturation to the phagolysosomal stage. Subsequently, it disrupts the phagosome membrane by a hemolysin-mediated process and enters the cytoplasm. In the cytoplasm, the bacteria start to multiply and become surrounded by actin filaments, which later form an actin tail at one end of each bacterial cell. The actin tails allow the bacterial cells to move in the cytoplasm until some of them reach the host cell membrane. When a bacterium presses against the cell membrane, a protrusion with the bacterium at the tip is formed. The protrusion penetrates a neighboring tissue cell. The bacterium and the protruded membrane become engulfed in a secondary phagosome so that the bacterium is surrounded with a double membrane. Dissolution of these membranes frees the pathogen to begin a new intracellular cycle.

Virulence Factors

Numerous genes and virulence factors contribute to the pathogenicity of *Listeria*. Virulence factors involved in infection are hemolysin (LLO); two phospholipase C enzymes, PC-PLC, and PI-PLC; internalins A (InIA), B (InIB), and C (InLC); and the protein ActA. Although InIA interacts with E-cadherin to mediate *L. monocytogenes* entry into epithelial cells, InIB facilitates its entry into a much broader range of cell types including hepatocytes, fibroblasts, and epithelioid cells. InLC may promote invasion of neighboring host cells. The vacuole formed around *L. monocytogenes* when it invades a cell is lysed by LLO and the two phospholipase C enzymes. LLO not only mediates lysis of the primary phagosome formed during uptake of the bacterium, but it is also required for escape from the double membrane vacuole that is formed during cell-to-cell spread of *L. monocytogenes*. The pores or membrane lesions caused by LLO probably facilitate access of the phospholipases to their substrates, leading to total dissolution of the phagosome. The intracellular mobility and cell-to-cell spread of *L. monocytogenes* require another surface protein, ActA, which is cotranscribed with PC-PLC. ActA mediates the formation of the polarized actin tail that propels the bacterium within the cytoplasm.

The adaptive, stress-mediated response of *L. monocytogenes* following exposure to sublethal hot, acidic, cold, or starvation conditions can increase its resistance to subsequent stressful conditions. Such stress hardening, particularly that caused by the acid condition that can be encountered in some foods and in the stomach, may also affect pathogenicity. Antioxidants such as catalase and superoxide dismutase play

a key role in the pathogen's defense against the oxygen-dependent microbiocidal mechanisms of the host.

Epidemiology of Meatborne Listeriosis

Contamination of Meat Processing Environments and Meats

Listeria monocytogenes is ubiquitous in the environment (see Figure 3), and is commonly present in food plants, including red meat and poultry abattoirs, and meat processing facilities. The organism can colonize equipment, walls, floor surfaces, and drains, as it adheres to and can form biofilms on stainless steel, glass, rubber, and polypropylene surfaces. The widespread presence of *L. monocytogenes* on raw materials and in processing environments leads to relatively frequent contamination of meat products with the organism. However, the strains found in meat products are a limited group of the strains present in animals, raw materials, or food manufacturing environments.

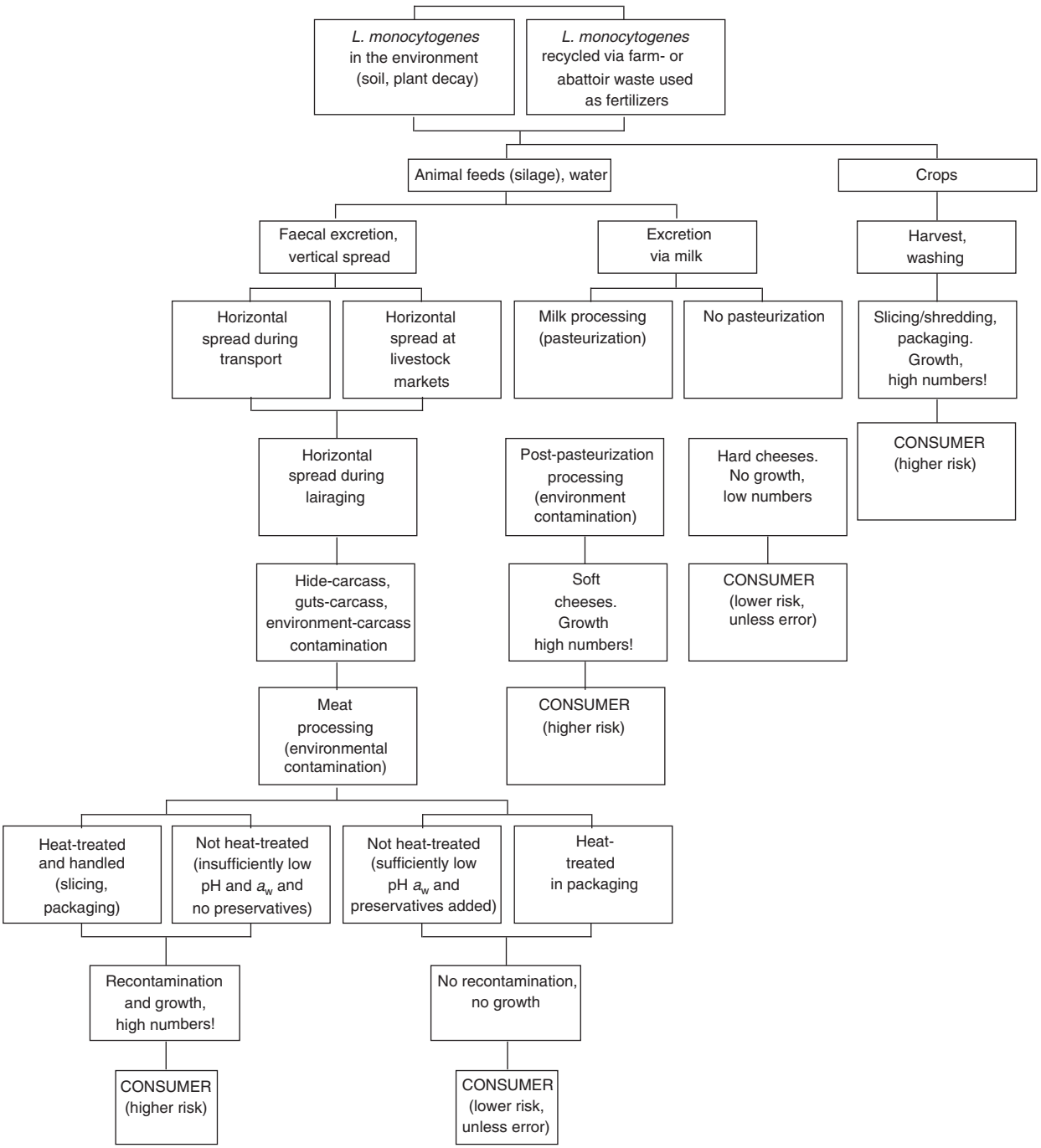

Figure 3 General epidemiology of foodborne listeriosis.

Incidence, Growth, and Survival on Meats

Reported prevalences of *L. monocytogenes* in meats (Table 2) vary greatly owing to differences in isolation methods, sizes of samples, product type, place and time of sampling, sanitation practices, production volume, season of the year, and temperature. Although *L. monocytogenes* is frequently located on product surfaces, it has occasionally been found within muscle tissue, indicating possible contamination of animal tissues before or during slaughter.

Listeria monocytogenes grows well on many foods, including meat, poultry and fish, but growth is dependent on temperature, pH, a_w, and the atmosphere around the food. The lower limits of temperature, pH, and a_w for growth of *L. monocytogenes* appear to be –0.4 °C, 4.4, and 0.90, respectively. The organism can grow in 10% NaCl. In foods, the temperatures, pH values, a_w values, and NaCl concentrations at which growth occurs are usually more restricted and are affected by food type, oxygen availability, and *Listeria* strain variability. Reported rates of growth of *L. monocytogenes* on meats are inconsistent, because of differences in the conditions used in studies; and because results obtained with different strains grown under the same condition can vary substantially.

Heat treatment is the most effective means of eliminating *L. monocytogenes* from meats. *Listeria monocytogenes* appears to be more heat resistant than other nonspore-forming foodborne pathogens, so cooking recommendations designed for rendering meat safe with respect to *Salmonella* might not be sufficient to eliminate *Listeria*. Table 3 illustrates the heat resistance of *L. monocytogenes* in meat substrates.

Table 2 Prevalence (percentage or percent range) of *Listeria monocytogenes* in meats from various countries of Europe, North America, and the Asia-Pacific region

Type of meat	*Europe (%)*	*USA/Canada (%)*	*Asia/Pacific (%)*
Raw meats			
Beef	5–35	6–77	0–60
Pork	3–29	0–95	10–67
Lamb/sheep	40–50	0	0–16
Poultry	12.5–100	0–70	25
Processed meats			
Precooked sausages	0–60	0–71	0–4
Fermented sausages	5–19	20	
Sliced, cooked	3–23	13	
Pate	2–85		0
Cooked poultry	0–25	2	

Source: Data from Jay, J.M., 1996. Prevalence of *Listeria* spp. in meat and poultry products. Food Control 7, 209–214 and Farber, J.M., Peterkin, P.I., 2000. *Listeria monocytogenes*. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food, vol. II. Gaithersburg: Aspen Publishers, pp. 1178–1232.

Listeriosis Associated with Meats

The incidence of listeriosis in human populations varies among countries. In the European Union (EU), the number of confirmed listeriosis cases in humans increased by 19.1% from 2008 to 2009. On the basis of the reported fatality rates and the total numbers of confirmed cases, it was estimated that in 2009 there were approximately 270 human deaths due to listeriosis in the EU. In the US, although *L. monocytogenes* is not among the five pathogens that cause most domestically acquired foodborne illnesses or hospitalizations, the number of deaths caused by this pathogen is relatively high. Although there were only 125 cases of foodborne listeriosis reported from 1996 to 2010, the case–fatality ratio of 12.8 was the highest among those pathogens that cause the most foodborne illnesses. In Canada, *Listeria* cause similar low rates of illness, but in 2008 a listeriosis outbreak caused by consumption of contaminated RTE meats claimed 23 lives.

Vehicles of infection in outbreaks of foodborne listeriosis have included dairy and various meat products (Table 4). Generally, it is accepted that *L. monocytogenes* levels at the time of consumption of < 100 cfu g^{-1} of food represent a very low risk for all populations. This limit relates not to dose but to a concentration, and is not based on a formal dose–response formula. Although this level might not be exceeded at the time of production of a RTE food, it might be exceeded after growth in the food. Risk management strategies are focused on those foods in which *L. monocytogenes* can multiply and are consumed without cooking.

Control and Preventive Measures

In some countries, such as the US, the total absence of *L. monocytogenes* from all RTE foods is required. In others, such

Table 3 Heat resistance of *Listeria monocytogenes* in meats

Meats	D-*value or range of* D-*values (minutes) at temperatures*					
	50 °C	*55 °C*	*60 °C*	*64–66 °C*	*70 °C*	*77 °C*
Raw beef	36.1–85	3.14–21	0.24–12.5	0.56–0.93	0.14–0.47	
Raw pork	109	9.8	1.14			
Raw poultry	100–179	13–14	5.6–8.7	0.52	0.11–0.13	
Sausages		20.1	7.3	1–2.08		0.84
Ham		17.8	1.82–3.48			

Source: Data from Farber, J.M., Peterkin, P.I., 2000. *Listeria monocytogenes*. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food, vol. II. Gaithersburg: Aspen Publishers, pp. 1178–1232 and Buncic, S., Avery, S.M., Rocourt, J., Dimitrijevic, M., 2001. Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes*? International Journal of Food Microbiology 65, 201–212.
Note: *D*-values are minutes producing 90% reduction.

Table 4 Foodborne illness outbreaks in the US caused by *Listeria monocytogenes*

Year	*Illness*	*Hospitalization*	*Deaths*	*Food vehicle*
1998	101	101	21	Hot dog, unspecified
1999	2	2	1	Deli meat, sliced ham; deli meat, sliced roast beef; and deli meat, sliced turkey
1999	4			Hot dog, unspecified
1999	5	5	1	Deli meat, unspecified
1999	11			Pate, unspecified
2000	12			Queso fresco, unspecified
2000	29	29	7	Deli meat, sliced turkey
2001	56	1	0	Potato salad
2001	28	0	0	Sandwich, deli
2002	54		8	Deli meat, sliced turkey
2003	12	12	1	Queso fresco, unpasteurized
2003	3			
2005	6	6	0	
2005	3	3	0	Chicken, grilled
2005	13	13	1	Deli meat, sliced turkey
2005	12	12	0	Queso fresco, unpasteurized
2006	3			Ham, unspecified
2006	2	1	1	
2006	2	0	0	Taco or nacho salad
2006	3	2	1	Other cheese, pasteurized
2007	5	5	3	Other milk, pasteurized; skim milk, pasteurized
2008	5	5	3	Tuna salad
2008	8	4	0	Cheese, Mexican style, pasteurized
2008	20	16	0	Sprouts
2009	6	1	0	
2009	2	2	0	Cheese
2009	8	3	0	Mexican style cheese
2011	146	142	30	Cantaloupes

Source: Data from Centers for Disease Control and Prevention (CDC), 2012. Foodborne outbreak online database. Available at: http://wwwn.cdc.gov/foodborneoutbreaks (accessed 06.11.13).

Table 5 Grouping of ready-to-eat (RTE) foods relative to control potential for *Listeria monocytogenes*

Categories	*Food related*	*Sampling*	*Analysis*	*Action level for* L. monocytogenes
1. RTE foods in which growth of *L. monocytogenes* can occur throughout the stated shelf life, for example, durable life date shown as a 'best before' date on the package	Deli meats, soft cheeses, hot dogs, and pâté	5 sample units (min 100 g or ml each), which are representative of the lot and the production conditions, taken aseptically at random from each lot	5×25 g analytical units are either analyzed separately or composited	Detected in 125 g
2. (a) RTE foods in which a limited potential for growth of *L. monocytogenes* to levels not greater than 100 cfu g^{-1} can occur throughout the stated shelf life (e.g., durable life date shown as a 'best before' date on the package)	Refrigerated gravlax/ cold-smoked rainbow trout and salmon, fresh-cut produce, etc.	5 sample units (min 100 g or ml each), which are representative of the lot and the production conditions, taken aseptically at random from each lot	5×10 g analytical units	>100 cfu g^{-1}
(b) RTE foods in which growth of *L. monocytogenes* cannot occur (i.e., <0.5 log cfu g^{-1} increase (validation may be needed)) throughout the stated shelf life, for example, durable life date shown as a 'best before' date on the package	Ice cream, hard cheese, dry salami, dried-salted fish, and varieties of prosciutto ham	5 sample units (min 100 g or ml each), which are representative of the lot and the production conditions, taken aseptically at random from each lot	5×10 g analytical units	>100 cfu g^{-1}

Source: Data from Health Canada, 2011. Policy on *Listeria monocytogenes* in ready-to-eat foods. Bureau of Microbial Hazards Food Directorate Health Products and Food Branch. Identification Number: FD-FSNP 0071. Available at: http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2011-eng.php (accessed 06.11.13).

as Canada, this is regarded as unattainable. Instead, foods are classified with respect to risks from *L. monocytogenes* according to the lethality of the process used, controls in place to prevent recontamination, and the potential for growth of *L. monocytogenes* during food storage. The categories currently used in Canada are presented in **Table 5**, but groupings are somewhat different in other countries. Packaged, long shelf life foods that are not cooked in the final package generally pose the highest risks with respect to foodborne listeriosis.

The control of *L. monocytogenes* in RTE meat and poultry products is extremely important given the ubiquitous presence of *Listeria* in the environment, its tolerance to unfavorable environmental conditions such as low pH and high NaCl levels, its ability to survive on equipment, its potential to contaminate products after processing, and its ability to multiply at cold temperatures. Commonly, thermal processing eliminates *L. monocytogenes* from products, but recontamination from food contact surfaces and aerosols generated during cleaning represent constant challenges.

To control *L. monocytogenes* on RTE meat and poultry products, the US Department of Agriculture requires adoption of one of the three processing alternatives: (1) the use of a postlethality treatment that reduces or eliminates *L. monocytogenes* and an antimicrobial agent or process that supresses or limits *L. monocytogenes* growth throughout product shelf life; (2) the use of either a postlethality treatment or an antimicrobial agent or process that achieves the same result; or (3) reliance upon a suitable sanitation program to control *L. monocytogenes*. End-product sampling requirements for *L. monocytogenes* increase with the risk inherent in the processing alternative chosen, the product type, and production volumes/unit time.

As freezing causes injury in surviving *L. monocytogenes* cells, it is not surprising that when combined with another antimicrobial hurdle, cells in thawed RTE meats grow relatively slowly at 4 °C. It is worth noting that the organic acids naturally present in products like pepperoni were more antilisterial during product temperature abuse (12 °C and 25 °C) than at 4 °C.

Regardless of whether foodborne illness from *L. monocytogenes* is caused by fresh produce (celery and melons) or packaged cured meats, processing plants are critical points where control can be achieved and must be applied. With processed meats, cooking (69–71 °C) eliminates the organism, but its survival and growth on downstream processing equipment, as well as on food contact surfaces used for any of these types of product must be constantly addressed to prevent cross-contamination arising as a result of biofilm development.

See also: Biofilm Formation. Hazard Analysis Critical Control Point and Self-Regulation. Meat Marketing: Cold Chain. Microbiological Analysis: DNA Methods; Standard Methods. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Hurdle Technology; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Viruses; Yeasts and Molds; *Yersinia enterocolitica*

Further Reading

Buncic, S., Avery, S.M., Rocourt, J., Dimitrijevic, M., 2001. Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes?* International Journal of Food Microbiology 65, 201–212.

Curtis, G.D.W., Lee, W.H., 1995. Culture media and methods for the isolation of *Listeria monocytogenes*. International Journal of Food Microbiology 26, 1–13.

Donnelly, C.W., 1999. Conventional methods to detect and isolate *Listeria monocytogenes*. In: Ryser, E.T., Marth, E.H. (Eds.), Listeria, Listeriosis, and Food Safety. New York: Marcel Dekker, pp. 225–260 (Chapter 7).

European Commission, Health and Consumer Protection Directorate-General, 1999. Opinion of the scientific committee on veterinary measures relating to public health on *Listeria monocytogenes*. Directorate B – Scientific Health Opinions, Unit B3 – Management of Scientific Committees II, pp. 1–44. Available at: http://ec.europa.eu/food/fs/sc/scv/out25_en.html (accessed 06.11.13).

Farber, J.M., Peterkin, P.I., 2000. Listeria monocytogenes. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food, vol. II. Gaithersburg: Aspen Publishers, pp. 1178–1232.

Gilmour, M.W., Graham, M., Domselaar, G.V., *et al.*, 2010. High-throughput genome sequencing of two *Listeria monocytogenes* clinical iso lates during a large foodborne outbreak. BioMed Central Genomics 11, 1–15. doi:10.1186/1471-2164-11-120.

Graves, L.M., Helsel, L.O., Steigerwalt, A.G., *et al.*, 2010. *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. International Journal of Systematic and Evolutionary Microbiology 60, 1280–1288.

Jay, J.M., 1996. Prevalence of *Listeria* spp. in meat and poultry products. Food Control 7, 209–214.

Kang, D.H., Fung, D.Y.C., 1999. Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*. Journal of Food Protection 62, 1346–1349.

Leclercq, A., Clermont, D., Bizet, C., *et al.*, 2010. *Listeria rocourtiae* sp. nov. International Journal of Systematic and Evolutionary Microbiolog 60, 2210–2214.

Low, J.C., Donachie, W., 1997. A review of *Listeria monocytogenes* and listeriosis. Veterinary Journal 153, 9–29.

Roberts, A., Nightingale, K., Jeffers, G., *et al.*, 2006. Genetic and phenotypic characterizations of *Listeria monocytogenes* lineage III. Microbiology 152, 685–693.

Rocourt, J., 1999. The genus *Listeria* and *Listeria monocytogenes*: Phylogenetic position, taxonomy, and identification. In: Ryser, E.T., Marth, E.H. (Eds.), Listeria, Listeriosis, and Food Safety. New York: Marcel Dekker, pp. 1–20 (Chapter 1).

Silk, B.J., Date, K.A., Jackson, K.A., *et al.*, 2012. Invasive listeriosis in the foodborne diseases active surveillance network (FoodNet), 2004–2009: Further targeted prevention needed for higher-risk groups. Clinical Infectious Diseases 54 (S5), S396–S404.

Vasquez-Boland, J.A., Kuhn, M., Berche, P., *et al.*, 2001. *Listeria* pathogenesis and molecular virulence determinants. Clinical Microbiology Reviews 14, 584–640.

Zunabovic, M., Domig, K.J., Kneifel, W., 2011. Practical relevance of methodologies for detecting and tracing of *Listeria monocytogenes* in ready-to-eat foods and manufacture environments – A review. Food Science and Technology 44, 351–362.

Relevant Websites

http://www.cdc.gov/foodborneoutbreaks
CDC – Centers for Disease Control and Prevention.

http://www.cdc.gov/outbreaknet/reports.html
Centers for Disease Control and Prevention.

http://www.efsa.europa.eu
EFSA – European Food Safety Authority.

http://www.hc-sc.gc.ca
Health Canada.

Pathogenic *Escherichia coli*

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Glossary

Bacteremia The presence of bacteria in the blood.
Bacteriophage Any virus that can infect bacteria.
Epithelium A tissue that lines surfaces and cavities in the body.
Fimbriae A thin surface appendage produced by many bacteria.
Pathogenicity island A horizontally transferred group of genes that confer virulence to a bacterium.
Plasmid A deoxyribonucleic acid molecule that is not part of the chromosome and can replicate independently of it.

Introduction

Most *Escherichia coli* live as harmless commensals in the intestines of warm-blooded animals, but certain types can cause disease in their hosts; these latter organisms are referred to as pathogenic *E. coli* (PEC). The diseases that might be caused by PEC are varied and depend on the site of infection.

Extraintestinal PEC (ExPEC) cause diseases outside the gastrointestinal tract, the most common of which are urinary tract infections resulting from infection with uropathogenic *E. coli*. ExPEC have also been associated with sepsis, meningitis, pneumonia, and wound infections. The sources of ExPEC are believed to be the intestines of humans, although evidence is emerging that animals might be a source of at least some ExPEC.

Of greater current concern with respect to food are *E. coli* that cause diseases of the gastrointestinal tract. Gastroenteritis can be caused by a variety of different types of PEC. The major types are described in Table 1. Symptoms range from diarrhea, which can be either acute or chronic, to more severe complications that can result in death. These pathogenic types are grouped on the basis of the diseases they cause and on specific virulence factors, which are involved in the disease process. Those factors that are known to be important in gastrointestinal diseases tend to be associated with attachment of the organisms to the intestines and, in some cases, the production of toxins.

Enteroinvasive *E. coli* are closely related to *Shigella* spp. They cause a disease similar to shigellosis, with invasion of the intestinal epithelium and inflammation of the intestines. The genes that confer invasiveness are carried on a plasmid referred to as pInv. Enterotoxigenic *E. coli* (ETEC) are the major cause of traveler's diarrhea. Infection is initiated through attachment to and subsequent colonization of the intestines. Attachment involves the production of fimbriae called colonization factors. Once the intestines have been colonized, ETEC produce heat-stable (ST) and heat-labile toxins, which cause a watery diarrhea that might be mild, short lived, and self-limiting or severe and possibly fatal, as in patients with cholera.

Enteroaggregative *E. coli* (EAEC) have been associated with persistent diarrhea (i.e., lasting more than 14 days) in infants in developing countries, but EAEC can also cause acute diarrhea in adults and infants and are the second greatest cause of traveler's diarrhea. This group of *E. coli* is diverse with respect to virulence factors but generally display aggregative adherence (AA), i.e., the cells attach to the gut wall and form layers with one another in a stacked-brick appearance. Most EAEC produce AA fimbriae, which are involved in AA and can also be important in attachment to the host tissues. EAEC can also

Table 1 The diseases and major virulence factors of pathogenic *Escherichia coli* associated with intestinal illnesses

Type	*Time to illness*	*Disease*	*Major virulence factors*	
			Attachment	*Toxins*
Enteroinvasive *E. coli*	8–24 h	Watery diarrhea and dysentery with blood and mucus	Invasion-related plasmid (pInv)	
Enterotoxigenic *E. coli* (ETEC)	8–44 h	Watery diarrhea and traveler's diarrhea	Colonization factors	Heat labile and heat stable
Enteroaggregative *E. coli* (EAEC)	8–48 h	Watery diarrhea, acute and persistent diarrhea, and the second greatest cause of traveler's diarrhea after ETEC	Aggregative adherence fimbriae	EAEC heat-stable enterotoxin
Enteropathogenic *E. coli*	12–36 h	Watery diarrhea with mucus and infant diarrhea	Locus of enterocyte effacement (LEE) and bundle-forming pilus	
Enterohemorrhagic *E. coli* and Shiga toxin-producing *E. coli*	3–4 days	Diarrhea, bloody diarrhea, hemolytic uremic syndrome, and death	LEE and others	Shiga toxins (Stx1 and Stx2)

produce the enteroaggregative ST toxin, which is similar to ST found in ETEC, and can possess genes for a variety of other possible virulence factors.

Enteropathogenic *E. coli* (EPEC) are a leading cause of diarrhea in infants in developing countries. EPEC cause attaching and effacing (A/E) lesions in the intestines as a result of the intimate attachment that occurs between the bacteria and the host cells. The cell components required for this intimate attachment are encoded by genes located on a pathogenicity island termed the locus of enterocyte effacement (LEE). Most EPEC can also produce fimbriae called bundle-forming pili, which are responsible for bacterium–bacterium attachment and can be involved in the initial attachment of EPEC to intestinal cells.

Enterohemorrhagic *E. coli* (EHEC) can cause bloody diarrhea (hemorrhagic colitis) or more severe disease, such as hemolytic uremic syndrome (HUS) that can lead to death. EHEC are a subset of the broader group of Shiga toxin-producing *E. coli* (STEC) that can produce Shiga toxins (Stx). EHEC is the term given to STEC that have caused clinical disease, although STEC is often used more broadly when referring to EHEC. The term verotoxin-producing *E. coli* is an earlier synonym of STEC that is still sometimes used in the literature and refers to the toxicity of these strains against Vero monkey kidney cells *in vitro*. In addition to Stx, most EHEC also carry LEE or other adherence factors that assist in colonization of the host's intestines. EHEC of serotype O157:H7 have been responsible for large foodborne outbreaks, many of which have been linked to the consumption of meat and meat products. For this reason, in 1994, *E. coli* O157:H7 was declared an adulterant of ground beef by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA). The term STEC has been used to refer to this group of strains in its broader sense throughout most of this article as opposed to the more specific EHEC.

Other *E. coli* that are able to cause diarrhea include diffusely adherent *E. coli*, cell-detaching *E. coli*, and cytotoxic necrotizing *E. coli*, but little is known about the significance and impact of these *E. coli* as human pathogens or their relationships with animals used for meat production. Many of the virulence factors of PEC are mobile and can move between strains of *E. coli* to produce new pathogenic types. Thus, the *E. coli* O104:H4 outbreak in Germany and other parts of Europe during 2011, which affected more than 4000 people, with more than 900 cases of HUS and at least 50 deaths, was caused by a strain of serotype O104:H4 that was essentially an EAEC which had acquired Stx and multiple antibiotic resistances. New combinations of virulence factors in *E. coli* are most likely to lead to new pathogenic types arising in the future.

All gastrointestinal PEC can be spread from human to human, either directly or through food and water that has been contaminated by humans. In addition to human sources, STEC have animal reservoirs and can be transmitted to humans through consumption of foods made from contaminated meat or by direct contact with contaminated animals. Ruminants, particularly cattle, are the major reservoir of STEC, and milk and meat have been implicated in outbreaks. For this reason the focus of the remainder of this article is on STEC as they are the most significant PEC associated with meat.

Clinical Significance

Human infection with STEC can cause a spectrum of diseases ranging from asymptomatic carriage to death. Worldwide, *E. coli* O157:H7 is the most common cause of HUS. However, in several outbreaks, the *E. coli* O157:H7 outbreak strain was isolated from stools of asymptomatic persons as well. The normal signs of *E. coli* O157:H7 gastroenteritis include abdominal pain, nonbloody diarrhea followed by bloody diarrhea after 1–4 days, absence of fever, and five or more bowel movements per day. HUS develops 5–13 days after initial diarrhea in 10–15% of patients, most commonly children who are less than 5 years of age. HUS is characterized by an acute onset of kidney damage. Additional severe extrarenal complications, including neurological impairment, increased pancreatic enzymes, edema, necrosis of the colon wall, and myocardial and central nervous system damage, can result from HUS and often lead to death. STEC infections generally do not result in bacteremia, indicating that the systemic complications of HUS are attributable to circulating Stx. The cell surface receptor glycosphingolipid globotriaosylceramide (Gb3) is present on cells in the human kidney. Stx binds to Gb3 and is internalized by endocytosis, resulting in protein synthesis inhibition through disruption of the 28S ribosomal subunit.

Molecular Aspects of Pathogenicity

Production of Stx is essential for the pathogenesis of bloody diarrhea and HUS caused by both *E. coli* O157:H7 and non-O157 STEC. Two main Stx types are encoded by bacteriophages integrated into the chromosomes of STEC/EHEC strains. Stx1 is closely related to the Stx of *Shigella dysenteriae*, but Stx2 shows only 55–60% deoxyribonucleic acid (DNA) and amino acid similarity to Stx1. Although a variety of genetic subtypes of stx_1 and stx_2 genes have been described, expression of the stx_2 gene is most strongly associated with significant clinical manifestation of bloody diarrhea and HUS. In addition to Stx production, all *E. coli* O157:H7 and most non-O157 STEC (e.g., serotypes O26, O45, O103, O111, O121, and O145) also encode the LEE pathogenicity island. However, HUS is also caused by some LEE-negative serotypes (e.g., O91, O104, and O113), indicating that LEE is not essential for HUS causation and that additional virulence factors might contribute to the severe illnesses caused by these serotypes.

There are phylogenetic and geographic variations among *E. coli* O157:H7 strains in the potential for disease causation and the severity of the illness they cause. Three lineages of *E. coli* O157:H7, LI, LII, and LI/II, have been identified. Although all lineages are found in the bovine O157 reservoir, LI and LI/II are overrepresented among human O157:H7 isolates. LI predominates in North America and Japan, whereas LI/II is most prevalent in the Netherlands. Stx bacteriophage association with the different lineages are also frequently observed: LI isolates carry stx_1 and stx_2 bacteriophages; LII isolates carry stx_{2c} bacteriophage; and LI/II isolates carry stx_2 alone or stx_2 and stx_{2c} bacteriophages. Eight virulence clades were identified among North American human isolates and these clades were correlated with historical *E. coli* O157:H7 outbreaks occurring

in the United States. Infections caused by clades 1, 2, and 3 (also typed as LI) isolates from 1982 and 1993 hamburger outbreaks were less severe than clade 8 (also typed as LI/II) isolates from the 2006 spinach and lettuce outbreaks.

Ecology

STEC shed in animal feces can remain viable in the environment for up to several months. Animal manure and wastes that carry STEC can enter water sources. Contaminated water used for irrigation contaminates fresh produce. Swimming in or drinking contaminated water can lead to human illness, as can direct contact with animals on farms or at petting zoos.

Most of what is known about the ecology of STEC comes from the study of *E. coli* O157:H7. Much less is known about STEC of other serotypes. The major reservoir of *E. coli* O157:H7 are ruminant animals, particularly cattle. *Escherichia coli* O157:H7 has also been isolated from sheep, deer, and goats. Nonruminant animals, such as pigs, horses, rabbits, birds, and flies, have also been found to carry *E. coli* O157:H7 on occasion. *Escherichia coli* O157:H7 does not cause disease in cattle. It is found in the intestines of healthy animals and, if so, is shed in feces, which commonly contaminate animal hides. The organism is also found in the mouths of animals. The prevalence of *E. coli* O157:H7 in herds of cattle can vary over time. In the Northern Hemisphere, the highest prevalence is observed in the warmer summer months. Not all animals within a herd may shed *E. coli* O157:H7 at the same time, and those animals which do, mostly shed only very low numbers. Occasionally, an animal may shed high numbers ($>$ 10 000 cfu g^{-1}) of *E. coli* O157:H7. These animals, which have been termed super shedders, are thought to pose the greatest risk for meat contamination during slaughter and processing. The reasons why some animals on occasion shed high numbers of *E. coli* O157:H7 are unknown. Various means of preventing shedding of *E. coli* O157:H7 have been proposed with a view to reducing contamination of meat and meat products. However, no method has as yet been shown to have consistent and substantial effects in practice.

STEC generally are commonly found in ruminant and other animals. STEC other than *E. coli* O157:H7 can account for more than half of STEC illnesses in humans. Non-O157 STEC are transmitted to humans in the same manners as are *E. coli* O157:H7.

Presence and Survival on Meat

Most published data on STEC in meat and meat products are for the prevalence of *E. coli* O157:H7. The prevalence of *E. coli* O157:H7 on carcasses and on retail product is generally less than 0.3%. However, some studies have reported prevalence on carcasses and retail product $>$30% and $>$3%, respectively.

To cause foodborne disease, STEC need to survive and possibly grow on meat products. There is very little evidence to suggest that PEC on meat products behave much differently from *E. coli* generally. On raw chilled or frozen meats, *E. coli* O157:H7 and other STEC can survive for extended periods but do not grow. Although some decrease in numbers of the pathogen might occur during storage under refrigeration, this is not usually sufficient to eliminate them. Their presence on refrigerated products is of concern because of their relatively low infectious doses and because cross-contamination of other food products may occur. STEC is eliminated in properly cooked products. The survival and growth of STEC in processed meats is dependent on factors such as the pH, water activity, and the presence of preservatives in the products. Control of these qualities in some products, such as certain types of salami, is critical for preventing the growth of these pathogens. Although it has been suggested that *E. coli* O157:H7 are more resistant to acidic conditions than other *E. coli*, the available evidence indicates that this is not the case. Control of PEC growth can generally be achieved by the same measures that control the growth of generic *E. coli* (see Section 'Control' below).

Detection

There are many challenges associated with the detection and isolation of PEC in meat and meat products. These include low prevalence, low numbers if present, and the presence of other bacteria, including non-PEC in meat and meat products. Sampling plans and test methods have been progressively developed to counter these challenges. An example of this is the microbiological testing program for *E. coli* O157:H7 in beef developed by the USDA FSIS. This was introduced in 1994 when US legislation categorized *E. coli* O157:H7 as an adulterant of ground beef. There have been several changes made since then to improve the sensitivity of the methods, for example, adoption of improved detection methods and increasing the amount of meat tested from 25 to 375 g. The general approach for detection of PEC in meat involves enrichment, screening to determine whether the target *E. coli* is present, and then isolation and confirmative identification of the pathogen. Enrichment is used to increase the numbers of PEC; however, this also increases the numbers of other bacteria. Selective agents, such as antimicrobials or other chemicals, may be added to inhibit competing bacteria. After enrichment, samples may be screened, using molecular methods (such as polymerase chain reaction) or immunological methods (such as enzyme immunoassays), for the presence of virulence factors, such as Stx and the *E. coli* attaching and effacing gene (*eae*) component of LEE, and specific serotypes, such as O157, O26, and O111.

If a molecular screening test is positive, the specific *E. coli* of interest must be isolated and confirmed. Within a single sample, the target serotype and individual virulence factors might each reside in a different *E. coli*. For example, a sample might test positive for Stx (or *stx*), *eae*, or LEE and serotype O111 on screening, implying that non-O157 STEC of serotype O111 is present in the sample. However, the *stx* gene might be carried in a nonpathogenic STEC, the *eae* gene in a different *E. coli*, and the O111 strain might be in yet another *E. coli*. In this case, the sample is negative for non-O157 STEC of serotype O111. The isolation of PEC from enriched samples can be difficult because of the presence of large numbers of other bacteria. Immunocapture can assist isolation of specific serotypes of *E. coli* through concentration of the target

organisms. For this, antibodies specific to a serotype are coated on magnetic beads or other particles. These antibodies bind cells of the target serotype by forming antibody–bacteria complexes. The coated particles can then be removed from the sample. In the case of magnetic particles, a magnet is used to remove the particles. This process is termed immunomagnetic separation. The antibody–bacteria complexes may then be tested further for the presence of the target serotype using selective and differential plating media or molecular detection methods.

Differentiating target *E. coli* from other *E. coli* on selective and differential media presents challenges as most *E. coli* share common phenotypic properties. A range of selective and differential media have been tested for the isolation of *E. coli* O157:H7 and non-O157 STEC. Most *E. coli* O157:H7 are phenotypically different from other *E. coli* in that they are unable to ferment sorbitol. Consequently, most *E. coli* O157:H7 produce colorless colonies on Sorbitol MacConkey Agar (SMAC), whereas other *E. coli* produce pink colonies. Cefixime and tellurite are commonly added to SMAC to improve its selectivity by inhibiting the growth of competing bacteria. *Escherichia coli* O157:H7 are also unable to produce the enzyme glucuronidase, a trait that differentiates them from most other *E. coli*. Such variations in biochemical abilities of different *E. coli* strains have been exploited to produce a range of chromogenic media for detection of different strains of *E. coli*. These media contain substrates that change color when cleaved by enzymes produced by bacteria. Unlike *E. coli* O157:H7, the non-O157 STEC are not easily differentiated from other *E. coli* on the basis of phenotypic differences. Once suspect colonies have been obtained, confirmation is necessary to ensure that the organism is an *E. coli* that carries the specific virulence factors and belongs to the serotype of interest. A range of biochemical, molecular, or immunologically methods can be used for confirmation.

There are many commercial test kits (including molecular and immunological) for detection of *E. coli* O157:H7. Methods for the non-O157 STEC are becoming commercially available in response to six of the non-O157 STEC serotypes being declared adulterants of ground beef by the USDA FSIS. Ongoing research into the pathogenic mechanisms of *E. coli* can identify more specific targets and lead to improvements of detection and isolation methods in the future.

Control

Numerous methods have been developed and applied in an attempt to control PEC on meat in general and beef in particular. These methods are applied at points in the production and supply chain from on the farm through primary processing to preservation of retail products. Some of the methods used and found to be effective not only control PEC but also affect generic *E. coli* and other bacteria as well. General approaches to hygiene and preservation fall into this category. More specific approaches to the control of PEC in meat have tended to focus on the control of *E. coli* O157:H7.

Results obtained with on-farm methods for the control of *E. coli* O157:H7 generally have been inconclusive, with many being found effective under some conditions but not under others. Given the diversity of agricultural practices associated with the production of meat, this is not surprising. Manipulation of diet has been one approach with, for example, high-fiber/low-energy and low-fiber/high-energy diets reportedly resulting in different prevalence and shedding rates of *E. coli* O157:H7 in various meat animal species. In particular, it has been postulated that the ratios of volatile fatty acids in and the pH of the rumen, which change with different diets, contribute to this effect. The gastrointestinal tracts of ruminants are, however, very complex and many factors might influence their functioning. Thus, no clear recommendations on the best diet to use to reduce *E. coli* O157:H7 carriage and shedding can be made. Vaccines represent another type of intervention that has been investigated for the control of *E. coli* O157:H7 in cattle. Although showing some promise, none of the vaccines developed so far can eliminate or consistently reduce carriage of this pathogen. Other methods that have been investigated to control *E. coli* O157:H7 on farm have been the supplementation of the feed or water of livestock with probiotics (live microbes with beneficial effects), bacteriophages (viruses that infect and kill specific bacteria), and chemicals, such as sodium chlorate. Although these methods have proved effective in trials, their practical value has generally been shown to be not as great as initially thought. On-farm interventions have included attempts to control PEC in the farm environment in order to restrict cross-contamination and reinfection of animals. Increased sanitation of equipment, such as water troughs, is an example of this form of control. Although such interventions are commendable and may reduce spread of a range of bacteria, they are unlikely to greatly reduce the risks posed by these bacteria.

A very wide range of methods have been applied for control of PEC (particularly *E. coli* O157:H7) during primary processing and in retail ready products. Many of these are general antimicrobial techniques that have either been tested on, or refined for effect on, PEC. Interventions that have been applied include steam pasteurization and treatment of carcasses, cuts trimmings, and other forms of meat with ionizing radiation, ozone, ultraviolet light, and chemical antimicrobials, such as chlorine and organic acids. Many of the methods have been shown to be effective in reducing both prevalence and numbers of, in particular, *E. coli* O157:H7 on carcasses and in retail products. None of the techniques have been shown to be completely effective for eliminating this pathogen under all conditions. In addition, many of the methods used have drawbacks associated with them, including the presence of potential harmful residues or a reduction in the quality of the meat product to which they have been applied. In most cases, a series or combination of rigorously tested and approved (by regulation) treatments used in combination have been shown to reduce the risks associated with these pathogens but not eliminate them.

The control of PEC throughout the food system is an area of active and ongoing research. Progress has been made in controlling PEC, particularly *E. coli* O157:H7, but the recent emergence of new serotypes and strains of concern to public health has complicated this endeavor. The search for effective means of controlling an ever-increasing array of pathogenic strains of *E. coli* will, undoubtedly, continue.

See also: Foodborne Zoonoses. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: Indicator Organisms in Meat. Parasites Present in Meat and Viscera of Land Farmed Animals. Spoilage, Factors Affecting: Microbiological

Further Reading

Bach, S.J., McAllister, T.A., Veira, D.M., Gannon, V.P.J., Holley, R.A., 2002. Transmission and control of *Escherchia coli* O157:H7 – A review. Canadian Journal of Animal Science 82, 475–490.

Ferens, W.A., Hovde, C.J., 2011. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. Foodborne Pathogens and Disease 8, 465–487.

Holck, A.L., Axelsson, L., Rode, T.M., *et al*., 2011. Reduction of verotoxigenic *Escherichia coli* in production of fermented sausages. Meat Science 89, 286–295.

Karama, M., Gyles, C.L., 2010. Methods for genotyping verotoxin-producing *Escherichia coli*. Zoonoses and Public Health 57, 447–462.

Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Review 11, 142–201.

Rhoades, J.R., Duffy, G., Koutsoumanis, K., 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. Food Microbiology 26, 357–376.

Schmidt, M.A., 2010. LEEways: Tales of EPEC, ATEC and EHEC. Cellular Microbiology 12, 1544–1552.

Relevant Website

http://www.fsis.usda.gov

Food Safety and Inspection Service – United States Department of Agriculture.

Prions

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Glossary

Astrogliosis An abnormal increase in the number of astrocytes, which are large, star-shaped cells in tissues of the nervous system, due to the destruction of nearby neurons.

Lymphoreticular system The tissues of the lymphoid and reticuloendothelial systems are regarded together as one system. It comprises primary lymphoid organs (bone marrow, bursal tissue, and the thymus), which are responsible for the production of lymphocytes, and the secondary lymphoid organs (lymph nodes, spleen, and gut-associated lymphoid tissue such as the tonsils and Peyer's patches), which function to provide an environment where lymphocytes can react to antigens from the tissue fluid, blood, and mucosal surfaces. The main functions of the lymphoreticular system are the removal of dying cells and the production of immune cells.

Neuropil A network of interwoven dendrites and axons and neuroglial cells in the gray matter of the central nervous system.

Introduction

Prion proteins are naturally occurring cell surface-anchored glycoprotein cellular proteins (PrP^Cs) that are found in fish, amphibians, birds, and mammals including humans. The biochemical function of PrP^C is not clear, but PrP^Cs are known to protect cells from oxidative damage, control circadian rhythms and sleep, and to be associated with the formation of memories. When PrPs are present with an abnormal conformation they can be infectious and recruit other PrP^Cs to change shape to the disease-forming isoform PrP^D. PrP^Ds are unique infectious agents that cause fatal neurogenerative diseases in humans and animals. In contrast to bacteria, viruses, and parasites, PrP^Ds do not contain nucleic acid, they are extremely difficult to destroy, and disease takes years to develop. Stanley Prusiner received the Nobel prize in 1995 for isolating proteinaceous infectious particles, which he termed 'prions,' and recognizing that prions are able to proliferate in the absence of nucleic acid. PrP^D is very resistant to degradative enzymes and cannot be broken down by the nerve cells. Nerve cells that incorporate PrP^D are eventually cleaved into fragments to leave cell-free holes in the brain. The cell fragments aggregate and precipitate to form plaques in the brain tissue, which eventually results in death. The spongiform damage, which is characteristic of prion-infected brain tissue, gave rise to the formal name 'transmissible spongiform encephalopathy (TSE)' for the disease condition caused by prions. TSEs include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in elk and deer, transmissible mink encephalopathy in mink, and feline spongiform encephalopathy in cats. Human TSEs include Creutzfeldt–Jakob disease (CJD), fatal familial insomnia (FFI), Gerstmann–Sträussler–Scheinker syndrome (GSSS), and kuru. BSE is the only animal TSE known to be transmissible to humans.

An epidemic outbreak of BSE, also known as mad cow disease, occurred in the 1980s in the UK. The outbreak was apparently due to the practice of supplementing cattle feed with rendered animal byproducts that included bovine tissues carrying BSE prions. The outbreak had a devastating economic impact on the beef industry worldwide when concerns were raised about the microbiological safety of meat derived from animals infected with BSE as a result of a reported increase in new variant CJD (vCJD) in humans. This suggested that dietary exposure to BSE prions might in time give rise to a major epidemic of vCJD. Before 1986, TSEs were not regarded as agents of foodborne disease in humans and little was known about the mechanism of prion diseases. Since then, much research effort has been invested in the detection, origin, distribution, pathogenicity, transmission, control, and eradication of, and assessment of risks from, animal and human TSEs.

Biochemical and Biophysical Properties

The amino acid sequence of PrP^D is identical to that of PrP^C. The conversion of PrP^C to PrP^D involves a change in the secondary structure of a part of the protein, from α-helix to β-sheet. Thus, human PrP^C contains 42% α-helix and 30% β-sheet, whereas PrP^D contains 30% α-helix and 43% β-sheet. The β-sheet conformation is more hydrophobic than the α-helix conformation, which leads to aggregation of PrP^D and the formation of amyloid plaques. It is unclear if host-encoded cofactors are involved in the conversion of PrP^C to PrP^D. The mechanism of conversion between PrP^C and PrP^D and the three-dimensional conformation of PrP^D have not yet been resolved.

PrP^C is totally degraded by proteinase K and is soluble in detergents, whereas PrP^D is only partially degraded by the proteinase and is insoluble in detergents. PrP^D is extremely resistant to inactivation by chemical or physical methods. Total inactivation of materials contaminated with PrP^D can be achieved by incineration, autoclaving at 134 °C for 4.5 h, exposure to 1 M NaOH in combination with autoclaving, or exposure to 2% sodium hypochlorite, which is very corrosive. Materials treated with formaldehyde are more difficult to disinfect as formaldehyde cross-links and further stabilizes the

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protein. PrP^D infectivity can be reduced by exposure to protein denaturing compounds, such as phenolics, guanidium thiocyanate, urea, and acidic 5% sodium dodecyl sulfate, or high doses of proteases, such as trypsin or proteinase K. BSE prions from cattle appear the most resistant to inactivation.

New techniques such as protein misfolding cyclic amplification, a concept similar to polymerase chain reaction (PCR) for amplification of nucleic acids, have been successful in detecting minute quantities of PrP^D from a wide range of species and matrices and could be a promising tool for screening tissues and blood of asymptomatic animals and humans.

Pathogenesis of Transmissible Spongiform Encephalopathies

Animal TSEs are transmitted primarily by the oral route. The minimum infectious dose is estimated at approximately 6 PrP^D monomers. Initially, PrP^D crosses the mucous membranes and is detected in the tonsils, Peyer's patches, and other gut-associated lymphoid tissue. PrP^D can be detected in the lymphoid tissue as early as 6 weeks and 3 months after exposure in CWD and scrapie, respectively. Replication of PrP^D can occur over months or years in the lymphoreticular system; however, with BSE and some scrapie cases, replication of PrP^D apparently does not involve the lymphoreticular system. The pathway and mechanism involved in the movement of PrP^D from the lymphatic tissues to the central nervous system (CNS) is not well understood. After a prolonged period of replication, PrP^D accumulates in the brain tissue, causing fatal neurodegenerative changes. TSEs are characterized by the accumulation of PrP^D in neurons and glial cells, with consequent vacuolation or spongiform changes in the neuropil and neuronal bodies in the gray matter of the CNS. In addition, some changes that may or may not be observed include neuronal death, astrogliosis, and the formation of amyloid plaques. As the amino acid sequence of a PrP^D is identical to that of the PrP^C that is encoded by the host, it is not recognized as a foreign protein by the immune system and an inflammatory response against PrP^D is not initiated.

Interspecies transmission of prion diseases is restricted by differences in the amino acid sequences of PrP^C from different species. However, PrP^D from a different species can infect and adapt to a new host. As the disease progresses, the concentration of host PrP^D increases which results in a more effective and efficient infection and transmission of disease. When the amino acid sequence of the acquired PrP^D prion closely matches that of the host PrP^C, there is a relatively high probability of the host developing the disease. When there is a lack of homology between the infectious prion and the host PrP^C, the probability of infection is reduced or the incubation period of the disease increases.

Animal Transmissible Spongiform Encephalopathiess

Bovine Spongiform Encephalopathy

The first case of BSE was diagnosed as a scrapie-like spongiform encephalopathy, in the UK in 1986, on examination of the brain tissue of an animal with neurological symptoms. Subsequent analysis indicated that BSE was present in cattle in the UK in 1985. Approximately 190 000 cases of BSE in farmed cattle have been reported worldwide to date, with more than 180 000 cases being in the UK. The outbreak peaked in 1992 in the UK, with 37 280 reported cases. Other affected countries include most European countries, Israel, Japan, Canada, and the USA. In 2011, there were 7 confirmed cases of BSE within the UK and 22 confirmed cases of BSE elsewhere.

Based on epidemiological evidence, the outbreak of BSE has been attributed to cattle feed which contained meat and bone meal (MBM) that was contaminated with BSE prions. Although the origin of BSE is unknown, it is widely suspected that the source of BSE prions was a cow that acquired BSE by a spontaneous conversion of PrP^C to PrP^D. The infected carcass was rendered into MBM and the PrP^D-contaminated MBM was served to cattle. The UK implemented a ban on feeding ruminant-derived MBM to ruminants in 1988. Owing to concerns about cross-contamination of ruminant feeds with nonruminant feeds that might contain ruminant proteins, the ban was extended in 1994 to the feeding of any mammalian proteins to ruminants; and further extended in 1996 to a complete ban on feeding mammalian MBM to any farmed livestock, including horses and fish. Cases of BSE occurred mainly in Europe because cattle producers in North America typically use plant-based dietary supplements rather than MBM.

A cow infected with BSE can spread PrP^D to her offspring, but embryos used in embryo transfers do not appear to carry or transmit BSE to the host or offspring. BSE does not appear to be transmitted directly from animal to animal and cattle do not appear to have a genetic disposition for infection with BSE prions. Young cattle are more susceptible than older cattle to infection with BSE prions, with cattle 0.5–1.5 years old being the most susceptible. The incubation period for naturally occurring BSE in cattle ranges from 2 to 8 years and the mean incubation period is estimated to be 4.5–5.5 years.

The pathway and mechanisms involved in the movement of PrP^D from the gut to the CNS are not well understood. Studies with mice have demonstrated that infectious BSE PrP^D accumulate in the Peyer's patches of the gut-associated lymphoid tissue. In cattle, there is little PrP^D replication in the lymphoid tissue, and PrP^Ds are thought to infect the CNS via the nerve endings in the intestinal tract. In cattle with clinical signs of BSE, infectious BSE particles are found in the brain, spinal cord, and dorsal root ganglia but not in peripheral nerves, lymphatic tissues, the gastrointestinal tract, reproductive and other organs, muscle, blood, or milk. BSE is difficult to detect as the incubation period is long, symptoms are apparent only toward the end of the incubation period, and cattle are often slaughtered before the onset of disease. Clinical symptoms include apprehension, nervousness, increased sensitivity to sensory stimuli such as light and noise, reluctance to be milked, aggression toward humans and animals, abnormal posture, lack of muscle control, difficulty in rising from a lying position, decreased milk production, and weight loss despite a normal appetite. The symptoms may persist for 2 weeks to 6 months before the animal dies, if it is not destroyed. There is no treatment or vaccine available for BSE.

Definitive diagnosis and confirmation can currently only be obtained postmortem by microscopic examination, immunohistochemistry, western blotting, and animal bioassays of the brain tissue. The classic diagnosis of BSE is the observation of characteristic sponge-like changes in sections of brain tissue, followed by confirmatory testing using immunohistochemistry and western blotting. Atypical strains of BSE have recently been identified by western blotting. These atypical strains have been distinguished as H-type and L-type based on relative higher (H) and lower (L) molecular mass banding patterns of the unglycosylated isoform of PrP^D in comparison with the classic BSE (C-type) associated with the original UK outbreak. The discovery of the L- and H- types, mainly in animals that were 8 years or older, has raised concerns about the existence of sporadic or spontaneous BSE. The L-type has been detected in cattle in Belgium, Italy, Canada, and Japan, whereas the H-type has been detected in cattle in France, Germany, the UK, Sweden, Canada, and the USA. Transmission studies in cattle and mice have shown that both H- and L-type BSE have biological characteristics that are different from those of the classical BSE agent. Sampling procedures may play an important role in recognizing and discriminating atypical strains from the classical strains of BSE, as discrimination by immunohistochemistry of atypical forms of BSE was apparently more reliable when tissue samples were obtained from the cerebellum instead of the medulla or brainstem. There is, as yet, a lack of knowledge about the zoonotic potential of atypical cases of BSE. However, experiments with transgenic mice that carry the human prion gene suggest that the L-type of BSE is capable of causing disease in humans.

In addition to the ban on feeding MBM, extensive surveillance programs, and rapid response, prevention, and control measures for BSE have been put in place. These include targeted enhanced testing programs for high-risk animals; surveillance of incidents of clinical neurological disease; strict control measures on the importation of cattle; routine screening tests at slaughter; removal during carcass dressing of specified risk material (SRM) such as the brain, spinal cord, thymus, and spleen for animals older than 6 months, and in addition the vertebral column with dorsal root ganglia for animals older than 30 months (OTM); exclusion of SRM from animal feeds; transparent communications in the event of BSE; livestock identification and traceability; and appropriate disposal of affected cattle. Animals that are OTM are excluded from the food chain if they are sourced from regions where there is risk of cattle developing or harboring BSE; and if not excluded from human consumption they are slaughtered in a facility that is approved for slaughter of OTM cattle. Meat from OTM cattle slaughtered anywhere in the European Union (EU) may only be released for consumption after brain tissue from each carcass has been tested and found negative for BSE.

BSE is a reportable disease. When it is detected, the entire herd and all contacts and offspring are destroyed. Worldwide, control of BSE has resulted in the destruction of more than 4.4 million animals. Outbreaks of BSE can have a devastating impact on the livestock sector and the overall economy of a country, as in the UK. The overall costs of even small outbreaks can be in billion dollars. Thus, the costs associated with an outbreak of BSE in Canada, with 17 confirmed cases since 2003, were estimated at US$6–US$8 billion as a result of loss of international markets for live animals and meat products, contraction of domestic markets, and loss of 75 000 jobs. In addition, increased rates of suicide, depression, and divorce were observed in cattle-rearing rural regions of Canada.

BSE is reported to have naturally infected goats as a result of their ingesting feed containing contaminated MBM, but there are no known cases of naturally acquired BSE in sheep or pigs. Even so, there is concern that endemic scrapie could mask BSE in sheep populations. When goat and sheep were experimentally infected with BSE, the neuropathological changes in the brain tissue were similar to BSE but distinct from scrapie. Furthermore, with increasingly sensitive diagnostic methods, PrP^D has been detected in the livers of sheep that were naturally infected with scrapie or experimentally infected with BSE. This raises concerns about potential risks to human health from BSE in sheep because sheep accumulate infective PrP^D in the blood during the preclinical and clinical stages of the disease. Precautionary measures to reduce the risk of BSE-infected sheep meat from entering the food chain are in place in the EU. SRM for sheep include the skull, brain, eyes, tonsils, and spinal cord of animals over 12 months old, and the spleen and ileum of all sheep, but not offal such as liver.

When pigs were infected with BSE by the parenteral route, the neuropathological changes in the brain tissue were similar to BSE but the distribution of lesions in pigs was different from that in cattle. Domestic cats and large members of the cat family and exotic ruminants in zoos in the UK developed feline spongiform encephalopathy and ungulate spongiform encephalopathy, respectively, during the epidemic outbreak of BSE, presumably from the consumption of BSE-infected meat or MBM. When deer were infected by intracerebral inoculation with BSE, the neuropathological changes and clinical signs were similar to CWD.

Scrapie

Scrapie was first described in the eighteenth century as a fatal infectious disease affecting flocks of sheep and goats in Europe. Scrapie has long been known to be widespread in Europe, Asia, and America, but it was thought to be absent from Australia and New Zealand. The spread of the disease to America has been attributed to the importation of infected animals. In 1978, Iceland's attempt to eradicate scrapie by destroying all indigenous sheep and goat flocks and importing scrapie-free animals from New Zealand several years later was unsuccessful. The appearance of classic scrapie in some of the imported flocks suggested that scrapie is persistent for a long period of time in pastures and could be difficult to eradicate. Scrapie is a reportable disease. To remain competitive and maintain market access, scrapie control programs are in place or being developed in many countries to reduce the incidence of scrapie and, possibly, eradicate it.

Scrapie is transmitted through the placenta of infected females to their offspring or, at birth, to other exposed animals in the flock. Males can contract scrapie but they do not transmit the disease to other animals. Scrapie is transmitted between sheep and goats under natural conditions. The susceptibility of sheep to scrapie is associated with a polymorphism of the *PrP* gene at codons 135, 154, and 171 where an increased susceptibility is observed when alleles are

homozygous for valine at codon 136 and glutamate at codon 171. Suffolk sheep, a common breed in North America, are homozygous for glutamate at codon 171 and susceptible to scrapie. The Suffolk genotype likely has been transmitted to other breeds. Selective breeding programs for scrapie-resistant genotypes containing dominant negative polymorphisms, such as arginine at codon 171 of the *PrP* gene, are being implemented as part of control strategies.

Clinical signs are observed in animals between 2 and 5 years of age. Once clinical signs are apparent, animals typically die within 2 months. The clinical condition progresses more rapidly in goats than in sheep but the symptoms are similar. Symptoms vary between cases of scrapie but typically include severe weight loss, poor appetite, itchiness, wool loss, depression, and neurological signs such as uncoordinated muscle movements and inability to keep up the head. PrP^D accumulates in the tonsils, lymph nodes, spleen, and gut-associated lymphoid tissue as well as in the placenta and peripheral nervous system. The peripheral distribution of PrP^D is dependent on the strain of scrapie and genotype of the host. The neuropathological lesions in the brain tissue are similar as those reported for BSE, but scrapie can be differentiated from BSE by western blotting. The molecular weight of the unglycosylated band for scrapie is higher than that of BSE. Furthermore, scrapie reacts with the $MAbP4^b$ antibody against a specific N-terminal sequence of the PrP protein, whereas BSE does not.

There is no epidemiological evidence to suggest that classical scrapie causes disease in humans. However, concerns are being raised about the potential risk to humans and animals from atypical strains of scrapie in sheep and goat that have been detected in a number of countries, including New Zealand and Australia, as a result of active disease surveillance. These atypical strains have been found in sheep of a PrP genotype that confers a high resistance to classical scrapie. In addition, atypical scrapie does not seem to be transmitted between animals in a herd. The neuropathological lesions observed for atypical scrapie differ from those seen with classical scrapie and BSE. The atypical scrapie strains isolated from different countries appear to represent a uniform type of scrapie prion. Atypical scrapie can be transmitted orally, and infectivity but not PrP^D can be detected in gut tissues after 12 months. Current surveillance methods may be inadequate for reliable detection of atypical scrapie. More knowledge on the origin, pathogenesis, transmission, and zoonotic potential of atypical scrapie are required before the risk it may pose to human health can be properly assessed.

Chronic Wasting Disease

CWD is a TSE that affects members of the Cervidae family. CWD has been detected in white-tailed deer, black-tailed deer, mule deer, moose, and elk in 14 states of the USA and 2 provinces in Canada, in both free-ranging and captive herds, and also in captive elk in Korea. CWD was first described in 1967 and recognized as a TSE in 1978. The origin of CWD is unknown. CWD in North America is concentrated in certain regions that are separated by large distances.

Prp^D is present in high levels in tonsils and Peyer's patches of infected cervids. The presence of CWD in free-ranging animals presents a significant obstacle to control the disease because it is impossible to eliminate infectious CWD prions from grazing areas and soil that are deposited in saliva, fecal material, and decomposing carcasses. Horizontal transmission of CWD is highly efficient. Thus, the prevalence of CWD is 30% among free-ranging deer populations and up to 100% in captive herds. Vertical transmission of CWD has not been investigated. The highly contagious nature and geographical spread of CWD has raised concerns about the potential transmission of CWD to livestock, because cattle graze on land that is frequented by infected cervids. When cattle were challenged with CWD by oral or contact exposure, infectivity was not detected. When cattle were challenged with CWD by intracerebral inoculation, 38% of cattle developed prion infection but spongiform encephalopathy was not observed. In contrast, when cattle were challenged with scrapie by intracerebral inoculation, 100% of cattle developed neurological disease and plaque deposits were observed in the brain tissue.

In addition to concerns about CWD spreading to livestock, concerns have been raised about the possible zoonotic transmission of CWD to humans through the ingestion of game meat. Infectious prions and Prp^D seeding activity have been detected in skeletal muscle of infected cervids. The ability of CWD prions to infect human is uncertain but, based on *in vitro* and transgenic mice studies, the risk is considered to be very low or nonexistent. As much remains to be discovered about the transmission barrier, infectivity, and disease progression of CWD, it is advised that no parts of animals infected with CWD should be used for animal or human food.

CWD prions appear to disseminate via the lymphatic system and Peyer's patches before entering the CNS. Clinical signs are nonspecific and difficult to detect, or may be absent altogether. Clinical signs include depression and separation from other animals, difficulty swallowing, excess salivation, excessive thirst and urination, lack of coordination, lowered head position, pneumonia, and weight loss. CWD is a reportable disease which can only be confidently diagnosed post-mortem by testing of brain tissue or tonsils. The neuropathological lesions in the brain tissue are similar as those reported for BSE in cattle and scrapie in sheep.

Human Transmissible Spongiform Encephalopathies

Kuru

Kuru is an acquired prion disease in humans. It was transmitted among the Fore people of Papua New Guinea at epidemic levels during the 1950s and 1960s as a result of ritual cannibalism that included the consumption of infectious brain tissue of deceased people. Once the practice of cannibalism was discouraged, the disease progressively declined and has now mostly disappeared. Kuru was the first human prion disease that was experimentally transmitted to animals.

Gerstmann–Sträussler–Scheinker Syndrome

GSSS is an extremely rare inherited prion disease. It is found in only a few families around the world. The onset of the disease usually occurs between the ages of 35 and 55 years. The

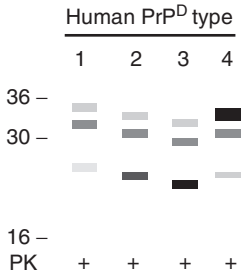

Figure 1 Differential mobility of proteinase K digestion products for di-, mono-, and unglycosylated PrP^D for sporadic CJD (1), iatrogenic CJD (2), kuru (3), and vCJD (4) by western blot. Adapted from Wadsworth, J.D.F., Collinge, J., 2012. Molecular basis of prion diseases. In: Brady, S., Siegel, G., Albers, R.W., Price, D. (Eds.). Basic Neurochemistry: Principles of Molecular, Cellular and Medical Neurobiology. New York, NY: Academic Press, pp. 872–885.

disease is characterized by a lack of muscle coordination followed by a prolonged period of dementia. Symptoms usually last from 2 to 10 years and result in death.

Fatal Familial Insomnia

FFI is an inherited prion disease caused by a missense mutation at the polymorphic codon 178 of the *PrP* gene. FFI typically appears in middle to late adulthood, although onset in people under 30 years old has been reported. FFI is characterized by disturbances of the wake and sleep cycle, autonomic dysfunction, and dementia (Figure 1).

Creutzfeldt–Jakob Disease

CJD is a rare prion disease that can be either sporadic or inherited. Approximately 85% of cases of CJD are sporadic with a prevalence of approximately 1–3 cases/million people/year and an estimated lifetime risk of 1:50 000. Sporadic CJD occurs in people between 55 and 65 years of age with a rapid increase in dementia that leads to death within 6 months of onset. The cause of sporadic CJD is unknown but it is speculated that PrP^C may spontaneously convert to PrP^D. In addition, approximately 300 accidental outbreaks of iatrogenic CJD have been linked to neurosurgical procedures, such as dura matter grafts and corneal transplants, treatment with human growth hormone harvested from the pituitary glands of human cadavers, and using contaminated instruments or equipment. Susceptibility to sporadic CJD has been linked to homozygous alleles encoding either methionine or valine at the polymorphic codon 129 of the *PrP* gene. Inherited CJD has been linked to missense or nonsense mutation in the *PrP* gene.

Between 1995 and 1996, a number of atypical cases of CJD were reported in a much younger than the usual age group, with distinct neuropathology and biochemical properties that were different from those of sporadic CJD but similar to those of BSE. The disease was recognized as vCJD in 1996 and linked to exposure to infectious BSE prions. Whereas the infectivity of sporadic CJD is confined to the CNS, infectivity of vCJD is detected in peripheral lymphoid organs as well, which raised concerns about iatrogenic transmission of vCJD through routine surgical procedures. Furthermore, transmission of vCJD through blood transfusions from infected donors has been reported. Between 1996 and 2011, 175 cases of vCJD in the UK and 49 cases in other countries have been confirmed. Since 2000, the number of cases in the UK has steadily declined. The total impact of the BSE outbreak on vCJD cases is not known as knowledge of the incubation time and susceptibility factors for vCJD are lacking. Incubation periods for acquired TSEs in humans may be longer than 50 years. To date, almost all patients with vCJD were homozygous for methionine at codon 129 of the *PrP* gene. The prevalence of the allele in the northern European population is 38%.

See also: Foodborne Zoonoses. Manure/Waste Management: Waste Management in Europe. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbial Contamination: Decontamination of Fresh Meat. Microbiological Safety of Meat: Emerging Pathogens. Residues in Meat and Meat Products: Feed and Drug Residues. Risk Analysis and Quantitative Risk Management

Further Reading

Aguzzi, A., 2009. Transmissible spongiform encephalopathies. In: Richman, D.D., Whitley, R.J., Hayden, F.G. (Eds.), Clinical Virology. Washington, DC: ASM Press, pp. 1307–1324.

Everest, S.J., Ramsay, A.M., Chaplin, M.J., *et al.*, 2011. Detection and localization of PrP^{SC} in the liver of sheep infected with scrapie and bovine spongiform encephalopathy. PLoS One 6, e19737.

Gavier-Widén, D., Stack, M.J., Baron, T., Balachandran, A., Simmons, M., 2005. Diagnosis of transmissible spongiform encephalopathies in animals: A review. Journal of Veterinary Diagnostic Investigation 17, 509–527.

Harman, J.L., Silva, C.J., 2009. Bovine spongiform encephalopathy. Journal of the American Veterinary Medical Association 234, 59–72.

Prusiner, S., 1982. Novel proteinaceous infectious particles cause scrapie. Science 216, 136–144.

Sigurdson, C.J., Aguzzi, A., 2007. Chronic wasting disease. Biochimica Biophysica Acta-Molecular Basis of Disease 1772, 610–618.

Simmons, M.M., Moore, S.J., Konold, T., *et al.*, 2011. Experimental oral transmission of atypical scrapie to sheep. Emerging Infectious Diseases 17, 848–854.

Tyler, J.W., Middleton, J.R., 2004. Transmissible spongiform encephalopathies in ruminants. Veterinary Clinics of North America: Food Animal Practice 20, 303–326.

Wadsworth, J.D.F., Collinge, J., 2012. Molecular basis of prion diseases. In: Brady, S., Siegel, G., Albers, R.W., Price, D. (Eds.), Basic Neurochemistry: Principles of Molecular, Cellular and Medical Neurobiology. New York, NY: Academic Press, pp. 872–885.

Relevant Websites

http://www.cdc.gov/ncidod/dvrd/prions/index.htm
Centers for Disease Control and Prevention.

http://www.oie.int/en/animal-health-in-the-world/bse-portal
World Organization for Animal Health.

Salmonella spp.

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Glossary

Enzyme-linked immunosorbent assay (ELISA) method A detection method in which antigens or antibodies are fixed in wells. Antigen–antibody complexes are formed after addition of a sample containing the corresponding antibody or antigen. Complexes are labeled with enzymes and visualized by an enzymatic color reaction.

Hazard analysis critical control point (HACCP) systems A scientific approach to establishing plant- or process-specific control systems in the food industry.

Host-adapted The adaptation of a microorganism to a specific host species. Therefore, the microorganism is found mostly in the host species and only infrequent in other species.

Lipopolysaccharides (LPS) An antigenic part of the outer cell membrane of Gram-negative bacteria.

Macrophage A cell that is specialized for engulfing foreign material and microorganisms that gain access to the body. Macrophages break down the foreign substances and present them to the immune system.

Muscle fluid The fluid obtained from thawed meat (also called meat juice).

Salmonellosis A general term for clinical disease caused by *Salmonella*, but frequently restricted to clinical infections from nontyphoid *Salmonella* serovars.

Sequelae The secondary clinical conditions following a primary disease.

Subclinical infection An infection without recognizable clinical symptoms.

Zoonoses The diseases that can be transmitted between animals and humans.

Introduction

Salmonella is a pathogen, the main reservoir of which is the gastrointestinal tract of warm-blooded animals. Among numerous serovars of *Salmonella enterica* subsp. *enterica* (*S.*), serovar Typhi and the serovars Paratyphi A and B infect only humans and are not spread from animals. The remaining (nontyphoid) *Salmonella* serovars originate from the animal reservoir. Through fecal contamination of meat during slaughter, meat animals are among the most important sources of human salmonellosis.

The effort to reduce human salmonellosis is challenged by a widespread, mostly subclinical occurrence of *Salmonella* in a variety of meat animals together with an ability of *Salmonella* to adapt to and survive changing environmental conditions.

Since the early 1990s, many new detection and typing methods, surveillance programs, and control methods for *Salmonella* in the farm-to-fork continuum have become available and have been implemented in an increasing number of countries worldwide. The growing awareness of a global food market has led to international initiatives toward global control of *Salmonella*.

Characteristics of *Salmonella*

The genus *Salmonella* belongs to the family *Enterobacteriaceae*. *Salmonella* are Gram-negative, facultatively anaerobic, motile rods that are catalase positive and cytochrome oxidase negative, produce gas from glucose, and are able to reduce nitrate. *Salmonella* and *Escherichia coli* are closely related and are believed to share a common ancestor. During evolution *E. coli* has acquired the ability to utilize lactose through being closely associated with mammals, whereas *Salmonella* is unable to utilize lactose and is more associated with reptiles and birds. The acquisition of pathogenicity islands, of which SPI-1 and SPI-2 are the most prominent, conferred virulence on *Salmonella*.

The genus *Salmonella* is comprised of two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is composed of more than 2500 serovars, but *Salmonella bongori* has only 22 serovars. *Salmonella enterica* is divided into 6 subspecies. Subsp. I (*enterica*) is comprised of approximately 1500 serovars and is particularly associated with infections in warm-blooded animals (Table 1).

Seroagglutination of outer membrane O-antigens (LPS) and flagella H-antigens define the serovar according to the Kaufmann–White scheme. For example the antigenic structure O:1,4,5,12 and H1/H2 b:1,2 defines *Salmonella enterica* subsp. *enterica* serovar Typhimurium (in short *Salmonella* Typhimurium or *S.* Typhimurium).

A few serovars are termed host specific or host adapted. *Salmonella* Typhi, *S.* Paratyphi A, *S.* Paratyphi C and *S.* Sendai infect only humans. *Salmonella* Choleraesuis is associated with pigs and *S.* Dublin is adapted to cattle but both serovars can cause serious infections in humans. *Salmonella* Gallinarum and *S.* Pullorum are specific for poultry. The factors that determine host specificity have not been clarified. In general, the remaining serovars of subspecies 1 are zoonotic and have a wider host range. The two most prominent serovars in human disease are *S.* Typhimurium, which in particular has a broad host spectrum, and *S.* Enteritidis, which can infect many hosts but has a predilection for poultry.

In general *Salmonella* grow between 5 °C and 46 °C with growth being optimal at temperatures between 35 °C and 37 °C. Physical conditions such as temperature, salinity, pH, and water

Table 1 Taxonomy of the genus *Salmonella*[a] and top five serovars of *S. enterica* subsp. *enterica* reported to World Health Organization, Global Foodborne Infections Network (GFN), 2009. Isolates reported to GFN are dominated by reports from Europe

Genus	*Species*	*Subspecies (subspecies number)*	*Number of serovars*	*Global top five serovars reported in humans 2009*[b] (% of isolates)
Salmonella	*S. enterica*	*S. enterica* subsp. *enterica* (I)	1531	*S.* Enteritidis (69.0) *S.* Typhimurium (14.0) *S.* Infantis (5.7) *S.* Virchow (1.3) *S.* Newport (1.0)
		S. enterica subsp. *salamae* (II)	505	
		S. enterica subsp. *arizonae* (IIIa)	99	
		S. enterica subsp. *diarizonae* (IIIb)	336	
		S. enterica subsp. *houtenae* (IV)	73	
		S. enterica subsp. *indica* (VI)	15	
	S. bongori		22	

[a]Grimont, P.A.D., Weill, F.-X., 2007. Antigenic formulae of the *Salmonella* serovars. 9th ed., WHO Collaborating Center for Reference and Research on Salmonella. France: Institut Pasteur.

[b]World Health Organization, Global Foodborne Infections Network. Country Databank. Available at: http://thor.dfvf.dk/portal/page?_pageid=53,1&_dad=portal&_schema=PORTAL (accessed 30.03.12).

activity will affect the growth rate. *Salmonella* can grow between pH 4.5 and pH 9.0 with optimal growth at pH 6.5 to 7.5. *Salmonella* do not grow at water activities below 0.93. Although the generation time for *Salmonella* is rather long at low temperatures, significant growth in fresh meat can occur at temperatures above 5 °C, which may pose a consumer risk. Although the background flora in meat can be numerous and interactions with the meat flora can reduce the growth rate of *Salmonella*, they will not stop *Salmonella* growing. A range of *Salmonella* serovars have been shown to survive freezing for months without any substantial reductions in numbers.

Isolation and Identification of *Salmonella*

Conventional Culture Detection

Several media have been developed for culture and isolation of *Salmonella* from food. Owing to low numbers of *Salmonella* in meat, direct plating of samples on selective agars lacks sensitivity. *Salmonella* detection in foods requires three culturing steps:

1. Preenrichment, to allow recovery and growth of injured and uninjured cells. Typical preenrichment media are buffered peptone water (BPW) and lactose broth (LB).
2. Selective enrichment in a broth medium that suppresses growth of most bacteria but supports growth of *Salmonella*. Typical media are Rappaport–Vassiliadis broth (RV), selenite cystine broth (SC), or tetrathionate broth (TB). From the RV medium a modified semisolid agar has been developed (MSRV), which allows detection due to swarming of motile *Salmonella*.
3. Plating on indicative media. The indicative media take advantage of biochemical features such as the ability to grow in the presence of bile salts and fermentation of sucrose or xylose, but not lactose. Examples are brilliant green agar (BGA); bismuth sulfite agar (BSA), and xylose lysine deoxycholate agar (XLD).

Suspect colonies are subcultured on nonselective media that allow seroagglutination and verification of the serovar. International standardization committees such as the International Standards Organization (ISO), the American Association of Analytical Chemists (AOAC), the International Dairy Federation (IDF), and the Nordic Committee on Food Analysis (NMKL) provide procedures for *Salmonella* detection to support international harmonization.

Rapid Detection Methods

As culture detection of *Salmonella* requires 3–5 days to provide a positive result, rapid detection methods have been developed. They are typically DNA based, e.g., polymerase chain reaction (PCR), or immunological- (antibody) based methods, e.g., enzyme-linked immunosorbent assay (ELISA). Although termed rapid, preenrichment is often necessary to obtain the required numbers of *Salmonella*, 10^4–10^5 cells ml^{-1}, needed for detection. These methods have reduced detection times to between 12 h and 24 h, which enable release of meat for shipment direct from cooling facilities at the slaughterhouse.

Immunological methods are developed based on interactions between *Salmonella* antigens and specific antibodies raised against *Salmonella*. The format can vary. The linking of an enzyme to the antibodies allows antigen–antibody complexes to be detected by conversion of a substrate of the enzyme with development of a visible color, light, or fluorescence when *Salmonella* is present.

For DNA-based methods, PCR methods are predominant. The principle of PCR is an enzymatic-driven multiplication of a *Salmonella*-specific portion of the *Salmonella* genome. The reaction is exponential and can produce a positive result in less than 2 h. However, the reaction takes place in a very small volume and a preenrichment step is usually needed to obtain sufficient cells from which the required amount of DNA (or RNA when relevant) can be extracted. With the recent development of real-time PCR, a robust and sensitive DNA-based method of detection has been made available.

Typing Methods for Epidemiology and Outbreak Investigation

Phage typing

Phage typing of *Salmonella* is based on the ability of specific bacterial viruses, i.e., bacteriophages, to destroy *Salmonella*. In phage typing, a number of bacteriophages are spotted onto agar plates with a confluent culture of the isolate to be typed. If a phage is able to infect and destroy the isolate, a spot cleared of cells appears. The pattern of spots determines the phage type. A phage typing scheme has been developed for a range of *Salmonella* serovars, and it has been part of the classical characterization for *S.* Enteritidis and *S.* Typhimurium for half a century (Figure 1).

Pulsed-field gel electrophoresis (PFGE)

In these methods, the bacterial chromosome is cut into large fragments by restriction enzymes and the fragments are separated by electrophoresis in a pulsing electric field. Bacteria showing the same bands on the gel are considered to be the same. PFGE has been widely used and has long been the standard method for investigation of *Salmonella* outbreaks (Figure 1). Other novel technologies are now replacing PFGE. These include multilocus sequence typing, multiple loci variable number of tandem repeats analysis and full genome sequencing.

Multilocus sequence typing (MLST)

MLST is based on sequencing of internal fragments of seven housekeeping genes. Different base sequences (alleles) can occur in each of the seven genes. Sequences for all seven genes are stored in an international database. To obtain a MLST type, the seven gene sequences determined for an isolate are forwarded to the database and a sequence type is returned. If sequences for two isolates are identical they will be classified as the same MLST type. MLST is becoming increasingly prominent in *Salmonella* epidemiological and outbreak investigation.

Multiple loci variable number of tandem repeats analysis (MLVA)

MLVA typing is based on the occurrence of short, repetitive base sequences in the DNA of the chromosome. The numbers of sequences vary, and strains with an identical number of sequences are considered identical. Each of five regions of the chromosome is analyzed for the number of repetitive sequences. The method has become important for typing of *Salmonella* (Figure 1).

Detection of Antibodies to *Salmonella* by Enzyme Immunoassay

Since the mid-1990s detection of specific antibodies to *Salmonella* LPS in blood samples, muscle fluid, milk, or egg yolk from food animals has been possible. Despite the inherent delay in antibody response (1–2 weeks after infection), an association between seropositivity and shedding of *Salmonella* has been documented. Serology is a convenient, inexpensive and sensitive method for monitoring and classification of *Salmonella* infection in groups of animals, but it is less suitable for testing individual animals. ELISA test kits are commercially available. Serological assays tailored to targeted *Salmonella* serovars are parts of *Salmonella* surveillance programs and are used for research worldwide.

Characteristics of Salmonellosis

Salmonellosis in Meat Animals

Most *Salmonella* infections in meat animals are subclinical; but infections with the host-adapted *Salmonella* serovars can

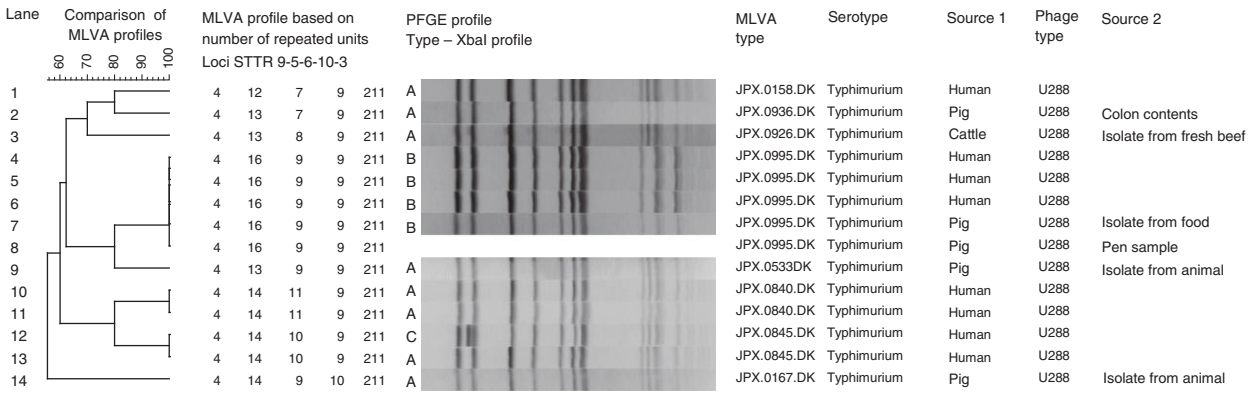

Figure 1 Subtyping methods applied to a selection of five *S.* Typhimurium isolates from an investigation of a human outbreak of *S.* Typhimurium phage type U288 in Denmark, Norway, and Sweden in 2008[a] (lane 4–8). All had identical MLVA type (JPX.0995.DK), and four were PFGE-typed and had identical PFGE types (type B). Lane 4–6: Three human outbreak isolates from Danish cases. Lane 7: Food isolate from Danish raw pork sausage meant for heat treatment. Lane 8: Isolate from a pen fecal sample from a Danish sow herd. The two food and animal isolates of MLVA JPX.0995.DK were epidemiologically related to a specific pig slaughterhouse and cutting plant identified as the site of contamination. Isolates in lane 1–3 and 9–14 are added for comparison and were not related to the outbreak. Source 1: Animal species or human origin. Source 2: Sample type of nonhuman isolates. Printed with permission from Sørensen, G., Diagnostic Engineering, Division of Food Microbiology, National Food Institute, Technical University of Denmark. [a]Bruun, T., Sørensen, G., Forshell, L.P., *et al.*, 2009. An outbreak of *Salmonella* Typhimurium infections in Denmark, Norway and Sweden, 2008. Eurosurveillance 14, 1–6.

cause severe, acute, clinical diseases. Outbreaks of such diseases can have high mortality rates and cause heavy economic losses. Symptoms are mainly related to septicemia (fever, weakness, loss of appetite), but enteritis is also common, and pneumonia, reproductive failure, and abortion may occur.

Non host-adapted serovars of *Salmonella* are considered potentially capable of infecting most meat animals, but certain serovars are more commonly isolated from certain meat animal species (e.g., *S.* Enteritidis in poultry, *S.* Derby in pigs) (Table 2). Infection of meat animals with the non host-adapted serovars may occasionally cause herd outbreaks of mainly gastrointestinal infections (diarrhea, fever, and dehydration) in young animals. Compared to infections with host-adapted serovars, infections with non host-adapted serovars generally have a lower mortality.

Table 2 Annual incidence of notified human salmonellosis (confirmed cases) in European Union (EU) Member States, 2010, and detection of *Salmonella* in broilers, turkeys, and slaughter pigs in baseline studies conducted in EU Member States between 2005 and 2007. Top five *Salmonella* serovars from humans and meat animals are listed below

EU member state	*Human salmonellosis 2010*[a,b] *(cases per 100 000)*	Salmonella *detected by culture in EU baseline studies*		
		Broilers 2005–2006[e] *(% of flocks)*	*Turkeys 2006–2007*[f] *(% of flocks)*	*Slaughter pigs 2006–2007*[g] *(% of intestinal lymph nodes)*
Austria	26.0	5.4	25.5	2.1
Belgium	29.2	12.4	17.8	13.0
Bulgaria	15.2	–	0.0	19.9
Cyprus	16.9	9.1	57.6	13.1
Czech Republic	78.1	19.3	42.7	5.8
Denmark	29.1	1.6	4.0	8.0
Estonia	28.4	2.0	–	6.4
Finland	45.3	0.1	0.0	0.0
France	11.1	6.2	13.3	18.5
Germany	30.4	15.0	9.2	12.7
Greece	2.6	24.0	16.5	21.2
Hungary	59.4	68.2	78.5	11.6
Ireland	7.8	27.6	27.6	15.4
Italy	4.5	28.3	38.8	16.4
Latvia	39.2	6.2	–	5.4
Lithuania	58.9	2.9	5.3	1.7
Luxembourg	42.0	–	–	16.0
Malta	38.7	–	–	–
Netherlands	13.6[c]	7.5	14.1	8.5
Poland	24.3	58.2	26.9	6.4
Portugal	1.9	43.5	6.3	23.7
Romania	6.0	–	–	–
Slovakia	91.1	5.7	22.9	7.8
Slovenia	17.7	1.6	21.1	6.3
Spain	38.4[d]	41.2	56.3	30.7
Sweden	38.7	0.0	0.0	1.5
United Kingdom	15.6	8.2	32.2	21.8
EU Total	**21.5**	**23.7**	**30.7**	**13.9**
Top five serovars (% of isolates)	*S.* Enteritidis (45.0) *S.* Typhimurium (22.4) *S.* Infantis (1.8) *S.* Typhimurium 4,[5],12:i:-(1.5) *S.* Newport (0.9)	*S.* Enteritidis (33.8) *S.* Infantis (22.0) *S.* Mbandaka (8.1) *S.* Typhimurium (3.0) *S.* Hadar (3.7)	*S.* Bredeney (16.5) *S.* Hadar (12.9) *S.* Saintpaul (10.9) *S.* Derby (9.8) *S.* Kottbus (7.5)	*S.* Typhimurium (40.0) *S.* Derby (14.6) *S.* Rissen (5.8) *S.* Typhimurium 4,[5],12:i:-(4.9) *S.* Enteritidis (4.9)

[a]European Food Safety Authority, European Center for Disease Prevention and Control, 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. The EFSA Journal 10(3): 2597 (442 pp). doi:10.2903/j.efsa.2012.2597.

[b]Reporting systems for human cases vary considerably. Human cases related to travel abroad are included.

[c]Sentinel system, calculated from estimated population coverage.

[d]Calculated from estimated population coverage.

[e]The European Food Safety Authority, 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in broiler flocks of *Gallus gallus*, Part A. The EFSA Journal 98, 1–85.

[f]The European Food Safety Authority, 2008a. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in turkey flocks, Part A. The EFSA Journal 134, 1–91.

[g]The European Food Safety Authority, 2008b. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part A. The EFSA Journal 135, 1–111.

Salmonellosis in Humans

Nontyphoid salmonellosis in humans is one of the most common gastrointestinal bacterial zoonoses worldwide. Salmonellosis peaks in warm seasons and the annual incidences per 100 000 population varies between regions and countries (e.g., Australia, 2009, 43.6; Canada, 2009, 18.0; European Union (EU), 2010, 21.5 (Table 2); New Zealand, 2010, 26.2; USA, 2010, 17.6). The incidence is probably underestimated by a factor of between 5 and 20, and comparisons between countries are confused by different sensitivities of the surveillance systems.

All *Salmonella* are considered potentially pathogenic to humans, but worldwide the serovars *S.* Enteritidis and *S.* Typhimurium are predominant in human disease (Table 1). The infective dose for healthy adult persons is believed to be approximately 100 000 cells, but it can be as low as 10 cells depending on the susceptibility of the person, the food vehicle, and the strain of *Salmonella*.

Typically, onset of symptoms is 12–72 h after exposure, and the duration of the illness is 4–5 days, often followed by a period of fatigue. Symptoms are mainly gastrointestinal, often accompanied by fever, headache, and muscle or joint pains. The infection is usually self-limiting and clinically indistinguishable from other common bacterial gastrointestinal infections. Sequelae are observed in 1 to 2% of the patients, and the mortality rate with many serovars is a few percent. In contrast, human infections with the host-adapted serovars (e.g., *S.* Dublin and *S.* Choleraesuis) often leading to septicemia, require antibiotic treatment, and show mortalities of 20–30%.

Food vehicles, commonly associated with sporadic and outbreak-related salmonellosis, are table eggs, and fresh pork, beef, and poultry meats. Increasing attention is being paid to nonanimal foods (fruit, vegetables, fresh herbs, sprouts) as vehicles for *Salmonella*. Various models have been developed to relate human cases to *Salmonella* reservoirs.

Mechanisms of Pathogenicity

Six serovars carry virulence (*spv*) genes on virulence plasmids. These include the host specific/adapted zoonotic serovars, and *S.* Typhimurium and *S.* Enteritidis.

Infection Cycle

Salmonella has both an animal and an extra-animal phase. *Salmonella* can survive in humid environments (soil, slurry) for months and in dry matter for years. Once ingested, *Salmonella* has to survive passage through the stomach. Although the stomach is perceived as presenting a harsh acidic environment, recent studies suggest that a pH gradient allows substantial fractions of ingested *Salmonella* to pass to the duodenum alive. On entering the small intestine the pH increases and the bacteria face intestinal proteases, bile salts, the gut microbiota, and the release of inhibitory antimicrobial peptides. Furthermore, *Salmonella* has to pass the mucus layer and overcome secreted antibodies (mainly IgA) before invasion of the intestinal epithelium provides the organisms with a relative safe intracellular compartment. Multiplication in the epithelial lining leads to shedding of *Salmonella* in feces.

Virulence Mechanism

Salmonella controls its own invasion of enterocytes and M-cells associated with intestinal lymphoid tissue. The uptake in M-cells occurs in close connection with macrophages, which process and exposes *Salmonella* antigens directly to the immune system. *Salmonella* is transported in macrophages to the lymph nodes. From there *Salmonella* enters the lymphatic system and the blood stream, from which it is taken up by macrophages in liver and spleen. If *Salmonella* growth exceeds its uptake by macrophages, generalized infection results. *Salmonella* can also be taken up directly from the intestinal lumen by dendritic cells (DC). Loosening of tight junctions enables DCs in the submucosa to protrude through the intercellular space and take up *Salmonella* directly from the lumen.

Epidemiology

Reservoirs

The primary reservoir for nontyphoid *Salmonella* is the gastrointestinal tract of animals and humans. Fecal counts of 10^6 cfu g^{-1} and 10^{12} cfu g^{-1} are common in the first week of subclinical and clinical infections, respectively. Asymptomatic, intermittent shedding may continue for months. *Salmonella* may also be isolated from intestinal lymph nodes, the oral cavity, and tonsils of infected animals and from blood culture of septicemic individuals.

Birds and wild animals, including rodents, particularly in the environments of infected farms and near dumps with human waste, may harbor *Salmonella*. Manure, slurry, or human sludge used as fertilizer and irrigation water of poor hygienic quality can contaminate crops used for animal feed or human consumption.

Transmission to Meat Animals

In populations of farmed animals in which *Salmonella* is prevalent, exposure of meat animals to *Salmonella* occurs mainly through direct or indirect fecal–oral transmission from animals brought in from outside the herd, or from other animals in the herd as a result of insufficient cleaning, drying, and disinfection of pens. In poultry production there is an additional vertical transmission from hens to chickens from internal contamination of eggs (often by *S.* Enteritidis). The serovars *S.* Typhimurium, *S.* Infantis, *S.* Derby, *S.* Dublin, and *S.* Enteritidis are examples of serovars that are mainly isolated from and transmitted by animals. Introduction of *Salmonella* into herds and maintenance of *Salmonella* infection within herds may also occur via infected wild birds or rodents and contaminated equipment or personnel. When *Salmonella* is not prevalent in farmed animals, the importance of transmission of *Salmonella* from contaminated animal feed or feed ingredients increases.

Salmonella in Animal Feed

In animal feed, *Salmonella* is found mainly as a contaminant of oil seeds (soy and canola) and in feed of animal origin, but the prevalence in commercial heat-treated feed often is below 1% of samples. The most common serovars in meat animals are rarely contaminants of feed whereas other serovars, often referred to as 'exotic' or 'feed-borne' serovars (e.g., *S.* Livingstone, *S.* Mbandaka, *S.* Putten, *S.* Rissen, and *S.* Senftenberg), are rare in meat animals and humans but common in feed.

Because sampling of the huge amounts of feed used for meat animals is necessarily limited, monitoring of *Salmonella* in feed by culture methods is inadequate for control of *Salmonella*. In many countries end product monitoring is supplemented with process control based on processors' own check programs and hazard analysis critical control point (HACCP), and in several countries heat treatment or acidification of commercial feed is part of *Salmonella* control programs.

Salmonella in Meat Animals

Monitoring of *Salmonella* in meat animals may be conducted in herds or at slaughter, by detection of *Salmonella* in fecal material, dust, or internal organs, or by detection of antibodies against *Salmonella* in blood samples, egg yolk, muscle fluid, or milk.

Poultry

Salmonella is commonly isolated from broiler chickens worldwide, although large differences between countries exist. In a baseline study in 23 EU Member States during 2005–06, *Salmonella* was detected in 23.7% of the flocks (Table 2). More than 50% of the isolates were *S.* Enteritidis or *S.* Infantis. Targeted mandatory surveillance programs for *S.* Enteritidis and *S.* Typhimurium in the EU have led to reduced flock prevalence, determined in routine monitoring to be 4.1% in 2010 (0.4% target serovars). *Salmonella* is also commonly isolated from other poultry. An EU baseline study in 2006–2007 found *Salmonella* in 30.7% of turkey flocks (Table 2). Common serovars in turkeys are *S.* Hadar, *S.* Heidelberg, and *S.* Saintpaul. Commercial flocks of geese and ducks frequently harbor a wide variety of *Salmonella* serovars.

Pigs

With the exception of some, mainly North European countries, *Salmonella* is widespread in pig populations. In an EU baseline study in 2006–2007, 13.9% of intestinal lymph nodes from slaughter pigs were positive for *Salmonella*, with *S.* Typhimurium and *S.* Derby being the predominant serovars (Table 2). Prevalence of *Salmonella* in slaughter pigs is as high as 40%, as measured by various sampling techniques, have been reported. An EU baseline study found boars and sows culture positive in approximately 30% of breeder and production units. An increasing number of countries worldwide have implemented surveillance programs for *Salmonella* in pigs. The Danish integrated program, which covers the entire production continuum, is among the most comprehensive of these. The pig herds are classified on the basis of serology and microbiology, enabling risk-based prevention and control actions to be taken in herds and at slaughter.

Cattle

The occurrence of *Salmonella* in cattle is often low (0–1%) but variable, with reports of 5–10% of animals shedding *Salmonella* being rare. *S.* Dublin and *S.* Typhimurium are the predominant serovars in cattle. Owing to the severe illnesses from *S.* Dublin infection in humans and cattle, this serovar deserves special attention as a target for control efforts. *Salmonella* in cattle is mainly detected as a result of animals showing clinical symptoms. Comprehensive monitoring programs in the primary stages of production are rare. In Denmark, a *S.* Dublin eradication program was initiated in 2007. It is based on routine serological monitoring of milk or blood samples from all cattle herds in the country. The program has significantly reduced the prevalence of seropositive dairy and other herds.

Salmonella at Slaughter

Salmonella in poultry slaughterhouses

In many countries, decontamination of carcasses is part of normal slaughter procedures for broilers, for which there is a lack of effective slaughter hygiene measures. Decontamination has only recently been accepted in the EU. *Salmonella* carcass prevalence varies considerably between countries. In a US study, approximately 10% of carcasses were positive for *Salmonella*. In the EU a declining trend has been observed for *Salmonella* in broiler carcasses, parallel to the declining prevalence of *Salmonella*-positive broiler flocks, after targets for reduction of *S.* Enteritidis and *S.* Typhimurium in broiler flocks were set.

Salmonella *in pig slaughterhouses*

Salmonella is widespread in slaughter pigs in many countries. During transport and lairage *Salmonella* will be exchanged between pigs, which will add to the *Salmonella* load on the slaughter line. Recent epidemiological studies suggest that direct contamination between carcasses is not the main driver of carcass contamination but rather the many stages of processing at abattoirs, in particular the operations of carcass polishing and splitting. There are large variations between the extents of *Salmonella* contamination of carcasses at different plants, and many factors are likely to contribute to this. Between 1% and 8% of dressed carcasses have been reported to be *Salmonella* positive.

Salmonella *in cattle slaughterhouses*

S. Typhimurium and *S.* Dublin are often isolated from cattle. In an American study during 1998–2000, *Salmonella* was isolated from 70% of cattle hides, 13.3% of fecal samples, and 6.7% of carcasses, and most frequently during August to October. Seasonality in *Salmonella* prevalence has been reported from Europe also.

Salmonella in Cutting Plants

Meat can be cross-contaminated during fabrication of cuts, and too high temperatures may allow growth of *Salmonella* on meat and meat plant equipment. HACCP-based own control

systems in cutting plants should enable the industry to maintain product hygiene. Dutch data suggests that if *Salmonella* contamination is high on meat delivered for cutting, only little control over contamination of product with *Salmonella* can be expected from the plants' own HACCP-based control systems.

Salmonella at Retail

The microbiological status of retailed meat is largely determined by the hygienic adequacy of handling practices and temperature control during distribution. At retail, the mincing process in particular adds to cross contamination and possibly allows growth of *Salmonella*. Minced beef and pork both present risks to consumers from *Salmonella*. In Ireland in 2007, a *Salmonella* prevalence of 5% has been reported as compared to 1.8% in Denmark in 2006, whereas a prevalence up to 50% was estimated for minced meats in The Netherlands (1998). The prevalence of *Salmonella* in meat cuttings from Danish butcher shops was 8.1% compared to a prevalence in meat cuttings from supermarkets of 2.6%.

Control and Preventive Measures

Control and Preventive Measures on Farms

The purpose of control strategies for *Salmonella* in meat animal farming is to reduce or eliminate *Salmonella* in animals that are presented for slaughter. The need for preharvest control depends on subsequent post harvest control. Focus of preharvest control varies with the animal species, but the basic principles of preharvest control are common for all. These are:

- Prevent introduction into the herd
- Prevent transmission within the herd
- Reduce prevalence and shedding in infected populations.

Table 3 Main risk factors and recommendations for reduction of subclinical *Salmonella* in slaughter pig herds

	Risk	*Protective factors/Recommendations*
Purchase of animals	Purchase of infected pigs[a]	Purchase from noninfected supplier herd
	High number of supplier herds[b]	
Management	Continuous production	Strict batch production (all-in/all-out)[b,c]
	Mixing of pigs	One-way flow of pigs – no mixing[d]
	Slurry flooding	Keep low levels in slurry pits[c]
Hygiene	Insufficient cleaning of pens	Thorough cleaning and desiccation and proper choice and use of disinfectant[c]
	Transmission from tools/boots	Separate tools and boots for each unit[c]
	Transmission from rodents/herd environment	Rodent control and other biosecurity[b,c]
Feed and feeding	Dry feed[d,e,f]	Wet feed – preferably fermented to pH < 4.5
		Alternatively acidification of feed (0.5–1% formic- or lactic acid[g] or whey[b])
	Finely grinded feed corn[h]	Coarser grinding of feed corn
	Commercial pelleted feed[b,d,f,h]	Home-mixed meal feed
		Alternatively 25% of corn fed as nonheat treated, coarsely grinded[i]
	Low % of barley in feed corn[j]	> 25% barley of feed corn
Herd size	Increasing herd size[a,d,e] but less *Salmonella* in the largest herds[d]	Practical and financial potential to implement the reduction means above

[a]Kranker, S., Dahl, J., Wingstrand, A., 2001. Bacteriological and serological examination and risk factor analysis of *Salmonella* occurrence in sow herds, including risk factors for high *Salmonella* seroprevalence in receiver finishing herds. Berliener und Münchener Tierarztliche Wochenschrift 114, 350–352.

[b]Lo Fo Wong, D.M.A., Dahl, J., Stege, H., *et al*., 2004. Herd-level risk factors for subclinical infection in European finishing-pig herds. Preventive Veterinary Medicine 62, 253–266.

[c]Alban, L., Baptista, F.M., Møgelmose, V., *et al*., 2011. *Salmonella* surveillance and control for finisher pigs and pork in Denmark – A case study. Food Research International 46, 656665. Available at: http://dx.doi.org/10.1016/j.foodres.2011.02.050 (accessed 08.11.11).

[d]Dahl, J., 1997. Cross sectional epidemiological analysis of the relations between different herd factors and *Salmonella* seropositivity. Epidemiologie et Sante Animale, 31–32, 1–3.

[e]van der Wolf, P.J., Wolbers, W.B., Elbers, A.R.W., *et al*., 2001. Herd level husbandry factors associated with the serological *Salmonella* prevalence in finishing pig herds in The Netherlands. Veterinary Microbiology 78, 205–219.

[f]Bager, F., 1994. *Salmonella* in Danish pig herds. Risk factors and sources of infection. In: Proceedings from the XVII Nordic Veterinary Congress, pp. 79–82. Reykjavik, Iceland: The Icelandic Veterinary Association.

[g]Dahl, J., Wingstrand, A., Baggesen, D.L., Nielsen, B., Thomsen, L.K., 1996. The effect of a commercial organic acid preparation on seroprevalence and shedding of *Salmonella* in finishing pigs. In: Proceedings of the International Symposium on the Epidemiology and Control of Salmonella, p. 178. Italy: Bologna.

[h]Jørgensen, L., Dahl, J. Wingstrand, A. 1999. The effect of feeding pellets, meal and heat treatment on the *salmonella*-prevalence in finishing pigs. In: Monetti, P.G., Vignola, G. (Eds.), Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, pp. 308–312. Washington, DC: USA.

[i]Dahl, J., Jørgensen, L., Wingstrand, A., 1999. An intervention study of the effect of introducing *Salmonella* controlling feed strategies in *Salmonella* high prevalence herds. In: van der Wolf, P.J. (Ed.), Proceedings of the 4th International Symposium on the Epidemiology and Control of Salmonella and Other Food Borne Pathogens in Pork, pp. 340–342. Germany: Leipzig.

[j]Jørgensen, L., Kjærsgaard, H.D., Wachmann, H., Jensen, B.B., Knudsen, K.E.B., 2001. Effect of wheat bran and wheat:barley ratio in pelleted feed on Salmonella prevalence and productivity of finishers. In: Proceedings of the 4th International Symposium on the Epidemiology and Control of Salmonella and Other Food Borne Pathogens in Pork, pp. 112–114. Germany: Leipzig.

Control in poultry farms

In poultry production the very efficient vertical dissemination of *Salmonella* through the breeding system demands a top–down eradication control strategy. Successful elimination of *Salmonella* from broiler flocks has taken place in many countries. Good farming practices, with strict batch production and biosecurity in confined production systems, and heat treatment of commercial poultry feed have been important elements. Thus, the flock prevalence in Danish broiler flocks was reduced from approximately 70% in 1990 to few percent since 2001. Reductions of *Salmonella* in other types of poultry have been achieved with similar procedures.

Control in pig farms

Eradication of *Salmonella* from pig farms has taken place in only a few North European countries with an a priori low prevalence, but monitoring and reduction of *Salmonella* in pig populations in which it is endemic have become more common. Risk factors for high prevalence of *Salmonella* in pig herds are well known, and it has proven possible to reduce high infection levels to moderate or low levels by purchase of noninfected pigs, strict all-in/all-out production, improved hygiene, and biosecurity. The use of certain feed and feeding practices can cause microbiological and chemical changes that reduce proliferation of *Salmonella* in the gastrointestinal tract (Table 3).

Control in cattle farms

In the last decade, evidence-based interventions to control *S.* Dublin in cattle herds have been developed. Introduction of *Salmonella* from purchased animals must be avoided, and introduction from equipment and herd environments may be prevented by strict hygiene and biosecurity measures. In infected herds, the risk of triggering an outbreak of salmonellosis can be reduced by good hygiene in calving and calf units. Infection from cow to calf through contact after calving should be avoided by supplementing calves with colostrum from a colostrum bank, and a one-way flow of animals in the farm should be established.

Vaccination against *Salmonella* is mainly used with poultry and may under some circumstances and for some serovars reduce *Salmonella* in the flocks. Serological vaccine reactions in routine monitoring assays should be taken into account when vaccination is considered.

Antibiotics should never be used to control subclinical infections with *Salmonella* due to the risk of development of antimicrobial-resistant *Salmonella*.

Control and Preventive Measures Postharvest

Salmonella cannot be dealt with through classical meat inspection practices as production animals mostly are asymptomatic carriers. In many countries physical (e.g., steam) and chemical (e.g., lactic acid or chlorine) decontamination of carcasses is used to reduce pathogen levels. Laboratory and in-line investigations of decontamination effects show that hot water and steam on average reduce *Salmonella* levels by 100- to 1000-fold, whereas the effect of chemical decontamination often is within the range of 10- to 30-fold. The use of two or more decontamination methods is common and can further improve efficacy. In Denmark, serological classification of herds has been used to direct 1% of all pig carcasses to hot-water decontamination. Plant control systems are often in place with routine monitoring of slaughter hygiene. This can also be required in relation to export to countries such as the USA. In the USA, *Salmonella* performance standards at different levels in the meat chain have been mandated, and in the EU process hygiene criteria have been instituted.

See also: Animal Breeding and Genetics: Traditional Animal Breeding. Conversion of Muscle to Meat: Slaughter-Line Operation and Pig Meat Quality. Cutting and Boning: Traditional. Foodborne Zoonoses. Hazard Analysis Critical Control Point and Self-Regulation. Manure/Waste Management: Manure Management. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: DNA Methods; Standard Methods. Modeling in Meat Science: Microbiology; Refrigeration. Nutrition of Meat Animals: Pigs. Risk Analysis and Quantitative Risk Management. Slaughter-Line Operation: Cattle; Pigs; Poultry. Species of Meat Animals: Cattle; Pigs; Poultry

Further Reading

Alban, L., Baptista, F.M., Møgelmose, V., *et al.*, 2011. *Salmonella* surveillance and control for finisher pigs and pork in Denmark – A case study. Food Research International 46, 656–665. Available at: http://dx.doi.org/10.1016/j.foodres.2011.02.050 (accessed 08.11.11).

Department of Agriculture (1996). Pathogen reduction: Hazard analysis and critical control points (HACCP) systems; Final Rule. Federal Register. Department of Agriculture, Food Safety and Inspection Service. USDA.

Anonymous, 2005. Commission regulation (EC) No 2073/2005 of November 2005 on microbiological criteria for food stuffs. European Commission. Official Journal of the European Union.

Bager, F. 1994. *Salmonella* in Danish pig herds. Risk factors and sources of infection. In: Proceedings from the XVII Nordic Veterinary Congress, pp. 79–82. Reykjavik, Iceland: The Icelandic Veterinary Association.

Berends, B.R., Knapen, F.V., Mossel, D.A.A., Burt, S.A., Snijders, J.M.A., 1998. Impact on human health of *Salmonella* spp. On pork in the Netherlands and the anticipated effects of some currently proposed control strategies. International Journal of Food Microbiology 44, 219–229.

Borch, E., Nesbakken, T., Christensen, H., 1996. Hazard identification in swine slaughter with respect to food borne bacteria. International Journal of Food Microbiology 30, 9–25.

Dahl, J., 1997. Cross sectional epidemiological analysis of the relations between different herd factors and *Salmonella* seropositivity. Epidemiologie et Sante Animale 31–32, 1–3.

Dahl, J., Jørgensen, L., Wingstrand, A., 1999. An intervention study of the effect of introducing *Salmonella* controlling feed strategies in *Salmonella* high prevalence herds. In: van der Wolf, P.J. (Ed.), Proceedings of the 4th International Symposium on the Epidemiology and Control of Salmonella and Other Food Borne Pathogens in Pork, pp. 340–342. Germany: Leipzig.

Dahl, J., Wingstrand, A., Baggesen, D.L., Nielsen, B., Thomsen, L.K., 1996. The effect of a commercial organic acid preparation on seroprevalence and shedding of *Salmonella* in finishing pigs. In: Monetti, P.G., Vignola, G. (Eds.), Proceedings of the International Symposium on the Epidemiology and Control of Salmonella, p. 178. Italy: Bologna.

European Food Safety Authority, European Centre for Disease Prevention and Control, 2012. The European summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. EFSA Journal 10, 442.

Grimont, P.A.D., Weill, F.-X., 2007. Antigenic formulae of the *Salmonella* serovars, 9th edition France: WHO Collaborating Centre for Reference and Research on Salmonella. Institut Pasteur.
Hald, T., Vose, D., Wegener, H.C., Koupeev, T., 2004. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Analysis 24, 255–269.
Hald, T., Wingstrand, A., Swanenburg, M., Altrock, A., Thorberg, B.-M., 2003. The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses. Epidemiology and Infection 131, 1187–1203.
Hansen-Wester, I., Hensel, M., 2001. *Salmonella* pathogenicity islands encoding type III secretion systems. Microbes and Infection 3, 549–559.
Jørgensen, L., Dahl, J., Wingstrand, A. 1999. The effect of feeding pellets, meal and heat treatment on the *salmonella*-prevalence in finishing pigs. In: Bahnson, P.B. (Ed.), Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork, pp. 308–312. Washington, DC, USA: Biomedical Communications Center, College of Veterinary Medicine, University of Illinois at Urbana-Champaign.
Jørgensen, L., Kjærsgaard, H.D., Wachmann, H., Jensen, B.B., Knudsen, K.E.B., 2001. Effect of wheat bran and wheat: barley ratio in pelleted feed on Salmonella prevalence and productivity of finishers. In: Proceedings of the 4th International Symposium on the Epidemiology and Control of Salmonella and Other Food Borne Pathogens in Pork, pp. 112–114. Germany: Leipzig.
Kranker, S., Dahl, J., Wingstrand, A., 2001. Bacteriological and serological examination and risk factor analysis of *Salmonella* occurrence in sow herds, including risk factors for high *Salmonella* seroprevalence in receiver finishing herds. Berliener und Münchener Tierarztliche Wochenschrift 114, 350–352.
Kühler, H., Sakaguchi, T., Hurley, B.P., *et al*., 2007. *Salmonella enterica* serovar Typhimurium regulates intercellular junction proteins and facilitates transepithelial neutrophil and bacterial passage. American Journal of Physiology: Gastrointestinal & Liver Physiology 293, G178–G187.
Lo Fo Wong, D.M.A., Dahl, J., Stege, H., *et al*., 2004. Herd-level risk factors for subclinical infection in European finishing-pig herds. Preventive Veterinary Medicine 62, 253–266.
O'Connor, A.B., Denagamage, T., Sargeant, J.M., Rajic, A., McKean, J., 2008. Feeding management practices and feed characteristics associated with *Salmonella* prevalence in live and slaughtered market-weight finisher swine: A systematic review and summation of evidence from 1950 to 2005. Preventive Veterinary Medicine 87, 213–228.
Smid, J.H., Heres, L., Havelaar, A.H., Pielaat, A., 2012. A biotracing model of *Salmonella* in the pork production chain. Journal of Food Protection 75, 270–280.
The European Food Safety Authority, 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in broiler flocks of *Gallus gallus*, Part A. The EFSA Journal 98, 1–85.
The European Food Safety Authority, 2008a. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in turkey flocks, Part A. The EFSA Journal 134, 1–91.
The European Food Safety Authority, 2008b. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part A. The EFSA Journal 135, 1–111.
The European Food Safety Authority, 2009. Analysis of the baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs, in the EU, 2008, Part A: *Salmonella* prevalence estimates. EFSA Journal 7(12), 1377 (93 pp).
Threllfall, E.J., Wain, J., Lane, C., 2011. Salmonellosis. In: Palmer, S., Soulsby, L., Torgerson, P., Brown, D.W.G. (Eds.), Zoonoses. Biology, clinical practice and public health control, 2nd ed. New York: Oxford University Press Inc., pp. 992.
van der Wolf, P.J., Wolbers, W.B., Elbers, A.R.W., *et al*., 2001. Herd level husbandry factors associated with the serological *Salmonella* prevalence in finishing pig herds in The Netherlands. Veterinary Microbiology 78, 205–219.
World Health Organization, Global Foodborne Infections Network. Country Databank. Available at: http://thor.dfvf.dk/portal/page?_pageid=53,1&_dad=portal&_schema=PORTAL (accessed 30.03.12).

Relevant Websites

http://www.cdc.gov/
Centers for Disease Control and Prevention (CDC) USA.
http://ecdc.europa.eu/en/Pages/home.aspx
European Centre for Disease Prevention and Control (ECDC), Sweden.
http://www.efsa.europa.eu/
European Food Safety Authority (EFSA), Italy.
http://www.ozfoodnet.gov.au/
OzFoodNet, Australia.
http://www.nml-lnm.gc.ca/NESP-PNSME/index-eng.htm
Public Health Agency of Canada, National Enteric Surveillance Program.
http://www.surv.esr.cri.nz/index.php?we_objectID=2933
Public Health Surveillance, Information for New Zealand Public Health Action.
http://thor.dfvf.dk/portal/page?_pageid=53,1&_dad=portal&_schema=PORTAL
World Health Organization, Global Foodborne Infections Network, Country Databank.

Staphylococcus aureus

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Glossary

a_w (water activity) The available water in a given substrate. Values range from 0 to 1, where 1 is a_w of pure distilled water.

D-value The decimal reduction time, or the duration of an antibacterial process (such as heat or irradiation) at a given intensity which results in a 90% reduction in the bacterial population.

Fail-safe Used in predictive microbiology to represent a situation where a model predicts growth but none occurs. The model has failed but in a safe manner.

Humectant An additive that binds water and controls water activity.

Microflora The naturally occurring microorganisms in a given environment.

Introduction

The first link between *Staphylococcus aureus* and food poisoning was made following an outbreak associated with eating cheese, and the first recognized meat-related outbreak, involving a fatality, was reported in 1894. The organism has subsequently been incriminated in incidents involving a wide range of food vehicles including meat, poultry, dairy, cream-filled bakery, and egg products as well as salads and canned mushrooms.

Staphylococcal food poisoning is an intoxication caused by the consumption of one of a variety of enterotoxins. A number of species of *Staphylococcus* are able to produce these toxins but only one species, *S. aureus*, is commonly associated with foodborne disease. It is regarded as an organism that competes poorly with the natural microflora of raw meats but is able to grow at low water activity (a_w) values. These two characteristics are reflected in the types of meat products normally associated with staphylococcal foodborne disease: i.e., foods that have been cooked and hence have no competitors present; and low a_w foods, often with high salt concentrations, which are conducive for the growth of *S. aureus*.

The toxin produced is heat stable and can survive commercial canning. *Staphylococcus aureus* may, therefore, be absent from a food, yet the food can still contain the toxin and cause disease. Good food handling practices (e.g., good personal hygiene, prevention of cross-contamination and temperature control) are the most effective way of preventing staphylococcal intoxications.

Characteristics of the Organism and its Toxin

The Organism

Staphylococcus aureus is a Gram-positive, catalase-positive coccus, 0.5–1.5 μm in diameter, which forms clusters of cells appearing as characteristic 'bunches of grapes' when viewed microscopically. In laboratory media it has an optimum temperature for growth of 37 °C with a range of 7–48 °C. However, when growing in food these limits may be reduced. For example, limited growth of *S. aureus* occurred on vacuum-packed ham and turkey, but not on chicken stored at 10 °C. The optimum pH for growth is 7.0–7.5 with a range of 4.2–9.3. The lower pH value at which growth occurs is raised when organic acids such as acetic and lactic acids are used as the acidulant, and no growth occurs at room temperature in vacuum-packaged fermented meat products of pH ≤ 5.3. Growth is optimal under aerobic conditions but the organism can also grow anaerobically. Of note is the organism's ability to grow at low a_w values. Growth can occur at a_w values as low as 0.86 (equivalent to 20% NaCl) depending on the humectant used (e.g., NaCl, sucrose, etc.), and the organism grows well in the presence of 7–10% NaCl.

Staphylococcus aureus is moderately heat resistant for a nonspore-forming foodborne pathogen, with a mean *D*-value (i.e., time for a 90% reduction) of approximately 5–7 min at 60 °C. However, in salty foods its thermal resistance is much greater. For example, the mean *D*-value at 60 °C increased from 6 to 25 min when the NaCl content of meat macerate was increased from 0% (w/v) to 8.4%. Microwave heating to 65 °C results in reductions of numbers of the order of 1.7–2.5 $\log_{10}$ cfu ml^{-1} or g^{-1} in a variety of foods. *D*-values for irradiation are in the range of 0.3–0.6 kGy depending on the atmosphere, temperature and nature of the food substrate in which the organism is irradiated. However, *D*-values as high as 0.86 kGy have been reported.

For a nonspore-forming organism, *S. aureus* is very resistant to adverse conditions and it can survive long periods of desiccation. For example, the organism can survive for more than 1000 days on a dry plastic surface. Survival during frozen storage is good at temperatures ≤10 °C, although there can be some loss of viability during freezing.

Staphylococcus aureus is frequently found in meat products, but usually at low concentrations. Some examples are given in Tables 1 and 2. Data are presented for detection of the organism and toxin by conventional methods, but reported prevalence rates are higher when molecular methods such as the polymerase chain reaction (PCR) are used.

An emerging concern with *S. aureus* is the acquisition of methicillin resistance and the roles that food production and

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Table 1 Examples of the prevalence of *Staphylococcus aureus*[a] on or in meat products, and the prevalence of enterotoxin-positive strains among isolates from such products

Product	*Proportion samples +ve (%)*	*Isolates enterotoxin +ve (%)*
Raw pork, France	57.7	34.6
Retail smoked ham, France	11.1	0
Fresh meat, Italy	26.1	21.4
Minced meat/burgers, Italy	31.2	46
Fresh meat preparations, Italy	10.6	53.7
Fermented sausages, USA	ND	0
Fish products, India	21	41
Ready-to-eat meat, Korea	2.1	100
Raw fish, Korea	19.8	50
Deboned beef, South Africa	15.8–24.4	ND
Beef, USA	20.5	ND
Chicken, USA	25.0	ND
Turkey, USA	24.6	ND
Turkey, USA	77	ND
Pork, USA	42	ND
Chicken, USA	41	ND
Beef, USA	37	ND
Boneless beef trim, USA	4.2	ND
Boneless beef trim, Australia	4.0	ND
Boneless beef trim, New Zealand	8.2	ND
Boneless beef trim, Uruguay	29.5	ND

[a]Or coagulase-positive staphylococci.

Abbrevation: ND, Not determined.

Source: Atanassova, V., Meindl, A., Ring, C., 2001. Prevalence of Staphylococcus aureus and staphylococcal enterotoxins in raw pork and uncooked smoked ham-a comparison of classical culturing detection and RFLP-PCR. International Journal of Food Microbiology 68, 105–113; Bhargava, K., Wang, X., Donabedian, S., *et al*., 2011. Methicillin-reistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. Emerging Infectious Diseases 17, 1135–1137; Bosilevac, J.M., Guerni, M.N., Brichta-Harhay, D.M., Arthur, T.M., Koohmarie, M., 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. Journal of Food Protection 70, 440–449; Levine, P., Rose, B., Green, S., Ransom, G., Hill, W., 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. Journal of Food Protection 64, 1188–1193; Normanno, G., Firinu, A., Virgilio, S., *et al*., 2005. Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. Inernational Journal of Food Microbiology 98, 73–79; Oh, S.K., Lee, N., Cho, Y.S., *et al*., 2007. Occurrence of toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. Journal of Food Protection 70, 1153–1158; Shale, K., Lues, J.F.R., Venter, P., Buys, E.M., 2005. The distribution of *Staphylococcus* sp. On bovine meat from abattoir deboning rooms. Food Microbiology 22, 433–438; Simon, S.S., Sanjeev, S., 2005. Prevalence of enterotoxigenic *Staphylococcus aureus* in fishery products and fish processing factory workers. Food Control 18, 1565–1568; and Waters, A.E., Contente-Cuomo, T., Buchhagen, J., *et al*., 2011. Multidrug resistant *Staphylococcus aureus* in US meat and poultry. Clinical Infectious Diseases 52, 1–4.

Table 2 Examples of data for concentrations of *Staphylococcus aureus* in cooked sliced meats (A), ready-to-eat pies and pastries (B) and other cooked meat products (C)

Count (g^{-1})	*Fraction of samples (%) positive*		
	A	*B*	*C*
ND	91.2	96.6	100
$20 \leq 10^2$	7.4	3.0	0
$10^2 \leq 10^3$	0.9	0.3	0
$10^3 \leq 10^4$	0.4	0	0
$10^4 \leq 10^5$	0	0.2	0
$10^5 \leq 10^6$	0.1	0	0
$10^6 \leq 10^7$	0.1	0	0

ND <20 CFU g^{-1}.

Source: Little, C.L., de Louvois, J., 1998. The microbiological examination of butchery products and butchers' premises in the United Kingdom. Journal of Applied Microbiology 85, 177–186.

consumption may have in infections of people with methicillin-resistant *S. aureus* (MRSA). Testing of Canadian feedlot cattle just before slaughter failed to detect MRSA; but MRSA has been detected in other food animals such as pigs, and in a variety of retail meat products. Examples are given in Table 3. Widespread or emerging resistance to other antibiotics is also a cause for concern.

Staphylococcal Enterotoxins

The organism causes disease through the production of 1 of a group of at least 20 structurally similar protein toxins. Only five subclasses of these toxins are commonly involved in human disease, i.e., staphylococcal enterotoxins (SE) types A, B, C, D, and E, with type C toxins being further divided into types C_1, C_2, and C_3. Human disease usually involves SE-A. Toxin production occurs under more restrictive conditions

Table 3 Examples of data for the prevalence of methicillin-resistant *S. aureus* (MRSA) in meat

Country	*Samples (number tested)*	*Number +ve for MRSA/Number tested*	*Percentage positive*
The Netherlands[a]	Beef	42/395	10.6
	Veal	39/257	15.2
	Pork	33/309	10.7
	Lamb/mutton	20/324	6.2
	Chicken	83/520	16.0
	Turkey	41/116	35.3
	Fowl	4/118	3.4
	Game	4/178	2.2
USA	Beef	2/156	1.3
	Chicken	3/76	3.9
	Turkey	1/57	1.7
USA	Minced pork	1/300	0.3
	Minced beef	0/198	0
	Minced turkey	0/196	0
Germany[a]	Fresh chicken	6/24	25.0
	Chicken meat products	4/19	21.1
	Fresh turkey	11/22	50.0
	Turkey meat products	11/21	52.4

[a]These studies used an enrichment and so were more sensitive than others using direct plating.

Source: Bhargava, K., Wang, X., Donabedian, S., *et al*., 2011. Methicillin-reistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. Emerging Infectious Diseases 17, 1135–1137; de Boer, E., Zwartkruis-Nahuis, J.T.M., Wit, B., Huijsdens, X.W., *et al*., 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. International Journal of Food Microbiology 134, 52–56; Feßler, A.T., Kadlec, K., Hassel, M., *et al*., 2011. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. Applied Environmental Microbiology 77, 7151–7157; and Kelman, A., Soong, Y.-A., Dupuy, N., *et al*., 2011. Antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats. Journal of Food Protection 74, 1625–1629.

than those that allow growth. It occurs between 10 and 45 °C and at pH 4.8–9.0, and is optimal at temperatures between 35 and 40 °C and pH values between 5.3 and 7.0. Toxin production is greater under aerobic than under anaerobic conditions, and occurs at a_w values ≥0.90. The toxin is very resistant to heat. For example, the *D*-value for SE-B is 100 min at 149 °C and an a_w of 0.99, and 225 min when the a_w is 0.90. The toxin is also resistant to the proteolytic enzymes that occur in the gastrointestinal tract, dehydration, gamma irradiation, and extremes of pH.

Because the toxin is heat stable, it is possible for a food to be free of viable organisms yet still be the cause of SE poisoning. This may occur if the required population was reached, enterotoxin was produced, and the cells were then inactivated by a process such as cooking.

Ingestion of less than 1.0 μg of toxin can result in illness, but this level is reached only when the population of *S. aureus* exceeds 10^5 g^{-1}. Concentrations as high as 1.5×10^{10} cfu g^{-1} have been reported in outbreak-related foods and ingested levels of 1–5 μg of toxin are normally associated with outbreaks, although children have become sick at a dose of 17 ng.

Not all isolates of *S. aureus* produce toxin. For example, one study of coagulase-positive food isolates found that only 30.5% were unequivocally enterotoxin positive.

Isolation and Identification

Enumeration and Detection

Because relatively high numbers of the organism are required to cause illness, in most cases an estimate of the concentration of the organism in a sample is needed rather than a sensitive detection method.

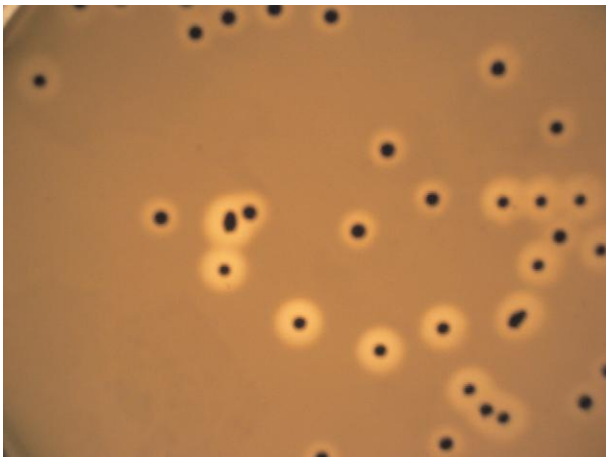

Figure 1 Typical colonies of *S. aureus* growing on BP agar.

There is a good overall agreement with regard to the methods that are appropriate for *S. aureus* (see ISO 6888-1 and ISO 6888-2). Baird-Parker (BP) agar is used as a plating medium in the ISO and many other methods. Typical colonies on BP agar appear 2–3 mm in diameter, black and shiny, surrounded by a white edge and a clear zone (Figure 1). Other media such as Rabbit Plasma Fibrinogen Agar (RPFA) are available. In this case, the medium identifies coagulase- and thermonuclease-positive colonies. With these media, the sample is prepared and dilutions either spread onto them (BP) or added to pour plates (RPFA),

the plates are incubated and suspect colonies counted and confirmed. A Most Probable Number method using enrichments of sample dilutions in Giolitti and Cantoni broth followed by plating to BP agar is recommended by the American Public Health Association (APHA). A number of other proprietary media are available in conventional agar and film formats.

For detection of *S. aureus* in foods in which cells may be injured, for example, in processed foods, the APHA recommend a short enrichment in a nonselective medium followed by a period of incubation with NaCl present as a selective agent. Isolates are then obtained on BP agar.

Several PCR methods that allow detection of *S. aureus* have now been published and commercial systems based on PCR are also available. Because the methods target different genes, they vary in their suitability for various tasks. General methods target genes such as *nuc* which encodes thermostable nuclease production. Others more specifically target toxin or antibiotic resistance genes. PCR methods are available as real-time applications for the detection of the organism in foods, and such techniques offer the possibility of quantifying rather than just detecting the presence of *S. aureus*.

Identification

Staphylococci are usually distinguished from other Gram-positive facultatively anaerobic cocci by the presence of catalase in *Staphylococcus*. Distinction between *S. aureus* and the micrococci is more difficult but can be achieved using the tests shown in Table 4. *Staphylococcus aureus* is characteristically positive for the following tests; thermostable nuclease, coagulase, clumping factor, yellow pigment, acetoin production, and hemolysis. The presence in *S. aureus* of clumping factor can be used to distinguish it from other coagulase-positive *Staphylococcus* species.

Not all *S. aureus* isolates are capable of producing toxin and it may be necessary to demonstrate the toxin-forming capability of food isolates. Because *S. aureus* may have grown, produced toxin, and then have been inactivated by cooking, a ready-to-eat food containing low numbers of the organism may not be safe. Toxin testing is therefore necessary in outbreak investigations where there is potential for enterotoxin to have been produced in the food before a bactericidal treatment.

Rapid test kits for the detection, identification, and confirmation of *S. aureus* are readily available from diagnostics manufacturers. In addition, kits are available for the detection of the SE. While being too numerous to detail, a list of approved test kits, along with their status in terms of recognition is maintained by AOAC International.

Table 4 Biochemical tests that discriminate between *S. aureus* and Micrococci

Test	S. aureus	*Micrococci*
Lysostaphin resistance	S	R
Coagulase production	+	−
Lysozyme resistance	R	R/S
Thermostable nuclease production	+	−
Anaerobic glucose fermentation	+	−
Anaerobic mannitol fermentation	+	−
Modified oxidase	−	+
Erythromycin resistance (0.4 μg ml^{-1})	R	S

Abbreviations: R, resistant; S, sensitive.

Typing

Numerous methods have been developed for the typing of *S. aureus*. Some of the methods that have been applied to meat products or outbreak investigations are briefly mentioned here.

Isolates may be assigned to different biotypes/ecovars according to staphylokinase, *β*-hemolysin, coagulase, and crystal violet agar growth tests. These ecovars are largely host-specific being grouped into human, poultry-like, bovine, ovine, and nonhost-specific ecovars. Molecular typing produces data that also tend to reflect a clonal association of *S. aureus* types and animal hosts. A few tests may therefore be useful in identifying the animal origin of *S. aureus* isolates, although a significant proportion of isolates may be of unspecified origin.

Phage typing is useful for the differentiation of isolates, and most enterotoxin-producing isolates have been shown to belong to a restricted range of phage types. However, the level of expertise required to use the methodology is likely to preclude small or routine testing laboratories from using it. Coagulase typing has also been used.

Pulsed field gel electrophoresis (PFGE) is a highly discriminatory molecular typing technique that has been successfully used to type *S. aureus* isolates. The primary restriction enzyme used was *Sma*I, with *Ksp*I being used subsequently to further evaluate indistinguishable isolates. *Sma*I digests produced profiles with 10–20 fragments in the 20–700 kb range. Clusters produced by PFGE correspond to those produced by biotyping, but not phage typing. PFGE has been shown to be more discriminatory than amplified fragment length polymorphism analysis. Staphylococcal protein A (*spa*) typing and multilocus sequence typing are other techniques that have been applied.

Characteristics of Foodborne Illness

Symptoms are caused by the production of a toxin which is thought to act by causing a signal to be sent from intestinal tract receptors to the medullary emetic center of the brain. The precise area in the abdomen that is stimulated has not been identified.

Symptoms normally occur 0.5–6 h (usually 2–4 h) after ingestion of the toxin and are most often nausea, frequent vomiting, and abdominal cramps for up to 48 h. Diarrhea is less common but can occur in a significant proportion of cases, and always occurs in conjunction with vomiting. In severe cases, headaches, fever, and collapse may occur. Recovery is usually swift, occurring over a few hours to 1 day, and generally no treatment is required. Death is not a common consequence of staphylococcal intoxication, but has been reported in the young and elderly. The organism may also be associated with autoimmune disorders. Estimates from the USA for illness associated with this organism are a hospitalization rate of 6.4% and a case fatality rate of $<0.1\%$.

Outbreaks of illness are often caused by contamination of foods by food handlers with uncovered infected wounds. It may also be transferred to food from the nose, where it is a commensal in 30% of the population, or other moist parts of the body, for example, mucous membranes and the skin. Enterotoxin production seems to be linked with isolates of human origin, further strengthening the link between food handling and food poisoning. An exception is the ovine biotype, isolates of which also frequently produce enterotoxin. PFGE has been used to type isolates from a meat processing plant and the results support the view that *S. aureus* on beef carcasses originate primarily from the hands of workers engaged in carcass dressing.

The organism is generally regarded as a poor competitor with other foodborne microorganisms. It, therefore, generally does not grow and cause problems in raw meats. Because of its tolerance to low water activity, its growth is favored in foods that are salted or dried, such as cured and fermented meats. It may also grow in foods such as cooked meats in which a competing flora has been reduced during processing.

Epidemiology

Staphylococcal intoxication contributes substantially to the burden of illness acquired from contaminated food. In 2008, in the EU, there were 291 outbreaks suspected as being attributable to staphylococcal intoxication, accounting for 5.5% of the total number of outbreaks of foodborne disease/intoxication. Of these outbreaks, 27.8% were verified. Two patients died. It is generally thought that, as symptoms are often self-limiting, only 10% of people with staphylococcal intoxication seek medical treatment.

Typical outbreaks occur through postcooking contamination by food handlers, with subsequent temperature abuse of the food that allows growth of and toxin production by *S. aureus*. For example, in an American outbreak where ham was the implicated vehicle, contamination probably occurred when an infected food handler removed casings from the hams which were subsequently stored at temperatures above 10 °C for more than 15 h.

Control and Preventative Measures

An important preventative measure centers around the training and personal hygiene of staff who handle food. Staff should cover wounds with sticking plasters and gloves where appropriate, and refrain from touching their noses, hair, or other parts of their body while working with food. If such events occur, then hands should be washed immediately.

Because the organism will grow well on foods with a background microflora reduced by cooking, it is essential to avoid cross-contamination of cooked foods by bacteria from raw foods in which staphylococci are likely to be present. Holding foods at a temperature less than 7 °C will ensure that the organism cannot grow and produce toxin.

Product formulations can be manipulated to prevent the growth of the organism by using multiple 'hurdles.' For example, pH and a_w may be used in combination at levels which alone would not prevent growth of the organism. Preservatives such as potassium sorbate and potassium nitrite may also be used to inhibit growth of the organism, especially when used in combination with another controlling factor. As an example, the inactivation rate in the presence of 2.5% potassium sorbate increases with increasing concentrations of CO_2 in packed atmospheres. Less is understood about the use of preservatives to prevent toxin formation specifically. Naturally occurring compounds, such as plant essential oils, are now also being considered as hurdles.

Predictive models, which model the growth rate of the organism given various input parameters such as pH and temperature, have been reported to overpredict growth. Therefore, they are generally, but not always, fail-safe. Other models predict the growth/no growth boundary in particular meat products. These may be useful tools for evaluating shelf stability.

In general, proper cleaning and sanitizing are effective in removing the organisms from manufacturing plants because the organism is sensitive to sanitizers used in the food industry.

See also: Microbiological Analysis: DNA Methods; Indicator Organisms in Meat; Standard Methods. Microbiological Safety of Meat: *Bacillus cereus*; Hurdle Technology. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Modeling in Meat Science: Microbiology

Further Reading

Balaban, N., Rasooly, A., 2000. Staphylococcal enterotoxins. International Journal of Food Microbiology 61, 1–10.

Cretenet, M., Even, S., Le Loir, Y., 2011. Unveiling *Staphylococcus aureus* enterotoxin production in dairy products: A review of recent advances to face new challenges. Dairy Science and Technology 91, 127–150.

De Buyser, M.L., Lombard, B., Schulten, S.M., *et al.*, 2003. Validation of EN ISO standard methods 6888 Part 1 and Part 2: 1999-Enumeration of coagulase-positive staphylococci in foods. International Journal of Food Microbiology 83, 185–194.

International Commission on Microbiological Specifications for Foods, 1996. Staphylococcus aureus. In: Roberts, T.A., Baird-Parker, A.C., Tompkin, R.B. (Eds.), Micro-organisms in Foods 5. Microbiological Specifications of Foodborne Pathogens. London: Blackie Academic, pp. 299–333.

Johler, S., Stephan, R., 2010. Staphylococcal food poisoning: A current review. Archiv für LebensmittelhygieneLebensmittelhyg 61, 197–236.

Kluytmans, J.A.J.W., 2009. Methicillin-resistant *Staphylococcus aureus* in food products: Cause for concern or case for complacency? Clinical Microbiology and Infection 16, 11–15.

Lancette, G.A., Bennett, R.W., 2001. *Staphylococcus aureus* and staphylococcal enterotoxins. In: Downes, F.P., Ito, K. (Eds.), Compendium of Methods for the Microbiological Examination of Foods, fourth ed. Washington, DC, USA: American Public Health Association, pp. 387–403.

Seo, K.S., Bohach, G.A., 2007. Staphylococcus arueus. In: Doyle, M.P., Beuchat, L. R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers. Washington, DC, USA: ASM Press, pp. 493–518.

Stewart, C.M., 2003. *Staphylococcus aureus* and staphylococcal enterotoxins. In: Hocking, A.D. (Ed.), Foodborne Microorganisms of Public Health Significance, sixth ed. Sydney, Australia: AIFST (NSW Branch) Food Microbiology Group, pp. 359–379.

Relevant Websites

http://www.aoac.org/testkits/tested methods.html
AOAC International.
http://www.combase.cc/index.php/en/
ComBase.
http://www.fda.gov/food/scienceresearch/laboratorymethods/bacteriologicalanalyticalmanualbam/ucm073674.htm
FDA Bacteriological Analytical Manual: Staphylococcal Enterotoxins: Micro-slide Double Diffusion and ELISA-based Methods.
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071429.htm
FDA Bacteriological Analytical Manual: *Staphylococcus aureus*.
http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/ucm070015.htm
FDA Bad bug book.
http://www.foodsafety.govt.nz/elibrary/industry/Staphylococcus_Aureus-Science_Research.pdf
Foodborne pathogens data sheet: *Staphylococcus aureus*.
http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/res-rech/mfhpb21-eng.pdf
Government of Canada method for the enumeration of *S. aureus* in foods.
http://pmp.arserrc.gov/PMPOnline.aspx
Predictive models: Pathogen modelling program (PMP) online.
http://www.textbookofbacteriology.net/staph.html
Todar's online textbook of bacteriology.

Thermotolerant *Campylobacter*

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Introduction

Campylobacters are a major cause of foodborne gastrointestinal disease, especially in the developed world, with foods of animal origin and fecally contaminated waters being the most common sources of infection. For humans, infection usually causes self-limiting diarrhea (campylobacteriosis) but occasionally more serious extraintestinal disease develops. The majority of reported campylobacteriosis is caused by thermotolerant species, principally *Campylobacter jejuni*, followed by *Campylobacter coli* and, to a much lesser extent, *Campylobacter lari*. Thermotolerant Campylobacters occur over a wide geographic range and have been isolated from the intestines of many warm-blooded animals. Although large numbers of *Campylobacter* are shed in feces, the bacteria are susceptible to environmental stresses and the health risk is usually associated with consumption of undercooked food or untreated drinking water. As thermotolerant *Campylobacter* spp. do not multiply at temperatures of less than 30 °C; appropriate temperature control and good food hygiene practices, including preventing cross-contamination, are important barriers to infection.

Characteristics of Thermotolerant *Campylobacter*

Thermotolerant *Campylobacter* belongs to the family *Campylobacteraceae* (from the Greek word for curved rod). Cells are slender, spirally curved rods typically 0.2–0.5 μm wide and 0.5–5 μm long with up to four 'windings.' After exposure to unfavorable conditions cells may round up to a coccoid shape. A typical curved *Campylobacter* cell is shown in Figure 1.

Members of the genus *Campylobacter* are Gram-negative motile bacteria that typically have a single flagellum, at one or both ends of the cell that drives their characteristic corkscrew darting motion. They are oxidase positive and have a respiratory type of metabolism. Energy is derived from either amino acids or tricarboxylic acid cycle intermediates but carbohydrates are not used. Although oxygen is required for respiration, thermotolerant *Campylobacter* are fastidious microaerophiles and do not grow, or grow poorly in air and do not grow under anaerobic conditions. Optimal growth occurs in microaerobic atmospheres usually comprising 5% oxygen, 10% carbon dioxide, and 85% nitrogen. In addition to the thermotolerant species that have an optimal growth temperature of 42 °C, other species with temperature optima in the 30–37 °C range have been linked to human illness.

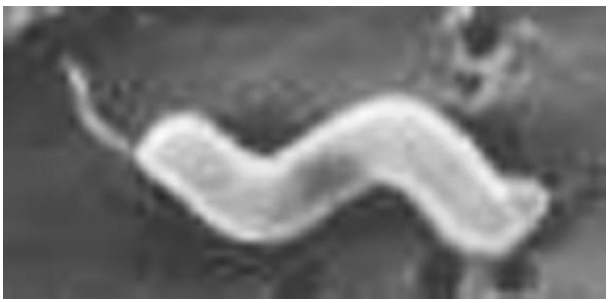

Figure 1 Scanning electron microscope image of *C. jejuni* showing characteristic spiral shape (typical dimensions 0.5 x 2.0 μm).

Campylobacter cells are fragile and sensitive to a wide range of stress factors including UV light, hydrogen peroxide, superoxide anions, low pH (<pH 5.0), drying (except under refrigeration), salt (>2%), and temperatures between 10 °C and 30 °C or above 55 °C.

Isolation and Identification

Traditional Methods

Standard protocols for the isolation of thermotolerant *Campylobacter* spp. from food, water, and feces by laboratory culture have been published by the International Standards Organization and the US Food and Drug Administration. These protocols are based on research results obtained over many years.

Skirrow was the first worker to demonstrate the link between contaminated food and campylobacteriosis, by plating fecal and food samples directly to a *Campylobacter*-specific agar. Plates were incubated in a microaerophilic atmosphere at 42 °C for at least 48 h. Although there have been modifications to Skirrows's agar recipe, direct plating is still widely used for the analysis of highly contaminated samples in both clinical laboratories and laboratories analyzing samples from the poultry industry.

The small size and motile nature of campylobacters are exploited in the passive filtration technique. A sterile cellulose acetate filter with either a 0.45 or 0.65 μm pore size is placed on an agar plate and several drops (approximately 100 μl) of test sample suspension carefully applied. The plate is left upright at either room temperature or 37 °C, in an ambient atmosphere, for approximately 45 min. The filter is then removed and the plates inverted and incubated in a microaerophilic atmosphere at 42 °C for 48 h. Nonselective blood agar plates are used for isolation of campylobacters sensitive to selective agents but selective agars are usually favored for fecal samples.

In contrast, isolation from food and water is more difficult because samples usually contain small numbers of cells that

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have been injured by exposure to various stresses. Analytical techniques for these types of samples include procedures that concentrate the bacteria into a suitably sized volume for inoculation and that maximize the recovery of sublethally damaged cells. The essential components of *Campylobacter* media are peptone growth substrates, oxygen quenching agents that include lysed horse blood, and antibiotics to suppress competing microorganisms. Inoculated liquid media can be incubated in air if containers are filled to the top.

Homogenates of solid foods are produced by gentle mixing to avoid excessive oxygenation, whereas liquid samples require concentration by centrifugation (milk) or filtration (water). Aliquots of homogenate, centrifuge pellet, or filters are inoculated into liquid *Campylobacter* enrichment medium that is then incubated at a reduced temperature for a time to enhance the recovery of injured cells. The most widely used resuscitation procedure is incubation for 4 h at 37 °C before enrichment for a further 44 h at 42 °C. However, extending the resuscitation period to 12–24 h at 37 °C followed by incubation for 24 h at 42 °C is used for water samples with low levels of contamination. Following incubation, enrichments are subcultured onto *Campylobacter* selective agar that has the same basic components as the liquid media, to obtain isolates that can subsequently be identified to a species/subspecies level.

The most widely used selective agars contain charcoal instead of blood. Charcoal and blood are both effective oxygen quenching agents but agar that contains charcoal has a longer shelf life. Inoculated plates are placed in a gas-tight vessel that is filled with an appropriate microaerophilic atmosphere and incubated at 42 °C for at least 24 h. *Campylobacter* colonies can vary in morphology, mostly depending on the degree of hydration of the plates. On moist plates colonies are grayish in color and spreading whereas on drier plates colonies are often small (1–2 mm diameter) and discrete.

Modern risk management protocols designed to ensure production of safe food and drinking water are based on quantitative data. Therefore, laboratories are required to enumerate thermotolerant *Campylobacter* in test samples. For fecal samples from infected individuals direct plating of known amounts of sample can be used, although the spreading nature of colonies can make counting difficult. However, the more reliable multitube most-probable-number (MPN) method is used for food and water samples

Species identification

The identities of presumptive *Campylobacter* colonies on selective agars need to be confirmed. A series of basic biochemical tests to do this includes: Gram stain, oxidase reaction, and microscopic examination of a wet mount for cell morphology and motility. The characteristic features confirmative for *Campylobacter* are Gram negative, oxidase positive, 'seagull-'shaped cells, and a rapid corkscrew motion.

Species identification is carried out on selected isolates that have been grown in pure culture. A range of biochemical tests is required to differentiate species of thermotolerant *Campylobacter* with the most basic including microaerophilic growth at 25 °C and 42 °C, hippurate hydrolysis, the presence of catalase, resistance to 30 μg ml^{-1} nalidixic acid, and resistance to 30 μg ml^{-1} cephalothin (reactions for the most common species are shown in Table 1). A large number of carefully standardized biochemical tests are required to accurately identify isolates to the species level.

Table 1 Typical biochemical reactions of three commonly isolated species of thermotolerant *Campylobacter* (+, positive; –, negative; S, sensitive; R, resistant)

Characteristics	Campylobacter jejuni	Campylobacter coli	Campylobacter lari
Growth at 25 °C	–	–	–
Growth at 42 °C	+	+	+
Nalidixic acid	S	S	R
Cephalothin	R	R	R
Catalase	+	+	+
Hippurate hydrolysis	+	–	–

Subspecies identification by serotyping

For epidemiological tracing of an infectious source, pathogens need to be identified to a level below that of species. For thermotolerant campylobacters this has traditionally been done by serotyping using heat-stable antibodies (Penner serotyping) or heat-labile antibodies (Lior serotyping). These methods have enabled tracing the source of outbreaks of infection but they are increasingly being replaced by molecular typing methods.

Molecular Methods

Specialist laboratories are adopting molecular techniques to identify the presence of specific species of *Campylobacter*, or to actually enumerate them, directly in samples. These methods are based on amplifying specific target gene sequences by the polymerase chain reaction (PCR) using primers that are specific for target species of thermotolerant *Campylobacter*. Enumeration is done either by adapting the identification to an MPN-type format or increasingly by the use of 'real-time' PCR.

Subspecies identification by pulsed-field gel electrophoresis

Molecular techniques for identification at a subspecies level allow rapid source tracing of the large numbers of isolates associated with sporadic cases of campylobacteriosis. A widely used method in clinical laboratories is pulsed-field gel electrophoresis (PFGE) in which large fragments of DNA are generated by a chosen restriction enzyme (e.g., *Sma*I) and separated by a specialized (pulsed field) electrophoretic technique. Classification is achieved by using computer programs to compare the DNA restriction fragment patterns of an isolate to those of reference markers. Some laboratories use more than one restriction enzyme for a more precise classification.

Multilocus sequence typing analysis of Campylobacter jejuni *populations*

Recently the diversity and relationships within populations of specific *Campylobacter*, most commonly *C. jejuni*, have

been defined by the technique of multilocus sequence typing (MLST). In this technique, variations and similarities within the fragments of several (typically seven) key genes required for metabolic function are identified by sequencing. The clonal complexes defined by computer analysis have been found very useful for tracing sources of infection through the food chain. MLST typing has demonstrated the close relationship between populations causing human disease and those commonly found on poultry. International sharing of information is facilitated by a publicly accessible database hosted by the Department of Zoology at Oxford University, UK. (pubmlst.org/*Campylobacter*).

Characteristics of Campylobacteriosis

Thermotolerant campylobacters are highly infectious and the infective dose for *C. jejuni* can be as low as 500 cells. Unlike with many enteric pathogens, there is usually limited spread of these *Campylobacter* within families and the main reservoirs of infection are animals. Although humans can be infected directly from animals, most disease is associated with consumption of contaminated food or water. In developed countries the routes of infection, in approximate order of frequency are undercooked chicken, red meat, raw milk, fecally contaminated water, bird-pecked bottled milk, cross-contaminated ready-to-eat foods, and puppies and kittens.

Symptoms appear between 1 and 7 days after infection. Thermotolerant *Campylobacter* infection can lead to a variety of disease scenarios. The onset is often abrupt, with cramping abdominal pains followed by diarrhea. The diarrhea may mimic that of either, or sometimes both, cholera (*Vibrio*), with copious amounts of water excreted in response to toxin production by the bacteria, or bacillary dysentery (*Shigella*) with mucus and blood present in the stool. Although nausea is a frequent symptom only a small proportion of patients actually vomit. A particular feature of *Campylobacter enteritis* is abdominal pain, which can mimic acute appendicitis. Some patients suffer a nonspecific influenza-like phase with one or more symptoms of fever, headache, and nausea. These patients may develop a more severe illness than those whose illness starts with diarrhea. Death due to *C. enteritis* itself is rare and is usually confined to elderly patients or those already suffering from another serious illness. However, a number of more serious conditions occasionally follow infection. These include the paralytic Guillian–Barré syndrome, an autoimmune disease that affects the peripheral nervous system, and reactive arthritis or Reiter's syndrome, which cause inflammation of the joints.

Patients who are not treated with antibiotics continue to excrete *Campylobacter* in their feces for several weeks after they have clinically recovered. Antibody studies have identified very high infection rates in young children in both developed and developing countries. Most adults in the Netherlands, rural and urban, tested positive for *Campylobacter* antibodies in spite of an antibody half-life of only approximately 2 years. It may be that many people are exposed to multiple infections and that some of these cause no or only very mild gastrointestinal disease. Although disease can occur in animals, in many cases infected animals that shed thermotolerant *Campylobacter* in large numbers are free from any symptoms.

Mechanism of Pathogenicity

Whether or not disease follows infection by thermotolerant *Campylobacter* depends on the susceptibility of the person and the relative virulence of the infecting strain. The rapid corkscrew motion of campylobacters facilitates penetration of the thick viscous mucus barrier of intestinal cells and this is followed by attachment to the enterocyte cells of the small intestine. Adhesion to cells involves surface components including flagella and lipopolysaccharides, outer membrane protein antigens, and carbohydrate moieties.

Once colonization has been established *Campylobacter* multiply rapidly and may produce a range of toxins, the most common of which are enterotoxins and cytotoxins. Enterotoxins cause watery, cholera-like diarrhea that is the most frequently occurring symptom of campylobacteriosis in developing countries. In developed countries the most commonly observed symptom is due to cytotoxins that cause microlesions of enterocytes leading to bloody diarrhea. During invasion, the host immune system is activated with various classes of immunoglobulins produced. One class, IgA, can cross the intestinal wall and immobilize *Campylobacter* cells resulting in short-term immunity whereas other immunoglobulins can act on bacteria entering the bloodstream, preventing bacteremia.

Recently, an alternative model of pathogenicity has been developed to account for cases with which correlation between human disease and the production of functional toxins is lacking. This 'immunopathogenic' model proposes that penetration and disruption of the intestinal mucosa by *Campylobacter* activates an immune response that can lead to a release of cytokines that are in fact responsible for the symptoms of diarrhea. If this is the case then most strains of *Campylobacter* have potential to cause disease and the outcome of infection will depend on the specific immune response of the infected individual.

Epidemiology

Thermotolerant campylobacters are constituents of the commensal intestinal microflora of birds, cattle, sheep, goats, and pigs and they seldom cause disease in these hosts. *C. jejuni* is particularly associated with birds and is also commonly isolated from the feces of farmed animals and pets.

Many developed countries have reported increasing rates of campylobacteriosis from the latter years of the twentieth century up to the early years of the twenty-first century. The reasons for this could include both improved detection and isolation techniques and increasing consumption of poultry and 'fast-foods.' Campylobacteriosis is a foodborne disease rather than food poisoning. The commonest causes of infection are considered to be insufficient cooking of meat, especially poultry, and cross-contamination from raw meats to ready-to-eat food. Although campylobacteriosis remains a major reported infectious disease recent data demonstrate an encouraging decline in rate. For example, New Zealand rates (per 10^6 population) declined from 384 cases in 2006 to 168 in 2008. This reduction has been linked to measures introduced by the poultry industry from 2007. However, ever since

Table 2 Reported occurrence of thermotolerant campylobacters on retail meats in seven countries

Country	*Food type*	*Sample number*	*% positive*	*Reference*
UK	Beef			Little *et al.*, 2008
	Muscle	1514	4.7	
	Offal	49	6	
	Lamb			
	Muscle	744	7.4	
	Offal	161	36.6	
	Pork			
	Muscle	1309	5.0	
	Offal	131	18.3	
	Poultry	758	55.5	Fricker & Park, 1989
	Chicken	198	83.3	Kramer *et al.*, 2000
Pakistan	Chicken	492	48	Hussain *et al.*, 2007
	Mutton	462	5.1	
	Beef	451	10.9	
USA	Chicken	212	70.7	Zhao *et al.*, 2001
	Turkey	194	14.5	
	Pork	209	1.7	
	Beef	210	0.5	
Poland	Chicken	203	80.3	Kwiatek *et al.*, 1990
	Duck	200	48.0	
	Goose	200	38.0	
	Turkey	236	3.0	
	Pork	105	2.9	
	Beef	114	0.9	
Ireland	Chicken	890	49.9	Whyte *et al.*, 2004
	Duck	24	45.8	
	Turkey	88	37.5	
	Lamb	262	11.8	
	Pork	197	5.1	
	Beef	221	3.2	
New Zealand	Chicken	230	89.1	Wong *et al.*, 2007
	Veal	90	10	
	Pork	230	9.1	
	Lamb and mutton	231	6.9	
	Beef	230	3.5	
Japan	Beef	51	0	Ono and Yamamoto, 1999
	Pork	55	0	
	Poultry	155	45.6	

2008 the New Zealand rate remains higher than that in many other developed countries such as Australia (108), England and Wales (92), EU (45), and USA (13).

Campylobacter is sensitive to dehydration, especially at room temperature and does not usually survive cooking or pasteurization. However, viable campylobacters were recovered from artificially contaminated beef roasts that had reached an internal temperature of 50–53 °C. Although generally considered sensitive to freezing, viable cells have been recovered from the surface of both poultry and beef after several weeks of frozen storage. Equally disturbing is the survival of *C. jejuni* on vacuum or carbon dioxide packaged beef stored at −1.5 °C for 41 days.

Unlike other foodborne pathogens such as *Salmonella*, *Campylobacter* do not have specific environmental survival mechanisms. The survival of campylobacters is related to the large numbers of cells that are released in the feces of carrier animals. A consequence of this is that most cases of campylobacteriosis are sporadic. Seasonal peaks are also common, particularly in rural areas. There is a relationship between peaks in the numbers of *Campylobacter* shed by dairy cows in spring and autumn and the seasonal peaks of human infection that occur in rural areas. Where large outbreaks of infection occur the origin has been traced to a common source such as highly contaminated water, milk, or poultry.

Thermotolerant *Campylobacter* has been recovered from retail meats in a number of countries throughout the developed world as shown by the data in Table 2. Although the reported numbers are always higher for poultry, especially chicken, direct DNA techniques have demonstrated that other meats can also be sources of infection. Risk assessment modeling suggests that control and preventative measures need to be applied at all stages from production to consumption.

Control and Preventative Measures

Control of the transmission of thermotolerant *Campylobacter* requires the implementation of food safety management

systems from production to consumption. The Codex Alimentarius Commission (i.e., the body responsible for developing international food standards and codes of practice and guidelines on behalf of the Food and Agriculture Organization of the United Nations and the World Health Organization) has endorsed the use of food safety management systems such as Hazard Analysis and Critical Control Points (HACCP) to control the spread of foodborne campylobacteriosis. Within the production system quality assurance programs include good agricultural practices (GAP) and good manufacturing practices (GMP), and involve the understanding, analysis, and control of food production practices. These management systems need to be supported by control measures in animal rearing facilities.

Control Strategies on Farms

As thermotolerant campylobacters are components of the normal commensal intestinal microflora of healthy animals they are not considered an animal health/welfare issue that impacts on production. However, at least for poultry and pigs, efforts are increasingly being made to reduce the level of *Campylobacter* on a whole flock/herd basis. Animal housing presents a significant risk factor for the introduction and spread of campylobacters and needs careful management. In addition, transport of animals and their assembly for slaughter are stressful and can increase shedding leading to environmental contamination and cross-infection.

Although animal housing is a risk factor, most poultry production is based on all-in all-out intensive production that can be rigorously controlled. In contrast, the grazing of open grassland by sheep and cattle or free-range rearing of poultry and pigs are more challenging environments for elimination of *Campylobacter* from farms.

Control in Poultry Houses

Poultry have a well-described association with campylobacteriosis and good hygiene and biosecurity measures are essential to protect poultry from acquiring *Campylobacter*. Contamination of flocks has been identified as the major source of infection in poultry. Chickens can become campylobacter-positive after transfer to growing houses, which suggests an environmental source of the organism. To control infection, thorough routine cleaning and disinfection of the houses is needed, especially between flocks. Drinking water should be of high quality; and adequate biosecurity measures such as the exclusion of wild birds, flies, rodents and pets (dogs and cats) and wearing dedicated in-house shoes and clothing is essential. Interventions that show promise for inhibiting *Campylobacter* include adding a bacteriocin (a toxin produced by another species of bacteria) to drinking water a few days before slaughter, oral administration of bacteriophage (specific for *C. jejuni*) or feeding the supplement caprylic acid.

Control in Meat Processing Plants

Campylobacter that enter processing plants on the skin, hide, or feathers or in feces of carrier animals have the potential to be spread within the plant. For poultry, in particular, prevention of gross carcass contamination by feces is a high priority because processing techniques include scalding, plucking, and eviscerating and can include cooling carcasses in tanks, all of which will inevitably lead to cross-contamination of carcasses. Cross-contamination is less likely during processing of carcasses that are dressed on an individual basis such as cattle, sheep, and pigs, although pig carcasses can be cross-contaminated during mechanical dehairing. For all processing, good butchering skills together with good personnel hygiene and sanitation of equipment and moving surfaces are essential to reduce the likelihood of contamination to a minimum. Although good hygiene is important it has been shown that some strains of *Campylobacter* can survive routine cleaning and decontamination procedures. It has also been shown that *Campylobacter* can establish in biofilms in, for example, water distribution systems, and potentially survive disinfection. The USA Department of Agriculture has approved chemical disinfection of carcasses by application of various antimicrobial solutions. However, chemical disinfection is not permitted in Europe (under European Union rules). Pasteurization of beef carcasses with hot water or steam, and spraying carcasses with 5% lactic acid are now common and effective decontaminating treatments used in North American beef packing plants. However, there is little specific information on the effects of these treatments on campylobacters.

Freezing is a control technique used in some countries. A reduction in campylobacteriosis in Iceland has been associated with freezing carcasses and a number of Scandinavian countries now require carcasses from flocks that test positive for *Campylobacter* to be frozen for several weeks. Crust freezing, in which carcasses are exposed to a stream of air at −30 °C, shows promise in pathogen reduction although the costs are even higher than for carcass freezing.

Control during Food Distribution

It is not possible to guarantee that raw meats will be pathogen free when they leave a processing plant, so distribution chains need be designed and operated to prevent cross-contamination or multiplication of bacteria. Cross-contamination can occur as a result of pack leakage. To minimize this, raw animal products should to be packaged in approved packaging films. The use of 'leak-free' packaging for raw chicken is considered to have made a major contribution to the recent reduction in campylobacteriosis in New Zealand. To avoid cross-contamination, ready-to-eat foods should be transported and stored in separate containers or compartments to those used for raw foods. Good temperature control is required throughout the distribution chain to maintain an acceptable microbiological condition for meat that reaches retail outlets.

Butcher shops and retail outlets

Most countries have laws aimed at ensuring that food offered to consumers is safe to eat. Usually, such legislation requires that food business employees are suitably trained and that food safety management systems, based on scientific principles, such as the HACCP system are implemented. *Campylobacter* may be present on a variety of foods, especially

raw poultry and other raw meats. Given its low infectious dose, care in handling such foods is needed to minimize the risk to consumers. However, growth of *Campylobacter* would not be expected. Handling practices must prevent both recontamination and cross-contamination. Essential components of the HACCP system include suitable premises and equipment, high-quality potable water, very high standards of employee hygiene, and constant attention to hygienic food handling practices.

Restaurants and fast food outlets

Where cooked foods are offered for sale the same type of HACCP system as is employed in butcheries is required. In restaurants, a variety of different types of foods are brought together with a consequent high potential for cross-contamination. To address this, cooking procedures should be sufficient to destroy any *Campylobacter* present. After cooking, subsequent handling practices must ensure that there is no possibility of recontamination.

Control in the Home

Preparation of food in the home kitchen has been identified as an important factor affecting the prevalence of foodborne disease. Householders need to handle meats with the same attention to hygiene and cooking practices as are required by restaurants. Meats need to be kept under refrigeration and stored and prepared in such as way as to avoid cross-contamination. Cooking procedures need to be sufficient to destroy any residual bacteria, especially when prepared foods are reheated. During meal preparation care is needed to avoid contamination, by ensuring that hands are thoroughly washed after handling raw meats and by keeping knives, chopping boards, cooking and serving utensils clean. Ideally, different sets of equipment should be used for raw and ready-to-eat products. To protect people from avoidable diseases such as campylobacteriosis and to constantly reinforce the need for care, public information to promote effective hygiene in the kitchen needs to be easily accessible and readily understood.

See also: Foodborne Zoonoses. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat. Microbiological Analysis: DNA Methods; Standard Methods. Modeling in Meat Science: Microbiology. Risk Analysis and Quantitative Risk Management

Further Reading

Ayling, R.D., Johnson, L.E., Evans, S, Newell, D., 1996. PCR/RLFP and PFGE subtyping of thermotolerant *Campylobacter* isolates from poultry epidemiological investigations. In: Newell, D., Ketley, J.M, Feldman, R.A. (Eds.), Campylobacters, Helicobacters and related organisms. New York: Plenum Press.

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P., Blaser, M.J., 1988. Experimental *Campylobacter jejuni* infection in humans. Journal of Infectious Diseases 157, 472–478.

Corry, J.E., Post, D.E., Colin, P., Laisney, M.J., 1995. Culture media for the isolation of campylobacters. Intrnational Journal of Food Microbiology 26, 43–76.

Cox, J.M., Pavic, A., 2010. Advances in enteropathogen control in poultry production. Journal of Applied Microbiology 108, 745–755.

Fricker, C.R., Park, R.W., 1989. A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. Journal of Applied Bacteriology 66, 477–490.

Hussain, I., Mahmood, M.S., Akhtar, M., Khan, A., 2007. Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. Food Microbiology 24, 219–222.

Jacobs-Reitsma, W., Lyhs, U., Wagenaar, J., 2008. *Campylobacter* in the food supply. In: Nachamkin, I., Szymanski, C.M., Blaser, M.J. (Eds.), *Campylobacter*, third ed. Washington, DC: ASM Press, pp. 627–644.

Kramer, J.M., Frost, J.A., Wareing, D.R.A., Bolton, F.J., 2000. *Campylobacter* contamination of raw meat and poultry at retail sale, identification of multiple types and comparison with isolates from human infection. Journal of Food Protection 63, 1654–1659.

Kwiatek, K., Wojton, B., Stern, N., 1990. Prevalence and distribution of *Campylobacter* spp. on poultry and selected red meat carcasses in Poland. Journal of Food Protection 53, 127–130.

Little, C.L., Richardson, J.F., Owen, R.J., de Pinna, E., Threfall, E.J., 2008. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antibiotic resistance pattern, 2003–2005. Food Microbiology 25 (3), 538–543.

Maiden, M.C.J., Bygraves, J.A., Feil, E., 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences USA 95, 3140–3145.

Maiden, M.C.J., Dingle, K.E., 2008. Population biology of *Campylobacter j ejuni* and related organisms. In: Nachamkin, I., Szymanski, C.M., Blaser, M.J. (Eds.), Campylobacter, third ed. Washington, DC: ASM Press, pp. 27–40.

Nachamkin, I., Blaser, M.J., 2000. *Campylobacter*, second ed. Washington, DC: ASM Press

Ono, K., Yamamoto, K., 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. International Journal of Food Microbiology 47, 211–219.

Park, P., 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. International Journal of Food Microbiology 74, 177–188.

Rowe, M.T., Madden, R.H., 2000. *Campylobacter*. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.), Encyclopedia of Food Microbiology. San Diego, CA: Academic Press.

Sanad, Y.M., Kassem, I.I., Abley, M., *et al.*, 2011. Genotypic and phenotypic properties of cattle-associated *Campylobacter* and their implications for public health in the USA. Plos One 6 (10), e25888. Available from www.plosone.org.

Skirrow, M.B., 1977. *Campylobacter enteritis*: A "new" disease. British Medical Journal 2, 9–11.

Wassenaar, T.M., 2011. Following an imaginary *Campylobacter* population from farm to fork and beyond: a bacterial perspective. Letters in Applied Microbiology 53, 253–263.

Whyte, P., McGill, K., Cowley, D., *et al.*, 2004. Occurrence of *Campylobacter* in retail foods in Ireland. International Journal of Food Microbiology 95, 111–118.

Wong, T.K., Hollis, L., Cornelius, A., *et al.*, 2007. Prevalence, numbers and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. Journal of Food Protection 70 (3), 566–573.

Zhao, C., Ge, B., De Villena, J., *et al.*, 2001. Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the greater Washington D.C. area. Applied and Environmental Microbiology 67 (2), 5431–5436.

Relevant Websites

http://www.cdc.gov/mmwr/....
Centre for Communicable Diseases USA: Statistics for morbidity and mortality.

http://www.efsa.europa/eu/en/efsajournal/
European Food Safety Authority: Analysis of trends for zoonotic agents in Europe.

http://www.hpa.org.uk/....
Health Protection Agency England & Wales: *Campylobacter* epidemiology.
http://www.foodsafety.govt.nz/elibrary/industry/general/...
New Zealand Food Safety Authority: *Campylobacter* RM strategy: 2010–2103.
http://pubmlst.org/campylobacter
Oxford University: MLST database.

Viruses

TH Jones, Agriculture and Agri-Food Canada, Lacombe, AB, Canada

Glossary

Gnotobiotic Animals that are born under aseptic conditions and raised in a sterile environment to permit investigations of the effects of exposing animals to microorganisms under controlled conditions.
Seroprevalence The frequency of individuals in a population that has antibodies to a specific pathogenic microorganism in their blood serum.
Vertical transmission Infectious diseases that are naturally transmitted from mother to infant during the period immediately before and after birth. Transmission routes include crossing the placenta, breast milk, or through direct contact during or after birth.
Viremia The presence of viruses in the bloodstream.
Zoonotic transmission Infectious diseases that are naturally transmitted between vertebrate animals and humans. Transmission occurs at the human–animal interface through direct or indirect human exposure to animals, their products and/or their environments.

Introduction

Until recently, the contribution of viruses to foodborne disease associated with the consumption of meat has largely been overlooked and underappreciated. However, enteric viruses are responsible for the majority of foodborne disease outbreaks worldwide and are often suspected when the causative agent is unknown or cannot be identified. Enteric viruses have a very high acid resistance, which allows them to survive the low pH of stomach acid. Unlike bacteria, viruses require a living host cell for multiplication and therefore viruses are unable to multiply in food. The infectious dose of enteric viruses, however, can be extremely low. Thus 10 or less infectious particles may be sufficient to cause disease. Enteric viruses are excreted in high numbers ($>10^{10}$ infectious particles per g of feces) by infected humans and animals. Furthermore, enteric viruses are extremely stable at low and freezing temperatures and are generally more resistant to environmental stresses than bacteria. As a result, current strategies to reduce bacterial pathogens in food may not be fully effective against viruses. Although the inability of viruses to multiply in foods is favorable from a food safety perspective, the low number of virus particles present in foods presents a challenge for their detection. Most enteric viruses cannot be cultivated and their detection is therefore restricted to molecular techniques, which have their own limitations as both infectious and noninfectious particles will be amplified if primer sets are sufficiently robust to detect and amplify the targeted genetic sequence.

The number of documented foodborne virus outbreaks is increasing due to advances in molecular detection and characterization methods and sample processing methods, as well as a rise in the consumption of raw and minimally processed foods, the globalization of trade between countries with widely different standards of hygiene and sanitation, and increasing vulnerable populations of elderly and immunocompromised individuals in some countries. The majority of foodborne illnesses are caused by viruses of human origin such as norovirus (NoV), hepatitis A virus (HAV), rotavirus (RV), and astrovirus, and are associated with the consumption of ready-to-eat foods, vegetables, fruits, and shellfish that are contaminated by infected food handlers, contaminated water, or by cross contamination of food. However, there are increasing concerns over the zoonotic transmission of NoV and RV through undercooked meat products due to an increasing evidence of the existence of animal strains of NoV and RV that are closely related to human strains; whereas the zoonotic transmission of hepatitis E virus has been demonstrated.

Hepatitis E Virus

Virus Characteristics

Hepatitis E virus (HEV) is the only member of the genus *Hepevirus* of the newly proposed *Hepeviridae* family. HEV is a small, nonenveloped virus with a diameter of 27–34 nm and positive sense 7.2-kb ribonucleic acid (RNA) genome. The genome contains a short 5′noncoding region, three open reading frames (ORF1, ORF2, and ORF3), a 3′ noncoding region, and a poly(A) tail. ORF1 encodes a polyprotein that is presumed to cleave into nonstructural proteins involved in viral replication that include a methyltransferase, RNA-dependent RNA polymerase, and helicase. ORF2 encodes the major capsid protein that is the target for vaccine development, whereas ORF3 encodes a small phosphoprotein that is thought to be a viral regulatory protein involved in intracellular signal transduction and the assembly of new virus particles.

HEV is classified into five genotypes: genotype 1 contains human epidemic strains circulating in Asia and Africa; genotype 2 contains human strains circulating in Mexico and Africa; genotype 3 strains are distributed worldwide and have been isolated from humans in sporadic cases of acute hepatitis E as well as from pigs in North America, Europe, and Japan; genotype 4 contains strains circulating in humans and pigs in Asia; and genotype 5 contains strains of avian HEV, isolated from chickens in North America, which are genetically very different from mammalian HEV. Although extensive sequence variation exists among the five genotypes, all belong to a single

serotype. Antibodies against HEV have been detected in pigs, cattle, chickens, sheep, goats, deer, wild boar, mongooses, dogs, cats, primates, and rats. Recent progress in the cultivation of HEV in a cell culture system may advance the understanding of HEV pathogenesis and permit research on virus stability and inactivation.

Disease

The incubation period of HEV is relatively long, 2–10 weeks, which is problematic when attempting to identify the source of infection. The disease is usually self-limiting, lasting for 1–4 weeks, and generally does not result in chronic hepatitis. Typical symptoms include jaundice, hepatomegaly (i.e., swollen liver), malaise, abdominal pain, anorexia, vomiting, fever, viremia, and dark urine. The primary site of HEV replication has not been identified but the intestinal tract is a likely candidate. After HEV finds its way to and infects the liver, HEV enters the bloodstream, accumulates to high concentrations in bile, and is subsequently excreted in feces. The immune response, initiated after 2–3 weeks of infection, is primarily responsible for the liver damage. The mortality rate in the general population is between 0.5% and 4% but increases dramatically to as high as 42% for pregnant women. Women in the third trimester of pregnancy are particularly at risk, possibly due to changes in hormone levels. Mortality may be up to 75% for patients with underlying chronic liver disease. Person-to-person transmission of HEV among family members of patients that have contracted HEV is rare, with rates estimated to be approximately 0.7–8.0%. Vertical transmission of HEV from a pregnant mother to her infant has been documented.

A treatment for HEV does not exist but the first vaccine against HEV, HEV 239, was approved by China in 2012 and may soon be commercially available for protection against HEV. The persistence of anti-HEV IgG antibodies is thought to provide long-term immunity to HEV. HEV is identified and diagnosed by the detection of IgM and IgG antibodies against recombinant HEV antigens or by reverse transcriptase (RT)-polymerase chain reaction (PCR) and nucleic acid sequence analysis for detection and genotyping HEV RNA in serum or feces.

Zoonotic Transmission

HEV genotypes 1, 2, and 4 are associated with epidemic and sporadic cases of acute viral hepatitis in humans in the regions of Asia, Africa, and Central America where the virus is endemic. Most of these outbreaks are associated with fecal contamination of drinking water. According to World Health Organization estimations there are approximatelyt 14 million symptomatic cases, more than 300 000 deaths and 5200 stillbirths annually associated with HEV. As sanitation and access to good quality water improve and vaccines become commercially available, such outbreaks will likely be reduced dramatically.

HEV genotype 3 is associated with sporadic cases of locally acquired HEV in industrialized countries where epidemics are not reported. The number of sporadic cases appears to be increasing in Europe, due to either an increase in surveillance or real increases in the disease. Although some cases of HEV are travel related the proportion of nontravel-related cases is increasing, especially in older men, and may be traced to zoonotic origins. Immunocompromised individuals, such as transplant patients, appear to be particularly vulnerable to chronic infection with HEV genotype 3. HEV is endemic in Japan, Australia, USA, and Europe, and seroprevalence as high as 36% has been reported. HEV is now widely accepted as a zoonotic disease that is carried by domestic pigs, wild boars, and deer. Infection in pigs generally occurs between the age of 2 and 3 months and HEV is shed in the feces for approximately 3–7 weeks. The majority of pigs over 6 months of age do not shed HEV in their feces but the seroprevalence of HEV in commercial pigs can be as high as 80%.

Swine veterinarians and persons handling swine have a higher frequency of IgG antibodies to HEV than the general population. Similar strains of HEV appear to circulate within the animal and human population. Swine HEV readily cross-reacts with an antibody to the capsid of human HEV. Furthermore, the genetic sequences of swine HEV closely resemble those of human strains, human HEV can infect swine, and swine HEV can infect nonhuman primates.

The consumption of undercooked or raw meat from animals that are reservoirs for HEV is a major risk factor. In Japan, four cases of HEV were directly linked to the consumption of raw deer meat, with the identical strain of HEV being isolated from the infected patients and the meat. Furthermore, several cases of HEV have been linked epidemiologically to eating undercooked pork liver or wild boar meat. Figatellu, a pig liver sausage that is traditionally consumed raw in France, has also been implicated as a source of HEV infection. In surveillance studies, HEV RNA was detected in 2%, 7%, and 11% of pig livers sold at the retail level in Japan, the Netherlands, and the US, respectively. Furthermore, infectious HEV has been recovered from liver in the US. The infectious dose required to cause disease is not known but it is thought to be high as the seroprevalence in human and swine populations is quite high, whereas the number of reported cases of HEV is very low.

Survival of Hepatitis E Virus in Food

There is little information on the stability of HEV in food. Heating a fecal suspension of HEV diluted to a final concentration of $<5\%$ in phosphate buffer solution (PBS) for 60 min at 56 or 60 °C was not sufficient to inactivate all the virus particles. HAV is more heat stable than HEV; after 1 h at 66 °C infectious HAV particles were not detected, suggesting that HEV would be inactivated under those conditions. The inactivation of HEV in PBS is likely greater than inactivation in meat tissue held at the same temperature for the same time as viruses will be protected by proteins in the liver and muscle matrix. The United States Department of Agriculture recently reduced the recommended temperature to be achieved during cooking of pork from 71 to 63 °C. This is of concern with respect to inactivation of HEV as the virus may be exposed to such temperatures for only a very short period of time. HEV was

unable to infect susceptible pigs when naturally contaminated liver was cooked to an internal temperature of 71 °C by stir-frying for 5 min or when liver was boiled in water for 5 min. However, when the infected liver was incubated at 56 °C for 1 h, a temperature representative of the minimum temperature attained by medium-rare meat, 80% of pigs became infected. These limited data suggest that pork must be thoroughly cooked to control the risk of foodborne transmission of HEV, particularly to the most vulnerable and susceptible segments of the population.

Caliciviruses

Virus Characteristics

Caliciviruses are nonenveloped viruses with a diameter of 27–40 nm and positive sense RNA genome ranging in size from 6.4 to 8.5 kb. The family *Caliciviridae* contains five recognized genera to date. These are the established genera *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*; and a new genus *Nebovirus* that contains the bovine strains Newbury agent 1 and Nebraska and a number of unclassified caliciviruses, including rhesus enteric calicivirus (Tulane virus), St. Valérien-like viruses in swine, and chicken caliciviruses. Caliciviruses cause a variety of diseases and have a broad host range. NoVs and sapoviruses (SaVs) are linked to the majority of outbreaks of food or waterborne, acute, nonbacterial gastroenteritis in humans worldwide and the close genetic relationship between human and animal strains has raised concerns about their zoonotic potential (Figure 1).

On the basis of the complete sequence of the capsid gene, NoVs are classified into five genogroups (GI–GV). Human NoV strains are located in GI, GII, and GIV, whereas porcine NoV strains are also found in GII. NoVs in GIII infect cattle and sheep, whereas GV infect mice. The genogroups are subdivided further with 8 genotypes for GI, 19 genotypes for GII, 3 genotypes for GIII, and 1 each for GIV and GV to date. The porcine GII NoVs belong to 3 genotypes that are distinct from human GII genotypes. SaVs too are classified into 5 genogroups. Human SaV strains are located in GI, GII, GIV, and GV, whereas porcine SaV strains are located in GIII. It is expected that a number of porcine strains that do not fit in GIII will be classified into new genogroups GVI and GVII as more genotypes are discovered.

The genome organization of NoV differs from SaV; the genome of NoVs contains three ORFs, whereas the genome of SaVs contains two ORFs. For NoVs, ORF1 encodes six nonstructural proteins required for viral replication, ORF2 encodes the major capsid protein VP1, and ORF 3 encodes a minor capsid protein VP2. VP1 folds into a shell domain and a protruding (P) domain, which is further subdivided into a P1 and P2 domain. The P2 domain is the hypervariable region of the capsid and contains both the immune and cellular receptor sites. For SaVs, ORF1 encodes the nonstructural proteins and VP1, whereas ORF2 encodes the minor capsid protein VP2. The RNA genome is replicated by the RNA-dependent RNA polymerase, which has no proofreading activity and causes a high error rate in transcription. This makes the caliciviruses highly flexible and diverse.

Disease

NoV is the leading cause of acute gastroenteritis from contaminated food or water. Approximately 25% of illnesses of human NoV in the US are attributed to food; however, the majority of outbreaks are associated with person-to-person transmission, particularly in populations with close contact, such as those of nursing homes, educational institutions, day cares, cruise ships; and in populations after natural disasters when personal hygiene and sanitation may be compromised. Outbreaks occur most frequently during the winter months. NoV is transmitted not only via the fecal–oral route but also via aerosols generated from vomit and contaminated surfaces. The number of outbreaks globally has increased dramatically since 2002, possibly due to changes in the predominant circulating strain. NoVs shed in the stool at levels $>10^8$ genome copies per g, and may persist for 3–4 weeks after clinical symptoms have disappeared from a resident population. NoV is extremely persistent in the environment and resistant to disinfectants; and the infectious dose is extremely low. The incubation time is short, from 12 to 72 h with a median of 33–36 h, and the symptoms typically last for 1–3 days. Symptoms include projectile vomiting, watery nonbloody diarrhea, abdominal cramps, and nausea. Low grade fever, headache, and muscle aches may also occur. Although the disease is described as self-limiting in the healthy population, the young, elderly, and immunocompromised individuals are particularly vulnerable to complications such as dehydration and possibly death. Owing to the short incubation time and rapid spread of secondary infections, it may be difficult to trace the outbreak back to its original source. Prior infection with NoV does not provide long-term immunity and the high antigenic variability of NoV makes it difficult to develop an effective vaccine. The most effective control is through proper handwashing and isolation of infected individuals. Most outbreaks in humans are caused by NoV GII genotype 4 (GII.4) and it appears that new GII.4 subtypes replace prevalent subtypes every 2–3 years. Sporadic cases of NoV are most often associated with diverse strains belonging to GII, with a smaller contribution from strains belonging to GI.

Human SaV infections, which mainly affect children under 5 years of age, do not occur as frequently as NoV infections and do not follow a seasonal pattern. The symptoms include diarrhea, fever, and vomiting, but projectile vomiting does not occur. SaV are excreted for approximately 2 weeks. NoV and SaV infections are thought to involve the small intestine, but little is known about the mechanisms of the disease as a cell culture system does not exist for these caliciviruses. NoV and SaV are detected by RT-PCR. Real time RT-PCR is increasingly being used for the detection of NoV as it is faster and more sensitive than conventional RT-PCR. Multiple primer and probe sets are required for NoV and SaV detection as, due to their genetic diversity, it is currently not possible to detect all known NoVs or SaVs with a single assay.

Zoonotic Transmission

The status of NoV and SaV as zoonotic agents is uncertain. Porcine NoV GII are closely related to human NoV GII. Porcine NoVs have been detected in Asia, Europe, and North

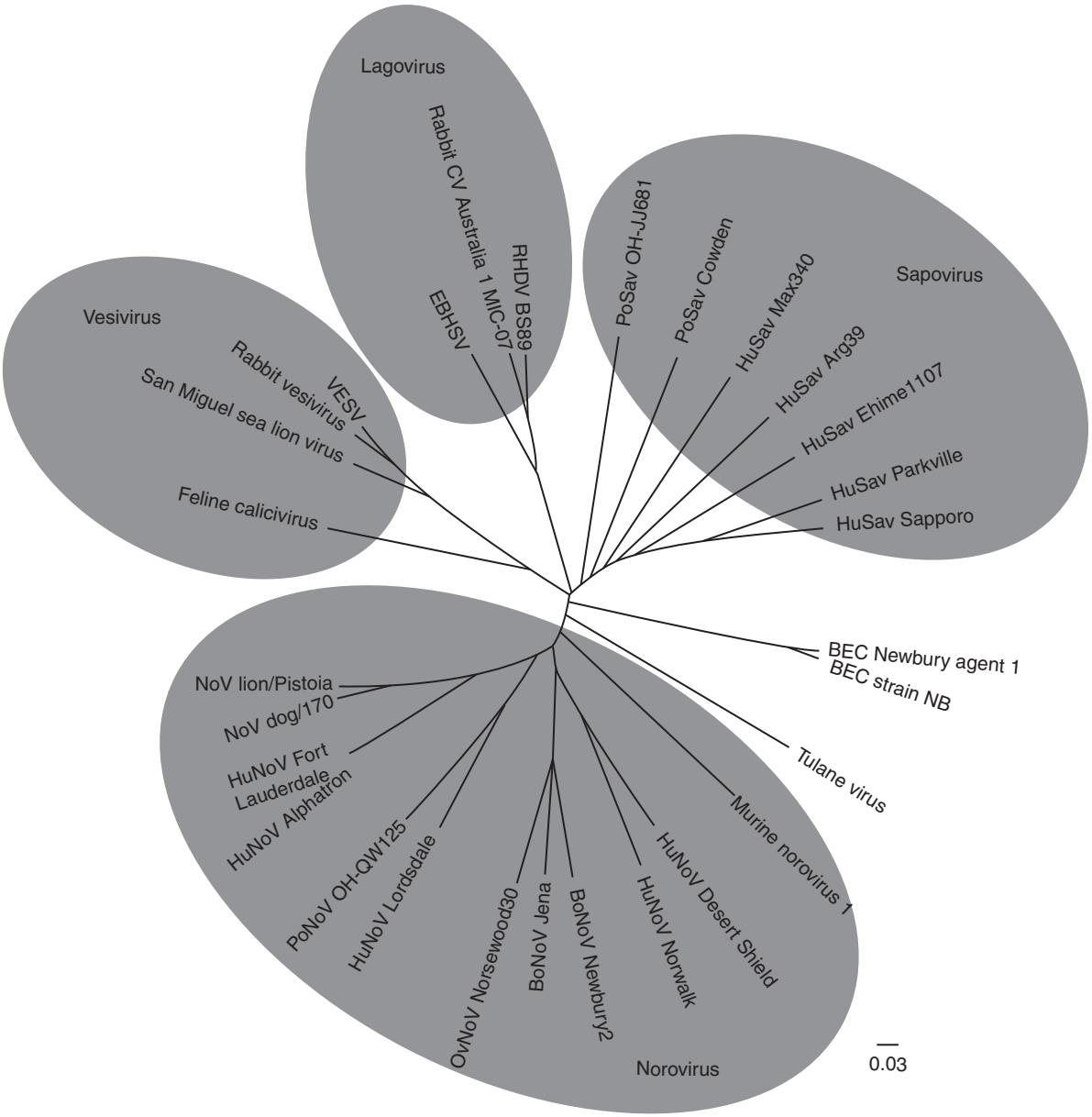

Figure 1 Classification of caliciviruses based on the nucleotide sequences of the complete capsid protein. Reproduced with permission from Greening, G.E., Wolf, S., 2010. Calicivirus environmental contamination. In: Hansman, G.S., Jiang, X.J., Green, K.Y. (Eds.), Caliciviruses: Molecular and Cellular Virology. Norfolk, UK: Caister Academic Press, pp. 25–44.

America and cause mild or asymptomatic infections in pigs. When gnotobiotic pigs were infected with human NoV GII.4, 74% of pigs developed mild diarrhea and 59% became seropositive. Furthermore, antibodies against human NoV GI and GII have been detected in swine. These findings suggest that swine could be a reservoir for human NoV. In addition, NoV GII.4-like RNA was detected in swine and bovine manure as well as in raw pork at the retail level. It is unknown if the source of the GII.4-like strain on the retail meat was of animal or food handler origin. Porcine SaV have been detected worldwide and cause mild or asymptomatic infections in young and adult pigs. The concerns about the zoonotic potential of SaV are lower than for NoV as the limited number of porcine SaV that have been identified to date are genetically different from human SaV.

Bovine GIII NoV infections can cause diarrhea or are asymptomatic in calves and cattle. A closely related NoV GIII was recently detected in fecal material from asymptomatic sheep. Although animal NoVs have not been detected in human stool samples, veterinarians have a higher frequency of IgG antibodies to NoV than the general population, which suggest that bovine NoV may infect humans. The ability of human NoV to infect cattle has not been demonstrated.

Concerns about the zoonotic potential of animal caliciviruses revolve around coinfection and interspecies

transmission. If either animals or humans are coinfected with both human and animal strains, then a potential recombinant animal/human NoV could emerge with an increased host range or increased virulence. Recombination occurs regularly in NoV and SaV, mainly within genogroups but intergenogroup recombinations have been reported. Furthermore, if animal caliciviruses adapt sufficiently to acquire the ability to infect humans, there will be major health implications. As NoV is found in the intestinal tract, NoV could be a likely contaminant on the surfaces of carcasses and meat as a result of fecal contamination during carcass processing. Considering that NoV GII.4-like RNA has been detected in swine, cattle, and retail meat, more surveillance and research is clearly required to address the concerns about the zoonotic potential of animal caliciviruses and to develop effective control or preventative measures.

Survival of Caliciviruses in Food

Human NoV cannot be grown in cell culture, therefore survival studies are typically carried out with cultivable surrogates. The results of such studies need to be interpreted with caution as murine NoV and feline calicivirus do not appear to be good models for human NoV in heat inactivation studies. Data suggest that time/temperature conditions recommended for pasteurization, such as 72 °C/15 s for milk or 70 °C/2 min for other foods are insufficient to inactivate GII.4 NoVs and consequently the risk of transmission of NoVs when such recommendations are used may be underestimated. On the basis of temperature inactivation profiles obtained with surrogate viruses, it was suggested that pasteurizing treatments of 63 °C/30 min or 70 °C/2 min were more effective than the high-temperature short-time pasteurization treatment of 72 °C/15 s. Murine NoV is more heat labile than human NoV but more heat resistant than *Escherichia coli*. Murine NoV and *E. coli* were reduced by 1.9 and 3.9 log units, respectively, after 30 s at 65 °C in raspberry puree. As caliciviruses are potentially present on meat surfaces, cooking recommendations adequate for inactivating caliciviruses should be identified and the potential risk of foodborne transmission of human caliciviruses by ground meat products should be assessed.

Rotavirus

Virus Characteristics

RVs belong to the genus *Rotavirus* and are a member of the *Reoviridae* family. RVs are nonenveloped viruses with a diameter of 60–80 nm. They contain a 16–27 kb double-stranded RNA (dsRNA) genome that comprises 11 segments surrounded by three protein layers. Each segment of RNA encodes a single structural or nonstructural protein, whereas two segments encode for two proteins. RVs are classified into seven serogroups (A–G) based on the viral capsid protein VP6 located in the middle protein layer. In addition, RVs are subdivided into 23 glycosylase or G-genotypes and a minimum of 32 protease sensitive or P-genotypes, which are based on the outer capsid proteins VP7 and VP4, respectively. VP7 and VP4 are encoded by separate gene segments. Serogroups A–C cause diarrhea in humans, whereas all serogroups (A–G) cause diarrhea in animal species. Group A RV (GARV) is associated with a wide range of mammals and birds; group B RV(GBRV) is primarily associated with pigs, cattle, sheep, and rats; group C RV (GCRV) with pigs, cattle, and dogs; groups D, F, and G mainly infect poultry, whereas group E has been associated with swine.

Disease

GARV has been linked to 90% of RV illness, which is an acute gastroenteritis occurring mostly in young children less than 5 years old. Clinical symptoms typically appear 1–2 days after infection and include vomiting, abdominal pain, and watery diarrhea caused by RV enterotoxin that put infants and children at risk of dehydration, and viremia that last for 3–8 days. RV infections are generally self-limiting but estimates suggest that GARV are responsible for more than 500 000 deaths annually in developing countries. The strain prevalence usually changes on a yearly basis. Strains that are prevalent in 1 year may be undetected in the subsequent year. Vaccines have recently become commercially available and appear to be highly effective in controlling RV disease. GBRV infects mainly adults and GCRV infects people of all ages. Approximately 100% of children are seropositive for GAVR by the age of 5 years, whereas approximately 60% of the population is seropositive for GCRV by the age of 60 years. GCRV is considered an emerging pathogen. RVs are typically shed in the feces for 5–7 days but shedding may last for several months in immunocompromised patients. The infectious dose is low, with 10–100 particles being sufficient to cause disease. Estimates suggest that 1% of RV cases are foodborne, whereas the majority are spread by person-to-person contact.

Zoonotic Transmission

GARV has been recognized as the major cause of diarrhea in calves and piglets and results in large economic losses for producers arising from treatment, reduced weight gain, and the death of animals. Animal strains are typically distinct from human strains but there is increasing evidence that GARV is being transmitted from animals to humans. Non-GARV infections are more predominant in animals than in humans. GCRV infections are widespread in swine and also occur in cattle. There are concerns about the zoonotic potential of GCRV due to an increase in seroprevalence of antibodies to GCRV in rural human populations. Unusual animal-like GCRVs are being detected in humans but direct transmission from animal to humans has not been reported. RVs are genetically diverse and are continually evolving into new genotypes, serotypes, and viral species as a result of point mutations, genetic reassortments, genomic rearrangements, or intragenic recombination. When animals or humans are coinfected with animal and human strains, RVs could potentially reassort into a novel strain during replication if one strand of the dsRNA genome was acquired from human RV and the other from animal RV.

Survival of Rotavirus in Food

RV is very stable in the environment and can survive for months at 4 °C and at freezing temperatures. Although limited data are available, RV is apparently more heat labile than other enteric viruses. A suspension of RV was rapidly inactivated at 60 °C or higher and *D* values of 8 and 5.8 min were reported for heating at 50 and 55 °C, respectively. As RV is associated with viremia as well as with the intestinal tract, meat must be thoroughly cooked to control the risk of foodborne transmission.

See also: Foodborne Zoonoses. Microbial Contamination: Decontamination of Fresh Meat; Microbial Contamination of Fresh Meat. Microbiological Safety of Meat: Emerging Pathogens

Further Reading

Aggarwal, R., Naik, S., 2008. Enterically transmitted hepatitis. In: Koopmans, P.G., Cliver, D.O., Bosch, A. (Eds.), Foodborne Viruses: Progress and Challenges. Washington, DC: ASM Press, pp. 65–85.

Bank-Wolf, B.R., König, M., Thiel, H.-J., 2010. Zoonotic aspects of infections with noroviruses and sapoviruses. Veterinary Microbiology 140, 204–212.

Colson, P., Borentain, P., Queyriaux, B., *et al.*, 2010. Pig liver sausage transmission as a source of hepatitis E virus transmission to humans. Journal of Infectious Diseases 202, 825–834.

Goyal, S.M. (Ed.), 2006. Viruses in Foods. New York, NY: Springer.

Martella, V., Banyai, K., Matthijnssens, J., Buonavoglia, C., Ciarlet, M., 2010. Zoonotic aspects of rotavirus. Veterinary Microbiology 140, 246–255.

Mattison, K., Shukla, A., Cook, A., *et al.*, 2007. Human noroviruses in swine and cattle. Emerging Infectious Diseases 13, 1184–1188.

Meng, X.J., 2010. Hepatitis E virus: Animal reservoirs and zoonotic risk. Veterinary Microbiology 140, 256–265.

Saif, L.J., Jiang, B., 1994. Nongroup A rotaviruses of humans and animals. Current Topics in Microbiology Immunology 185, 339–371.

Scipioni, A., Mauro, A., Vinjé, J., Thiry, E., 2008. Animal noroviruses. The Veterinary Journal 178, 32–45.

Topping, J.R., Schnerr, H., Haines, J., *et al.*, 2009. Temperature inactivation of feline calicivirus vaccine strain FCV F-9 in comparison with human noroviruses using an RNA exposure assay and reverse transcribed quantitative real-time polymerase chain reaction-A novel method for predicting virus infectivity. Journal of Virological Methods 156, 89–95.

Wilhelm, B.J., Rajić, A., Greig, J., *et al.*, 2011. A systematic review/meta-analysis of primary research investigating swine, pork, or pork products as a source of zoonotic hepatitis E virus. Epidemiology and Infection 139, 1127–1144.

Relevant Websites

http://www.cdc.gov/norovirus/
Centers for Disease Control and Prevention, USA.

http://www.who.int/mediacentre/factsheets/fs280/en/
World Health Organisation, Hepatitis E Factsheet.

http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index1.html
World Health Organisation, Initiative for Vaccine Research, Diarrohoel Diseases.

Yeasts and Molds

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Introduction

Yeasts and molds are important microorganisms related to human welfare and food resources. They contribute greatly to the food industry in areas such as wine making, single cell protein production, brewing, baking, vitamin production, etc. However, under special conditions they can act as potential spoilage organisms in food, especially in processed, preserved, and refrigerated food. The enumeration and identification of yeasts and molds from foods are of great importance in understanding the true value of these organisms in various food systems. Although yeasts and molds are normally not a major concern to most producers and processors of meats, under conditions such as drying, curing, freezing, and the use of preservative agents, yeasts and molds can compete effectively with bacteria and become the dominant microflora. A knowledge of how certain yeasts and molds colonize meat products and of the their effects on the meat products is essential in order to prevent economic losses during spoilage or to maximize desirable fermentation of certain cured meat products by yeasts and molds. Occasionally, some pathogenic yeasts and molds may occur in meat and meat products and can pose food safety issues in these products.

Yeasts and molds are ubiquitous microbes in our environment and play an important role in the entire cycle of processes that influence the balance of energy of all living and nonliving matter. Along with other microbes, they can be very beneficial to humans through their roles in the various geochemical cycles such as the phosphorus cycle, the carbon and oxygen cycles, the nitrogen cycle, and the sulfur cycle. Without their influence, the earth would not be habitable by humans. They are also important in various fermented foods such as wine, cheese, beer, vinegar, bread and soybean products, and in the production of industrially important acids, solvents, antibiotics, steroids, and enzymes. They can even be eaten as foods such as mushrooms, yeasts, and single-cell protein. On the other hand, they can spoil our food supplies and cause devastating diseases in animals and humans that, if unchecked, could actually destroy the human race.

Yeasts and molds belong to the eukaryotes since they have defined nuclear membranes surrounding the nucleic acids, as opposed to bacteria, which belong to the prokaryotes and do not have defined nuclear membranes. In the ranking of biological systems, yeasts and molds belong to the Division Eumycetes in the Phylum Thallophyta. Collectively, yeasts, molds, and mushrooms are termed fungi.

Yeasts are single-celled fungi. The cells are usually oval in shape and divide by budding in the asexual cycles or by formation of ascospores in the sexual cycles. Molds are multicellular, complex organisms that produce sexual and asexual spores. They grow by germination of sexual or asexual spores and elongation of the thallus (a complete cell) into hyphae, which may be septated or nonseptated. The intertwined hyphae will form a complex called mycelium, which appears as fuzzy and cottony growth in the environment and on meats, foods, clothing, walls, paper, and other materials.

Both yeasts and molds have sexual and asexual cycles. There are four types of asexual spores. Sporangiospores are enclosed in the sporangium. Conidiospores are released with microconidia and macroconidia. Chlamydospores are thalli that form a thick wall and become asexual spores. Arthrospores are formed by fragmentation of a septated mycelium. There are also four types of sexual spores. Oospores are formed by mating of a smaller 'male' thallus with a larger 'female' thallus. After exchange of genetic materials, sexual spores are formed. Zygospores are formed by the mating of two homogamous thalli. After exchange of genetic materials, sexual spores are formed. Ascospores are formed when two sexual spores mate and then, after exchange of genetic materials, the sexual spores are formed in a sac (ascus). Basidiospores are formed after exchange of genetic materials; sexual spores are formed at the basidium of very complex structures found in mushroom development.

A synopsis of the four classes of fungi is as follows:

- Phycomycetes: Sexual spores are free zygotes. Asexual sporangiospores are enclosed in the sporangium, which is developed from the sporangiophore. Mycelia are nonseptate (coenocytic). Important genera include *Mucor* (Figure 1), *Rhizopus* (Figure 2), and *Absidia*.
- Ascomycetes: Sexual spores in asci. Asexual spores are formed at the end of the conidiophore and are released. These structures are very distinctive for different genera of molds. Filamentous cells belong to the mold group and nonfilamentous cells are true yeasts. Important molds in this class include *Aspergillus* (Figure 3), *Penicillium* (Figure 4), *Fusarium* (Figure 5), *Alternaria* (Figure 6), *Botrytis* (Figure 7), *Cladosporium* (Figure 8), *Geotrichum* (Figure 9), and *Stemphylium* (Figure 10). Important yeasts in this class include *Saccharomyces cerevisiae, Candida albicans, Schizosaccharomyces, Hansenula*, and *Kloeckera*.
- Basidiomycetes: Sexual spores on basidium. Asexual spores are very rare. Mycelium is septated. Mushrooms and toadstools belong to this class.
- Deuteromycetes (Fungi imperfecti): These are the 'imperfect fungi' since only sexual or asexual cycles, but not both cycles, are observed. This is a catchall group. When both sexual and asexual cycles are observed, the organisms become assigned to one of the other three classes, usually into the *Ascomycetes* class. More detailed description of yeasts and molds related to food science and meat science can be found in *Modern Food Microbiology* by JM Jay (see Further Reading).

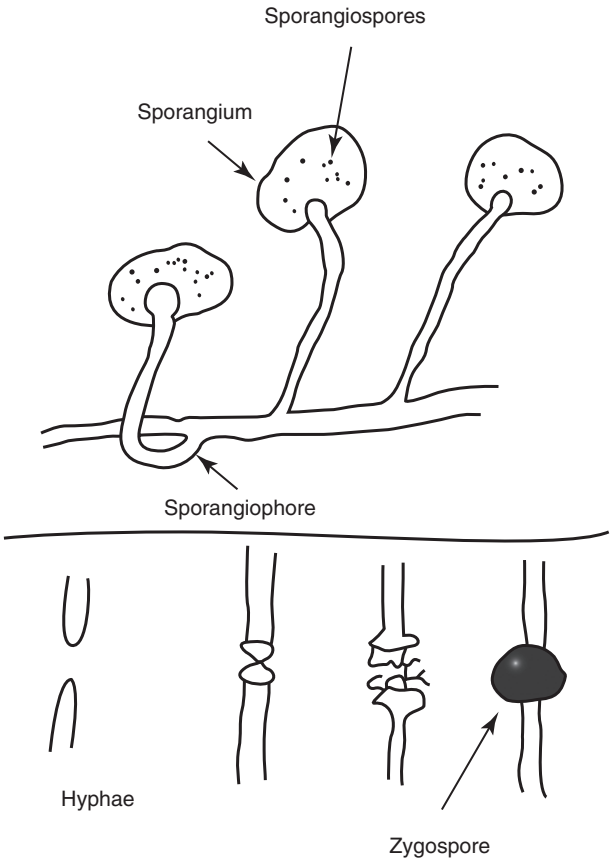

Figure 1 *Mucor* is very widespread and appears as a messy gray mass.

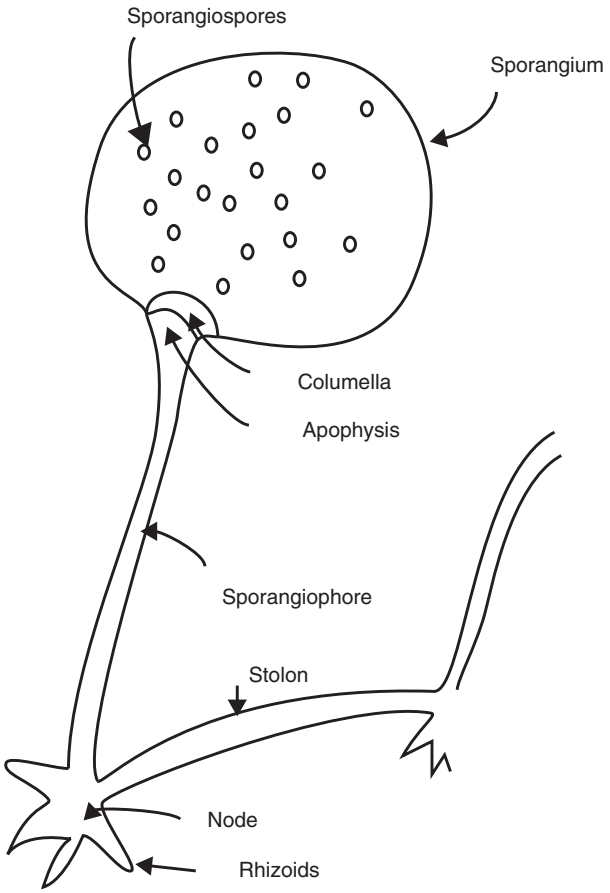

Figure 2 *Rhizopus nigricans*. This mold is very widespread and grows on fruits, preserves, and bread (the classic 'bread mold'). Sporangia are black.

Identification and Enumeration of Yeasts and Molds in Foods

Yeasts and molds are relatively large microbes and can be observed at 400 times magnification under the compound microscope. They can be tentatively identified to the genus level by experienced mycologists according to the type of hyphae, the shape, the size of sexual and asexual spores, and the morphology of various fruiting bodies of the organisms. Some special structures of yeasts and molds are described in the previous section. For further identification of the organisms, a variety of morphological and physiological tests must be made.

For identification of yeasts, some of the following criteria can be used:

1. Cultural characteristics on or in a variety of liquid and agar media such as glucose–yeast extract–peptone water, surface assimilation media, malt agar plus 2% calcium carbonate, morphology agar, etc.
2. Vegetative reproduction characteristics such as formation of asexual endospores, chlamydospores, germ tubes, and ballistospores.
3. Sexual characteristics such as formation of ascospores and observation of the life cycle.
4. Physiological characteristics such as fermentation or assimilation of carbohydrates and other carbon compounds, breakdown of arbutin, assimilation of nitrate and nitrite, acid production from glucose, starch formation, breakdown of fat, etc.

Recently, however, commercial 'rapid systems' have been developed that allow the identification of a large number of yeast isolates using convenient multichamber units containing various essential growth media and reagents. After growth and application of reagents, the data collected can be matched with known databases, manually or electronically, to ascertain the identity of the isolates. The Uni-Yeast-Tel (Flow Lab Inc., McLean, VA), Analytab (API-bioMerieux, Hazelwood, MO), and Minitek (Becton Dickinson, Sparks, MD) systems are some of the useful diagnostic kits for rapid yeast identification. The most ambitious automated system is the Vitek system (bioMerieux, Hazelwood, MO), which utilizes a plastic card with 30 tiny wells containing reagents for growth and biochemical reactions of a pure culture isolate of interest. There are 26 tests for each isolate. After the pure culture is injected into the plastic card, the card is incubated at 30 °C for 24 h and the results and interpretation of the identity of isolates are then produced automatically by the instrument. Vitek can also identify many types of pathogenic and nonpathogenic bacteria of especial importance in medical microbiology.

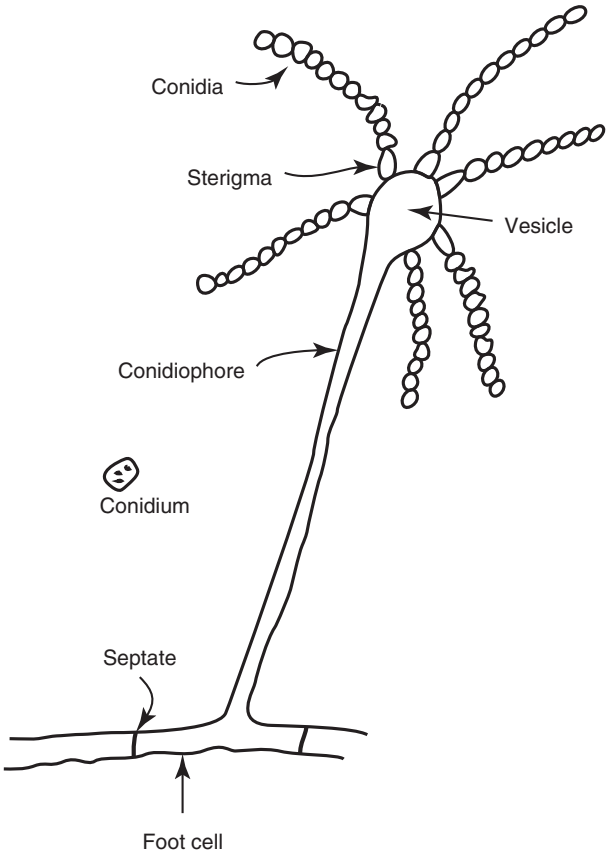

Figure 3 *Aspergillus*. The mold is yellow, green, or black; it grows on many foods. Some species produce aflatoxins.

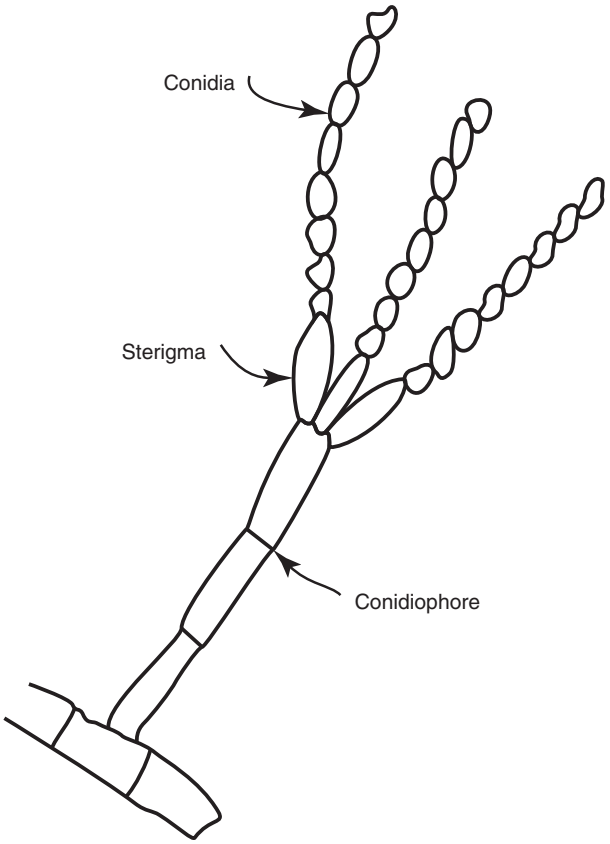

Figure 4 *Penicillium* (=‘a brush’). The mold is blue or blue-green.

For identification of molds, the following criteria can be used:

1. Septate or nonseptate hyphae.
2. Presence or absence of rhizoids (roots).
3. Presence or absence of fruiting bodies and arrangements of asexual and sexual spores.
4. Arrangement of conidia (microconidia and macroconidia).
5. Color and morphology of various molds in specialized agar, etc.

It takes an experienced mycologist to recognize a fungus to the genus by the above tests. To obtain species-level identification, a variety of physiological, immunological, and genetic tests may have to be made. At present there is no ‘rapid method’ for mold identification. The techniques of polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are being investigated by various research groups for rapid identification of molds.

Enumeration of yeasts and molds in liquid or food can be made by observation under the microscope using a specialized slide with grids, such as the Petroff–Hauser counting chamber (for individual yeast cells) or the Howard mold-counting chamber (for observation of mold filaments). After obtaining the average number of yeast cells in a few grids (usually 10), the number can be multiplied by a microscope factor (usually 6–7 log) to obtain the number of yeast cells per milliliter or per gram of liquid or solid. Similarly, mold filaments can be counted and converted to mold filaments per milliliter or per gram of food.

For enumeration of viable (living) yeast and mold cells, the best way is first to make a 1:10 dilution of the food and homogenize the mixture thoroughly; serially dilute (1:10) the sample in sterile buffer solution and place a known volume of the diluted sample (e.g., 1 ml) into a Petri dish; then pour a warm selective agar such as Yeast and Mold Agar (YM Agar, DIFCO, Detroit, MI) at pH 3.5 into the Petri dish. After incubation at 21 °C for 3 days, typical yeast and mold colonies, if present, can be counted and reported as yeast or mold count per milliliter or per gram. Yeast colonies on the agar are usually white, smooth and round. For molds, a variety of morphologies can occur with different colors, shapes and sizes. Prolonged incubation will give erroneous results as the spores of the first mold colony can be discharged and land on another part of the agar plate, greatly increasing the number of mold counts.

There is no accepted safe standard for yeasts and molds in liquid or solid food. Suggested appropriate ranges for bacterial, yeast, and mold counts for consideration of acceptance or rejection of food items, and in air, are given in Table 1.

Of course, these numbers do not indicate the presence of pathogens. Any pathogen such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, etc. will not be acceptable. There is no yeast and mold standard that would render the food totally unacceptable at low numbers.

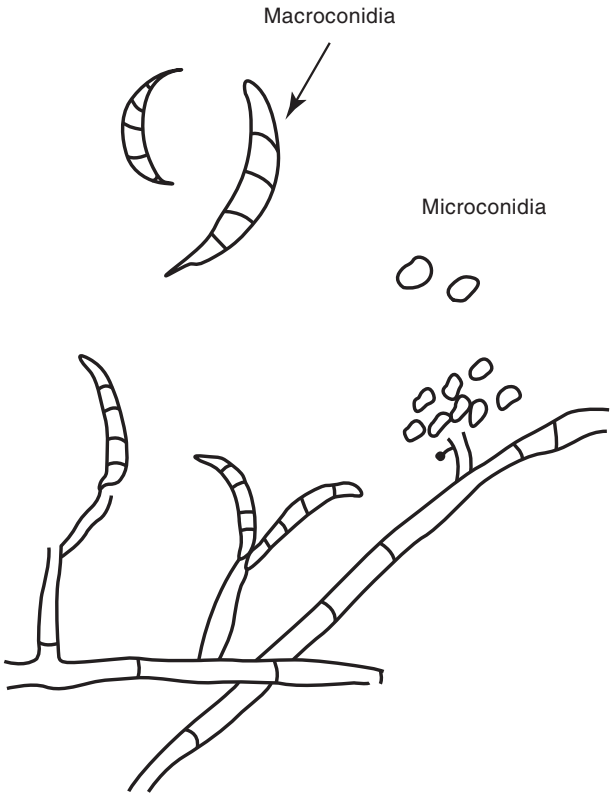

Figure 5 *Fusarium*. Grows on fruits (bananas) and vegetables; causes neck rot; has boat-shaped macroconidia.

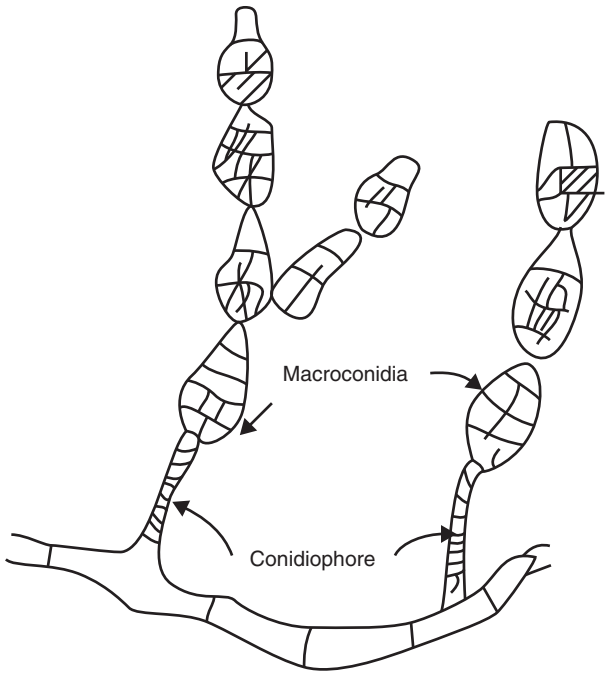

Figure 6 *Alternaria*. The appearance is dark yellow, green, or black. The mold damages plants.

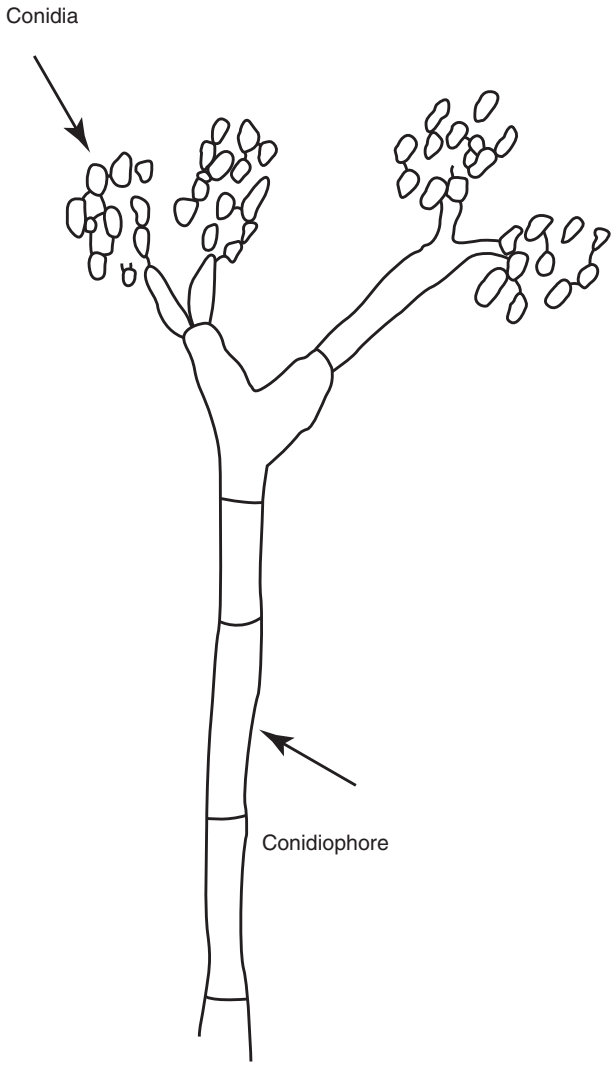

Figure 7 *Botrytis*. The appearance is gray. The mold spoils various foods.

Importance and Occurrence of Yeasts in Meats

According to NJW Kreger-van Rij there are 60 genera and 500 species of yeast. Foodborne yeasts account for approximately 43 genera and 220 species. This is because various foods provide extremely wide ecological environments for yeasts.

JM Jay has indicated that common yeasts found in fresh and refrigerated meats include *Candida*, *Cryptococcus*, *Debaromyces*, *Hansenula*, *Pichia*, *Phodotorula*, *Saccharomyces*, *Torulopsis*, and *Trichosporon*. The following are examples of the presence and activities of yeasts in various meat systems.

HK Dalton and colleagues isolated and identified 383 yeasts in a comparative study of yeast flora in British fresh sausage and minced (ground) beef. The majority yeast genera were *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia*, *Rhodotorula*, and *Torulopsis*. *Debaryomyces hansenii* was the most commonly isolated yeast from most samples, followed by *Candida zeylanoides*, and *Pichia membranaefaciens*. The sulfide in sausages did not appear to affect the numbers and kinds of yeasts present.

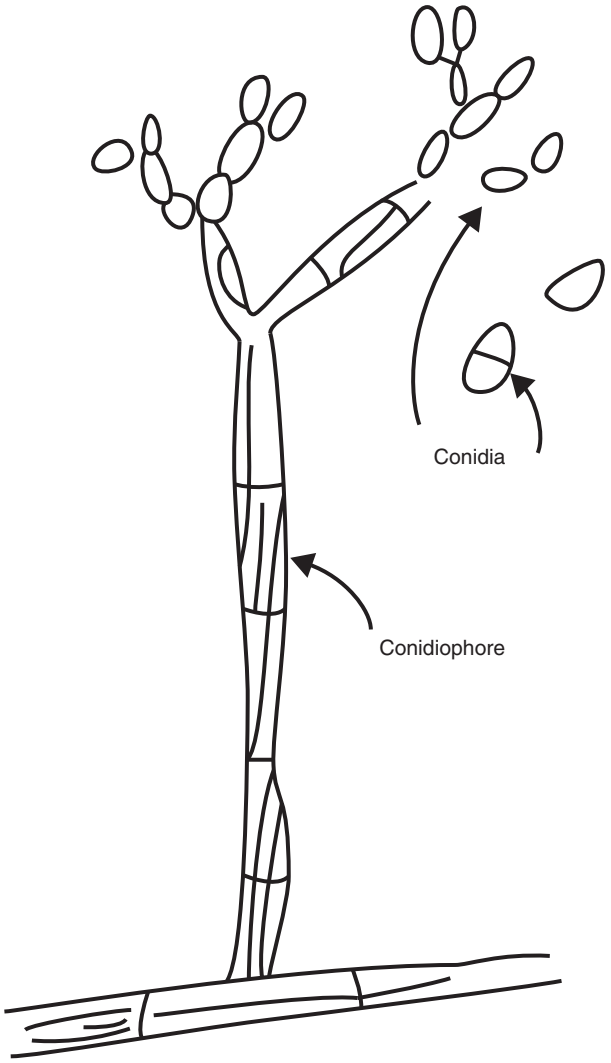

Figure 8 *Cladosporium*. The appearance is of dark conidiophores and green mold. Causes 'black spot' on beef.

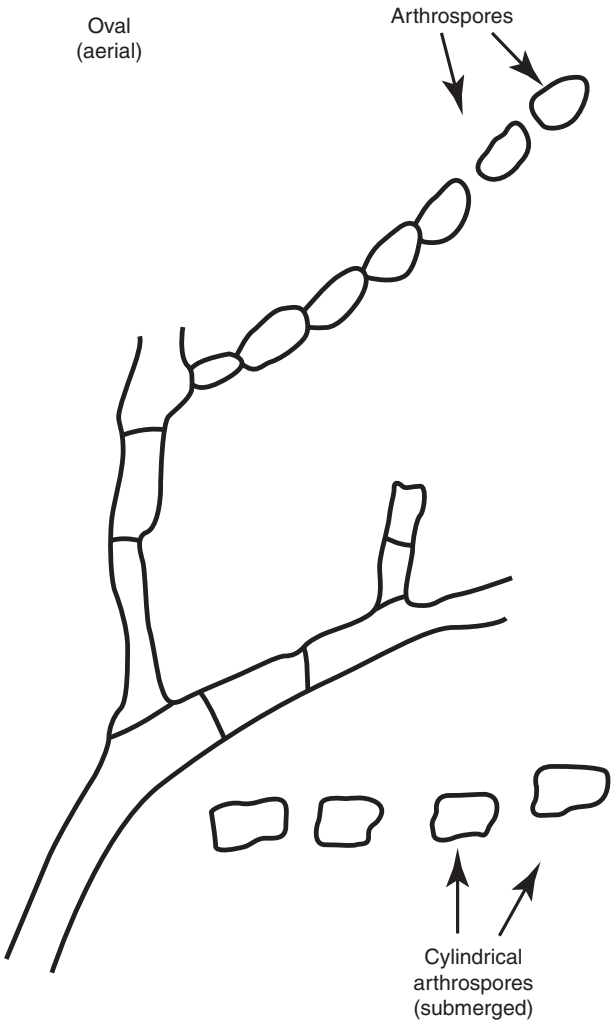

Figure 9 *Geotrichum*. Forms arthrospores. This 'dairy mold' imparts flavor and aroma to cheeses.

E Monte and colleagues studied the fungal profiles of Spanish country-cured hams. From 160 surface samples of 40 hams, yeast counts were between 4 log and 5.4 log CFU per gram (CFU = colony forming unit) and filamentous fungi counts were from 2.7 log to 4.4 log CFU per gram. *Debaryomyces marama* isolated from these samples could grow at 16% NaCl. Various filamentous fungi, such as *Eurotium repens, Pencillium expansum, Pencillium cyclopium, Pencillium viridicatum, Pencillium brevicompactum,* and *Pencillium simpliciissimum* were identified. G Comi and colleagues researched total yeast counts on 150 samples of fresh and refrigerated meat obtained from various locations in Italy. They found that most fresh samples had a yeast count of 0–100 CFU per gram, with 80% in the range of 0–3 log CFU per gram; 60% of the samples stored under refrigeration for 7 days had counts of 5–6 log CFU per gram; and after 14 days 60% had 6–7 log CFU per gram. To study the growth of yeast during refrigerated storage (2 °C; 80% relative humidity), 40 meat samples were classified into five groups, depending on initial count level, and stored for 18 days, with yeast count determined every 3 days. The results for the composition of the yeast flora on fresh and refrigerated meat samples revealed that *Torulopsis* spp. were predominant (35% of total) on fresh meat, followed by *Trichosporon* (25%). After 7 days of refrigerated storage, *Trichosporon* spp. predominated (45%), followed by *Candida* spp. (20%). *Debaryomyces hansenii, Endomycopsis platypodes,* and *Lipomyces starkeyi* were present on fresh samples but absent from refrigerated samples. *Rhodotorula* spp. and *Cryptococcus* spp. increased on refrigerated samples.

DY Hsieh and JM Jay characterized and identified 194 yeasts isolated from 28 samples of fresh and 4 samples of spoiled minced beef. Seventy-nine strains were from five genera, with the genus *Candida* accounting for 82% of the strains and 61% of the identified species. Other genera found were *Rhodotorula, Torulopsis, Trichosporon,* and *Cryptococcus. Candida lipolytica* was the most frequently isolated species in their study, and *Ca. zeylanoides* was more indigenous to minced beef than any of the other 21 species identified.

E Johannsen and colleagues examined the yeast flora present in minced beef before and after irradiation. No reduction

in the number of yeasts was observed after the meat was irradiated at a dose of 2.5 kGy. A definite increase in the number of psychrotrophic yeasts was observed in irradiated meat after 14 days of storage at 4 °C. The recovered yeast flora comprised representatives of the following species: *Candida famata*, *Cryptococcus albidus*, *Cryptococcus infirmominiatus*, *Cryptococcus laurentii*, *Trichosporon cutaneum*, *Trichosporon pullulans*, *Rhodotorula minuta*, and *Rhodotorula rubra*.

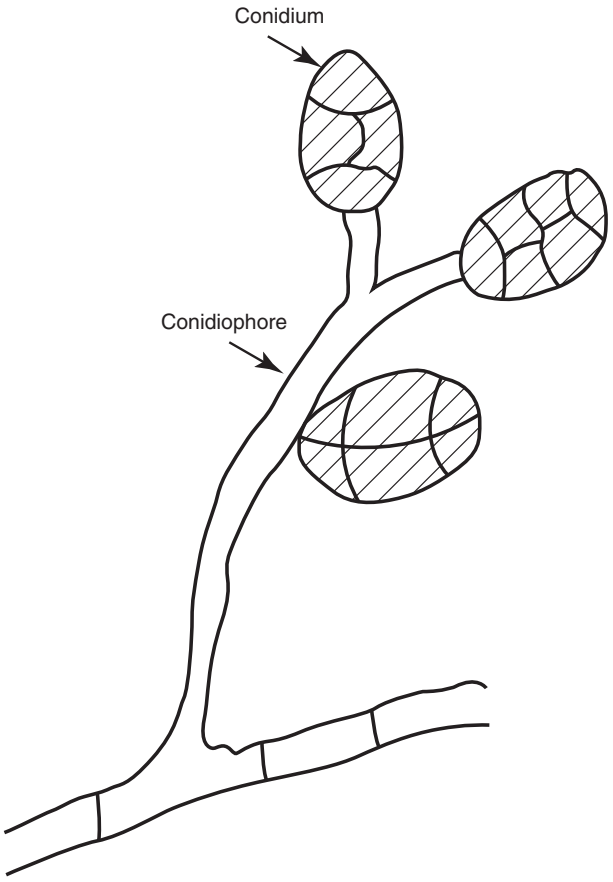

Figure 10 *Stemphylium*. Causes gray leaf spot.

PD Lowry and CO Gill studied the yeast flora on frozen lamb stored at −5 °C. Lamb loins wrapped in gas-permeable plastic film and stored at −5 °C developed a yeast flora with a maximum number of approximately 6 log CFU per cm^2) after 20 weeks. Yeasts were identified as *Cr. laurentii*, *Cr. infirmominiatus*, *T. pullulans*, and *Ca. zeylanoides*. No microbial growth was detected on lamb loins stored at −10 °C for 40 weeks.

CCS Lin and DYC Fung isolated and identified yeasts from various foods. *Ca. lipolytica*, *Ca. zeylanoides*, and *R. rubra* were isolated from beef; *Coleophora azyma*, *Ca. famata*, and *Ca. lipolytica* from ham; *Ca. famata* and *Ca. lipolytica* from hot dogs; *Ca. famata*, *Ca. lipolytica*, and *R. rubra* from turkey ham.

Studies have also been carried out on the effect of yeasts on the sensory properties and quality of meat products. G Comi and colleagues determined the lipolytic enzyme and esterase activity of yeasts from raw ham on various substrates. The highest lipolytic activity was shown by *Torulopsis* spp., *T. cutaneum*, and an unidentified *Trichosporon* species. For most species, the lipolytic activity of the yeasts was generally less than that of the bacterial genera *Lactobacillus* and *Micrococcus*. The results revealed that yeasts did not present a major problem in relation to lipolysis in raw ham. However, in another report by G Comi and colleagues, two endopeptidases from *Torulopsis* spp. isolated from raw ham were observed. The enzyme activity was related to the concentration of NaCl.

RJ Winger and PD Lowry made a sensory evaluation of lamb after growth of yeast at −5 °C. *Cryptococcus laurentii* was inoculated at various densities onto lamb loins, which were then frozen and stored at −5 °C for 10 weeks. During storage, yeast numbers increased by 2 log cycles. No foreign flavors associated with the high yeast numbers could be discriminated by a trained taste panel.

In a study by M Kobatake and H Kurata, proteolytic and lipolytic yeast species were widely distributed among the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Leucosporidium*, *Rhodotorula*, and *Trichosporon*. All yeasts tests were isolated from chilled household foods and raw seafood.

Table 1 Ranges of fungal/bacterial counts and their significance for acceptance/rejection/remedial action

Count[a]	*Significance/indication/action*
In foods (CFU per g, per ml, or per cm^2)	
$0–10^2$	Low count: no problem
$10^3–10^4$	Intermediate count: no problem but slight concern
$10^5–10^6$	Definite concern: corrective action to be taken
10^7	Index of spoilage
10^8	Odor will develop
10^9	Slime will form
10^{10}	Absolutely unacceptable
In air (CFU per m^3)	
$0–10^2$	Low count: no problem
$10^2–3 \times 10^2$	Intermediate count: no problem but concern
$>3 \times 10^2$	Definite concern: corrective action to be taken

[a]CFU = colony forming unit.

Note: The scales were developed by the author at Kansas State University.

SU Nwahakwu and TVI Akpata studied the utilization of carbohydrate and protein by *Ca. famata* during spoilage of snail meat. The results indicated that *Ca. famata* was a potential spoilage organism for snail meat. The shelf life of snail meat at room temperature could be increased by eliminating *Ca. famata*.

These are, of course, only some examples of the occurrence of yeasts in meat. Suffice to say that yeasts can spoil meat under unfavorable conditions and, since they can grow both aerobically and anaerobically, control of the contamination of meat with yeasts should be considered a priority in food safety and preservation.

Importance and Occurrence of Molds in Meats

There are literally hundreds and thousands of species of mold on record. JM Jay has indicated that common molds found in fresh and refrigerated meats include *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Monascus*, *Monilia*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus*, *Sporitrichum*, and *Thamnidium*. The following are examples of the presence and activities of molds in various meat systems.

The ubiquity of molds in the preparation, handling and storage environments of fresh meats is such that their presence in these products should be expected. However, molds are rarely involved in spoilage of fresh meats, except under special circumstances, such as storage at a low temperatures or use of preservatives. It has been established that molds can grow at pH values between 2 and 11, at water activities (a_w) between 0.62 and 0.995, at temperatures between $-1\,^{\circ}C$ and around 60 °C, and over a wide range of nutrient limitations. Some mold chlamydospores, conidia, and vegetative cells are known to retain viability more than 22 years, while spores may retain viability for more than 30 years. However, molds are not as versatile as bacteria in their capacity to grow over wide ranges of oxidation–reduction potentials. Most foodborne mold isolates require aerobic conditions or positive Eh values, unlike many bacteria, which can proliferate under negative Eh conditions. Under natural conditions that permit the growth of a variety of bacteria and molds, bacteria invariably outgrow the molds, and no fungal growth is observed on fresh and processed meats. In fact the mycology of most meats has not received much attention until recently, owing to the capacity of these foods to support rapid bacterial growth. Nevertheless, when meat-preservation processes such as aging, curing, drying, or freezing are employed, molds can compete effectively with bacteria and become the dominant microflora. Researchers have noted the presence of molds on fresh meat since the 1920s. FT Brooks, CG Hansford, and MA Wright studied the condition of beef quarters known as 'black spot,' 'whiskers,' and 'white spot' and showed them to be caused by molds such as *Cladosporium*, *Sporotrichum*, and *Thamnidium*. The growth of these molds on the surface of beef occurs due to a lack of sufficient moisture to permit bacterial growth. Fat decomposition, 'green patches,' off-odor, and stickiness are other spoilage manifestations of moldy meat. For minced beef and pork, mold counts range from 70 to 480 000 CFU per gram. Counts for fresh and frozen fish range from 600 to 270 000 CFU per gram. Determinations for chicken meat range from 100 to 97 000 CFU per gram or CFU per cm^2, while sausage samples were found generally to contain lower numbers.

According to RA Hart, a total of 20 genera of mold have been reported on cured, fresh, processed, or stored meats. The most frequently recorded species belong to the genera *Aspergillus* and *Penicillium*. This may be a reflection of their generally higher incidence in meat environments. It may also be a reflection of the greater ease of their identification, of their ability to grow at low a_w, or of some other property that these two groups to adapt to meats with greater ease than other molds. **Tables 2** and **3** give incidences of fungi in different muscle foods and different muscle foods from which fungi have been isolated as reported by RA Hart.

Mold growth is favored over bacterial growth in country-cured hams and some dry sausage products. Thirteen genera of mold were isolated and identified by JC Ayres in 1967 in a mycological study of cured hams and sausage. The genera *Aspergillus* and *Penicillium* were isolated from both products more frequently than any others; *Cladosporium* and *Alternaria* were isolated from 14 samples of ham. *Aspergillus* spp. generally grow better in the low-a_w country-cured hams, while *Penicillium* predominates in higher-a_w fermented sausages.

Cold or frozen storage often tends to reduce the a_w of meat, thus simultaneously selecting for psychrotrophic and xerotolerant molds. None of these cold-storage molds are known to be pathogenic or mycotoxigenic. A dark discoloration known as 'black spot' is one of the most economically important forms of psychrotrophic mold spoilage. Beef, mutton, pork, veal, lamb, and rabbit are prone to this type of spoilage. Strains that can produce 'black spot' include *Cladosporium cladosporioides*, *Cladosporium berbarum*, *Penicillium hirsutum*, and *Aureobasidium pullulans*. *Cladosporium*, in addition to producing deep-seated black spots, is also proteolytic. 'White spot,' another type of meat spoilage, is caused by *Chryosporium pannorum* and occasionally by *Acremonium*, *Thamnidium*, *Mucor*, and *Rhizopus* cause the cottony gray–black growth found in beef stored in cold rooms, called 'whiskers.'

One of the 'whisker' molds, *Thamnidium elegans*, grows rapidly enough to compete successfully with psychrotrophic bacteria at or near 0 °C. Growth of whisker molds on meat is sometimes considered desirable, as some believe that the flavor and tenderness of beef is enhanced during the growth of these molds.

One of the major problems concerning molds in foods is the production of mycotoxins by several genera of molds. *Aspergillus flavus* and *Aspergillus parasiticus* can produce aflatoxins B_1, B_2, G_1, and G_2, which are carcinogenic to animals. B_1 can be converted to M_1 through dairy cows in milk. Aflatoxins are most likely to be found in stressed crops grown in arid climates such as in the southeastern United States. The toxic effects of aflatoxins on animals can have devastating consequences for human and animal health. Other mycotoxins include trichothecene, zearalenone, ochratoxins, sterigmatocystin, patulin, and *Alternaria* metabolites. Ochratoxin A can be produced by *Aspergillus ochraceus* and several strains of *Penicillium* in grain that is insufficiently dried at harvest and is transferred to pigs and poultry eating moldy grains. In Denmark, all pig carcasses with light or enlarged kidneys are analyzed for ochratoxin A and condemned if the content is above a threshold level.

Table 2 Reported incidence of fungi in different muscle foods

Isolates	*Bacon*	*Fish*	*Meats*	*Poultry*
Alternaria spp.	1	–	1	1
Aspergillus clavatus	–	–	1	–
Aspergillus glaucus	–	1	1	–
Aspergillus niger	–	–	2	–
Aspergillus spp.	1	1	1	1
Botrytis spp.	1	–	–	–
Cladosporium herbarum	–	–	2	–
Cladosporium spp.	–	–	1	1
Fusarium spp.	1	–	1	–
Geotrichum candidum	–	–	1	1
Geotrichum spp.	–	–	2	1
Monilia spp.	1	–	–	–
Monascus purpureus	–	–	1	–
Mortierella spp.	–	–	1	–
Mucor lusitanicus	–	–	1	–
Mucor mucedo	–	–	1	–
Mucor racemosus	–	–	1	–
Mucor spp.	1	–	3	1
Neurospora sitophila	–	–	1	–
Oidium spp.	1	–	–	–
Oospora spp.	1	–	–	–
Oospora nikitinskii	–	1	–	–
Penicillium asperulum	–	–	1	–
Penicillium frequentans	–	–	1	–
Penicillium expansum	–	–	2	–
Penicillium glaucum	–	–	1	–
Penicillium oxalicum	–	–	1	–
Penicillium spp.	1	1	2	2
Rhizopus stolonifer	–	–	1	1
Rhizopus spp.	1	–	3	–
Sporendonema epizoum	–	1	–	–
Sporendonema spp.	–	1	–	–
Sporotrichum carnis	–	–	1	–
Sporotrichum spp.	–	–	1	–
Thamnidium elegans	–	–	4	–
Thamnidium spp.	–	–	2	–
Zygorrhynchus spp.	–	–	1	–
Total species strains	9	7	31	8

Source: Reproduced from Hart, R.A., 1994. Development of selective dye media for food mold. PhD dissertation, Kansas State University.

Beneficial Aspects of Yeasts and Molds in Meat Systems

The benefits from yeasts and molds in fermented food around the world are enormous. *Saccharomyces cerevisiae* is probably the most economically important industrial yeast in the world, being the microorganism responsible for alcoholic fermentation of beer and wine and indirectly contributing to the distilled liquor industry. It is also used in bread making. The mold *Aspergillus oryzae* is as important in Oriental food fermentation as *Ca. cerevisiae* is in Western food fermentation. This mold is responsible for the huge soybean-based fermented food industry in the Orient. Many molds are responsible for fermentation of dairy products and legume products. However, yeasts and molds are not heavily involved in fermented meat products. Yeast has very little beneficial functions in fermented meat products. Molds are known to contribute to the quality of some dry European-type sausages and Italian salami. JC Ayres described nine species of penicillia and seven aspergilli on fermented sausages and attributed beneficial effects to these molds in the preservation of the sausages. JC Ayres also considered mold growth beneficial in dry-cured ham products in the southern United States. More recently, it was found that up to 96% of the mold in fermented sausages in northern Italy were penicillia and 4% are aspergilli. The initial flora of the sausage was made up of 95% yeasts, but after 2 weeks of fermentation the proportions of yeast and mold were equal. After 4–8 weeks, molds constituted more than 95% of the fungal flora. In other studies, *Penicillium camemberti* and *Penicillium nalgiovense* have been added to raw dry sausages to prevent the growth of mycotoxigenic molds.

Spoilage and Food Safety Issues of Yeasts and Molds

Yeasts and molds are very important in spoilage of all types of meat by the production of various enzymes that degrade protein and lipid structures and by the production of undesirable colors and unsightly visible mold colonies on meat surfaces and on the casings of sausages. Although these growths may not be harmful to health, the very presence of yeast and mold growth is reason for discarding these products, causing economic losses. Yeasts and molds can contaminate meat and meat products through human and animal contact, contact with equipment, water, or ingredients and even as a result of air contamination.

The real threat from molds in meat safety is the production of mycotoxins by some molds. The most important mycotoxins are the aflatoxins (B_1, B_2, G_1, and G_2) produced by *A. flavus* and *A. parasiticus* and the ochratoxins produced by *A. ochraceus* and several strains of *Penicillium*. These mycotoxins are usually produced in grains and plant products and seldom in meat products. Research performed in the author's laboratory has indicated that inoculated *A. flavus* and *A. parasiticus* cultures can produce detectable amounts of aflatoxins in experimental salami. However, due to the presence of antioxidants, such as combinations of butylated hydroxyanisole, butylated hydroxytoluene, tertiary butyl hydroxyquinone, and propyl gallate the amount of aflatoxin production is greatly suppressed.

Conclusions

Yeasts and molds are important microorganisms in meat and meat products, mainly as spoilage organisms and seldom as food-poisoning agents. In certain conditions they can be beneficial in meat fermentation. It is important to monitor the presence of yeasts and molds regularly in the food processing

Table 3 Fungi isolated from different muscle foods

Fungal species/strain	*Food source*
Alternaria spp.	Bacon, beef, country-cured hams, untreated poultry
Aspergillus clavatus	Meats
Aspergillus flavus	Country-cured hams, Italian-type salami, Spanish dry-cured hams
Aspergillus fumigatus	Meats, Spanish dry-cured hams
Aspergillus glaucus	Country-cured hams, meats, katsuobushi (fermented fish)
Aspergillus gracilis	Country-cured hams
Aspergillus niger	Chilled beef carcass, country-cured hams, fermented sausage, meats, moldy hams, salami
Aspergillus ochraceus	Moldy hams, salami
Aspergillus repens	Country-cured hams, moldy hams, salami
Aspergillus ruber	Country-cured hams, fermented sausages
Aspergillus tamarii	Moldy hams, salami
Aspergillus versicolor	Country-cured hams
Aspergillus wentii	Fermented sausage, moldy hams, salami
Aspergillus spp.	Bacon, country-cured hams, dry European sausage, fish protein concentrate, moldy hams, poultry meat, refrigerated beef, salami, smoked fish
Aureobasidium pullulans	'Black spot' of beef
Botrytis spp.	Bacon
Chrysosporium pannorum	Frozen lamb
Cladosporium herbarum	'Black spot' of beef
Cladosporium cladosporioides	'Black spot' of beef, frozen lamb
Cladosporium spp.	Chlortetracycline-treated poultry, country-cured hams, refrigerated beef
Fusarium spp.	Bacon, meats
Geotrichum candidum	Poultry
Geotrichum spp.	'White spot' of beef, chicken, fresh sausages
Monilia spp.	Bacon, refrigerated beef
Monascus purpureus	Meats
Mortierella spp.	Meats
Mucor mucedo	'Black spot' of frozen mutton
Mucor racemosus	'Whiskers' of beef, meats, untreated poultry
Mucor spp.	Bacon, 'black spot' of beef, refrigerated beef, 'whiskers' of beef, cured meats, untreated poultry
Mucor lusitanicus	'Whiskers' of beef
Neurospora sitophila	Meats
Odium spp.	Bacon 'black spot' of beef, meats
Oospora spp.	Salted fish, salted fish 'dun'
Penicillium chrysogenum	Meats
Penicillium expansum	Beef, green patches on beef, meats
Penicillium frequentans	Beef, moldy hams, salami, fermented sausages
Penicillium glaucum	'Black spot' of frozen mutton
Penicillium hirsutum	'Black spot' of beef
Penicillium oxalicum	Green patches on beef
Penicillium spp.	Bacon, dried beef, refrigerated beef, 'black spot' of beef, chicken, smoked fish, moldy hams, country-cured hams, chlortetracycline-treated poultry, salami, fermented sausage
Pulleteria pullulans	Fresh shrimp
Rhizopus nigricans	'Black spot' of beef, chlortetracycline-treated poultry
Rhizopus spp.	Bacon, refrigerated beef, 'whiskers' of beef, 'black spot' of frozen mutton
Scopulariopsis spp.	Dry European-type salami
Sporendonema spp.	'Dun' of dry-salted fish, salted fish

(*Continued*)

Table 3 Continued

Fungal species/strain	*Food source*
Sporotrichum carnis	'White spot' of beef
Sporotrichum spp.	Refrigerated beef
Thamnidium elegans	'Black spot' of beef, 'whiskers' of beef
Thamnidium chaetocladioides	Beef, 'whiskers' of beef, salted-minced pork
Thamnidium spp.	'Black spot' of beef, refrigerated beef
Zyghorrhynchus spp.	Frankfurters

Source: Reproduced from Hart, R.A., 1994. Development of selective dye media for food mold. PhD dissertation, Kansas State University.

environment to control their contamination of meat and meat products and food products in general.

Acknowledgment

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See also: Ham Production: Dry-Cured Ham. Sausages, Types of: Dry and Semidry

Further Reading

Beuchat, L.R., 1987. Food and Beverage Mycology. Westport, CT: AVI Publishing Company.
Deak, T., Beuchat, L.R., 1996. Handbook of Food Spoilage Yeast. Boca Raton, FL: CRC Press.
Erickson, L.E., Fung, D.Y.C., 1988. Handbook on Anaerobic Fermentations. New York, NY: Marcel Dekker.
Fung, D.Y.C., Bourgeois, C.M., Leveau, J.Y. (Eds.), 2003. Microbiological Control for Foods and Agricultural Products. New York, NY: VHS.
Fung, D.Y.C., Goetsch, S.J., 2002. Introduction of Food Microbiology. Manhattan, KS: Department of Animal Sciences and Industry, Kansas State University.
Jay, J.M., 1998. Modern Food Microbiology, fifth ed. Gaithersburg, MD: Chapman and Hall.

Yersinia enterocolitica

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Glossary

Apoptosis The biochemical events leading to cell changes and programmed cell death in a multicellular organism.
Cytotoxicity The ability to cause damage to cells.
Epidemiology The study of the prevalence of disease in populations.
Fermentation An anaerobic oxidation–reduction reaction where an organic substrate acts as the final electron acceptor instead of oxygen.
Heterogeneous Consisting of more than one form.
Pathogen An organism capable of inflicting damage.
Phenotype The observable characteristics of an organism as determined by genetic and environmental influences.
Psychrotroph An organism capable of growth at low temperatures but having an optimum growth temperature of $>15\,^{\circ}C$.
Virulence The potential ability of an organism to cause disease; determines pathogenicity.

Introduction

Yersinia enterocolitica is a particularly versatile foodborne pathogen, adept at survival in many habitats, both within and external to host animals. Pathogenic *Y. enterocolitica* organisms are significant causes of human disease in many parts of the developed world. Infections are most usually associated with pig meats, but all types of meats have been shown to harbor the bacterium.

The genus *Yersinia* belongs to the family Enterobacteriaceae and 11 species are currently recognized. A further six species have been proposed for inclusion into the genus. Three species are considered pathogenic to humans: *Y. enterocolitica*, which is primarily associated with food- and waterborne illness; *Yersinia pseudotuberculosis*, which is associated with enteropathogenic zoonotic infections; and *Yersinia pestis*, the cause of bubonic plague. A further seven of the remaining species (previously characterized as '*Yersinia enterocolitica*-like organisms'), although not considered pathogenic, share habitats with these organisms and so must be differentiated from them by testing laboratories.

Yersinia enterocolitica is differentiated by negative citrate and mucate tests, the ability to ferment sucrose, and an inability to ferment rhamnose, raffinose, and melibiose at 25 °C. *Yersinia enterocolitica* is motile and indole-positive below 30 °C, but not at 35 °C. The Voges–Proskauer reaction is positive at 22–28 °C, but negative at 37 °C for the majority of strains, which can be a problem when proprietary biochemical test kits are used for identification.

Even with the classification of separate species within the *Y. enterocolitica*-like group, *Y. enterocolitica sensu stricto* remains highly heterogeneous, and has been divided into subgroups on the basis of biochemical activity (biotypes, five currently recognized) and lipopolysaccharide O antigens (serotypes, at least 75 currently recognized). A connection between particular biotypes and serotypes is well documented, as is the correlation between these characteristics and the potential to cause human infection. Thus, biotype and serotype determination (expressed as bioserotype) provides one means of determining whether a strain is potentially pathogenic or of environmental origin. An association between serotypes and geographical niches has also been established, although with

Characteristics of the Organism

Yersiniae are Gram-negative, nonspore-forming, straight rods or coccobacilli, 0.5–0.8 μm in diameter and 1–3.5 μm in length (Figure 1). Variations in size or shape (i.e., pleomorphism) may occur, depending on culture medium and temperature. These bacteria are facultatively anaerobic organisms, catalase-positive, oxidase-negative, and ferment glucose with little or no gas, while lactose is not fermented. Phenotypic criteria are frequently influenced by temperature, optimum activity generally being between 25 and 29 °C, which create difficulty for identification by traditional biochemical tests.

Yersinia enterocolitica sensu stricto is one of the eight species currently designated within the '*Yersinia enterocolitica* group' of this genus. The biochemical differentiation between these species is shown in Table 1. The group is highly heterogeneous and some strains of different species overlap in their biochemical reactions, antigen expression, and ecological niches.

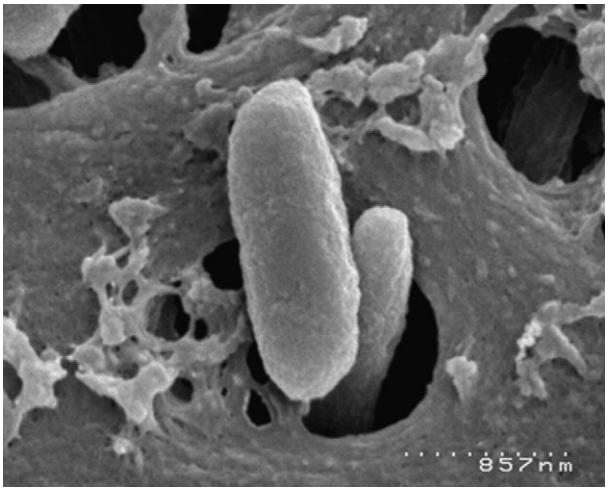

Figure 1 *Yersinia enterocolitica* biotype 3.

 doi:10.1016/B978-0-12-384731-7.00040-4

Table 1 The '*Yersinia enterocolitica*-like group'

Biochemical test[a]	Yersinia enterocolitica		Yersinia aldovae	Yersinia bercovieri	Yersinia frederiksenii	Yersinia intermidia	Yersinia kristensenii	Yersinia mollaretii	Yersinia rohdei
	Biotypes 1–4	*Biotype 5*							
Citrate (Simmons)	–	–	+	–	v	+	–	–	+
Mucate (acid)	–	–	v	+	+	+	v	+	–
Sucrose, acid	+	v	–	+	+	+	–	+	+
Melibiose, acid	–	–	–	–	–	+	–	–	v
Raffinose, acid	–	–	–	–	–	+	–	–	v
L-Rhamnose, acid	–	–	+	–	+	+	–	–	–
D-Trehalose, acid	+	–	+	+	+	+	+	+	+
Voges–Proskauer	+	+	+	–	v	+	–	–	–

[a]Incubation at 25–28 °C.

Note: +, positive reaction; –, negative reaction; v, variable reaction.

Source: Adapted from Barton, M., Robins-Browne, R., 2003. *Yersinia enterocolitica*. In: Hocking, A. (Ed.), Foodborne Microorganisms of Public Health Significance, 6th edn. Waterloo, Australia: AIFST Inc. (NSW Branch), pp. 577–595; Carniel, E., Autenrieth, I., Cornelis, G., *et al*., 2006. *Y. enterocolitica and Y. pseudotuberculosis*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes, 3rd edn, vol. 6. Singapore: Springer, pp. 270–398; and Weagent, S., Feng, P., 2007. *Yersinia enterocolitica*. In: Hammack, T., *et al*. (Eds.), Bacteriological Analytical Manual. Silver Spring, MD: US Food & Drug Administration (Chapter 8). Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

Table 2 Biotypes of *Yersinia enterocolitica*

Biochemical test[a]	*Biotype 1A*	*Biotype 1B*	*Biotype 2*	*Biotype 3*	*Biotype 4*	*Biotype 5*
Indole production	+	+	D	–	–	–
Esculin hydrolysis (<24 h)	+	–	–	–	–	–
Lipase (Tween esterase)	+	+	–	–	–	–
Pyrazinamidase	+	–	–	–	–	–
β-D-Glucosidase	+	–	–	–	–	–
D-Xylose, acid	+	+	+	+	–	v
Trehalose, acid	+	+	+	+	+	–
Nitrate reduction	+	+	+	+	+	–
Deoxyribonuclease	–	–	–	–	+	+

[a]Incubation at 25–28 °C.

Note: +, positive reaction; –, negative reaction; d, delayed reaction; v, variable reaction.

Source: Adapted from Nesbakken, T., 2005. *Yersinia enterocolitica*. In: Fratamico, M., Bhunia, A., Smith, J. (Eds.), Foodborne Pathogens – Microbiology and Molecular Biology. Wymondham, UK: Caister Academic Press, pp. 228–249; Carniel, E., Autenrieth, I., Cornelis, G., *et al*., 2006. *Y. enterocolitica* and *Y. pseudotuberculosis*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes, 3rd edn, vol. 6. Singapore: Springer, pp. 270–398; and Weagent, S., Feng, P., 2007. *Yersinia enterocolitica*. In: Hammack, T., *et al*. (Eds.), Bacteriological Analytical Manual. Silver Spring, MD: US Food & Drug Administration (Chapter 8). Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

time, this has become less valid, possibly owing to international trade in food products:

- Biotype 1A strains were previously considered to be not virulent; however, recent studies suggest that some are capable of causing human disease.
- Biotype 1B is considered to be highly pathogenic, and includes several pathogenic serotypes predominantly isolated from North America, of which O:8 is the most common.
- Biotypes 2 and 3 are classified as moderately pathogenic and contain pathogenic serotypes O:5,27 and O:9, the latter being most often isolated in northern Europe.
- Biotype 4 is classified as moderately pathogenic and is associated with serotype O:3; it has worldwide distribution.
- Biotype 5 strains appear largely restricted to animals, notably rodents and ruminants, although some human infections have been reported in Europe.

Biochemical differentiation of the biotypes of *Y. enterocolitica*, is outlined in **Table 2**.

Two subspecies have been identified from deoxyribonucleic acid (DNA)–DNA hybridization and examination of 16 S gene sequences. *Yersinia enterocolitica* subsp. *enterocolitica*, consisting of the highly pathogenic bioserotype 1B strains, and *Y. enterocolitica* subsp. *palearctica*, consisting of the

remaining biotypes 1A, 2, 3, 4, and 5, which are of varying pathogenicity.

For subtyping serotypes of *Y. enterocolitica* for epidemiological purposes, pulsed-field gel electrophoresis (PFGE), ribotyping (DNA restriction fragment polymorphism of ribosomal ribonucleic acid (rRNA)), and random amplification of polymorphic DNA (RAPD)-based fingerprinting have been used with some success. Schemes for phage typing have been developed but are restricted to a small number of reference laboratories.

Clinical Presentation

Infections with *Y. enterocolitica* present the clinician with a wide range of symptoms and outcomes. Yersiniosis, the disease caused by *Y. enterocolitica*, primarily affects the lymphatic system, particularly the glands associated with the gastrointestinal tract. The most common initial symptom of infection is a self-limiting diarrhea, but more serious symptoms are not uncommon, particularly in Scandinavia. The incubation period is usually between 24 h and 36 h, although postingestion intervals of up to 11 days have been reported.

In most cases, symptoms occur in children less than 5 years of age, typically diarrhea, usually accompanied by fever and abdominal pain (i.e., enteritis), which normally lasts for a few days, but sometimes up to 3 weeks. In rare cases, acute enteritis symptoms may be followed by more serious illness. In older children and adolescents, acute disease symptoms are similar to those of appendicitis.

Septicemia is a rare complication, usually associated with high-virulence type 1B strains, most often affecting immunocompromised people. If *Y. enterocolitica* become disseminated into the bloodstream, the consequences are serious and can include abscesses of the spleen, liver, or lungs.

Although most cases of disease are self-limiting and without long-term complications, 2–3% of infections with *Y. enterocolitica* are associated with a variety of conditions that follow the acute phase. Reactive arthritis is the most commonly encountered, typically following the onset of acute disease by 7–14 days. The joints most commonly affected are fingers, wrist, elbow, knee, ankle, and toes. The arthritis migrates, affecting one joint after another, in around 85% of affected patients.

The majority of gastrointestinal infections attributed to *Y. enterocolitica* are self-limiting so that antimicrobials are not usually prescribed. However, treatment is necessary in cases of severe enteritis, particularly in immunocompromised people and for patients with septicemia or other invasive infections. Antibiotic susceptibility varies among subgroups, but the organism is usually susceptible to aminoglycosides, cotrimoxazole, tetracycline, third-generation cephalosporins, and fluoroquinolones.

Mechanism of Pathogenicity

Only a proportion of *Y. enterocolitica* strains are capable of causing human disease. These pathogenic strains are ingested with contaminated food or water. They are able to pass through the stomach (which has low pH, <3) undamaged because they produce the enzyme urease, which releases ammonia, neutralizing the pH in the immediate vicinity of the bacterial cell. On reaching the small intestine, bacterial cells first bind to epithelial cells, then penetrate the intestinal mucosa via M (microfold) cells, colonizing the Peyer's patches, where they multiply. From the Peyer's patches, they infect the lymphatic system, frequently involving adjacent regions of the intestine and mesenteric lymph nodes and, in rare cases, the liver, spleen, and bloodstream. A capacity to resist nonspecific host defense mechanisms is shared with other members of the genus including *Y. pestis* and *Y. pseudotuberculosis*.

Pathogenic strains can be divided into a group of lower pathogenicity that is nonlethal to mice and comprises organisms from biotypes 2 to 4, and a highly pathogenic group of biogroup 1B strains that are lethal to mice. Strains of the low-pathogenicity group possess multiple virulence factors, some chromosomally mediated and some derived from a 70–75 kbp plasmid, pYV. Both plasmid- and chromosome-associated virulence factors are required for full expression of virulence. Strains of the high-pathogenicity group possess a further chromosomal locus, designated the high-pathogenicity island (HPI), which is shared by certain other species of Enterobacteriaceae. Nonpathogenic strains lack the pYV plasmid and chromosomally mediated virulence proteins.

The following are the major virulence mechanisms currently identified in *Y. enterocolitica*:

- Cell adhesion. Adhesion to host cells is the function of two proteins: YadA, encoded by the virulence pYV plasmid, which mediates bacterial autoagglutination and attachment to host cells; and the chromosomally encoded MyfA, which synthesizes a fibrillar structure.
- Cell invasion. Cell invasion is induced by the bacterial outer membrane protein, invasin, encoded by the chromosomal *inv* gene. A second bacterial chromosomal gene, *ail*, encodes an outer membrane protein, unrelated to invasin, which appears to have a secondary role, possibly contributing to persistence within the Peyer's patches.
- Toxin production. The bacterial chromosome encodes a heat-stable enterotoxin *Yst*, that causes diarrhea. The plasmid encodes a number of factors, collectively known as *Yops*., which are postulated to form a pore to enable passage through the eukaryotic cell membrane. The lipopolysaccharide cell wall has also been shown to influence virulence, possibly by increasing cell hydrophilicity, or in association with *ail* and *YadA*.
- HPI. Free iron is essential for bacterial growth; however, in the human host, it is actively bound by transferrin and lactoferrin, or incorporated into heme groups, and is thus present in only very low amounts. The chromosomal HPI associated with biotype 1B strains encodes synthesis, regulation, and transport of a high-affinity iron chelator (known as a siderophore), yersiniabactin, which enhances iron uptake by the cell. Other strains are, however, capable of making use of siderophores from other sources, hence the association between deferroxamine treatment and systemic infections. Biotype 1B strains possess a further pathogenicity island (Ysa-PI) that is associated with colonization of gastrointestinal tissues during the initial phases of infection.

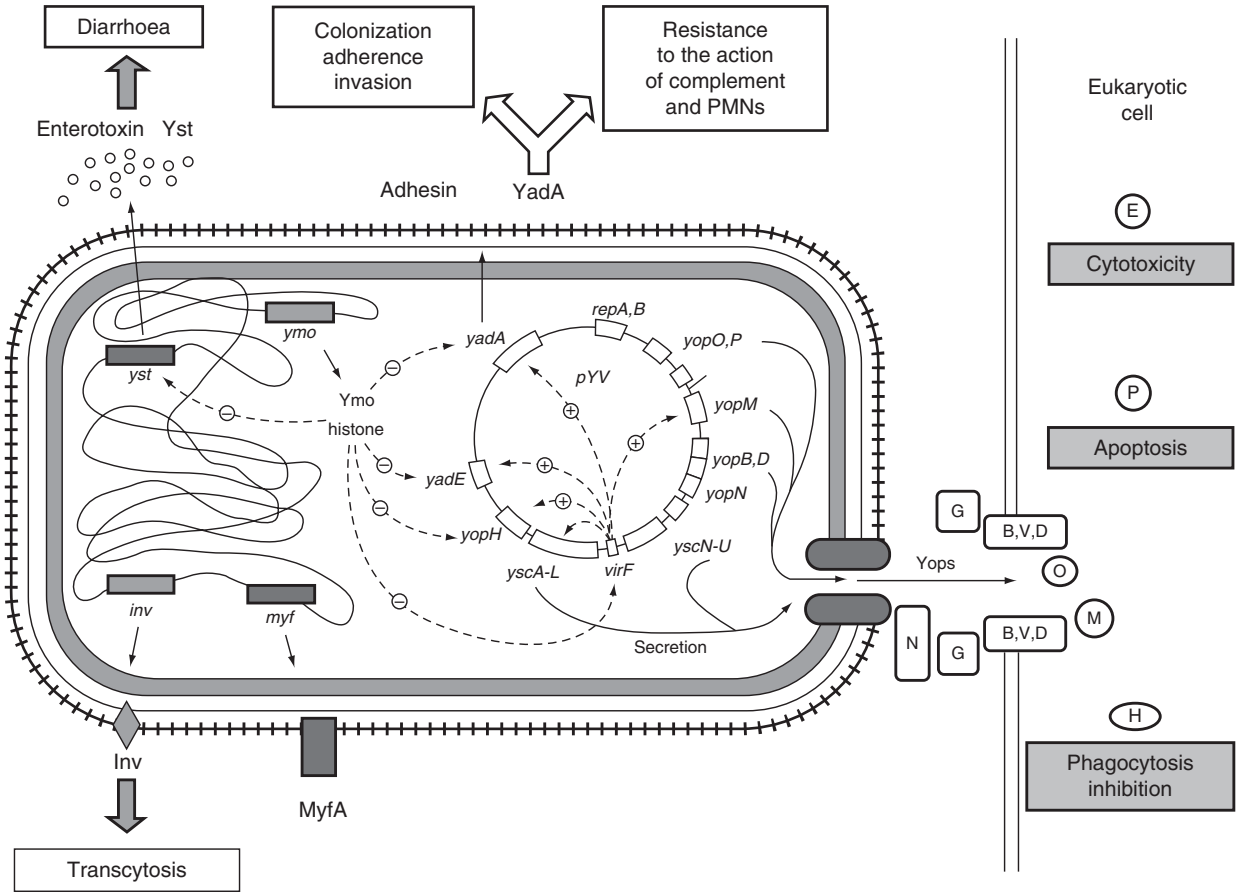

Figure 2 Simplified overview of the virulence factors of *Yersinia enterocolitica*. (PMN, polymorphonuclear cells). Reproduced from Sory, M., Cornelis, G., 2000. Virulence determinants of the bacterial pathogen *Yersinia enterocolitica*: mode of action and global regulation. In: Cary, J., Linz, J., Bhatnager, D. (Eds.), Microbial Foodborne Diseases – Mechanisms of Pathogenesis and Toxin Synthesis. Lancaster, PA: Technomic, pp. 131–155, with permission from CRC Press.

Environmental factors (temperature, pH, ionic strength) regulate optimal expression of these factors. To give examples, *Yersinia* outer-membrane proteins (*Yops*) and the adhesin YadA, are induced at 37 °C, whereas urease production is induced at 25 °C. A number of regulatory genes, such as plasmid *VirF* and chromosomal *ymo*, are involved in mediating this expression.

The interaction between these factors and the consequences for infection are graphically portrayed in Figure 2.

Isolation and Identification

Established methods for detection of *Y. enterocolitica* continue to rely on traditional enrichment and selective plating protocols. Owing to the heterogeneous nature of *Y. enterocolitica*, no individual enrichment scheme will be effective for isolating all serotypes or biotypes. Consequently, multiple enrichment and plating approaches are utilized. Nevertheless, conventional culture procedures are likely to significantly underestimate the presence of *Y. enterocolitica*. Factors that may influence this include background microbiota present on the meat/product being tested and the proliferation of this microbiota during sample enrichment, resulting in suppression of *Yersinia* spp. present in the sample. Certain culture conditions may also result in an inability to determine the pathogenicity of an isolate due to loss of the plasmids coding for virulence factors. Quantitative analysis is normally not performed from meat or meat environments, although modification of existing methods using multiple-tube techniques is feasible.

A number of molecular-based methods have been published in the scientific literature for the detection of *Y. enterocolitica* in foods. To date, however, only one has been formally gazetted as a reference method. Nordisk Metodikkomité for Næringsmidler method 163 has two options: one for detecting potentially pathogenic strains based on the *ail* gene (method A), and the other for detecting fully virulent bacteria based on *yadA* (method B). Method A is validated for detection of serotypes O:3, O:9, O:8, and O5,27. Method B is considered suitable for detecting all pathogenic variants.

Isolation and identification of an organism from meat as *Y. enterocolitica* is incomplete without determination of virulence expression. Although the plasmid essential for full virulence expression is stable at 25–28 °C, the physiological traits are only expressed at 37 °C, at which temperature the

plasmid becomes unstable *in vitro*. In consequence, detection is hampered by protocols employing 35 °C or 37 °C incubation temperatures for isolation, as is normal in clinical laboratories.

Sample Preparation

Initial suspensions of 1:5 weight to volume for meat samples, or a method to achieve a 1:2 ratio of surface area to volume for swabbed surfaces, are made in an appropriate diluent such as 0.01 mol l^{-1} phosphate-buffered saline (pH 7.6).

Enrichment

Various approaches have been adopted for enrichment of *Y. enterocolitica*. Advantage is taken of the psychrotrophic nature of yersiniae to outgrow mesophilic organisms at low temperatures. The concern with all cold enrichment protocols is the compromise, which must be made, between achieving maximum recovery and having a timely result available for appropriate action to be taken when a product is found to be contaminated. Other methods have, therefore, been developed incorporating antimicrobials (e.g., irgasan, ticarcillin) into more nutritious culture media. Finally, some protocols utilize the resistance of the organism to high pH.

Typical cold enrichment methods utilize nonselective media including phosphate-buffered saline (PBS), PBS plus 1% sorbitol, or tryptic soy broth. Incubation time depends on temperature; at 4 °C, incubation for 14–21 days is normally recommended, but this can be shortened to 3 days by increasing the temperature to 10 °C. Cold enrichment has also been applied with peptone sorbitol bile broth (American Public Health Association (APHA)/Food and Drug Administration (FDA)).

Selective antimicrobial enrichment broths include bile oxalate sorbose broth (BOS), irgasan ticarcillin cholate broth (ITC), and modified Rappaport medium (MRM). BOS is best-suited for recovery of serotype O:8, whereas the latter two media are more suited to isolation of serotype O:3 and O:9 strains. The enrichment broths are inoculated either direct from sample homogenates or from cold-enriched cultures at ratios of 1 volume inoculum to 100 volumes broth, with incubation typically being at 22–25 °C for 3 days (with BOS being further incubated for another 2 days and then subcultured again).

Treatment of cold enrichment cultures with alkali (1–9 parts 0.25% KOH in 0.5% aqueous NaCl, for 3–4 s) has been shown to significantly reduce background microbiota before plating. This procedure has also been applied to BOS cultures, but is not appropriate for ITC/MRM.

Isolation

Although yersiniae can be isolated on general enteric plating media (e.g., MacConkey agar), specific media have been developed for isolation of *Y. enterocolitica*. These include cefsulodin irgasan novobiocin agar (CIN) and Salmonella–Shigella agar modified by addition of desoxycholate and calcium (SSDC) agar. CIN and SSDC plates are routinely incubated at 30–32 °C for 24 h. On CIN agar, characteristic *Y. enterocolitica* colonies are small (≤1 mm), smooth, with red center, and translucent rim. On SSDC, they are also small (≤1 mm), but gray colored, with an indistinct rim.

Identification

A convenient initial screen involves the inoculation of Kliger iron agar (KIA) and urea agar slants, and incubating them at 25–28 °C for 24–48 h. Isolates that produce an alkaline slope and acid butt with little or no gas production in KIA, and are urease positive require further investigation.

Definitive identification of *Y. enterocolitica* to the biotype level requires a test for motility and battery of biochemical tests (see Tables 1 and 2 for reactions). These should all be performed at 25–30 °C. Commercially available identification systems may be utilized, but these should also be incubated at 25–30 °C; and the results should be evaluated critically, as the majority of the test systems available do not include all of the species in their databases, nor do they contain the tests necessary to discriminate between all of the species within the '*Y. enterocolitica*-like' group.

Serotyping is not called for in routine testing protocols; however, if it is required for epidemiological purposes, antisera are commercially available (Denka Senken antisera are available through Oxoid, Basingstoke, UK. Antisera can now also be sourced from SSI, Copenhagen, Denmark).

Tests of Pathogenicity

The most commonly used test for pathogenicity is that of Congo red/crystal violet binding, and calcium dependency, which is performed on Congo red magnesium oxalate agar (CR-MOX). A suspension that will give discrete colonies is spread-inoculated onto CR-MOX and incubated at 37 °C for 24 h. Pathogenic organisms produce tiny red colonies, indicative of Congo red binding and calcium dependency. Crystal violet binding is indicated by flooding this plate with 85 μg ml^{-1} aqueous crystal violet and decanting off the dye after 2 min. Pathogenic isolates display small, intensely purple colonies.

Other phenotypic indicators of pathogenicity include autoagglutination at 37 °C and negative esculin, pyrazinamidase, and salicin reactions. Polymerase chain reaction techniques have been devised for detection of virulence genes, but these are currently utilized only by reference and research groups.

Epidemiology

The *Y. enterocolitica* group is found worldwide and is distributed widely in the environment, owing in no small part to the organisms' ability to remain metabolically active at extremes of temperature, nutrients, and pH.

Yersinia enterocolitica has been isolated from lakes, streams, well water, soil and vegetables, animal sources including humans, pigs, dogs, cats, ruminants – particularly deer and goats, rodents, and monkeys, and invertebrates such as crabs, snails, and molluscs.

By far the most commonly identified source of pathogenic strains has been pigs. There is a particular association with pig tonsils, though studies have found offal meats, notably tongue, heart, liver, and kidney, together with minced pork, to carry significant levels of *Y. enterocolitica* contamination. *Yersinia enterocolitica* O:3 isolates from healthy pigs and human infections were found to be indistinguishable in Australian studies using a number of molecular fingerprinting techniques. It has been postulated that cross-contamination from pork occurs directly or indirectly by contact with equipment and foodhandlers or through the air in meat processing facilities, retail shops, and kitchens. As a psychrotolerant organism, *Y. enterocolitica* can multiply at all stages along the cold chain from meat producer to home refrigerator.

Household pets are possible intermediate hosts, particularly for transmission to small children. Direct human-to-human transmission may also occur via the fecal–oral route and via asymptomatic carriers.

Meat products associated with *Y. enterocolitica* contamination include pork, game meats, beef, lamb, and poultry. An association has also been found with washing of food in untreated water. Outbreaks of *Y. enterocolitica* infection have been associated with well water, streams, milk, pork, cheese, chocolate milk, chitterlings (pork intestines), and raw vegetables. It should be noted, however, that many of the studies outside of swine have failed to establish the pathogenicity of isolates.

Control Measures

The temperature range for growth of *Y. enterocolitica* is very wide; in optimal conditions growth has been recorded between −1.3 °C and 42 °C. The organism will also grow at pH values between 4.2 and 9.6 and at salt concentrations up to 5% NaCl. There is thus a high potential for growth on chilled, vacuum-packed meats and meat products. However, several studies have shown that pathogenic strains will multiply poorly in the presence of competing organisms such as *Hafnia alvei* and avirulent *Yersinia* spp. Yersiniae survive freezing and remain viable for long periods in frozen food and after thawing. The organism is also relatively resistant to chlorine, particularly in aquatic environments containing predatory protozoa.

Potassium sorbate (in mildly acidic conditions) is an effective inhibitor of *Y. enterocolitica* in preserved foods. Also some inhibition at 5 °C has been observed with use of 150 ppm sodium nitrite.

Yersinia enterocolitica is susceptible to heat, being destroyed by pasteurization at 71.8 °C for 18 s or 1–3 min at 60 °C.

Because pigs are implicated as a major source of infections (particularly with bioserotype 4, O:3, which is the type most commonly isolated worldwide from human infection), interest has centered on means of reducing this contamination. *Yersinia enterocolitica* is unlikely to be detected by traditional post-mortem inspection. Indeed, traditional inspection processes, such as submaxillary lymph node excision to detect tuberculosis, may result in transfer of *Y. enterocolitica* to other tissues. Control measures remain the subject of debate, but removal of the head, or at least the tongue and pharynx, as early as possible in the dressing process and certainly before evisceration, has been recommended, with these tissues being removed to a separate location for inspection away from the carcass. Stringent application of other good manufacturing practices, such as bung sealing and careful cleaning of the environment during slaughter and dressing, will further minimize the risk.

In retail environments, and kitchens, it is important to separate raw pork from ready-to-eat foods. Normal techniques for food and personal hygiene will also reduce the risk of infection. The eating of raw pork, which is customary in some countries, is to be discouraged.

See also: Chemical Analysis: Standard Methods. Foodborne Zoonoses. Meat Marketing: Cold Chain. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat; Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Analysis: DNA Methods. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium Perfringens*; Emerging Pathogens; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*. Modeling in Meat Science: Microbiology. Nutrition of Meat Animals: Pigs. Species of Meat Animals: Game and Exotic Animals; Pigs; Sheep and Goats

Further Reading

Barton, M., Robins-Browne, R., 2003. *Yersinia enterocolitica*. In: Hocking, A. (Ed.), Foodborne Microorganisms of Public Health Significance, 6th edn. Waterloo, Australia: AIFST Inc. (NSW Branch), pp. 577–595.

Bhagat, N., Virdi, J., 2011. The enigma of *Yersinia enterocolitica* biovar 1A. Critical Reviews in Microbiology 37 (1), 25–39.

Bottone, E., 1999. *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbes and Infection 1, 323–333.

Carniel, E., Autenrieth, I., Cornelis, G., *et al*., 2006. *Y. enterocolitica* and *Y. pseudotuberculosis*. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes, 3rd edn, vol. 6. Singapore: Springer, pp. 270–398.

Drummond, N., Murphy, B., Ringwood, T., *et al*., 2010. *Yersinia enterocolitica:* a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. Foodborne Pathogens and Disease 9 (3), 1–11.

Fàbrega, a., Vila, J., 2012. *Yersinia enterocolitica*: pathogenesis, virulence and antimicrobial resistance. Enfermedades Infecciosas y Microbiología Clínica 30 (1), 24–32.

Fredriksson-Ahomaa, M., 2007. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In: Simjee, S. (Ed.), Infectious Disease: Foodborne Diseases. Totowa, NJ: Humana Press, pp. 9–113.

Fredriksson-Ahomaa, M., Lindström, M., Korkeala, H., 2010. Yersinia enterocolitica and Yersinia pseudotuberculosis. In: Juneja, V., Sofos, J. (Eds.), Pathogens and Toxins in Food: Challenges and Interventions. Washington, DC: ASM Press, pp. 164–180. Chapter 11.

ICMSF, 1996. Yersinia enterocolitica. In: Roberts, T., Baird-Parker, A., Tompkin, R. (Eds.), Micro-organisms in Foods 5 – Characteristics of Microbial Pathogens. London: Blackie Academic and Professional, pp. 458–492.

Nesbakken, T., 2005. *Yersinia enterocolitica*. In: Fratamico, M., Bhunia, A., Smith, J. (Eds.), Foodborne Pathogens – Microbiology and Molecular Biology. Wymondham, UK: Caister Academic Press, pp. 228–249.

Perry, R., Fetherston, J., 2007. The Genus Yersinia. From Genomics to Function. Advances in Experimental Medicine and Biology, vol. 603. New York: Springer.

Robins-Browne, R., 2007. *Yersinia enterocolitica*. In: Doyle, M., Beuchat, L. (Eds.), Food Microbiology – Fundamentals and Frontiers. Washington, DC: American Society for Microbiology, pp. 293–321.

Skurnik, M., Bengoechea, J., Granfors, K., 2003. The Genus Yersinia. Entering the Functional Genomic Era. Advances in Experimental Medicine and Biology, vol. 529. New York: Kluwer Academic/Plenum.

Sory, M., Cornelis, G., 2000. Virulence determinants of the bacterial pathogen *Yersinia enterocolitica*: mode of action and global regulation. In: Cary, J., Linz, J., Bhatnager, D. (Eds.), Microbial Foodborne Diseases – Mechanisms of Pathogenesis and Toxin Synthesis. Lancaster, PA: Technomic, pp. 131–155.

Thisted Lambertz, S., Granath, K., Fredriksson-Ahoma, M., Johansson, K.E., Danielsson-Tham, M-L., 2007. Evaluation of a combined culture and PCR method (NMKL-163A) for the detection of presumptive pathogenic *Yersinia enterocolitica* in pork products. Journal of Food Protection 70 (2), 335–340.

Weagent, S., Feng, P., 2007. *Yersinia enterocolitica.* In: Hammack, T., *et al.*, (Eds.), Bacteriological Analytical Manual. Silver Spring, MD: US Food and Drug Administration (Chapter 8). Available from: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm

MICROORGANISMS AND RESISTANCE TO ANTIBIOTICS, THE UBIQUITY OF

Contents

Antibiotic Resistance by Microorganisms

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Glossary

Acquired resistance Resistance that is obtained as a result of exposure to the antibiotic being administered. It typically happens through spontaneous mutation, transformation (uptake of naked DNA), transduction (DNA transferred via bacteriophages), or conjugation (cell-to-cell contact).

Commensal bacteria Bacteria that are part of the normal flora that exist within the host without causing disease or gaining any real benefit from the host.

Intrinsic resistance Resistance that is inherently part of the bacterium through structural or functional properties of the bacterium itself. The bacterium has never been susceptible to an antibiotic to which it is intrinsically resistant.

Nontarget bacteria Bacteria in the host that are exposed to the antibiotic used in treatment of the target pathogen. These bacteria may develop resistance to the antibiotic as a consequence of residing in the host when the antibiotic was administered to treat a target pathogen.

Target pathogen The bacterium or disease complex for which the antibiotic was developed and marketed for treatment.

Zoonotic bacteria Bacteria that can be transferred between animals and man and vice versa.

Introduction

Antibiotics in Human and Veterinary Medicine

Antibiotic use in food-animal production can illicit great debate among scientists regarding the merit and contribution to increased public health costs and disease. Regardless of the discussion, the fact is that antibiotics are used, and are necessary, in both human and veterinary medicine, including food-animal production. Guidelines have been established in both the medical and veterinary professions, which underscore the need to educate patients and producers on the proper use of antibiotics. Consumers are also more aware and involved in the antibiotic dialog. They have increased their demand for meats produced without the use of antibiotics, which is noticeable in the increased share of space given to antibiotic-free meat in the marketplace.

Antibiotics were first developed for use in human medicine; related applications or compounds were developed later for use in veterinary medicine. As an example, tetracycline was first used in human medicine. Currently, oxytetracycline and chlortetracycline are most often used in veterinary medicine while doxycycline and minocycline are most often used in human medicine. Ceftriaxone, a third generation cephalosporin, was developed for use in human medicine; later, ceftiofur, also a third generation cephalosporin, was developed for use in veterinary medicine.

The vast majority of antibiotic compounds used today originated from the soil or fungi. As resistance developed to the original antibiotic, the 'next generation' antibiotic was often developed by manipulating the chemical structure of the initial compound. Subsequent generations were developed the same way. However, as resistance developed to the newest generation of antibiotics within a given class of antibiotics, resistance was usually also conferred to all lower generations of antibiotics within the same class. This can make an entire class of antibiotics ineffective against certain bacterial populations or bacterial strains.

Antibiotics and Use

Antibiotics can be developed for either narrow- or broad-spectrum activity against a particular microorganism or a wide range of microorganisms. Regardless of their activity, antibiotics are labeled and marketed for a specific use. This can be

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for a specific disease related to a single bacterium, multiple bacteria, a disease complex (i.e., bovine respiratory disease (BRD) in veterinary medicine), or some other use. Treatment of BRD can include the recommendation to use third generation cephalosporins, fluoroquinolones, florfenicol (a relative of chloramphenicol) or tilmicosin, a macrolide antibiotic. These are intended to treat bacterial pneumonias caused by *Pasteurella* species or *Haemophilus somnus*.

Fortunately, these bacteria do not cause disease in humans and are not transferred in meat. However, use of any antibiotic will confer resistance to nontarget bacteria as well as target bacteria throughout the host. This happens in human medicine as well; for example, when children are given an antibiotic for ear infections or adults are treated for sinus or respiratory infections. As treatment continues, and in concert with the host immune system, target bacteria are killed and eliminated from the host; the illness suppresses and the animal or human is cured and feels better. However, it is possible that as treatment continues resistance develops in the target bacteria and an unintended consequence develops as a resistant nontarget population of bacteria also develops. This nontarget resistant bacterial population can persist within the host and/or be shed into the environment. Also, the resistant genes in these bacteria can also be transferred to other nonresistant bacteria conferring resistance. One population of nontarget bacteria are commensal bacteria that are discussed below in the Section Commensal Bacteria.

Target versus Zoonotic Pathogens

As described above in the Section Antibiotics and Use, antibiotics in veterinary medicine are commonly used to treat specific bacterial diseases in animals that do not transfer to humans. One exception is *Salmonella*. More than 2500 serotypes of *Salmonella* have been named excluding antigenic formulas. Of these, some are known to be host-adapted, such as Dublin for cattle and Choleraesuis for swine. These host-adapted serotypes can cause serious disease within their respective host and antibiotic treatments are often used when disease arises. The other serotype that is not host-adapted but does cause serious illness among most food animals is *Salmonella* Typhimurium. Mostly, zoonotic pathogens typically do not cause disease in animals and behave in a commensal-like relationship; they persist within the host without causing overt disease. However, when animals are treated with antibiotics for other diseases meant for a specific target pathogen, zoonotic bacteria are also exposed to the antibiotic and can become resistant.

Antibiotic Treatment in Humans

As described in other articles, foodborne disease results in a large number of cases of human illness on a global, national, and local level costing hundreds of millions of dollars annually. However, gastroenteritis is typically self-limiting and resolves in 5–7 days, thus the use of antibiotics is contraindicated. However, antibiotic treatment might be warranted in the young, the elderly, the immunocompromised or when septicemia occurs. Antibiotics are always contraindicated for use in infection with Shiga-toxin-producing *Escherichia coli* (STEC).

Antimicrobial resistance is a global problem. Multiple-antimicrobial-resistance compounds the problem because treatment options can become severely limited. This has occurred in human medicine as observed with extensively drug-resistant tuberculosis. Once heralded as 'miracle drugs,' limited access to antibiotics could drastically impact the global population that is expected to reach approximately 10 billion people by 2050. Further, our food supply must grow to meet the protein demands of this population, doubling by 2050. Because no additional land mass will be available, as with current intensive food-animal production practices, future food animal production will also require the use of antibiotics, particularly for the prevention and treatment of disease. Therefore, the development of resistant bacteria in human and veterinary medicine is inevitable. Our ability to manage and control these antimicrobial resistant populations is critical.

Zoonotic Bacteria

Salmonella

Zoonotic bacteria can be transferred between animals and humans and are most often associated with meat. The most common zoonotic bacteria are *Salmonella* and *Campylobacter*. As described in other articles, these bacteria are ubiquitous in nature and have been recovered from nearly all vertebrate animals. As a result of their wide ecological exposure and association with food animals and humans, bacteria [including zoonotic bacteria] are exposed to natural and man-made antibiotics. Because many antibiotics originated from the soil, and as hospital waste water, industrial runoff, pasture runoff, animal manure, and waste treatment plants can contribute some level of active metabolite, the complexity associated with sorting out exposure levels and understanding (even in part) the diverse interaction within the ecosystem between bacteria, man, animal, and environment is critical.

Antimicrobial resistance in *Salmonella* has been studied widely. However, there are several critically important things to remember when interpreting antimicrobial resistance in *Salmonella*: (1) While one can obtain a 10 000-ft view by analyzing resistance using total '*Salmonella*' for any one antibiotic, it is paramount to assess resistance at the serotype level. This is critically important as the acquisition of resistance (acquired resistance) does not occur equally between serotypes. Some serotypes are resistant to many classes of antibiotics and many antibiotics within a class. They can also acquire resistance from mobile genetic elements (in particular plasmids and transposons), which appear to have carried resistance attributes for a very long period of time, perhaps before some antibiotics were used. *Salmonella* Typhimurium and its association with the Inc A/C plasmid and multiple-drug resistance is a good example of this. (2) The predominant serotypes vary among animal sources and they can vary among years. As a result, an analysis must be done within each food animal source. For instance, although the top two serotypes recovered from humans and animal sources may remain fairly consistent, changes within the top ten serotypes recovered

from year to year are often observed. (3) Within a particular serotype, genetic diversity is observed, which indicates that there can be heterogeneity among isolates within a serotype. This is particularly critical when one begins describing a 'serotype.' For instance, analysis of PulseNet or USDA VetNet or most molecular data associated with many different isolates within a particular serotype will identify differences in antimicrobial resistance attributes and/or molecular or virulence attributes and/or temporal, demographic, or source data that clearly indicate a difference between the isolates. Therefore, one cannot simply talk about 'all *Salmonella* Typhimurium or *Salmonella* Kentucky, or *Salmonella* Derby, etc.' as they may in fact, not have the same genotypic and phenotypic characteristics or respond the same to antimicrobial treatments or intervention and control measures.

Fluoroquinolones (ciprofloxacin) and extended-spectrum cephalosporins, particularly the third generation cephalosporin (ceftriaxone), are important in human medicine for treating complicated zoonotic infections. In the US, a fluoroquinolone is licensed for treating respiratory infections in swine and cattle and ceftiofur, a third generation cephalosporin, is licensed for use in food animal production. Recently, ciprofloxacin resistance has emerged in some *Salmonella* serotypes. Resistance to the cephalosporins has also begun to rise among certain serotypes in humans and food animals. In addition, extended-spectrum beta-lacatamases have also been detected in increasing frequency globally. Beta-lactamases are unique enzymes produced by some bacteria making them resistant to antimicrobials such as penicillins and carbapenems; carbapenem antibiotics may be a last line of treatment for some diseases.

In developing countries where antibiotics can be purchased over the counter for use in both human and veterinary medicine, or in countries where antibiotic use practices are not tightly monitored, resistance to the fluoroquinolone and cephalosporin classes of antibiotics can be quite high. The World Health Organization and other global bodies are working to develop programs to mitigate antimicrobial resistance in these instances and have taken steps to publish lists of critically important antimicrobials.

Multiple-drug resistance, most often defined as resistance to three or more classes of antibiotics, is also dependent on serotype. A most interesting factor is the emergence of large plasmids carrying multiple resistance genes that appear to easily move between and within *Salmonella* serotypes. A classic example is the emergence of definitive type (DT) 104 Typhimurium in England in the late 1990s. DT104 carries penta-resistance to five antimicrobials: Ampicillin, Chloramphenicol, Streptomycin, Sulfamethoxazole, and Tetracycline (ACSSuT). It was most often associated with carriage in cattle although it was also isolated from cats, horses, foxes, and other animals. Disease was noted in both humans and animals, rose to epidemic status, eventually resolved, and the strain, while recovered in low numbers from animals, humans and meats, ceased to cause significant disease. Although DT104 has been transmitted globally, in the US, the UK epidemic strain was never isolated although other strains of DT104 were recovered from food animals (primarily cattle and swine including retail meat) and were particularly predominant in the Pacific Northwest. In the US, *Salmonella* Newport and Dublin also acquired ACSSuT resistance through acquisition of a large plasmid emerging predominantly in isolates recovered from cattle and ground beef.

It is interesting to observe that for countries with *Salmonella*-monitoring/surveillance systems, antimicrobial resistance among *Salmonella* isolates originating from humans can often exhibit no resistance to all antimicrobials tested. When resistance does occur to one antibiotic it is most often to tetracycline. Multiple-drug resistance to three antibiotics is most often to tetracycline, streptomycin, and the sulfa antibiotics. These three antibiotics were the first developed more than 50 years ago and have been in use longer than the other classes of antibiotics. It is quite interesting to observe that it is unusual to encounter 100% resistance to these three antibiotics either alone or in combination in a population of bacteria unless they were acquired from a specific source and the antibiotic use practice was known.

Campylobacter

Antimicrobial resistance in *Campylobacter* has been studied primarily in *Campylobacter jejuni* and *Campylobacter coli*. *Campylobacter jejuni* is most often associated with disease in humans. Although *C. jejuni* can be recovered from all food animal species, it is most prevalent and disease is most often associated with consumption of poultry, both chicken and turkey. Antimicrobial resistance has been observed predominantly to tetracycline, the fluoroquinolones, and the macrolide antimicrobials. In the mid-2000s, the fluoroquinolones were withdrawn from use in the US poultry industry. Since then, resistance to ciprofloxacin in *C. jejuni* recovered from humans, US retail chicken breasts and chicken carcasses postslaughter has not changed significantly. Interestingly, resistance to erythromycin, the antimicrobial of choice when treatment is indicated, remained less than 2% for the same time period in humans. Recently, resistance to gentamicin has emerged in *C. jejuni*.

Campylobacter coli is less often associated with disease in humans and is the predominant species recovered from swine. Antimicrobial resistance is most often observed to tetracycline, the fluoroquinolones, the lincosamides, and the macrolides. However, in the US, resistance has also emerged to the aminoglycoside, gentamicin as mentioned above for *C. jejuni*. Historically, antimicrobial resistance has always been higher in *C. coli* when compared to *C. jejuni*. The reason for this phenomenon is unknown.

Aquaculture

Food animals have specifically implied beef cattle, dairy cattle, swine, chickens, and turkeys, whereas *Salmonella* and *Campylobacter*, as well as other zoonotic bacteria, have been implicated in seafood outbreaks globally. Although antibiotics for use in the industry are limited, resistance has been reported to tetracycline.

Commensal Bacteria

Commensal bacteria are part of the normal flora that exists within the host without causing disease or gaining any real

benefit from the host. Interestingly, with the exception of those serotypes of *Salmonella* that cause disease in some animals, *Salmonella* can have a commensal-like relationship in food animals as can *Campylobacter* species. Because animals show no overt sign of disease, it is difficult to determine if an animal is carrying a zoonotic pathogen, regardless if it is resistant or not, into the processing plant before the derived meat enters the retail market.

However, that is only part of the story of commensal bacteria. *Escherichia coli* and *Enterococcus* species are often referred to as commensal bacteria and they are often included in antimicrobial resistance monitoring systems. Their inclusion is predicated on evidence that they serve as reservoirs of resistance genes, which can be passed to both nonpathogenic and pathogenic bacteria including the foodborne zoonotic pathogens *Salmonella* and *Campylobacter*.

Escherichia coli are Gram-negative bacteria and often respond in the same manner to selective antimicrobial pressures as do *Salmonella*. As such, it is not unusual for it to be interchanged with *Salmonella* as the sentinel organism in antimicrobial monitoring systems. *Enterococcus* species are Gram-positive bacteria; they also respond well to selective antimicrobial pressures and provide antimicrobial resistance information for Gram-positive bacteria being studied. Both are ubiquitous in humans, animals, and the ecosystem and they are easy to isolate. No one 'serotype' or strain of *E. coli* is selected for study; these are referred to as 'generic' *E. coli*. For *Enterococcus*, however, the species of choice are *Enterococcus faecium* and *Enterococcus faecalis*, which are important in human illness.

The same antibiotics are monitored for *E. coli* as for *Salmonella*. For *E. faecium*, of particular interest is resistance to the human antibiotic quinupristin/dalfopristin since a comparable antibiotic, virginiamycin, was licensed for use in veterinary medicine as a feed additive to promote growth. For both *E. faecium* and *E. faecalis*, resistance to the human antibiotic vancomycin is also important as the comparable veterinary antibiotic, avoparcin, was also available for use (avoparcin was never used in the US). Depending upon the socioeconomic status of the country, resistance to both antibiotics can vary. In the European Union, use of antimicrobial growth promoters was banned approximately 15 years ago, including avoparcin and virginiamycin. Since then, low levels of resistance to quinupristin/dalfopristin continue to persist in some countries. Virginiamycin is still used in the US.

Other Bacteria Associated with Food Animals

Methicillin-Resistant *Staphylococcus aureus*

Although methicillin-resistant *Staphylococcus aureus* (MRSA) has been associated with both healthcare (healthcare-associated; HA-MRSA) and the community (community-acquired; CA-MRSA), there is much debate over the association with meat and meat products. One strain, CC398, is called livestock-associated MRSA (LA-MRSA) and appears to be different from previous strains recovered from humans. It has been recovered from swine, cattle, and poultry and its association with meat and meat products is also under study. However, once in the animal population, amplification occurs through production practices in which large numbers of animals are comingled thus making it nearly impossible to stop the spread of bacteria among animals (especially on the skin). This almost assures colonization and exposure for the farmer/owner or production worker once contact with the animal(s) occurs.

Clostridium difficile

Clostridium difficile has been recovered from neonatal swine and calves as well as mature swine and dairy cattle. While it has been suggested that community-acquired *C. difficile* associated disease (CA-CDAD) can be facilitated by contact with ill animals or consumption of contaminated meat, additional studies are needed to confirm this. Antimicrobial resistance among *C. difficile* has been evaluated and resistance has been observed to the lincosamides, macrolides, penicillins, and fluoroquinolones in particular. As observed for other bacteria, the species of food animal plays a role in resistance as isolates recovered from one animal source can be more or less resistant to some antibiotics than isolates recovered from another animal source. This would not be unexpected as antibiotic use is specific for production of a particular food animal.

Other Factors to Consider

Travel

Travel appears to play a major role in the acquisition of resistant bacteria from meat, food, and food stuffs. Developed countries tend to report lower levels of resistance among bacteria collected within their respective countries. However, for people who travel and return with a foodborne resistant infection, the resistant isolate is often attributed to the country of travel, which may not control the use of antibiotics in the same manner as the country of origin. Therefore, it is important to consider the type of food eaten and hand washing hygiene when traveling to minimize the risk of acquiring a resistant foodborne pathogen.

Proper Handling, Storage, and Cooking

Proper handling and storage are critical components to the control of foodborne infections regardless of the presence or absence of resistant bacteria. The importance of safe storage, not mixing raw and cooked products, separate processing areas in the kitchen for vegetables and meats, proper cleaning and sanitizing of surface areas and utensils, hand washing, and (most importantly) utilizing proper cooking temperatures cannot be over emphasized. The presence of resistant bacteria makes this even more critical.

Conclusions and Summary

Antimicrobial resistance is of global concern. It takes a minimum of 10 years from discovery of a potential new antibiotic through testing to market. Unfortunately, there are very few new antibiotics in development for the foreseeable future.

Therefore, as bacterial resistance to multiple antibiotics use increases, treatment options in both veterinary and human medicine become more limited, which impacts animal health, the wholesomeness of food, and human health. Further studies are needed to develop mitigation and control measures to arrest the current progression of antimicrobial resistance. Surveillance and monitoring efforts should be expanded to cover more countries, more bacteria, and provide timely global reporting. Finally, scientists should be encouraged to find newer, novel antibiotics and be less dependent upon the need to manipulate the chemical structure of existing antimicrobials. Today, scientists have developed fifth-generation cephalosporins. Bacteria have one purpose survival. If we keep exposing them only to chemical structural changes and nothing new or novel, they will continue to develop resistance mechanisms faster, shortening the useful availability of the antibiotic even further.

See also: Environmental Contaminants. Foodborne Zoonoses. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants. Microbial Contamination: Microbial Contamination of Fresh Meat; Microbial Contamination of Processed Meat. Microbiological Safety of Meat: Emerging Pathogens; *Listeria monocytogenes*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Potential Environmental and Wildlife Sources of Microorganisms in Meat. Quality Management: Abattoirs and Processing Plants. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

Further Reading

Carcia-Alvarez, L., Dawson, S., Cookson, B., Hawkey, P., 2012. Working across the veterinary and human health sectors. Journal of Antimicrobial Chemotherapy 67, i37–i49.

Folster, J.P., Pecic, G., Rickert, R., *et al*., 2012. Characterization of multidrug-resistant *Salmonella enterica* serovar Heidelberg from a ground turkey-associated outbreak in the United States, 2011. Antimicrobial Agents and Chemotherapy 56, 3465–3466.

Graveland, H., Duim, B., van Duijkeren, E., *et al*., 2011. Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. International Journal of Medical Microbiology 301, 630–634.

Green, A.L., Dargatz, D.A., Wagner, B.A., *et al*., 2010. Analysis of risk factors associated with *Salmonella* spp. isolated from US feedlot cattle. Foodborne Pathogens and Disease 7, 825–833.

Iovine, N.M., 2013. Resistance mechanisms in *Campylobacter jejuni*. Virulence 4, 230–240.

Jackson, C.R., Fedorka-Cray, P.J., Anandaraman, N., *et al*., 2007. Introduction to USDA VetNet: Status of *Salmonella* and *Campylobacter* databases from 2004 through 2005. Foodborne Pathogens and Diseases 4, 241–248.

Jackson, C.R., Lombard, J.E., Dargatz, D.A., *et al*., 2011. Prevalence, species distribution and antimicrobial resistance of enterococci isolated from US dairy cattle. Letters in Applied Microbiology 52, 41–48.

Lienaba, E., Carattoli, A., Coque, T.M., *et al*., 2013. Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals I An EU perspective of epidemiology, analytical methods, risk factors, and control options. Clinical Infectious Diseases 56, 1030–1037.

Lindsey, R.L., Frye, J.G., Fedorka-Cray, P.J., *et al*., 2011. Microarray-based analysis of incA/C plasmid-associated genes from multidrug-resistant *Salmonella enterica*. Applied and Environmental Microbiology 77, 6991–6999.

McEwen, S.A., Fedorka-Cray, P.J., 2002. Antimicrobial use and resistance in animals. Clinical Infectious Diseases 34 (Suppl. 3), S93–S106.

Molla, B., Byrne, M., Abley, M., *et al*., 2012. Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) of porcine origin. Journal of Clinical Microbiology 50, 3687–3693.

Nilsson, O., 2012. Vancomycin resistant enterococci in farm animals – Occurrence and importance. Infection Ecology and Epidemiology 2, 16959. Available at: http://dx.doi.org/10.3402/iee.v2i0.16959 (accessed 20.10.13).

Szmolka, A., Nagy, B., 2013. Multidrug resistant commensal *Esherichia coli* in animals and its impact for public health. Frontiers in Microbiology 4, 1–13.

Trott, D., 2013. Beta-lactam resistance in gram-negative pathogens isolated from animals. Current Pharmacology Design 19, 239–249.

Weir, M., Rajic, A., Dutil, L., *et al*., 2012. Zoonotic bacteria and antimicrobial resistance in aquaculture: Opportunities for surveillance in Canada. Canadian Veterinary Journal 53, 619–622.

Relevant Websites

http://www.cdc.gov/narms/index.html
Centers for Disease Control and Prevention.

http://www.cdc.gov/pulsenet/
Centers for Disease Control and Prevention.

http://cspinet.org/new/pdf/outbreaks_antibiotic_resistance_in_foodborne_pathogens_2013.pdf
Center for Science in the Public Interest.

http://www.efsa.europa.eu/en/topics/topic/amr.htm
European Food Safety Authority.

http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm059103.htm
FDA NARMS.

http://www.fao.org/food/food-safety-quality/a-z-index/antimicrobial/en/
Food and Agriculture Organization (FAO) of the United Nations.

http://www.phac-aspc.gc.ca/cipars-picra/
Public Health Agency of Canada.

http://www.ars.usda.gov/Main/docs.htm?docid=6750&page=1
USDA NARMS.

http://www.who.int/foodborne_disease/resistance/agisar/en/
World Health Organization.

http://www.oie.int/en/for-the-media/amr/
World Organization for Animal Health.

Potential Environmental and Wildlife Sources of Microorganisms in Meat

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Glossary

Antibiotic resistance The characteristic or ability of a microorganism to be unaffected by a particular class of chemotherapeutic agents.

Antimicrobial residue Trace amounts of chemicals (antibiotics) detectable in tissues or food products.

Zoonosis Diseases of animals transmissible to humans.

Introduction

There is growing concern that commensal bacterial flora and zoonotic pathogens which colonize food-producing animals are sources of antimicrobial-resistant organisms that not only contaminate the food supply but also serve as reservoirs of antimicrobial resistance genes that can be horizontally transferred to human pathogens. Theoretical and empirical evidence support the value of reducing the prevalence of zoonotic pathogens in live animals to improve food safety and human health. One proven method for the control of production-limiting diseases of food animals is through the application of biosecurity measures on the farm. Specifically, wildlife is known to contribute significantly to the epidemiology of many economically important infections of domestic animals, such as foot and mouth disease, influenza, and bacterial diseases of animals with zoonotic potential, such as brucellosis and tuberculosis. More recently, with the advent and application of modern molecular typing methods, the importance of wildlife–livestock interactions, as it relates specifically to food safety, has gained increased recognition and attention.

The species of animals raised, the production practices, and the location of the farming (or hunting) operation can all influence the likelihood of transmission of antimicrobial-resistant organisms between food animals and wildlife. For example, it is easier to restrict wildlife access in a confined indoor facility than it is to control interactions in an extensive outdoor production system. Likewise, types of wildlife and other domestic animals present in a particular geographic area may dictate the likelihood of contact. Notwithstanding, most animal rearing locations have at least some opportunities for contact with birds, rodents, or larger mammalian species, such as deer or feral pigs, or domestic animals such as dogs or cats. Transmission of microorganisms between wildlife and livestock and poultry should be considered bidirectional and may have significant implications for wildlife health and ecology, especially as it relates to organisms that cause morbidity and mortality in wildlife populations, or be of importance in the consumption of wild game. In addition, there are also viral (e.g., rabies and Nipah virus) and parasitic infections (e.g., *Toxoplasma gondii*) transmissible from wildlife to food animals that can render derived meat products unsafe. However, in this article, the authors consider only the available evidence to support transmission of antimicrobial-resistant organisms from environmental sources and aquatic, terrestrial, and avian animals.

Similarly, nonpathogenic bacteria carrying genetic determinants for resistance (e.g., integrons) can result in horizontal transfer of these determinants to pathogenic bacteria through a variety of means, including transduction, conjugation, and transformation.

Role of Wildlife

The role that wildlife play in the transmission of antimicrobial-resistant organisms to food animals depends on their capacity to become infected, maintain the infection, and infect subsequent hosts. In this regard wildlife may be classified as outlined (Table 1).

Environmental Exposure to Antimicrobial-Resistant Organisms

The composition and diversity of the intestinal microbial flora of animals is largely determined by their environmental exposures. Even as early as during the birthing process (or hatching), the inevitable presence of bacteria in the environment provide an abundant source of antimicrobial-resistant organisms, some of which may eventually colonize the host and persist long term. Other organisms may only be passed transiently through the digestive tract or contaminate the hide, skin, or feathers.

Many of the antibiotics in use today were originally derived from soil organisms. Typically, a gram of soil may harbor between 10^6 and 10^9 bacteria, the majority of which have not yet been cultured. It is, therefore, not surprising that antimicrobial resistance among soil bacteria is common. Even if these bacteria do not colonize animals or humans, soil bacteria serve as an enormous reservoir of antimicrobial resistance genes. Horizontal gene transfer occurs readily among several genera of bacteria of piscine, aquatic, avian, environmental, and mammalian origin. Metagenomic analyses of soil organisms and human pathogens further support the hypothesis that horizontal gene transfer is the origin of a large proportion of antimicrobial resistance genes present in human clinical pathogens.

In addition to endogenous antimicrobial-resistant organisms present in soil and aquatic sediments, anthropogenic actions may further contribute to, and select for, antimicrobial-resistant organisms in the environment. First, there

Table 1 Roles wildlife may play in the transmission dynamics of antimicrobial-resistant bacteria

Host type	*Definition*	*Comment*
Unsusceptible	Animals that may be exposed but do not become infected or colonized	Although these animals may be present or even predominant at the livestock–wildlife interface, they do not contribute to pathogen transmission
Maintenance	Animals in which the organism persists in the absence of transmission from other hosts	Wildlife may be a maintenance host for some bacterial organisms and not for others, depending on the fitness of the organism and its ability to persist in a novel environment. In contrast, other bacteria may be multihost organisms if they are adapted to several species
Mechanical vector	Transmission not related to infection, but simply external contamination	For example, conveyance of organisms on the feet, hooves, or paws
Spillover	A nonmaintenance host that is susceptible to infection following interspecies transmission	Wherein exposure is frequent, infection is persistent, or prevalence is high, a spillover host may have the appearance of being a maintenance host
Amplifier	A spillover host in which the organism replicates successfully	Increases the environmental load of the organism, favoring transmission
Dead end	A spillover host that is not capable of intra- or interspecies transmission	These animals do not contribute to the continued transmission of the organism, but in the case of food safety, they may be contaminated with the organism if harvested and consumed as food
Spillback	A term reserved for the infection of a maintenance host from a spillover host	

may be direct environmental contamination with antimicrobial-resistant organisms originating from human or animal wastes. Solid waste disposal and wastewater treatment sites may receive large numbers of bacteria, including resistant organisms. Runoff from these locations can contaminate waterways used for agricultural purposes, thus providing a conduit for colonization of livestock or the contamination of livestock feedstuffs. Moreover, these activities also attract wildlife and serve as a source from which wild animals and birds may become infected with antimicrobial-resistant organisms. The cycle is self-perpetuating as, in some ecosystems, wildlife is the primary cause of microbial impairment of watersheds.

In addition to the antimicrobial-resistant organisms present in animal manures that are used for fertilization of crops and occasionally aquaculture ponds, manure may contain unabsorbed antibiotics and bioactive metabolites that exert pressure for the selection of antimicrobial-resistant organisms in the environment, thereby increasing their fraction available to colonize animals in the environment.

Of particular relevance is the use of antibiotics in aquaculture. Similar to production systems for terrestrial animals, antibiotics are used in the production of finfish and crustaceans to treat and control bacterial infections. Information about the exact amount of antibiotics that are used around the world in aquaculture is scant. In some countries, antimicrobial use in aquaculture is highly controlled, but in other regions of the world, where regulations and oversight are lax, antibiotics are used liberally. The extent to which antibiotics end up in the environment depends on the production system (recirculating systems, indoor/outdoor systems, sea pens, etc.) and is still poorly defined. However, it is estimated that approximately 75% of the drugs used in aquaculture end up in the environment. As a result, the presence of antimicrobial-resistant organisms is higher in sediments collected near sea pens than that found in sediments farther away. Antimicrobial-resistant organisms can persist in the environment for extended periods, even in the absence of continued application of antimicrobial selection pressure.

Antimicrobial-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* and methicillin-sensitive *Staphylococcus aureus*, have been isolated from wild and captive marine mammals. In these cases, molecular subtyping indicated that the organisms recovered were indistinguishable from epidemic clones in the human population, indicating that the marine mammals were more likely spillover hosts from human maintenance hosts. Multiple-antimicrobial-resistant organisms were commonly isolated from stranded pinnipeds in northern California and predatory marine fishes (sharks and redfish). Collectively, antimicrobial-resistant organisms in water and fish pose a food safety and public health risk. At the same time they serve as sentinels for environmental contamination.

In summary, the high prevalence of antimicrobial-resistant organisms in the environment where food animals are raised provides ample opportunity for exposure to pathogens and commensal organisms that may colonize poultry and livestock. Contamination of feedstuffs, water, and natural animal behaviors, such as feeding (grazing, foraging, and pecking), grooming and preening, and nesting, can transfer environmental organisms from the environment to the animals and their digestive tracts. There is mounting evidence that preharvest strategies to reduce the carriage of foodborne pathogens by animals will have significant impacts on the contamination of meat with these organisms. Likewise, control of contamination of the environment with antimicrobial residues and hygienic practices that reduce the contact animals have with antimicrobial-resistant pathogens may provide strategies to reduce the probability that meats derived from animals raised in these conditions are likely to become

contaminated with antimicrobial-resistant organisms at the time of harvest.

Transmission of Antimicrobial-Resistant Organisms from Terrestrial Animals

Among emerging human pathogens, 20% are resistant to one or more antimicrobials, and this number has significantly been increasing with time. Indeed, a large number of bacteria are zoonotic – shared between domestic animals or wildlife and humans. For example, it is estimated that 70% of all emerging pathogens of humans originate from wildlife sources. The routes through which bacteria are transferred from wildlife sources and humans are complex and are not fully elucidated. Direct contact with wildlife or the wildlife environment is but one infrequent mechanism that humans may become exposed to wildlife microflora. In contrast, poultry and livestock, depending on the management system, have many opportunities to interact with wildlife and share a common environment with wildlife. Wildlife is attracted to available feed supplies, water, and shelter provided at animal production facilities. Furthermore, expansion of livestock production into areas previously used for wildlife habitat provides many opportunities for the indirect transmission of bacterial flora to humans from the environment and wildlife through various food-producing animal intermediates.

Increasingly, researchers are recognizing the threat terrestrial wild animals pose to food safety. A variety of foodborne pathogens, both antimicrobial sensitive and resistant, including *Salmonella*, *Escherichia coli* O157:H7, and *Campylobacter*, have been isolated from wildlife. The complex ecosystems involving these enteric pathogens and antimicrobial-resistant organisms are now suspected to involve more than humans and food animals, that is, they also include wildlife as potential reservoirs and agents for dispersal. Although antimicrobial-resistant bacterial intestinal microflora can be isolated even from populations of wild animals with limited exposure to domestic animals or human activity, it is recognized that the major source of these pathogens are humans or agricultural animals themselves. Nevertheless, because of their mobility, wildlife can disperse these pathogens among farms and contaminate water, animal feed, and crops intended for human and farm animal consumption, making wildlife a concern for on-farm biosecurity and public health.

The frequency and types of antimicrobial-resistant bacteria exchanged between terrestrial wildlife and livestock are governed by the species of animals raised, level of confinement, extent of farm biosecurity, and the types of wildlife in the proximity. Although some bacterial pathogens of domestic animals are restricted to certain closely related animal species, nearly 70% of organisms can infect multiple animal hosts. In addition, wildlife may also serve as mechanical vectors for the transmission of antimicrobial-resistant bacteria. In such cases, the organism need not infect or colonize the wild animal, but rather it may simply contaminate the skin, fur, or feathers and be transferred from one location to the other as the animal moves bacteria between farms or regions.

The exact mechanisms on how wildlife acquires antimicrobial-resistant flora and the extent to which they transmit these organisms to food animals are unknown. Clearly, like production animals, wildlife may become colonized with organisms present in their environment. Animals in proximity to human activities have been found to share resistant organisms with domestic animals and humans. Although wildlife sharing a common environment with livestock or humans is sufficient to result in shared pools of antimicrobial-resistant bacteria or genes, it is not a prerequisite for colonization. Some animals without contact with agricultural animals or other human activities carry intestinal flora with reduced susceptibility to a number of antibiotics. This indicates that there are various points for entry and factors for selection and maintenance of novel antimicrobial resistance organisms or genes that are independent of livestock and poultry.

It is assumed that the transmission of organism between wildlife and livestock occurs via direct contact, contact by domestic animals of fomites or environments previously contaminated by wildlife, or through the contamination of feed or water supplies. Specific behavioral interactions define transmission outcomes. A classical example is the case for tuberculosis transmission between badgers and possums and cattle. During the advanced stages of disease, these wildlife species express atypical behaviors, including the loss of fear of other animals. Cattle and deer are able to approach these animals and, due to such proximity, large numbers of organism can be transmitted via direct contact or aerosol. Another way that bacteria may be transmitted, for example, between wild bison and domestic cattle is at the time of calving. Finally, carnivores may become infected from maintenance host from scavenging fetal membranes or mortalities.

In recent years, there has been increasing encroachment of wildlife habitats for food animal production. Reciprocally, animal production facilities often attract wildlife. Additively, these factors have augmented the potential for livestock–wildlife interactions. Probably the most common route of interspecies transmission of antimicrobial-resistant organism is through sharing a common environment, including feed, feed bunks, and water sources. Wild animals that often frequent livestock production facilities and from which antibiotic-resistant organism have been isolated include grazing ungulates, raccoons, wild pigs, rabbits, and other rodents. However, the directionality of the transmission in these cases cannot be deduced from simple cross-sectional studies.

Transmission of Antimicrobial-Resistant Organisms from Avian Species

The literature on the carriage of foodborne pathogens by wild birds is rich with descriptive studies on *Salmonella enterica* and antimicrobial-resistant bacteria, including indicator species such as *E. coli*. A great deal of this focus reflects the public health impact associated with *Salmonella* and antimicrobial-resistant infections as well as the likelihood of detecting these organisms in wild avian species.

Bacteria resistant or carrying genes for resistance to drugs of primary importance to human health, including fluoroquinolones, vancomycin, and third-generation cephalosporins, have been isolated from wild birds along with isolates showing multidrug resistance.

Salmonella enterica and antimicrobial-resistant bacteria have been isolated from a diverse array of species across several orders of wild birds from around the world, including passerines, gulls, cormorants, pelicans, waterfowl, cranes, pigeons and doves, raptors, galliformes, psittacines, and even penguins. A number of large national reviews of avian wildlife mortality events have also demonstrated the diversity of birds that may potentially carry *Salmonella*. Interestingly, the diversity of serotypes obtained from apparently healthy birds appears to be far richer than those documented from birds that have mainly died of salmonellosis and often reflect the proximity to human or agricultural environments where these birds forage. In addition, the diversity and prevalence of serotypes of *Salmonella* and antimicrobial-resistant bacteria may be much greater from bacteria isolated from the feet and feathers of birds than from their feces or gastrointestinal tracts, suggesting that 'mechanical vectoring' may also be a concern for interspecies transmission.

It is important to recognize that although the diversity of birds that could potentially carry *Salmonella* or antimicrobial-resistant organism is quite high, the prevalence of carriage may vary with bird species and geographical region depending on the behavior of the bird species and the availability of human and agricultural sources of these bacteria. For instance, researchers mist netted and collected fecal samples from more than 500 barn swallows (*Hirundo rustica*) but failed to find any samples that tested positive for *Salmonella* despite sampling across multiple regions and periods in Sweden. Interestingly, however, even in remote areas such as Antarctica and the Arctic, *Salmonella* serovars and antimicrobial-resistant bacteria commonly found in humans and domestic animals have been isolated from wild birds, suggesting the possibility of a complex recycling process involving humans, marine wildlife, and marine sources of food and feed ingredients. In a study on antimicrobial resistance, it has been found that there is a high prevalence of antimicrobial-resistant genes and integrons in *E. coli* isolates from black-headed gulls (*Larus ridibundus*) located in three nesting colonies near industrial cities (1 colony) and areas of intense agriculture (2 colonies) in the Czech Republic; the researchers noted that the most frequent phenotypes of antibiotic resistance were consistent with the consumption of antimicrobial drugs in human and veterinary medicine in the Czech Republic.

Investigators conducting a study on multidrug resistance in wild birds in Germany, involving one rural and one urban sampling area, suggested that birds of prey, waterfowl, and passerines represent a notable source of multidrug-resistant *E. coli* and that the patterns were similar to those isolated from domestic animal. Interestingly, on-farm studies focused on different species often suggest conflicting views on the significance of wild birds as a potential reservoir for antimicrobial-resistant organisms. For instance, in a study of house sparrows (*Passer domesticus*) on two Czech dairy farms, there was no evidence to suggest that the birds were infected with resistant *E. coli* isolates with the same phenotypes as those found in cattle from the same farms despite close contact between the animals. In contrast, in another study of red-billed choughs (*Pyrrhocorax pyrrhocorax*) in Spain, strong evidence was found that these birds carried multidrug-resistant strains of bacteria that resembled the waste, including pig slurry and sewage sludge, which was used as fertilizer on the fields where these birds forage for soil invertebrates. Consequently, predictions concerning the role of birds in spread of *Salmonella* and antimicrobial-resistant organism in agricultural systems need to be species specific and recognize complex ecological processes.

Antimicrobial-resistant organisms have been obtained from both ill and healthy birds using microbiological analysis of fecal, cloacal, and entire gastrointestinal tracts and entire carcasses. In addition to the source of animals and material used among studies, microbiological methods, sample sizes, and the number of sites used in determining prevalence estimates are quite variable. Consequently, a great deal of caution should be used in making comparisons among species and orders of birds, across regions, countries, and continents.

Research Needs

The literature concerning the transmission of antimicrobial-resistant bacteria has been dominated by descriptive studies that focus on whether these bacteria are carried by wildlife or the subtypes of these organisms, based on molecular techniques or antibiogram profiles, match those found in agricultural animals. From these studies, it is clear that these organisms are shared between wildlife and agricultural animals but is less clear in many situations, for example, whether wildlife are having a significant impact on the carriage of these organisms in the farm environment. Epidemiological studies focusing on the agricultural and wildlife ecosystem of populations of farms are required to address a number of issues, including the relation between the degree of contact with wildlife species and the probability of agricultural animals carrying these resistant organisms or having their feed, water source, or environment contaminated with antimicrobial-resistant organisms. These types of studies are needed to establish that wildlife is having a quantifiable role in spreading these bacteria to agricultural animals and not merely acting as sentinels for organisms that are being shed by agricultural species. Furthermore, randomized control trials, cohort studies, and case-crossover studies examining the impact of wildlife control measures on the prevalence of antimicrobial-resistant organisms are needed to further establish the causal role of wildlife in spreading these organisms to agricultural species and in identifying the most effective control measures. In terms of development of quantitative risk assessment and infectious disease transmission models, a number of inputs remain largely unknown, especially regarding the movement of populations of wildlife among farms, the probability of wildlife acting as biological or mechanical vectors for these pathogens, quantitative measures of the amount of bacteria transmitted by wildlife carriers, and basic information concerning the survival of these organisms within wildlife and their survival in the farm environment. Consequently, integrative research activities between wildlife biologists, veterinarians, epidemiologists, microbiologists, and experts in risk assessment and mathematical modeling are required to understand and quantify the risk posed by various wildlife species and to develop intervention strategies in order to prevent the transmission, distribution, and/or multiplication of these antimicrobial-resistant organisms in agricultural animal species and

subsequent food animal products. However, it is important to remember that the nature of these processes may be very different depending on the species of bacteria, species of wildlife, agricultural species and production system, and the temporal and spatial scale at which these questions are being addressed.

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See also: Foodborne Zoonoses. Meat, Animal, Poultry and Fish Production and Management: Disease Control and Specific Pathogen Free Pig Production. Microbial Contamination: Microbial Contamination of Fresh Meat. Species of Meat Animals: Game and Exotic Animals

Further Reading

Blanco, G., Lemus, J.A., Grande, J., 2009. Microbial pollution in wildlife: Linking agricultural manuring and bacterial antibiotic resistance in red-billed choughs. Environmental Research 109 (4), 405–412.

Dolejska, M., Bierosova, B., Kohoutova, L., Literak, I., Cizek, A., 2009. Antibiotic-resistant *Salmonella* and *Escherichia coli* isolates with integrons and extended-spectrum beta-lactamases in surface water and sympatric black-headed gulls. Journal Applied Microbiology 106 (6), 1941–1950.

Fenton, A., Pedersen, A.B., 2005. Community epidemiology framework for classifying disease threats. Emerging Infectious Diseases. Available at: http://dx.doi.org/10.3201/eid1112.050306 (accessed 24.11.13).

Forsberg, K.J., Reyes, A., Wang, B., *et al*., 2012. The shared antimicrobial resistome of soil bacteria and human pathogens. Science 337 (6098), 1107–1111. doi:10.1126/science.1220761.

Relevant Websites

http://www.nature.com/nature/journal/v451/n7181/pdf/nature06536.pdf
Nature Publishing Group.

ftp://ftp.fao.org/ag/agn/food/aquaculture_rep_13_16june2006.pdf
The Food and Agricultural Organization.

MINCED MEATS

KO Honikel[†]

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Glossary

Colloidal systems Colloids are substances which are dispersed in small particles (10^{-5}–10^{-7} cm in diameter) in gaseous, liquid, or solid compounds. In meat science they are important: as a gas in fluids which are foams, as fluids in gas which are mists or sprays, as solids (meat proteins and as solid fat particles) in aqueous solutions they are suspensions, or fluids (oil or molten fat) and in aqueous solutions are emulsions. Meat batters in a bowl chopper are often called emulsions but their correct physical definition is a suspension.

Swelling of meat proteins Fibrillar proteins of meat attract each other because of their positive and negative charges of amino acid side chains. Salt (NaCl), dissolving immediately in the aqueous environment of water in meat, added to minced meat lets the ions move in between the protein chains, in this way reducing their attraction and permits the swelling of the protein structure by the influx of water.

Introduction

Meat mincing is a process in which meat cuts with mainly intact cellular structures are reduced in particle size, usually with the help of a meat mincer or sometimes even a bowl chopper. Flaking machines can also be employed, producing small but discrete muscle pieces. In former times mincing was done by hand with knives cutting the meat into small pieces. Later, rocking knives (end of nineteenth, first quarter of the twentieth century) were mounted on top of a rotating bowl being the prototype of a modern bowl chopper. With all the mechanical equipments only a part of the cellular structures are disintegrated.

In contrast to an intensive comminution in a bowl chopper or in emulsion mills, in which very sharp knives cut many thousand times per minute through myofibrillar and connective tissue proteins of the batter, the degree of reduction of particle size and cellular destruction in minced meat is limited. In a meat mincer, the precut pieces of meat (1–4 cm cubes or strips) are fed onto a rotating spiral shaft (screw auger) or a pump-type system that presses the meat through a static end plate with holes of 1.5–10 mm diameter and against a rotating knife. The knife rotates at the speed of the spiral shaft. The degree of cutting of myofibrillar and filamental structures is rather limited. A mincer is able to grind semifrozen (−5 °C and higher) or unfrozen meat. The temperature should not exceed +2 °C at the end of the mincing process mainly for the reason of preventing microbial growth.

Thus, mincing involves a crude disruption of cellular structures of muscle and fat cells together with a mechanical disruption of the well-ordered fibrillar structures of myofibres and connective tissue sheets. Cellular and subcellular membranes are only partially disrupted; connective tissue sheets are cut into smaller pieces, but pieces of tendon are still recognizable; and the myofibrillar structures (myofibers and fibrils, usually in the state of rigor mortis) are broken down only into smaller pieces. By legal requirements in many countries, no additives are permitted in minced meat sold as such in raw state, but the addition of up to 1% salt is permitted. More than 1% salt, the fibrillar structure of the muscle would alter recognizably.

These steps are required for processing of meat into hamburger products, restructured meat, or they are applied as the first step for manufacturing meat products.

Flaking machines slice small pieces of meat with a rotating or a falling knife but without the perforated plate that is used in mincing. Usually, the meat is semifrozen so that its stiffness permits a clear cut through the meat.

The consequences for the structure of the meat are similar to those of mincing, but to a lesser degree, except that connective tissues (tendons) are more thoroughly cut than they are by mincing. Mincing and flaking simulate the manual use of a knife.

Further comminution to a more or less homogenous batter is done by mills or bowl choppers. Milling and chopping are applied for processed meat products like sausages. They form colloidal systems of dissolved, swollen, and undissolved meat proteins and fat particles in an aqueous dispersion, a general expression of a colloidal system consisting of solid particles in another solid, fluid, or gaseous material. In both cases, fast-rotating sharp knives cut through the meat. In contrast to mincing or flaking mills, choppers permit the direct addition of such ingredients as salt, additives, extenders, etc. Homogenous batters in which fibrillar structures and fat globules of fat cells are disrupted into very tiny parts (<100 μm) usually for sausage manufacturing, are obtained as the end product.

[†]Deceased.

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Often these meat batters at ambient temperatures and below are called meat emulsions. Emulsions in its physical definition are colloidal systems of a dispersed fluid phase in a continuous fluid matrix, for example oil in water. This does not fully apply to a meat batter in a bowl chopper. In a meat batter water is the continuous fluid matrix with more than 50%, often exceeding 60%, in which a part of the meat proteins because of the addition of salt (2% and more, lower concentrations with additives like phosphates) are dissolved and solid particles of proteins (10–20%) and fat particles (up to 35%) are dispersed. Such a colloidal system is called a suspension. A part of the protein surfaces of the dissolved proteins have changed their three-dimensional structures and they have become partially more lipophilic. They try to form a protein 'net or skin' around the fat particles. The modified protein structures entrap the fat particles already during the chopping process. On heating the dissolved and undissolved (dispersed) protein molecules and particles form a three-dimensional network in which fat droplets and water are entrapped, bound, or immobilized. A complicated colloidal system is formed which cannot be called an emulsion in any state. As a consequence of the entrapped fat particles the meat batter becomes a heat stable three-dimensional network of which little or no water and fat cookout occurs (see **Table 1** E and F as batter-like structures in comparison to A through D as structures of minced meat).

In conclusion, mincing even the addition of water does not develop colloidal systems (see in **Table 1,** steps A and B).

Advantages of Mincing

Meat in the full state of rigor mortis – between 5 and 40 h postmortem, depending on species, preslaughter and slaughter stress, chilling, etc. – is tough because of the permanent cross-links of actin and myosin between filaments and the still intact longitudinal and transverse fibrillar structure of myofibres themselves. The various connective tissue sheets (endomysium, perimysium and epimysium) increase toughness, which increases with the age of the animal as a result of the increasing degree and maturity of cross-linking between the collagen molecules in the connective tissue.

Table 1 Water-holding (binding) capacity of beef (pH 5.5; 2% fat) after different processing steps, ending all with heating up to 75 °C; the mixtures were mixed for 1–2 min in a bowl by hand except for E and F, where a bowl chopper was employed

Status of processing	*Water (ml)/product (kg)*	*Cookout (%)*
Intact meat (100 g)	745	27
(A) Minced meat (3 mm plate)	745	22.5
(B) A+75 ml water	828	47.5
(C) A+2% salt	735	0.25
(D) C+75 ml water	822	9.1
(E) D+50% minced pork backfat	640	2.3
(F) E+0.2% diphosphate	640	0.5

Note: Owing to the low fat content of the beef in A to D no fat cookout occurred. In E a minor fat cookout of $<0.5\%$ was observed.

Mincing mechanically disrupts these protein structures and enhances the sensory tenderness. The disruption of cellular membranes allows a rapid distribution throughout the meat particles of subsequently added salt (usually in a mince or a Hamburger patty approximately 1%) or other additives for sausage manufacturing. The effect of higher salt contents in coarsely ground (minced) sausage products like salami-type sausages is that salt enhances the solubility of myofibrillar proteins as the salt is immediately available at the intracellular myofibrils, which are additionally fragmented into smaller pieces. The highly ordered and fixed postrigor fibrillar structures thus no longer exist to the same extent (see also **Table 1**).

The rapid action of salt in minced meat is also advantageous in prerigor (hot boned) meat. In the prerigor state, owing to the presence of higher concentrations of adenosine triphosphate (ATP), actin and myosin in the myofibrils are separated (there are no permanent cross-links). The addition of salt (1.5–4.5% NaCl) leads to a disintegration of the myofibres through swelling and dissolution of myofibrillar proteins. Rigor mortis with its actin-myosin cross-linking can no longer occur, and the meat remains structurally in a prerigor state despite the subsequent ATP depletion and pH fall. This prerigor salted meat has excellent water-holding capacity as swelling by salt does not necessarily promote an intensive disruption of the above-mentioned rigor structures. When 'emulsion-type' sausages are made with this prerigor salted meat, there is no need for additives such as phosphates or citrates, to enhance water binding. The salt contents can remain at a minimal level of approximately 1.6–1.8%. No jelly or fat cookout will be observed.

The effects of mincing and further processing are described together in **Table 1**. Mincing itself (A) enhances the water-binding capacity (WBC) slightly. It results in a lower cookout. Addition of 75 ml water to 100 g of meat (B) does not improve or change the WBC of the meat proteins. Proportionally more cookout is observed. However, the addition of 2% salt to meat (C) enhances the WBC and 75 ml water and 2% salt (D) exhibits a much higher WBC than (B). The use of diphosphate (F) enhances the WBC further. It will also be enhanced by further comminution and the addition of fat (E).

Mincing of meat permits mixing with other foods, allowing the extension of meats. Starch and other polycarbohydrates, nonmeat proteins, water, etc. are possible extenders. Use of extenders enhances the range of meat-containing products, from hamburgers to meat loaves and meat balls, in which meat may be a minor component.

Grinding/mincing also permits the formation of uniformly shaped restructured meats in moulds. Enhanced tenderness and a uniformly distributed flavor are easily achieved.

The nutritional quality of minced meats can also be enhanced by the addition of vitamins (e.g., antioxidative vitamins C and E) and changes in the fatty acid composition by the addition of mono- or polyunsaturated fatty acids are possible.

Disadvantages of Mincing

Mincing has two main disadvantages.

Oxidative Changes

Mincing permits the addition of substances that may enhance the microbial and sensory (rancidity) shelf-life, although mincing per se is counterproductive in terms of shelf-life as meat surfaces are tremendously increased. Oxygen and microorganisms can be distributed throughout the mince. In contrast, a solid meat cut is more or less sterile in its interior and also oxygen penetrates only a few millimeters deep.

The cellular membrane, essentially a lipid bilayer, contains in its phospholipids a considerable number of unsaturated fatty acids. Despite their tendency to oxidize easily, the unsaturated fatty acids are protected in intact muscle of a living being by the ordered bilayers and antioxidants such as vitamin E (tocopherols) between the phospholipids. Oxygen which is transported via the blood stream to the cell surface will penetrate through the bilayer into the intracellular space. The double bonds of the fatty acids will be the target of the oxygen on its way to the intracellular space. The tocopherols, however, are protecting the double bonds of these fatty acids from the oxygen radicals which in turn become oxidized. In a living organism, however, the oxidized antioxidant molecules are replaced but in postmortem tissues such a replacement cannot take place over long durations as the formation of reduced compounds need energy (anabolic pathways), which is exhausted with the onset of rigor mortis. Because grinding, usually carried out postrigor, disrupts the ordered cellular structures, irreversible oxidation can occur. In sensory terms, the oxidation of unsaturated fatty acids and the following formation of carbonyls (e.g., nonenal) lead sooner or later to rancidity. Rancidity does not occur over a period of a few hours, but even frozen or chilled storage in oxygen-permeable packages over time leads to slow but ongoing oxidation. These oxidative changes via oxygen radical formation or peroxides are a self-promoting process. It is a chain reaction which continuously enhances the concentration of rancid compounds. For this reason, the storage potential of frozen minced meat is rather limited. The fatter the minced meat is, the shorter its shelf-life, even if it is chilled or frozen.

Some countries permit the addition of antioxidants to minced meat, such as ascorbate/ascorbic acid (E 300–304), isoascorbates (E 315–316), tocopherols (E 306–309), or metal ions sequestering organic acids and their salt like citric acid/citrates and phosphates or chemical antioxidants like gallates, BHT, BHA (E- numbers 310 and 320 and 321) to minced meat to retard oxidation. Another possibility to reduce oxidation of minced meat is the immediate packaging of the mince after preparation into packaging with carbon dioxide and/or nitrogen. But in these packages the meat loses its attractive red color. In modified atmosphere packages (MAP), the color of the meat is highly dependent on the amount of oxygen. Initially, the bright oxymyoglobin layer is deep in the muscle, but after a time metmyoglobin layer can involve surface layers that become brown. With higher oxygen levels this is less likely but oxidized products can occur.

Microbial Spoilage

Meat itself in its composition is not only a well-balanced food for human beings, but also a good feeding medium for many microorganisms. In an intact muscle piece, microorganisms are usually on the surface only; the interior is more or less sterile. On mincing when the surface area is greatly increased, the microorganisms present on the meat surface are distributed throughout the mince. Immediate chilling from 0 to +2 °C retards or inhibits their further growth, but a common means of preventing microbial growth is to heat the mince immediately or within 24 h.

The use of antibacterial additives (no nitrite permitted) or salt also retards the microbial spoilage. However, in many countries minced meat must be sold without additives. In some countries, however, as mentioned above for retarding rancidity, the regulations permit a limited number of additives like sorbates/sorbic acid, borates, or benzoic acid.

See also: Microbiological Analysis: Standard Methods. Microbiological Safety of Meat: *Salmonella* spp. Modeling in Meat Science: Microbiology. Packaging: Technology and Films. Processing Equipment: Mixing and Cutting Equipment. Spoilage, Factors Affecting: Oxidative and Enzymatic

Further Reading

Dainty, R.H., Machey, B.M., 1992. The relations between the phenotypic properties of bacteria from chilled stored meat and spoilage processes. Journal of Applied Bacteriology 73 (Supplement), 103–114.

Dufy, G., Dolan, A., Burgess, C.M., 2009. In: Nollest, L.M.L., Toldra, F. (Eds.), Handbook of Muscle Food Analysis. Boca Raton, FL: CRC Press, pp. 593–610.

EU Council Directive 94/65/EC. 14.12.1994. Requirements for production and marketing of minced meat and meat preparations.

EU Regulation No. 853/2004 29.04.2004 laying down specific hygiene rules for food of animal origin.

Fung, D.Y.C., 2009. Methods for evaluating microbial flora in muscle foods. In: Nollest, L.M.L., Toldra, F. (Eds.), Handbook of Muscle Food Analysis. Boca Raton, FL: CRC Press, pp. 563–592.

Girard, J.P., 1992. Technology of Meat and Meat Products. Chichester: Ellis Horwood.

Honikel, K.O., 1990. The meat aspects of water and food quality. In: Hardman, T.M. (Ed.), Water and Food Quality. London: Elsevier, pp. 277–304.

Lawrie, R.A., 1998. Lawrie's Meat Science, sixth ed. Cambridge: Woodhead Publishing.

Mandigo, R.W., 1988. Restructured meat. In: Lawrie, R.A. (Ed.) Developments in Meat Science 4. London: Elsevier, pp. 297–316.

Norrung, B., Andersen, J.K., Buncic, S., 2009. Main concerns of pathogenic microorganisms in meat. In: Toldra, F. (Ed.), Safety of Meat and Processed Meat. New York: Springer Science, pp. 3–29.

Offer, G., Knight, P., 1988. The structural basis of water holding in meat. Part 1, general principles and water uptake in meat processing. In: Lawrie, R.A. (Ed.) Developments in Meat Science 4. London: Elsevier, pp. 63–172.

Pearson, A.M., Dutson, T.R. (Eds.), 1994. Quality Attributes and their Measurement in Meat, Poultry and Fish Products. Glasgow: Blackie Academic and Professional.

Varnam, A.H., Sutherland, J.P., 1995. Meat and Meat Products Technology, Chemistry and Microbiology. London: Chapman and Hall.

MODELING IN MEAT SCIENCE

Contents

Meat Quality

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Glossary

Causal model A qualitative or quantitative model that hypothesizes the relationships between two or more phenomena in a way that affirms the behaviour of at least one phenomenon to be directly caused by one or more other phenomena.

Component model A model that seeks to describe the behaviour of a single component within a system, typically by describing the relationships between several phenomena.

Statistical model A qualitative or quantitative model that hypothesizes the relationships between two or more phenomena in a way that affirms the behaviour of at least one phenomenon to be correlated with one or more other phenomena, but not necessarily implying that there is a direct causal relationship between them.

Introduction

To understand or design a meat processing operation, there is a wide range of potential processing options available. An incomplete list of the processing regimen characteristics that might affect meat quality attributes important to consumers would include:

- the muscles involved,
- the age, genetics, gender, and physical condition of the animal,
- the extent to which the animal was stressed, or not, before slaughter,
- the method of slaughter,
- the electrical treatments applied,
- the size and shape of the piece of meat,
- the way the meat is hung or otherwise supported,
- the temperature profile of the meat over time,
- the method of packaging,
- the method of storage,
- physical treatments during cooling and storage, including moisture loss and exposure to oxygen and other gases, and
- the method of preparation and cooking.

With such a large number of different characteristics to consider, it was difficult for managers and designers of meat processing operations to understand intuitively how combinations of these characteristics might affect meat quality attributes. This difficulty has been addressed by the development of mathematical models to predict meat quality as functions of the process and animal characteristics. These models have the further advantage that they have allowed managers and designers to make quantitative predictions of outcomes that previously required the expert judgment of a highly experienced meat scientist. It should be noted, however, that all models are simplifications of the real world, and this simplification is achieved by excluding those aspects of the world that are unimportant to the situation being studied. The decision as to which aspects are unimportant inevitably requires some judgment and experience in the field, and so it is desirable that a model user should have some knowledge of meat science before developing or applying the models described later in this article.

There are two broad approaches to modeling any problem. One approach is to develop a conceptual framework that shows some presumed causal links between the various phenomena involved in the system of interest, to express each link in the form of a model, and to find parameters for each model. This approach requires an understanding (or at least a hypothesis) of the causal relationships among the phenomena and detailed quantitative research to evaluate the parameters

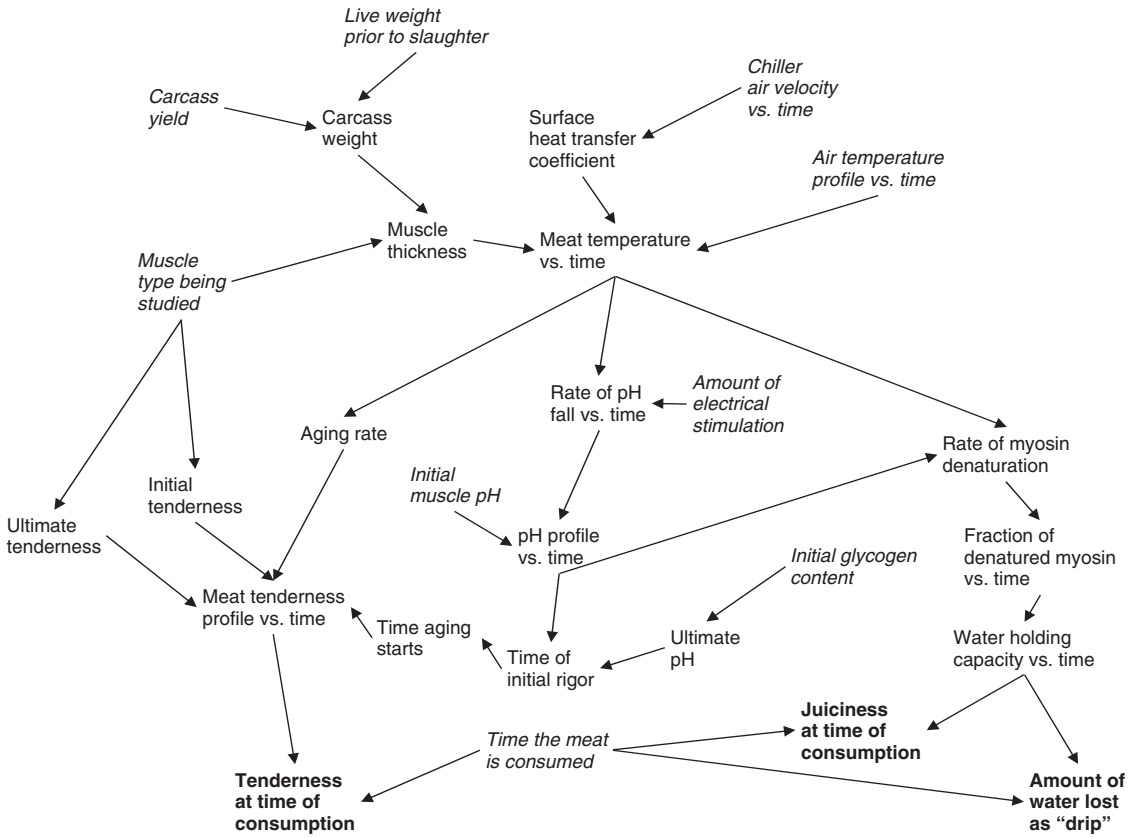

Figure 1 One of the many conceptual frameworks that could be used to understand the interactions among phenomena that affect a few measures of meat quality. Each arrow should be interpreted as saying that the phenomenon at the tail of the arrow appears to directly affect the phenomenon at the head of the arrow. Inputs that would have to be specified by any user of the model are shown in italics; outputs that would be predicted by the model are shown in bold. 'Time' is the amount of time since slaughter.

of those modeled relationships. Another approach is to use statistical techniques to relate system inputs and output phenomena, without hypothesizing specific causal relationships among them. This second approach requires a considerable amount of data for the inputs and outputs, with all relevant combinations of the input variables having to be included in the dataset if the resulting model is to be robust.

First, a causal model framework is considered that could be used to provide a qualitative description of the relationships among many phenomena that are relevant to meat quality. Second, component models of individual phenomena are considered that are relevant to meat quality. Third, a successful statistical model is examined that makes quantitative predictions of output variables from input variables.

An Example Causal Model Framework

The type of framework that is used, and the levels of phenomena and time scale on which it should operate will always depend on the purpose of the framework, that is, the behavior that it intends to illustrate or explain, or the outcomes that it intends to predict. In the area of meat quality, these behaviors and predictions could range from the submicroscopic scale of enzyme reactions to the macroscopic level of meat tenderness and drip loss.

An example model framework that attempts to illustrate the relationships among some of the macroscopic phenomena involved in the rigor and aging process is shown in Figure 1. Some points can be noted:

1. Although some values are indicated as inputs to this model, the selection of inputs depends on the selection of the model boundary. Thus, one could note that the live weight of an animal depends on its breed, gender, age, feeding regimen, and many other factors. This model is chosen to study the rigor and aging processes and not the process of animal growth; hence the live weight of the animal is considered immediately before slaughter.
2. The authors of this framework have decided that they are interested in predicting the tenderness of the selected muscle, its juiciness, and the total amount of water lost as drip, but have chosen not to examine the specific mechanism through which drip occurs, to predict the meat color, surface dryness or any of a number of other values that could have been of interest.
3. Within the model framework itself, the authors have chosen to simplify the relationships. The framework shown in Figure 1 does not include the effect on tenderness of low-temperature shortening (often called cold shortening) or

high-temperature shortening (often called heat or hot shortening, which also involves inhibition of tenderizing enzymes), or the effect of muscle type or fat content on juiciness. These simplifications may limit the applicability of the model, but they also reduce its complexity, and therefore make it easier to understand for the situations where it is applicable.

4. Where possible, the relationships have been limited to those where a cause can be tied directly to an effect by an understanding of the physics, biochemistry or sensory science behind the process being modeled. As an example, this model framework suggests that juiciness does not depend directly on the fraction of denatured myosin, but on water holding capacity.
5. The precise definitions of the variables within the model are important because they can often be measured in different ways. For example, drip might be measured in terms of the volume of liquid that is exuded by meat contained in a plastic bag. The amount of drip over time can also be measured by centrifuging a meat sample. The same phenomenon will be measured differently by the two techniques, though one would expect the two measures to be correlated.
6. Any model or model framework is a theory. While a good model will provide a very useful guide to the behavior of the system that it is designed to represent, any model is always an approximation to that system. Sometimes – especially in a rapidly developing field – a model is no more than a representation of a best guess as to how the phenomena in the system are related. One of the uses of the model, in this latter case, is to predict behavior that one should expect to see in the real system. By comparing the model's prediction with the behavior of the real system, one can discover how well the model embodies one's understanding of that system.

A model framework such as that shown in Figure 1 can allow one to draw conclusions about the system behavior without the need to make quantitative predictions. For example, one may ask if it might be expected that meat from larger animals of a species would be more tender than meat from smaller animals. This model framework suggests that it might be more tender, because the muscles on a larger animal will be thicker, hence they will cool more slowly, hence they will have more time to age, and will consequently have dropped closer to their ultimate tenderness by the time they are consumed than would the muscles from a smaller animal. In this way, a conceptual framework can be of considerable value by itself. To make quantitative predictions of the system outputs, however, a conceptual framework must be made more concrete by defining the relationships among the phenomena that it contains – usually through the use of mathematical equations.

Component Models of Meat Quality Attributes

Historically, the results of studies in meat science have been reported in graphical or tabular form, relating one or more manipulated variables to one or more response variables. Increasingly, however, these results are being formulated as mathematical equations that describe hypothesized relationships between causes and effects. The use of mathematical equations instead of tables or graphs can allow both a more concise description of the relationship and easy interpolation between the discrete conditions that were actually studied.

Examples of Algebraic, Differential Equation, and Computer Models

Where the relationships between phenomena apply at instants of time, they can be described using algebraic equations. For example, in 1977, Jeacocke measured the rate of pH change over time for beef muscles at various temperatures. He plotted his measured results in a graph that showed points between 0 and 35 °C, but this relationship could be expressed quite easily as a mathematical model if one fitted a convenient form of equation to the data. It turns out that eqn [1] fits Jeacocke's data very well:

$$r = 0.0837 - 0.0425T + 0.000225T^2 \qquad [1]$$

where r is the rate of pH fall in units per hour and T is the temperature in °C, and the model is applicable for temperatures between 0 and 35 °C. The data measured by Jeacocke and the fitted curve are shown in Figure 2.

Where the relationships between phenomena are rates of change, it is usually most effective to express them in the form of differential equations. To continue the example of Jeacocke's pH change measurements, a model to predict pH (as opposed to the rate of change of pH) could be expressed as shown in eqn [2].

$$dpH/dt = -0.0837 + 0.0425T - 0.000225T^2 \qquad [2]$$

While the transformation of a set of data points on a graph into eqn [2] is quite straightforward, this simple form of model construction can provide considerable predictive power. If one knew the initial pH, and knew (or could calculate) how the muscle temperature changed over time, one

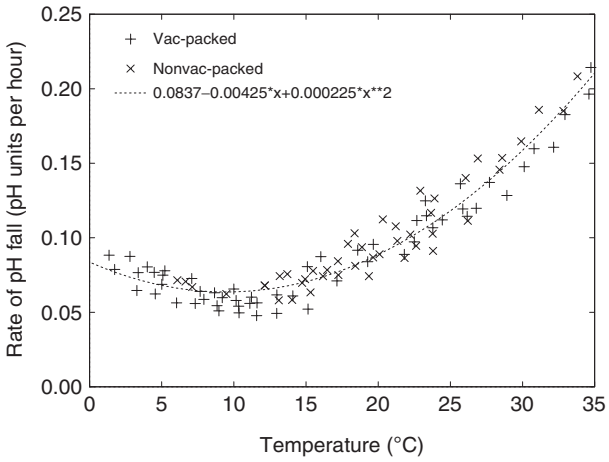

Figure 2 Measured rate of pH fall vs. temperature, with a fitted model curve, replotted from data collected by Jeacocke, R.E., 1977. The temperature dependence of anaerobic glycolysis in beef muscle held in a linear temperature gradient. Journal of the Science of Food and Agriculture 28, 551–556, with the curve added.

could solve this differential equation numerically to calculate a predicted pH at each point in time for that muscle. This would be impossible to achieve based only on the data points that were originally reported.

As discussed earlier, there are approximations and theories involved in any modeling process. In this case, by constructing the model shown in eqn [2], the authors have assumed that temperature is the only factor that affects the rate of pH fall and that, for example, the rate of pH fall is independent of the current pH of the meat. In fact, because any piece of meat has an ultimate level below which its pH will not drop, it is clear that eqn [2] will be incorrect once the pH drops below that level. A crude (but perhaps sufficiently accurate) way to deal with this problem might be to describe the pH profile by choosing between eqns [3] and [4], based on the current pH level.

$$\text{if pH} > \text{ultimate pH: } d\,pH/d\,t = -0.0837 + 0.0425T - 0.000225T^2 \quad [3]$$

$$\text{if pH} \leq \text{ultimate pH: } d\,pH/d\,t = 0 \quad [4]$$

This sort of choice can be made easily if the model is implemented in a computer program and, since that also makes it easy to solve the differential equations using numerical methods, computer programs have become the usual preferred method of implementing and evaluating most types of meat quality models.

A Model to Describe Formation of Pale, Soft, and Exudative Meat

One of the first models of meat quality phenomena was developed by Offer in 1991. Offer reported that "[although] the importance of pH and temperature conditions to the severity of the pale, soft and exudative (PSE) state has been much discussed, insufficient emphasis is often given to the importance of the time for which the carcass experiences these adverse conditions." Offer therefore used the dependence of the rate of denaturation of myosin *in vitro* on pH and temperature to predict the time-course of myosin denaturation in the prerigor period. To keep the focus of the model on protein denaturation, Offer used a linear rate of pH fall (rather than using a temperature-dependent model such as eqn [2]) and an exponential rate of temperature fall to specify the pH and temperature of the muscle at any given time during the rigor process. The exponential rate of temperature fall was modified by assuming that the heat produced by postmortem glycolysis was liberated at a constant rate.

Offer hypothesized that the denaturation of myosin was ultimately responsible for enhanced drip loss in the PSE condition, and that the rate of denaturation could be described using an Arrhenius equation. By reasoning from the combination of several earlier published results, Offer was able to estimate that the rate constant (k_d, in s^{-1}) approximately obeyed eqn [5].

$$k_d = 2.13 \times 10^{34} \exp(-43000/(R\,T)) \times 10^{-1.3pH} \quad [5]$$

where R is the gas constant and T is the temperature, in Kelvin. It was assumed that the rate of myosin denaturation would fall to zero when the muscle entered the rigor state because the myosin overlapped by actin filaments would bind to actin.

The model was then solved for a wide range of cooling rates and rates of pH fall. This allowed Offer to plot graphs to show the effects of time, pH fall rate, and cooling rate on the rate constant of the Arrhenius equation. Then the effects of these variables on the fraction of myosin denatured could be shown. The benefits of this modeling approach were summarized by Offer as follows:

> *The calculations described… help to rationalize several key aspects of processing practice and to explain certain previously puzzling phenomena connected with PSE. Seemingly unrelated features of processing to achieve optimal meat quality have been brought together under a reasonably simple and predictive model. This could be used to predict the amount of drip associated with various treatments of the carcass, thereby defining the critical conditions that have to be fulfilled if the severity of PSE is to be ameliorated.*

Additional Factors to Consider

As well as helping to explain relationships among observed data, modeling can also identify areas that need further research. For example, myosin, modified by pH and temperature prerigor, is not the only protein that changes during processing. During aging and tenderization postrigor, cytoskeletal proteins degrade and release free water (the rate depends on temperature, but the extent is relatively independent of temperature) that can contribute to drip, and this water needs to be included in the model.

Electrical stimulation not only accelerates rigor mortis and helps avoid cold shortening, but recent studies show that, in addition, stimulation improves the tenderness of tropical breeds so that they become more similar in tenderness to British and continental breeds and that both, in turn, are more tender after stimulation than unstimulated British breeds. There are opportunities to explore these sorts of relationships through cause–effect modeling or to quantify them through the sort of statistical modeling framework described in the following section.

An Example Statistical Modeling Framework

Where the relationships among phenomena to be modeled are indirect, or where each individual phenomenon cannot be separated out from the others, a statistical modeling approach might be most appropriate. This was the case for the Meat Standards Australia (MSA) beef grading system.

The MSA model sought to predict a meat quality (MQ) score using a variety of techniques, but mainly through a regression approach. In general terms, the model was as follows:

$$MQ\ \text{score} = (\text{formula } \textit{involving animal variables and treatment variables}) + (\textit{consumer variation}) \quad [6]$$

The researchers used data from 39 muscles or groups of beef muscles, each of which was cooked using up to five different methods (some or all of grill, roast, stir-fry, thin slice, slow cook). They collected the values of 140 variables for each of over 32 000 samples, including input data such as breed,

age, and feed regimen, processing inputs such as carcass suspension and electrical stimulation characteristics, marbling, fat depth and pH, position of the sample within muscle and days aged, and the cooking method. Output data included tenderness (*tn*), juiciness (*ju*), like flavor (*fl*), and overall liking (*ov*) scores evaluated by panels of 10 consumers per sample. The MQ score was constructed from the four sensory scores as follows:

$$MQ = 0.4\ tn + 0.1\ ju\ + 0.2\ fl + 0.3\ ov, \quad [7]$$

During the analysis, the researchers removed from the model variables that failed to predict eating quality either individually or in combination, and variables that were positively correlated with other variables that remained in the model.

The researchers found many relationships of interest in the model. For example, they found a negative correlation between eating quality and the percentage *Bos indicus* content in the animal. They also found a negative correlation between eating quality and ossification score. Both of these relationships were expected based on prior research.

A particularly interesting result apparent from the model was the effect of the method of carcass suspension and the eating qualities of different muscles. For example, the MQ scores of M. longissimus dorsi lumborum, M. biceps femoris, and M. semimembranosus were improved by suspension from the obturator foramen, while MQ scores of the M. psoas major and M. spinalis dorsi were made worse by this suspension method, when compared to suspension from the Achilles tendon.

Although considerable research has been done on the effect of aging on tenderness, much of that research has been carried out on a very limited range of different muscles. In contrast, the many different muscles used to develop the MSA model enabled the researchers to examine the relative effect of aging on tenderness for a range of muscles and for a variety of suspension methods.

When applied to the MSA dataset, the model was found to predict a consumer-evaluated grade (not satisfactory, three-, four-, or five-star) with an accuracy of between 50 and 70% and, if there was an error, the error was of the order of one grade. This allowed the model to be used as a grading tool, to provide feedback reporting to suppliers, and to be integrated with traceability and management systems. The MSA model for predicting eating quality is presently used to grade 2 million beef carcasses per year in Australia.

The Future of Meat Quality Modeling

The meat quality models developed to date have provided many benefits, in helping to predict meat quality attributes for the purposes of process design, in aiding understanding of the mechanisms that underlie those attributes, and in predicting consumer grades of meat with various characteristics. In the case of the MSA model for grading beef carcasses, which is used by the meat industry in Australia, the grading has resulted in increased returns to producers, processors, and retailers. These benefits will only be more important in the future as the understanding on which models are based and the need for quantitative predictions of product characteristics both increase over time.

See also: Conversion of Muscle to Meat: Aging. Electrical Stimulation. Modeling in Meat Science: Microbiology; Refrigeration. Tenderness Measurement

Further Reading

Devine, C., Wells, R., Lowe, T., Waller, J., 2014. Pre-rigor temperature and the relationship between lamb tenderisation, free water production, bound water and dry matter. Meat Science 96, 321–326.

Dransfield, E., Jones, R.C.D., MacFie, H.J.H., 1981. Quantifying changes in tenderness during storage of beef. Meat Science 5, 131–137.

Farouk, M.M., Lovatt, S., 2000. Initial chilling rate of pre-rigor beef muscles as an indicator of colour of thawed meat. Meat Science 56, 139–144.

Ferguson, D.M., Jiang, S.-T., Hearnshaw, H.R., Rymill, S.R., Thompson, J.M., 2000. Effect of electrical stimulation on protease activity and tenderness of M. longissimus from cattle with different proportions of *Bos indicus* content. Meat Science 55, 265–272.

Graafhuis, A.E., Lovatt, S.J., Devine, C.E., 1992. A predictive model for lamb tenderness. In: Johnstone, P.R. (Ed.), Proceedings of the 27th Meat Industry Research Conference, pp. 143–147. Hamilton, New Zealand: Meat Industry Research Institute of New Zealand.

Gursansky, B., O'Halloran, J.M., Egan, A., Devine, C.E., 2010. Tenderness enhancement of *Bos indicus* and *Bos taurus* cattle through electrical stimulation. Meat Science 86, 635–641.

Jeacocke, R.E., 1977. The temperature dependence of anaerobic glycolysis in beef muscle held in a linear temperature gradient. Journal of the Science of Food and Agriculture 28, 551–556.

Kołczak, T., Pospiech, E., Palka, K., Łącki, J., 2003. Changes of myofibrillar and centrifugal drip proteins and shear force of psoas major and minor and semitendinosus muscles from calves, heifers and cows during post-mortem ageing. Meat Science 64, 69–75.

Kristensen, L., Purslow, P., 2001. The effect of ageing on the water-holding capacity of pork: The zrole of cytoskeletal proteins. Meat Science 58, 17–23.

Lovatt, S.J., 1997. Modelling meat processing operations Proceedings of 43rd International Congress of Meat Science and Technology, pp. 114–117. Auckland, New Zealand: Meat Industry Research Institute of New Zealand.

McGlone, V.A., Devine, C.E., Wells, R.W., 2005. Detection of tenderness, post rigor age and water status changes in sheep using near infrared spectroscopy. Journal of Near Infrared Spectroscopy 13, 277–285.

Offer, G., 1991. Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. Meat Science 30, 157–184.

Ouali, A., 1990. Meat tenderization: Possible causes and mechanisms. A review. Journal of Muscle Foods 1, 129–165.

Polkinghorne, R., Thompson, J.M., Watson, R., Gee, A., Porter, M., 2008. Evolution of the Meat Standards Australia (MSA) beef grading system. Australian Journal of Experimental Agriculture 48, 1351–1359.

Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P. (Eds.), 2002. Numerical Recipes in C++: The Art of Scientific Computing. Cambridge, UK: Cambridge University Press.

Rosenvold, K., North, M., Devine, C., *et al*., 2007. The protective effect of electrical stimulation and wrapping on beef tenderness at high pre rigor temperatures. Meat Science 79, 299–306.

Watson, R., Gee, A., Polkinghorne, R., Porter, M., 2008. Consumer assessment of eating quality – Development of protocols for Meat Standards Australia (MSA) testing. Australian Journal of Experimental Agriculture 48, 1360–1367.

Watson, R., Polkinghorne, D.R., Thompson, J.M., 2008. Development of the Meat Standards Australia (MSA) prediction model for beef palatability. Australian Journal of Experimental Agriculture 48, 1368–1379.

Relevant Website

http://www.mla.com.au/Marketing-beef-and-lamb/Meat-Standards-Australia
Meat Standards Australia.

Microbiology

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Glossary

Combase A Combined dataBase for predictive microbiology

FSO Food Safety Objective, that is, the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).

GHP Good Hygienic Practice, refers to all practices which ensure that food is safe and suitable for consumption. GHP can be implemented at all stages of the food chain.

HACCP Hazard Analysis and Critical Control Points, is a systematic approach to food safety. Process steps, at which a food borne hazard would reach an unacceptable level, are identified and control measures/procedures for corrective actions implemented. In many countries, the compliance with HACCP principles is mandatory for businesses dealing with food of animal origin.

Model, deterministic A deterministic model will always produce the same output from a given starting condition or initial state.

Model, stochastic A stochastic process is a collection of random variables used to represent the evolution of some random value, or system, over time.

Monte Carlo simulation Monte Carlo methods are a class of computational algorithms that rely on repeated random sampling to compute their results.

Introduction

Contamination of food by pathogenic or spoilage bacteria is an important economic and public health issue worldwide. To estimate the risks of bacterial contamination of meat, it is necessary to know the bacterial responses (growth, survival, decay) in food matrices, and under food processing and storage conditions. Modeling of these microbial responses is based on the principle that growth or decay of microbial populations, even in the most complex organic commodities, is largely dependent on a few environmental factors only, such as temperature, pH, or availability of water, and growing bacterial cultures in adequately adjusted nutrient broths can be an appropriate *in vitro* simulation. Although, this is a more economical approach than testing artificially inoculated food, only a limited number of environmental conditions can be tested. When mathematical terms are employed to describe changes in microbial numbers as a response to the chosen factors, the responses can be interpolated. This allows the shelf life of food to be estimated and the microbial safety of novel product formulations to be assessed, and can be an integral part of safety assurance concepts.

The Need for Control of Microbial Activity in Meat and Meat Products

Both safety and shelf life of meat and meat products are strongly related to the presence and activity of microorganisms on, or in, these foods. Understanding of the dynamics of microbial populations in foods will allow the minimization of economic losses owing to food spoilage, but it is also a prerequisite to ensure equivalency in food safety in international trade. Changes in primary production (e.g., free-range animals), the preference for minimally processed foods with fewer preservatives (e.g., nitrite, sodium chloride), and different culinary preparation will influence the survival or growth of foodborne pathogens. Not only the exposure of consumers, but also the age distribution and immune status of the population may change, resulting in increased susceptibility.

Preventive Measures and Intervention Strategies

Three strategies are commonly used to improve and ensure the microbiological safety of meat and meat products:

1. Hygiene, i.e., prevention of microbial contamination, which increases shelf-life, but will not guarantee that a product is 100% free from pathogenic bacteria.
2. Antimicrobial treatment as a part of the production process (e.g., sterilization of cans, high-pressure processing) or as an 'additional treatment' (e.g., organic acids, irradiation). Such additional treatments are not allowed in all countries or for all food commodities.
3. Reduction of bacterial growth through control of the microbial environment (i.e., properties of the food and the conditions during processing and storage). These measures usually aim at generating suboptimal growth conditions.

Measures under (2) and (3) can be developed on a trial-and-error basis ('empirical'), which is rather expensive, inflexible (even minor changes in the process require a reassessment), and would ultimately mean that the assessment of safety would be based on testing the final product; however, even a large sample size cannot guarantee 100% safety.

The more scientific and economic approach is process-oriented, and in conformity with HACCP and risk assessment. Monitoring and control of the production process means control of the environmental conditions, and thus control of the growth and survival of contaminant microorganisms. This

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is based on two assumptions: first, that growth or survival is understood to be a consistent response of bacteria to environmental conditions, and second, that the relatively complex food environment can be reduced to a small number of factors, for example, temperature, available water, and pH. The benefits of this approach not only include the knowledge of why specified conditions are safe, but also allow compensation for changes in one factor by readjusting others. It should hence be possible to predict safe conditions, and facilitate the development of new products.

Understanding of Microbial Dynamics in Muscle Foods

Description of Microbial Growth

Multiplication of microorganisms will occur only after a certain adaptation (lag) phase. This is followed by accelerated and then exponential growth (exponential phase, or log phase). When the nutrients are limited, a plateau – where multiplication and decay are in equilibrium – is observed (stationary phase), which is followed by a decrease in microbial cell numbers (see Figure 1). An important dynamic variable to define such a growth curve is the (instantaneous) specific growth rate $\mu(t)$, which is defined as the (instantaneous) change of population per unit time divided by the number of microorganisms at that time. When the population size is expressed as the natural logarithm (ln) of the number of cells and this is plotted against time, the specific rate represents the (instantaneous) slope of the growth curve. The most important parameters of these sigmoid growth curves are the maximum specific growth rate, the initial and final population size, and the lag period, which is commonly defined as the time when the tangent to the inflexion of the growth curve (the slope of which is the maximum specific growth rate, μ_{max}) intercepts the level of initial population size.

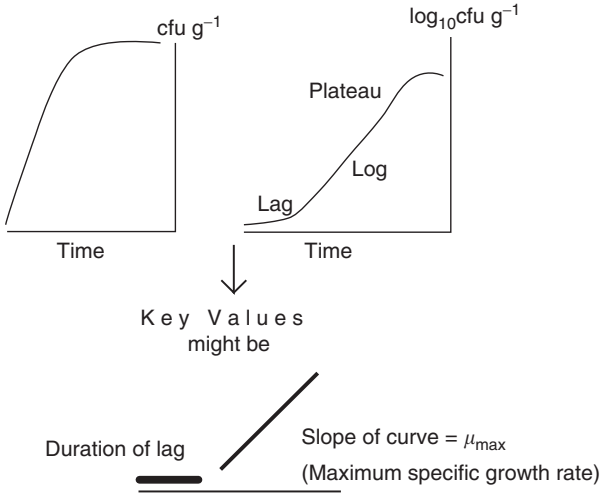

Figure 1 The microbial growth curve and derived key values. The upper-left diagram shows a typical 'growth curve', for example, generated from bacterial cultures grown in nutrient broth, where microbial numbers are plotted against time. Plotting the (natural) logarithms of the bacterial numbers against time will typically result in a sigmoid curve (see the upper-right diagram), which is composed of three distinct phases: a phase with practically no increase in bacterial numbers ('lag'), followed by a nearly linear increase ('log'), and then reduced multiplication ('plateau'). A tangent to the slope of the curve will represent the maximum multiplication rate. While the 'lag' represents the adaptation phase of the microbes to their 'new' environment, a plateau is only observed when the supply of nutrients is limited or microbial interactions reduce growth rate.

Microorganisms multiply by doubling, and, for a single cell, the time elapsed from birth to division is termed the 'generation time.' Although, this can be assessed by advanced image analysis systems, it is more common to specify the time a microbial population needs to double its numbers ('doubling time'). Whereas, the generation time has a frequency distribution in the microbial population, the doubling time is a single parameter meant as some sort of average value of the generation times of the single cells. This mean value is generally smaller than the arithmetical mean of the individual generation times, unless the cells divide synchronously.

Definition of Factors Controlling Microbial Growth

Factors influencing microbial growth in foods can be grouped as intrinsic (pH, water activity, structure), extrinsic (temperature, gaseous atmosphere), process-related (temperature regime, sterilization, chilling, high pressure), and implicit (properties of the bacteria of concern, microbial interactions).

Three approaches can be distinguished in studying the relative contributions of these factors to the production of safe and shelf-stable food products: first, the definition of growth boundaries; second, the qualitative description of time-intensity profiles of external factors constituting 'hurdles' for microbial growth; and finally, a quantitative approach, which results in mathematical models.

Growth boundaries

The range of temperature, pH, and water activity allowing growth is well known for a number of bacteria, and has been used for bacterial classification. Even with a very limited number of growth/no-growth observations, it is possible to set up tables like Table 1. This allows a quick assessment of risk/no-risk areas. Instead of a growth/no-growth criterion, decrease or increase limits in microbial numbers can be specified. For example, from these data it can be estimated that, at a temperature of 2 °C, a carbon dioxide content of 20% will

Table 1 Observations of growth/no-growth of a *Pseudomonas fragi* mixed culture (grown in Brain Heart infusion at pH 5.5, at various temperatures and with varying CO_2 content in the atmosphere). Own data

	100% CO_2	*50% CO_2*	*20% CO_2*	*Air*
20 °C	Ns	+(4.1)	+(2)	+(1.6)
10 °C	Ng	+(17.44)	+(6.1)	Ns
4 °C	Ng	+(45)	Ns	+(2)
2 °C	Ng	Ng	+(31)	+(6)

Ng, time to doubling >50 h; +, growth observed; doubling time in hours is given in brackets; Ns, condition not studied.

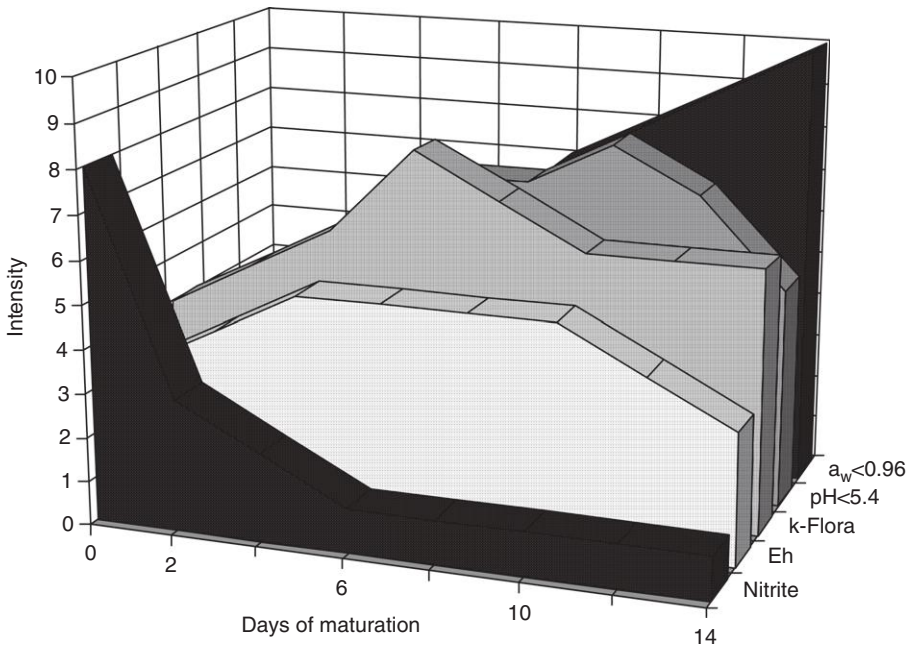

Figure 2 A time profile of the intensity of 'hurdles' during maturation of fermented sausage (a_w ... water activity; k-Flora ... , 'starter cultures'; Eh ... redox potential; Nitrite ... sodium nitrite added). This is a classical, but more qualitative, description of environmental factors influencing microbial growth. It clearly shows that there is a need for 'mathematical modeling' of microbial growth under varying, or fluctuating, conditions. Furthermore, the diagram raises the question as to whether these 'combined processes' are additive only or synergistic.

effectively retard *Pseudomonas* growth, allowing a mere doubling of numbers in 31 h.

Qualitative description

The intensity of the abovementioned intrinsic, extrinsic, etc. factors influences bacterial growth. Hence, it is possible to use a single factor for microbial control, with a lethal effect on microorganisms, as is the case for heat treatment in fully retorted canned food. However, such treatment may markedly affect the sensory and sometimes nutritional properties of foods and is not applicable to all types of meat products. A combination of two or more less intense control factors can function as one single 'effector'; fermentation and ripening of salami-type sausages is an often-cited example (Figure 2). Here a number of control factors or 'combined processes' with different time-intensity profiles interact, so that throughout the maturation period bacterial growth is controlled. This situation is more complex than the classical, original 'hurdle' concept.

Quantitative approach

Mathematical models are commonly classified as either empirical or mechanistic. The empirical, 'black box,' or descriptive approach aims to reproduce exactly particular data, while a mechanistic approach aims to identify the common structures behind various sets of observations. Thus, the term mathematical model is often explained not as a fixed (set of) formulae, but rather as an idea of how things might function. In the case of microbial growth, this is 'a set of basic hypotheses on microbial behavior.' In practice, a mixture of both approaches is used. Descriptive components are inflexible, as they represent a simple statistical representation of particular results and lack the ability to elucidate the underlying mechanisms. They must therefore be distinguished from 'real' models.

For some commodities, a few empirical data and quite simple mathematics will allow prediction of microbial activity. A classical example is thermal processing in the canning industry, which relies on mathematics for estimating the elimination of (spores of) the most infamous anaerobic organism, *Clostridium botulinum*. This is a typical empirical (statistical) approach. Notably, the decimal reduction time describes only one part of the microbial elimination curve; in practice a 'shoulder' is often observed before microbial cell numbers decrease in a log-linear way (Figure 3).

Another simple and straightforward example is a method for calculating the shelf-life of pasteurized milk (this concept can easily be applied to heat-treated meat products). The following assumptions are made: (1) a mere five spoilage bacteria are present in 1 l of milk ($=0.005\ ml^{-1}$ or $-2.3\ \log_{10}$ units ml^{-1}); (2) the standard deviation of microbial numbers is $s=0.4$ log cycles (per milliliter); (3) spoilage is observed at 7.5 $\log_{10}$ *Pseudomonas* sp. per milliliter of milk; (4) storage temperature is 4 °C; and finally (5) the doubling time for *Pseudomonas* sp. at this temperature is approximately 5.5 h. From these data, the difference between initial and final (spoilage) level is calculated to be 9.8 $(=7.5-(-2.3))$ $\log_{10}$ cycles. To reach a multiplication to the 9.8 $\log_{10}$ level, 32.5 doublings (i.e., $10^{9.8}\sim2^{32.5}$) of the initial five microorganisms are necessary. Consequently, the time to spoilage is 7.5 days ($32.5\times5.5=178.75$ h~7.5 days). Assuming there is a normal distribution, 95% of the initial contaminating flora are distributed in the range from 5 ± 2 times the standard deviation s,

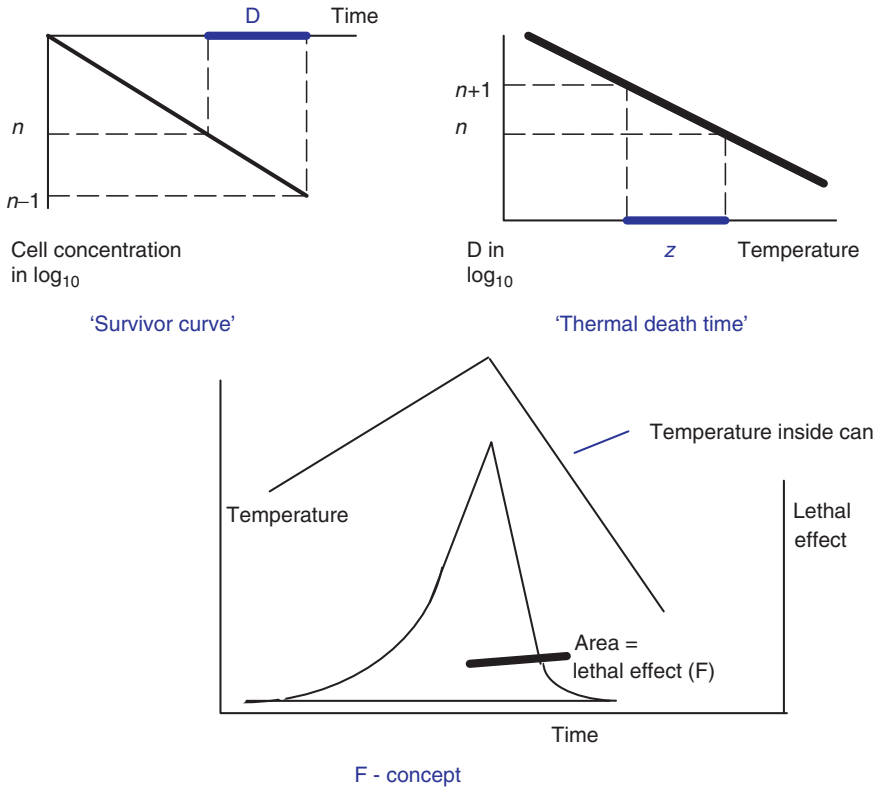

Figure 3 Model-building in thermally processed canned foods. Mathematical treatment of microbial processes became an issue in food canning technology around 1920. For practical purposes, a number of key values were derived: Decimal reduction time ('D'): In bacterial cultures exposed to 'lethal' temperatures at constant level, the reduction of the logarithm of viable cells per time will be nearly constant. The increase in temperature to effect a tenfold reduction in viable bacterial counts is termed 'z' value. However, these key values are not sufficient to estimate the total bactericidal effect during sterilization of cans, as the temperature inside the can varies with time (temperature rise – holding time – cooling). The lethal effect (F) is a sort of weighed time-temperature integral. This is a purely empirical, but effective, approach and implies some simplifications (e.g., during treatment with lethal temperatures, 'survivor curves' display an initial 'shoulder'). For pasteurization, similar mathematical treatment can be employed.

i.e., a range from 0 to approximately 31.5 microorganisms per liter ($= -1.5$ $\log_{10}$ units ml^{-1}) milk. As shelf life prediction should be set to this worst-case scenario, the difference between starting conditions and spoilage level is 9 ($=-7.5 - (-1.5)$] $\log_{10}$ units, which corresponds to approximately 30 doublings. For 30 doublings, 164 h, i.e., 6.8 days, are needed to reach spoilage levels.

Similar calculations for the spoilage of fish have been presented. It has been shown that the rate of growth (R) of spoilage bacteria at a given temperature t as compared to 0 °C can be expressed as $R=(1-0.1t)^2$. This simple equation represents a quantitative description of the well-known fact that a decrease in temperature rapidly reduces spoilage rate.

It should be noted that all the three examples mentioned above focus on one extrinsic or process factor. However, the problems faced by the meat industry are usually far more complex.

Variation of key factors

Given that there are tools available to describe microbial growth and key factors have been defined, it is feasible to create sets of data describing the different microbial response when a few well-defined key factors are varied.

Typically, a defined concentration of bacteria is inoculated in a series of adjusted nutrient broths, and during incubation at defined temperatures and atmospheric composition, concentrations of viable cells are determined by various methods. To these growth curve data, sigmoidal curves are fitted. This can be performed conveniently using computer software, and will result in a number of mathematical functions ('primary models'). The next step is to describe how the fitted parameters of each curve are affected by the various controlling factors. For this purpose, each curve is represented by a few parameters, most notably the maximum specific growth rate. This key value is plotted against the conditions under study, such as pH, sodium chloride content, and temperature, resulting in a response surface ('secondary model', see **Figure 4**). Often, polynomial functions of second order (i.e., quadratic) have been applied successfully.

From these functions, predictions of microbial behavior can be made and subsequently compared with independently acquired data, for example, those available from the literature. If this validation step is successful, one may consider applying the model to real situations. Ideally, the predictions from the simple model are accurate, i.e., they match observations from complex real food matrices. However, a systematic inaccurate overprediction often tends to give 'safe-side' results, which is

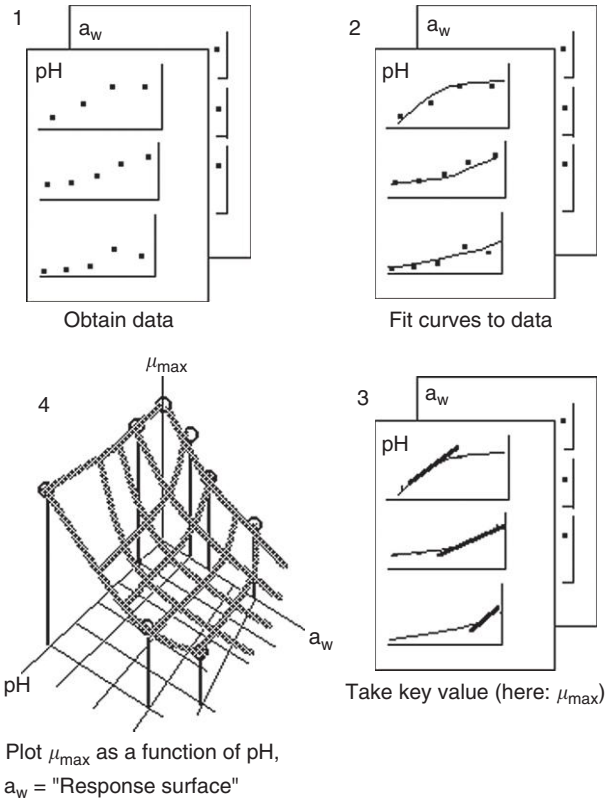

Figure 4 From growth curves to 'response surfaces.' Bacterial cultures are grown in parallel in a number of nutrient broths adjusted to different environmental conditions (pH, water activity, incubation temperature, atmosphere, etc.), and the bacterial numbers are plotted against time, with each data set representing the bacterial response to an individual environmental situation. To these data sets, a mathematical function is fitted (software packs are available for this purpose), from which key values can be derived (see also **Figure 1**). These key values can be plotted against the experimental conditions varied in the nutrient media (in this case, pH, and water activity). To these individual data points, a mathematical function can be fitted resulting in a 'response surface.' Thus, within the range of experimental conditions studied, it is possible to interpolate, for example, the μ_{max}, and to 'predict' microbial behavior for specific conditions that originally were not studied. Such response surfaces can be generated even from a very limited number of microbial experiments.

entirely acceptable, especially where pathogenic bacteria are concerned, for instance, growth rate in solid foods being lower than predicted by a model derived from experiments in nutrient broth, which is usually the case.

This analysis implies that first those variables need to be defined by which the food and microorganism under study can be described (e.g., temperature, pH, water activity, etc.). When these fall within the range of the model, it should be possible to predict situations where a risk of microbial activity may occur. Obviously, a great number of potential combinations of different growth conditions prevail under industrial circumstances. However, the availability of a model now enables us to focus attention on a significantly reduced number of conditions, i.e., those most likely to occur in practice. Only these have to be investigated via challenge tests. This approach reduces analytical effort considerably.

In addition to the logarithmic (exponential) phase of microbial growth, the relationship between lag time and fluctuations of environmental conditions, such as changes in temperature, may be of importance, as microorganisms react to these with an adaptation phase, during which metabolic or structural changes take place and multiplication is suspended. Changes in meat production and processing environment are mostly temperature changes. The mathematical description of the inactivation of microorganisms as a response to environmental conditions has been an issue in the canning industry for more than 80 years.

Limitations and Challenges

Mechanistic models are, by nature, a scientifically based, simplified, but versatile description of how, in our case, bacteria respond to various environmental conditions or changes thereof. Small discrepancies between observed and predicted results are inevitable; these discrepancies are termed 'errors.' Each step of model generation will contribute to these 'errors.'

The mathematical procedures will introduce numerical procedure errors. Simplifications of and assumptions about microbial behavior (for instance, assuming a homogeneous bacterial population and disregarding the natural biological variance) lead to homogeneity errors. For instance, different strains of the same bacterial species may respond quite differently to environmental conditions (intraspecies variability). In part, this can be explained by a different 'history' of the isolates, which will consequently influence the adaptation phase, but differences in the maximum specific growth rate have also been reported, for example, for *Listeria* and *Yersinia*. This is usually compensated when a mix of several strains is used for growth rate determination. The behavior of small numbers of cells is an issue when survival of low-infective dose pathogens (e.g., *Escherichia coli* O157:H7) has to be evaluated. When it comes to describing the behavior at an individual cell level, 'quantal microbiology' has been created, analogous to quantum mechanics in physics. By the same token, the mathematical concepts can be extended to a molecular biology level, i.e., modeling the expression of genes as a response to environmental factors.

Modeling on the basis of a few environmental key factors only necessarily implies incompleteness, and therefore results in completeness errors. The key factors have to be chosen in an appropriate way. Thus, when water activity is under study, the results depend not only on the final water activity, but also on the type of humectant (sodium chloride or carbohydrates).

Mathematical modeling of microbial growth and inactivation is still 'work in progress.' In particular, modeling of the food chain requires that fluctuating environmental conditions (e.g., temperature) and stochastic elements, as distributions of initial cell numbers, temperatures, etc. are considered. Monte Carlo simulations have been used for this purpose. For practical assessment of shelf life, time–temperature integrators have proven useful. Another issue is

that low cell concentrations and growth/survival near to the growth boundaries have not been sufficiently studied for all relevant bacteria.

Mathematical models as outlined above has become an indispensable tool in qualitative as well as quantitative microbial risk assessment, and the development of food safety objectives. Knowledge on extent and probability of changes in microbial numbers in the food chain is also an essential part of risk-ranking software.

Databases and User-Friendly Predictive Software Packages

Currently, a number of predictive software packages are available not only for use by food scientists, but also industry and food safety authorities. These include, but are not limited to, the 'Seafood Spoilage Predictor' of the Danish Institute for Fisheries Research (http://dtuaqua.dk), the Pathogen Modeling Program of the US Department of Agriculture (USDA) (http://www.ars.usda.gov/Services/docs.htmdocid=6786), the Growth Predictor and Perfringens Predictor of the Institute of Food Research (IFR) (UK) (http://www.ifr.ac.uk/Safety/GrowthPredictor/), Sym'Previus of the French ADRIA Developement company, France (http://www.symprevius.net/), and, finally, ComBase (http://www.combase.cc/). The latter software originated as a combination of the Pathogen Modeling Program of the USDA and the Food Micro Model from the UK. The Combase package (freely accesible on the internet or delivered as a stand-alone version) consists of a database containing >50 000 growth curves with detailed description of bacteria and experimental conditions; the database is regularly updated. Data sets can be filtered and retrieved by a built-in browser and growth curves can be fitted to selected data sets; in addition, growth prediction tools and tutorials are available.

See also: Canning. Hazard Analysis Critical Control Point and Self-Regulation. Microbiological Safety of Meat: *Aeromonas* spp.; *Bacillus cereus*; *Clostridium botulinum* and Botulism; *Clostridium perfringens*; Emerging Pathogens; Hurdle Technology; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; *Salmonella* spp.; *Staphylococcus aureus*; Thermotolerant *Campylobacter*; Yeasts and Molds; *Yersinia enterocolitica*. Modeling in Meat Science: Meat Quality; Refrigeration. Packaging: Modified and Controlled Atmosphere; Overwrapping; Technology and Films; Vacuum. Risk Analysis and Quantitative Risk Management

Further Reading

Baranyi, J., 2004. Predictive modelling to control microbial hazards in the food processing industry. In: Smulders, F.J.M., Collins, D.J. (Eds.), Food Safety Assurance and Veterinary Public Health, vol. 3. Safety assurance during food processing. Wageningen: Wageningen Academic Publishers.

Baranyi, J., Pin, C., 2004. Modelling microbiological safety. In: Tijskens, L.M., Hertog, M.L.A.T.M., Nicolai, B.M. (Eds.), Food Process Modelling. Cambridge: Woodhead Publishing.

Baranyi, J., Roberts, T.A., 1995. Mathematics of predictive food microbiology. International Journal of Food Microbiology 26, 199–218.

Baranyi, J., Roberts, T.A., 2000. Principles and application of predictive modelling of the effects of preservation factors on microorganisms. In: Lund, B., Baird-Parker, R., Gould, G. (Eds.), The Microbiology Safety and Quality of Foods. New York: Aspen Publishers Inc. (Chapter 18).

Baranyi, J., Tamplin, M., 2003. ComBase: A common database on microbial responses to food environments. Journal of Food Protection 67, 1834–1840.

Elfwing, A., Le Marc, Y., Baranyi, J., Ballagi, A., 2004. Observing the growth and division of large number of individual bacteria using image analysis. Applied and Environmental Microbiology 70, 675–678.

ICSMF, 1996. Microorganisms in Foods. Characteristics of Microbial Pathogens. London: Blackie Academic and Professional.

McKellar, R.C., Xuewen, L., 2004. Modeling Microbial Responses in Foods. Boca Raton, FL: CRC Press.

McMeekin, T.A., Olley, J.N., Ross, T., Ratkowsky, D.A., 1993. Predictive Microbiology: Theory and Application. New York: John Wiley and Sons.

McMeekin, T.A., Ross, T., 1996. Shelf life prediction: Status and future possibilities. International Journal of Food Microbiology 33, 65–83.

Pin, C., Baranyi, J., 2007. Kinetics of single cells: Observation and modeling of a stochastic process. Applied and Environmental Microbiology 72, 2163–2169.

Relevant Websites

http://www.combase.cc/
Combase.

http://www.ars.usda.gov/Services/docs.htm?docid=6786
Pathogen modeling program.

http://dtuaqua.dk
Seafood Spoilage Predictor.

http://www.symprevius.net/
Sym´Previus.

Refrigeration

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Glossary

Half-cooling time The time taken for the temperature of an object to drop from its initial temperature to a temperature half way between its initial and its ultimate temperature, which will be the temperature of its surrounding environment.

Introduction

Many factors affect the quality and safety of a meat product, but perhaps the most important single factor is a meat product's temperature profile over time, because of its direct and indirect effects on many of the other factors. Reducing the product's temperature, through refrigeration, has an important impact on the rates of microbial growth, enzymatic action, pH fall, and aging, among other factors, making the ability to predict and control meat temperature critical to the success of any high-quality meat processing operation.

The prediction of temperature change (for instance), whether in an existing process or in a new process to be designed, is one purpose of modeling. Another, perhaps more important, purpose of modeling is to aid the scientist or technologist in understanding the complex physical and biochemical processes that take place during meat processing, storage, and transport. Either of these purposes would be sufficient in its own right for modeling to become a key technique in the development of modern meat science.

The process of developing any model of a refrigeration process will commence with the fundamental principles of refrigeration described elsewhere in this encyclopedia. From there, the development path to a practical and useful model will depend on:

- the purpose for which the model is to be used, and hence, the outputs that are required;
- the accuracy of prediction required for those outputs;
- the scope of the model, in terms of time (the periods of the process that are of interest) and space (the distance away from the meat product that the model should include) – both of which have a substantial influence on the amount of effort required to develop the model; and
- the data available for model input, and to validate the model's accuracy.

There are many components in a refrigeration system and models have been developed for every component (see Further Reading), but this article focuses on models of the meat products, grouped by the boundaries of each model component in both space and time.

Model Boundaries

Three possible sets of model boundaries are shown in Figure 1. The individual component models, for example, the meat product or refrigerant evaporator models, have the tightest boundaries. The next set of boundaries group the models that estimate the thermal load on the refrigeration system (i.e., the heat that the refrigeration system must remove from the refrigerated space per unit of time) into a group of 'application models' and those that describe the refrigeration system itself into a group of 'refrigeration plant models.' The largest boundary in Figure 1 encompasses the application and refrigeration plant models to form an overall 'meat refrigeration facility model.' These three spatial sets of boundaries may be perceived as natural ways to divide the problem, but they are far from being the only ways. For some purposes, it may be necessary to model only a small part of the meat product (e.g., the loin of a lamb carcass), and thus the entire model would lie inside the 'cooling meat product' component shown in Figure 1. For other purposes, it may be necessary to model many processing plants together (e.g., to predict the total meat production or energy consumption of the entire industry), in which case even the outer boundary shown in Figure 1 would only be a small part of the whole model.

Figure 2 shows some ways in which models could be categorized along the time dimension. Depending on the purpose of the model, one could choose to examine the process with a greater or lesser granularity in time, as shown in the Figure 2. The granularity of a model in the time dimension is determined by the interval between model outputs.

In some cases, a very fine resolution is required, so that one can examine changes that occur over periods of seconds or minutes, such as moisture loss through evaporation or moisture uptake during spray-chilling. In other cases it may be important to model longer time intervals, such as chilled storage and transport, with a longer model output interval to predict (for instance) the slow aging of meat during transport, or the growth of anaerobic microorganisms.

The effort required to construct and validate a model and the amount of input data required to execute the model increase in rough proportion to the number of individual model components and the total number of model output intervals to be observed. This means that it is important to restrict the model scope in both space and time so that the effort required of the model developer and user is acceptable and realistic compared to the benefits to be gained from the model.

Much of a model's value results from the fact that the model is much simpler to understand than the real object or process that it represents. In a successful model, this simplification results from model developer's choices to exclude those parts of

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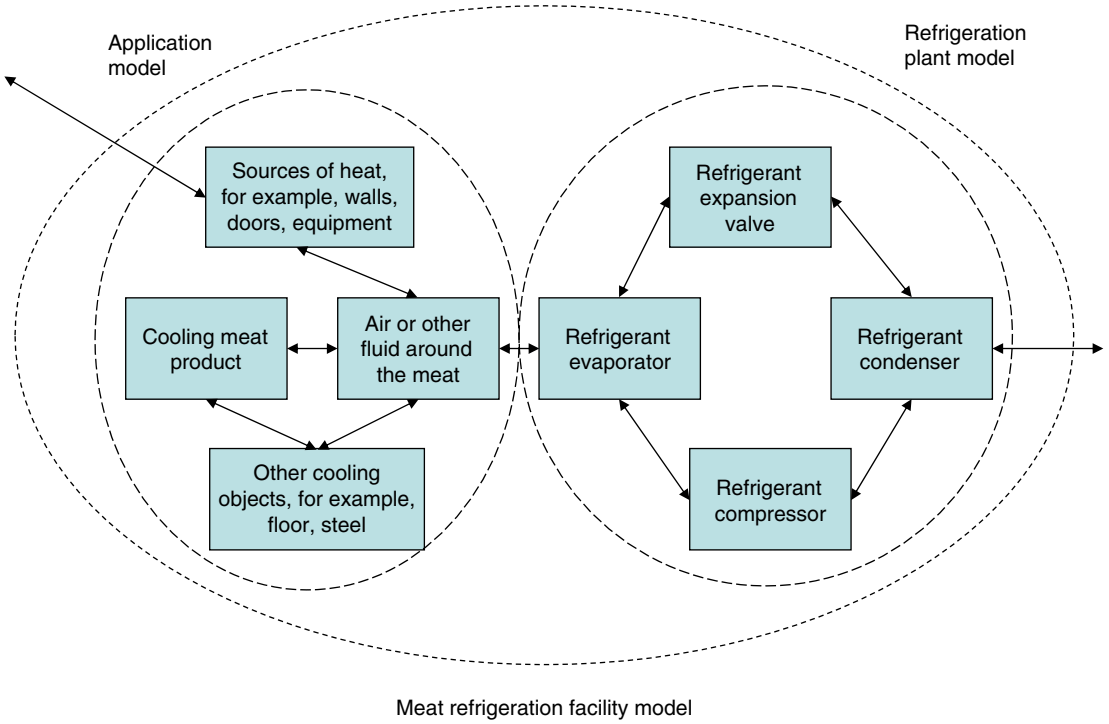

Figure 1 Conceptual division of a meat refrigeration model into an application model and a refrigeration plant model, and then into component models. Only one example of each component type is shown, though in most cases there would usually be many components of each type.

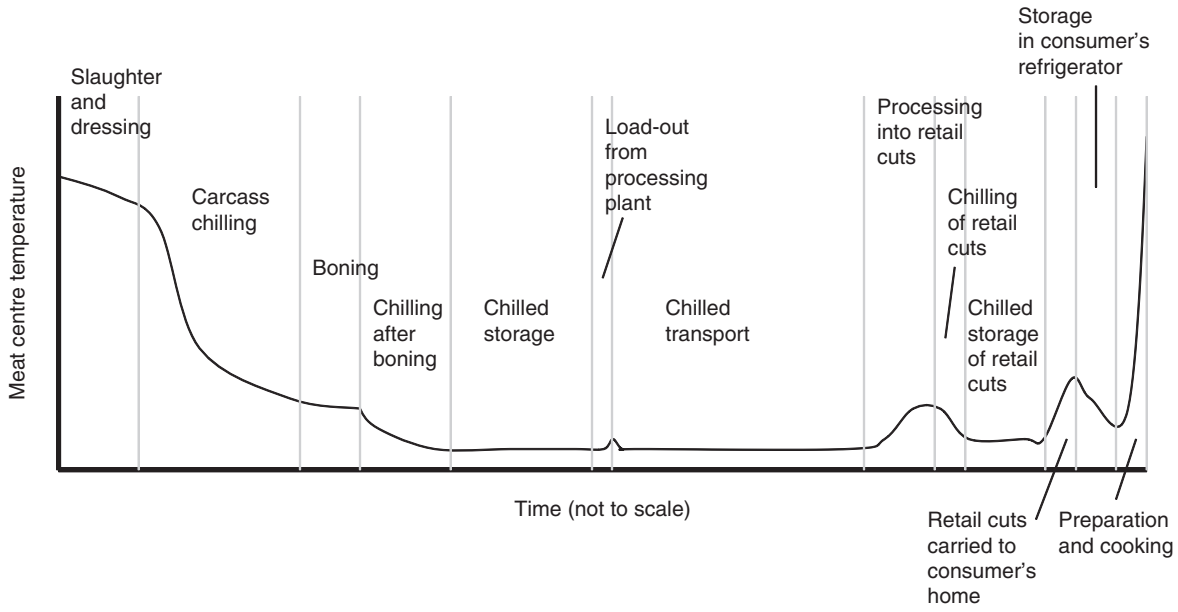

Figure 2 Conceptual division of a meat refrigeration model into components along the time dimension for a chilled meat process.

the real world that have little effect on the outputs of interest, or to include some parts only as averages or approximations.

For these reasons, scientists and engineers have developed many different models of meat refrigeration and its related processes, designed to gain insight into different aspects of the process, or to predict different outputs with more or less accuracy and, hence, effort required. Some of these models, or classes of models, are described in this article. Others can be found in the Further Reading section.

Meat Product Chilling and Freezing Times

Perhaps the simplest useful model of meat product chilling and freezing is the 'lumped-parameter' model, based on

Newton's law of cooling, which can be written as shown in eqn [1].

$$\frac{dT}{dt} = \frac{hA}{VC}(T_a - T) \quad [1]$$

where T, is the temperature of the meat product, °C; t, is time, s; h, is the heat transfer coefficient between the meat product and its surroundings, W m^{-2}K; A, is the surface area of the meat product, m^2; V, is the volume of the meat product, m^3; C, is the specific heat capacity of the meat product, J m^{-3}K; and T_a, is the temperature of the meat product's surroundings, °C.

This model has the advantage of a straightforward analytical solution for T in terms of t, if the other parameters are constant with time and temperature. If the other parameters change over time, this equation can be solved numerically. However, the model predicts only a single temperature for the meat product at any given time because it assumes that the temperature throughout the product is uniform. The validity of this assumption can be assessed by the value of the Biot modulus, Bi, shown in eqn [2].

$$\text{Bi} = \frac{h(V/A)}{k} \quad [2]$$

where k, is the thermal conductivity of the unfrozen meat product, W mK^{-1}.

A low value of Bi indicates that the relative resistance to heat transfer within the cooling object is low (and the rate of cooling is therefore controlled by the rate of convective heat transfer at the surface), whereas a high Bi value indicates that the relative resistance within the object is high (and the rate of cooling is therefore controlled by the rate of conduction through the object). If Bi is less than 0.1, it is often assumed that the lumped-parameter model is adequate; however, for larger values of Bi, a more sophisticated model is required to obtain reasonable accuracy.

The rate at which a meat product cools when conduction within the meat is important is defined by the Fourier equation, a partial differential equation describing the conservation of energy. For some cases, this equation can be solved analytically to predict how the temperature varies at any location in a cooling object; however, the analytical solutions only apply to objects with simple shapes, for example, an infinitely wide and long slab, an infinitely long cylinder, a sphere, or an infinitely long rod-like shape. Further, with a few exceptions, an analytical solution of the Fourier equation cannot be obtained in cases where the thermal conductivity and heat capacity of the object change significantly with temperature, as is the case for any meat product during freezing and for most cases where the conditions external to the cooling object change over time.

Although the Fourier equation is difficult to solve analytically for complex geometries and changing properties and conditions, there are several alternative modeling approaches that have simplified the problem by increasing the spatial and time resolution of the model. In terms of spatial resolution, if the object is not considered as a whole, but as many thin slices, elements, or volumes, each with a relatively simple shape, then this can make it possible to solve the Fourier equation for each of the slices, elements, or volumes (see Figure 3). Similarly, in terms of time resolution, the whole process can be divided into small intervals and each interval can be considered individually. In that case, it might be assumed that the temperature (and hence the temperature-dependent thermal properties) in each part of the object will not change significantly during an interval, or it might change according to a simple (e.g., linear) relationship with time, again making it possible to solve the Fourier equation for each time interval.

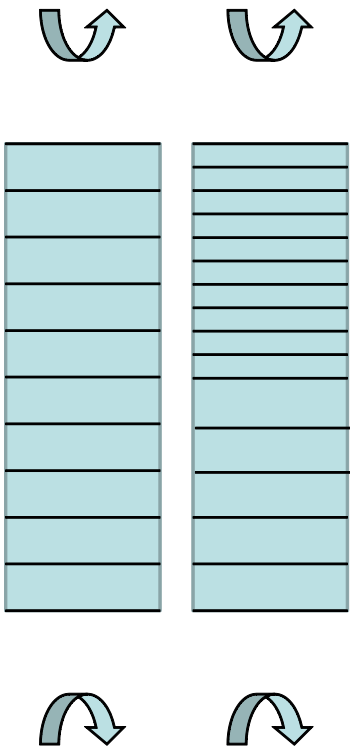

Figure 3 Two different ways of dividing a model of a slab-shaped meat product into slices so that Fourier's equation can be solved for each slice, with convective cooling at the top and bottom. The diagram on the left shows the model divided into equal-width slices, whereas the diagram on the right has thinner slices at the top to increase the accuracy of the computation at that end.

This type of reasoning has led to the development of many alternative models that can be applied to cooling meat products, of which the most popular have been the finite difference and finite element methods. If moisture diffusion is being considered as well as heat transfer, it may be necessary to use separate grids for heat and moisture transfer. Where modeling extends to the flow of fluid around the meat product, it may also be necessary to use computational fluid dynamics to evaluate fluid flow directions and velocities. These methods must generally be implemented in the form of computer software, and it is recommended that persons wishing to use them purchase or hire the use of specialist packages unless they have considerable time and effort to dedicate to the implementation. Even given the right computer software, the amount of data required to define complex meat product shapes for input to finite element software can be considerable and the amount of time required to collect the data can be substantial, especially if the modeling is to be carried out in all three spatial

dimensions. However, developments in the collection of three-dimensional shape data have made this task considerably easier.

In many cases, some simplification of the meat product shape is an acceptable compromise and this has led to the development of meat product cooling models that deal (approximately) with the whole meat product at once. These models can sometimes be solved analytically if the thermal properties and conditions do not change with temperature or time during the process. Alternatively, they can be evaluated with changing thermal properties and conditions by expressing them as ordinary differential equations (ODEs) and then dividing the process into short time intervals to solve the equations numerically. In either case, it is often satisfactory to use a single shape factor that adjusts the predicted chilling or freezing time upwards or downwards with respect to the freezing time of a reference shape with the same thermal properties as the meat product. One commonly used shape factor is the equivalent heat transfer dimensionality, E, developed by Cleland and Earle, which is dependent on both the shape and the Biot number. The Biot number dependence of E is complex but eqn [3] is a satisfactory definition at $\mathrm{Bi}=0$:

$$E=\frac{SX}{V},\quad \text{at}\quad \mathrm{Bi}=0 \qquad [3]$$

where X, is the radius, or half-thickness of the object, m.

The value of E varies between 1 and 3, with $E=1$ for an infinitely wide and long slab, $E=2$ for an infinitely long cylinder, and $E=3$ for a sphere at all values of Bi. For beef sides, a typical E value is approximately 1.3, for lamb legs $E\approx 2.1$, and for boxes of various shapes E might range from 1.2 to 1.5.

For chilling time, a popular model is that of Cleland and Earle, which can be expressed as shown in eqns [4] or [5].

$$\mathrm{Fo}_{1/2}=\frac{\mathrm{Fo}_{1/2,\mathrm{slab}}}{E},\ \text{for flat-sided objects (e.g., boxes)} \qquad [4]$$

$$\mathrm{Fo}_{1/2}=\frac{3\mathrm{Fo}_{1/2,\mathrm{sphere}}}{E},\ \text{for oval-shaped objects (e.g., carcasses)} \qquad [5]$$

where $\mathrm{Fo}_{1/2}$, is the half-cooling Fourier number (dimensionless time) for an irregularly shaped object, or a slab or sphere as indicated by the subscript.

The Fourier number is defined by eqn [6].

$$\mathrm{Fo}=\frac{kt}{CX^2} \qquad [6]$$

Because the rate of temperature change for an object exposed to constant external conditions follows an exponential function over time (after an initial time lag if the temperature of interest is that at the center of the object rather than the surface or mass average), the shape of that exponential can be defined by the time required for the temperature to fall half of the way from its initial value to its final value. This time is known as the half-cooling time, and the Fourier number calculated for the half-cooling time is known as the half-cooling Fourier number. The half-cooling Fourier number for a slab or sphere depends only on the Biot number. Values of $\mathrm{Fo}_{1/2}$ for a range of Biot numbers were tabulated by Cleland and Earle, or $\mathrm{Fo}_{1/2}$ could be calculated from the analytical solutions to the one-dimensional Fourier equation reported by Carslaw and Jaeger (see Further Reading). The fraction unaccomplished temperature change at the end of the chilling process, Y_{final}, is defined by eqn [7]:

$$Y_{\mathrm{final}}=\frac{T_{\mathrm{final}}-T_{\mathrm{a}}}{T_{\mathrm{initial}}-T_{\mathrm{a}}} \qquad [7]$$

where T_{initial} and T_{final}, are the initial and final product temperatures, °C.

The number of half-cooling times, $N_{1/2}$, required to chill the product to its final temperature can be calculated from eqn [8], unless the defined final temperature is at the center of the product, when the initial lag must be added:

$$N_{1/2}=\frac{-\ln(Y_{\mathrm{final}})}{\ln(2)} \qquad [8]$$

Equation [9] can then be used to calculate the total chilling time:

$$t_{\mathrm{c}}=N_{1/2}t_{1/2} \qquad [9]$$

where t_{c}, is the chilling time, s.

For freezing time, the model of Pham is both relatively accurate and convenient to use. Pham's model enhanced the converging freezing model described by Plank in 1941 (see Figure 4) by correcting Plank's assumption of a sharp freezing front and including the sensible heat content (i.e., the heat content removed as the product temperature is reduced) both above and below the freezing temperature, as well as the latent heat that is removed during ice crystal formation. Pham's model can be expressed as shown in eqn [5]

$$t_{\mathrm{f}}=\frac{1}{E}\left(\frac{\Delta H_1}{\Delta T_1}+\frac{\Delta H_2}{\Delta T_2}\right)\left(\frac{X}{h}+\frac{X^2}{2k_f}\right) \qquad [10]$$

where t_{f}, is the freezing time, s; ΔT_1, ΔT_2, are the temperature driving forces during chilling (1) and freezing (2), °C; ΔH_1, ΔH_2, are the enthalpy changes during chilling (1) and freezing (2), J m^{-3}; and k_{f}, is the thermal conductivity of the frozen meat product, W mK^{-1}.

The temperature driving forces and enthalpy changes were calculated based on the mean freezing temperature, T_{fm}, as shown in eqns [11–15].

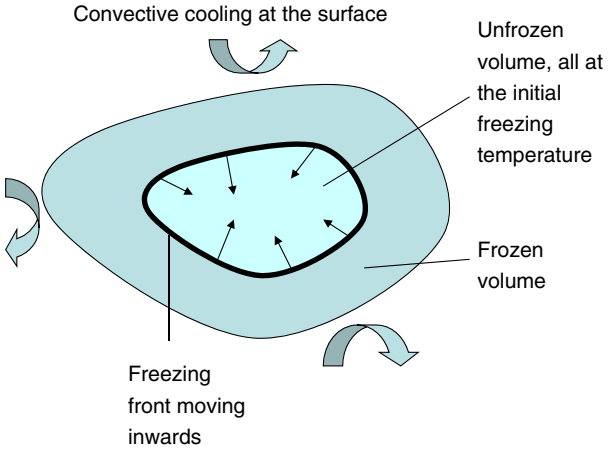

Figure 4 Plank's conceptual model of the freezing process, on which many freezing models are based.

$$T_{fm} = 1.8\ ^{\circ}C + 0.263T_c + 0.105T_a \quad [11]$$

$$\Delta T_1 = (T_{initial} + T_{fm})/2 - T_a \quad [12]$$

$$\Delta T_2 = T_{fm} - T_a \quad [13]$$

$$\Delta H_1 = C_u(T_{initial} - T_{fm}) \quad [14]$$

$$\Delta H_2 = L + C_s(T_{fm} - T_c) \quad [15]$$

where T_c, is the final center temperature, °C; C_u and C_s, are the specific heat capacities of the unfrozen and frozen product, J m^{-3}K; and L, is the latent heat capacity of the product, J m^{-3}.

These simplified methods for chilling and freezing time calculation can be expected to be accurate within ±10% of the chilling or freezing time, respectively, with 90% confidence.

Meat Product Chilling and Freezing Heat Loads

Models to predict chilling and freezing times produce a single value as an output – the time required to chill or freeze. When modeling the cooling of a meat product to predict the heat load that a product will place on the refrigeration system over time, however, these single values are not sufficient. In principle, the heat load of a chilling or freezing product can be derived from eqn [16].

$$\dot{Q} = hA(T_{surf} - T_a) \quad [16]$$

where $\dot{Q}$, is the heat load (or heat flux), W; and T_{surf}, is the surface temperature of the product at any given moment in time, °C.

Because T_{surf} varies during the course of the process, so does $\dot{Q}$, and models that predict the surface temperature of the product (such as the finite difference and finite element models described above, which predict temperatures throughout the product at each output interval) can therefore be used with eqn [16] to predict the heat flux. When another type of model has been used to calculate the cooling time, the mean heat load for a chilling process, $\dot{Q}_{mean}$, can be calculated from eqn [17].

$$\dot{Q}_{mean} = \frac{\Delta H_{process}}{t_c} \quad [17]$$

where $\Delta H_{process}$, is the amount of heat released as the product cools from $T_{initial}$ to T_{final}, J.

The variation in heat load during chilling can be modeled approximately by using the half-cooling time calculated by the method of Cleland and Earle, because one can calculate the mean heat load during the jth half-cooling period, Q_j, using eqn [18].

$$\dot{Q}_j = \frac{\Delta H_{total}}{2^j t_{1/2}} \quad [18]$$

where ΔH_{total}, is the total amount of heat that would be released if the product were cooled from $T_{initial}$ to T_a, J.

The differential equation model for the chilling heat load described by Lovatt *et al.* as shown in eqn [19] can be solved to provide a smooth curve of heat load versus time and allow for time-variable chilling conditions.

$$\frac{dH}{dt} = \frac{E}{3}\frac{V\beta_1^2 k}{X^2}(T_a - T_{ma}) \quad [19]$$

where H, is the specific enthalpy of the product, J m^{-3}; and β_1, is the first root of the equation $\beta \cot \beta + (\mathrm{Bi} - 1) = 0$. This bases the equation on the average product temperature instead of the surface temperature used in eqn [16]; T_{ma}, is the average temperature of the meat product, defined by $T_{ma} = \frac{H - H_f}{C} + T_f$ where H_f, is the specific enthalpy of the product at its initial freezing temperature, J m^{-3}; T_f, is the initial freezing temperature of the product, °C.

A similar model can be used to predict the heat load during the freezing process, using a modification of the converging freezing front concept developed by Plank. This model is shown in eqns [20] and [21]. Although the freezing time depends only on the time required for the slowest cooling point to freeze, the freezing heat load depends strongly on the overall shape of the meat product. As a result, this heat load model requires an additional shape factor, N, to define the way in which the volume of the frozen region, V, changes with the position of the freezing front, x_f.

$$\frac{dx_f}{dt} = \frac{T_a - T_{ma}}{L x_f^{E-1}\left[\frac{1}{hX^{E-1}} - \frac{\left(x_f^{2-E} - X^{2-E}\right)}{k_f(2-E)}\right]} \quad [20]$$

$$\frac{dV}{dx_f} = N\left(\frac{x_f}{X}\right)^{N-1}\frac{V}{X} \quad [21]$$

$$\frac{dH}{dt} = L\frac{dx_f}{dt}\frac{dV}{dx_f} \quad [22]$$

where L is the latent heat of freezing, defined by $L = H_f - C_f(T_f - T_{base})$ if freezing is assumed to start when T_{ma} is approximately equal to T_f, J m^{-3}; T_{ma} is the average product temperature, calculated from a suitable function that relates that average temperature to its enthalpy, °C; C_f, is the heat capacity of the frozen product, J m^{-3}K; and T_{base}, is the temperature at which H is defined to be zero, °C.

When chilling and freezing are described by separate ODEs, any freezing process that starts with the product being warmer than its initial freezing temperature is modeled initially as a chilling process (e.g., using eqn [19]), and then as a freezing process (e.g., using eqns [20–22]) once the surface actually starts to freeze. Criteria to determine when it is best to make the transition from the chilling model to the freezing model have been suggested in the literature, but they will not be discussed further here (see the Further Reading section).

Because they conceptually divide the meat product into small pieces, and model the behavior of each piece during each time interval, numerical models based on the finite difference or finite element methods (for instance) can predict heat loads and temperature profiles through the product with almost all combinations of external conditions that one

could envisage. In contrast, the ODE models described in eqns [19–22] assume that the temperature profile through a chilling product conforms to a consistent shape, or that the frozen volume of a freezing product can be defined by a single parameter – the position of the freezing front. As a result, one can easily define circumstances where the ODE models described here will predict heat loads poorly – for example, if the air temperature varied so much that it rose above the average product temperature, and thus the product started to warm up during part of the process. This highlights the importance of choosing a model that is simple enough to understand and evaluate, but not so simple that it fails to represent an important aspect of the process. Fortunately, the circumstances in which ODE models are unsatisfactory rarely occur in practical meat processing operations, with the result that ODE models are often used to design new industrial refrigeration systems and to evaluate the performance of existing systems.

See also: Modeling in Meat Science: Meat Quality; Microbiology. Refrigeration and Freezing Technology: Applications; Principles

Further Reading

Carslaw, H.S., Jaeger, J.C., 1959. Conduction of Heat in Solids, second ed. Oxford, UK: Oxford University Press.

Cleland, A.C., Earle, R.L., 1982. A simple method for prediction of heating and cooling rates in solids of various shapes. International Journal of Refrigeration 5, 98–106.

Crank, J., 1975. The Mathematics of Diffusion, second ed. Oxford, UK: Oxford University Press.

Crocombe, J.P., Lovatt, S.J., Clarke, R.D., 1999. Evaluation of chilling time shape factors through the use of three-dimensional surface modelling. In: Proceedings of the 20th International Congress of Refrigeration, vol IV, Paper 353. Sydney, Australia: The International Institute of Refrigeration.

Fricke, B.A., Becker, B.R., 2006. Sensitivity of freezing time estimation methods to heat transfer coefficient error. Applied Thermal Engineering 26, 350–362.

Hossain, M.M., Cleland, D.J., Cleland, A.C., 1992a. Prediction of freezing and thawing times for foods of regular multi-dimensional shape by using an analytically derived geometric factor. International Journal of Refrigeration 15, 227–234.

Hossain, M.M., Cleland, D.J., Cleland, A.C., 1992b. Prediction of freezing and thawing times for foods of three-dimensional irregular shape using a semi-analytical geometric factor. International Journal of Refrigeration 15, 241–246.

Hossain, M.M., Cleland, D.J., Cleland, A.C., 1992c. Prediction of freezing and thawing times for foods of two-dimensional irregular shape using a semi-analytical geometric factor. International Journal of Refrigeration 15, 235–240.

Lewis, R.W., Nithiarasu, P., Seetharamu, K.N., 2004. Fundamentals of the Finite Element Method for Heat and Fluid Flow. Chichester, UK: John Wiley & Sons.

López-Leiva, M., Hallström, B., 2003. The original Plank equation and its use in the development of food freezing rate predictions. Journal of Food Engineering 58, 267–275.

Lovatt, S.J., Pham, Q.T., Cleland, A.C., Loeffen, M.P.F., 1993. A new method of predicting the time-variability of product heat load during food cooling – Part 1: Theoretical considerations. Journal of Food Engineering 18, 13–36.

Pham, Q.T., 1986. Simplified equation for predicting the freezing time of foodstuffs. Journal of Food Technology 21, 209–219.

Pham, Q.T., Trujillo, F.J., McPhail, N., 2009. Finite element model for beef chilling using CFD-generated heat transfer coefficients. International Journal of Refrigeration 32, 102–113.

Santos, M.V., Vampa, V., Califano, A., Zaritzky, N., 2010. Numerical simulations of chilling and freezing processes applied to bakery products in irregularly 3D geometries. Journal of Food Engineering 100, 32–42.

Trujillo, F.J., Pham, Q.T., 2006. A computational fluid dynamic model of the heat and moisture transfer during beef chilling. International Journal of Refrigeration 29, 998–1009.

Welty, J.R., Wicks, C.E., Rorrer, G.L., Wilson, R.E., 2007. Fundamentals of Momentum, Heat, and Mass Transfer, fifth ed. New York, NY, USA: John Wiley & Sons.

Relevant Websites

https://www.ashrae.org/
American Society of Heating, Refrigerating, and Air-Conditioning Engineers.

http://www.iifiir.org/
International Institute of Refrigeration.

MUSCLE FIBER TYPES AND MEAT QUALITY

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Glossary

Adenosine triphosphatase (ATPase) Enzyme that catalyzes the hydrolysis of ATP to ADP, releasing energy that is used in the cell.
Muscle fiber Elongated muscle cells.
Myofibril Slender striated strand within skeletal muscle fibers, composed of bundles of myofilaments. Myofibrils occur in groups of branching threads running parallel to the cellular long axis of the fiber.
Myosin The commonest protein in muscle cells, forming thick filaments. Myosins are composed of one or two heavy chains and usually four light chains. Myosin and the protein actin form the contractile units (sarcomeres) of skeletal muscle. In the sarcomere, actin and myosin filaments slide past each other to cause the shortening of a muscle fiber.
Myosin heavy chain isoforms (MyHC isoforms) Heavy chains of myosin molecules with differences in several amino acid compositions.

Introduction

Skeletal muscle is essentially composed of muscle cells colloquially called 'muscle fibers' due to their elongated shape. Their diameter ranges from 10 to 100 μm, yet they can measure from a few mm to several cm in length. These muscle fibers are ensheathed in connective tissue (endomysium) and packed into fascicle groups of several dozen cells ensheathed in another layer of connective tissue (perimysium) to form fiber bundles.

Muscle fibers account for up to 90% of muscle volume. Muscle fibers share similar shape and appearance, but they are actually very different. Adult mammal muscle has four types of muscle fibers, called type I, type IIA, type IIX, and type IIB, which vary in terms of muscle metabolism and contractile speed. Muscles generally comprise various proportions of each muscle fiber type depending on the muscle's function.

Postmortem changes and the changes occurring during transformation processes also vary according to muscle fiber type composition, impacting meat, and meat product quality.

Muscle Fiber – General Structure and Composition

Muscle fibers contain alternating light and dark bands that give skeletal muscle its striated appearance. The muscle fiber is an elongated, spindle-shaped cylindrical filament measuring 10–100 μm in diameter but up to several centimeters long. Each fiber contains an outer shell packed with nuclei and is coated with a plasma membrane called the sarcolemma.

The sarcoplasm (muscle cell cytoplasm) houses the cellular organelles (Golgi apparatus, mitochondria, lysosomes, ribosomes, etc.) and the sarcoplasmic reticulum. It contains a medley of soluble proteins including glycolytic enzymes and the myoglobin that carries oxygen to the mitochondria and gives the cells a reddish pigmentation. It also hosts glycogen granules, which are the muscle cell's main energy store. The sarcoplasm also contains myofibrils measuring just 1–2 μm in diameter and which are arranged into bundles that fill almost all the intracellular volume (Figure 1).

These myofibrils are built from an assemblage of thin and thick myofilaments. The thin myofilaments are essentially composed of actin, troponin, and tropomyosin proteins.

The thick myofilaments are mainly composed of a complex of myosin molecules built of:

- four myosin light chains (MyLC; 20 000 g mol^{-1}) and
- two myosin heavy chains (MyHC; 230 000 g mol^{-1}), where the myosin heads host the adenosine triphosphatase (ATPase) activity that catalyzes the breakdown of ATP into adenosine diphosphate, thus releasing the chemical energy needed for muscle contraction.

Four different isoforms of MyHC have so far been identified, namely I, IIa, IIb, and IIx. These four isoforms are used to characterize not only the four main pure muscle fiber types – I, IIA, IIX, and IIB – but also hybrid fiber types containing several myosin isoforms, such as type I–IIA, type IIA–IIX, or type IIX–IIB fibers. These hybrid fibers are fibers whose properties are in the process of changing from a pure type into another type, driven by a change in age, physical activity levels, or exposure to prolonged stress. The type modification always follows the order I→I–IIA→IIA→IIA–IIX→IIX→IIX–IIB→IIB and back in reverse.

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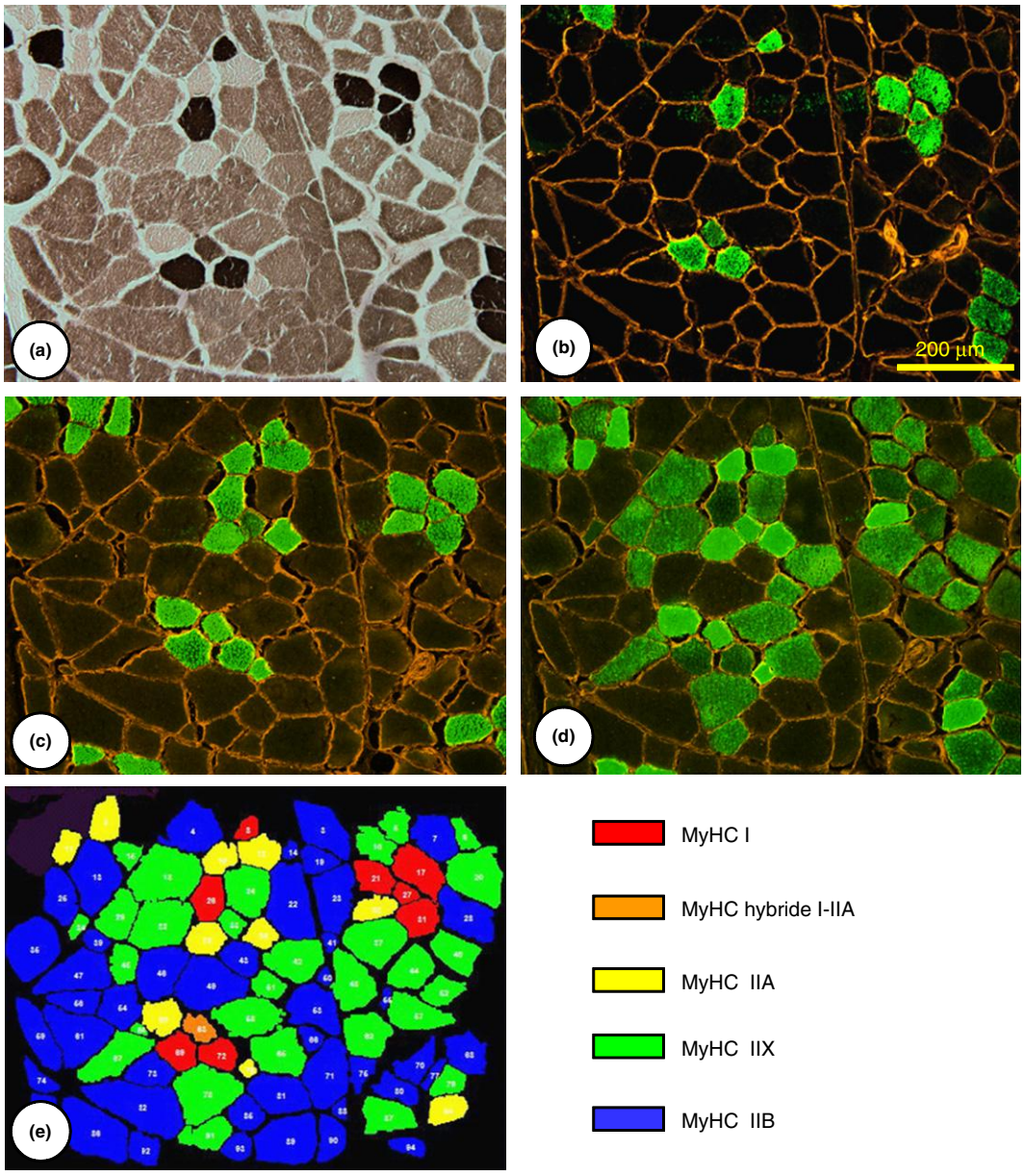

Figure 1 Histological fiber type identification on serial sections of pig longissimus dorsi muscle. (a) ATPase activity has been demonstrated by histoenzymology after section preincubation at pH 4.45. Fiber I: black, fiber IIA: white, and fiber IIB and IIX: gray. (b, c, d) Double labeling of laminin (orange) and MyHC: green by immunohistofluorescence. (b) BAD5 antibody labeling MyHC I myosin isoform (fibers I). (c) BF35 antibody labeling MyHC I + MyHCIIa myosin isoforms (fiber I + fiber IIA). (d) SC71 antibody labeling MyHC IIa (intense) and MyHC IIx (fibers IIA and IIX). (e) Image recombined accurately identifying each fiber type. The immunohistofluorescence is heavier to implement, but unlike the histoenzymology method, it allows to discriminate the IIX fibers from the IIB fibers and hybrid fibers containing mainly two isoforms.

Muscle Fibers – Characteristics

The four main muscle fiber types found in adult mammal skeletal muscle present different sets of characteristics, as listed below.

- Type I: Slow twitch, red, oxidative metabolism. These fibers are specialized for endurance activities such as maintaining posture. The neurons fire at low frequency, the fibers contract relatively slowly, and myosin ATPase activity is low. The fibers are dense with lipids, mitochondria, and myoglobin. Type I are the smallest-sized fibers.
- Type IIA: Fast twitch, intermediate metabolism. These fibers have high myosin ATPase activity, can use both oxidative and glycolytic metabolism, and are dense with mitochondria and myoglobin. They are solicited for rapid-movement activity but are less fatigue resistant than type I.
- Type IIB and IIX: Fast twitch, white, glycolytic metabolism. These fibers are dominantly force generating, are mobilized for activities such as sprinting or weightlifting that

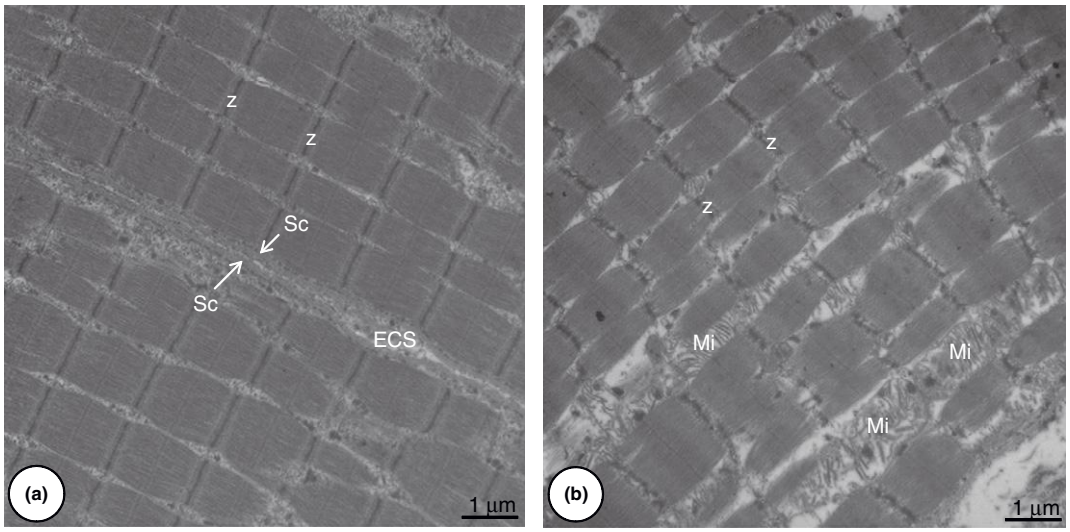

Figure 2 Ultrastructure of different bovine muscle fiber type: Z line (Z), Sarcolemma (Sc), Extracellular space (ECS), Mitochondria (Mi). (a) Two fast glycolytic fibers, probably IIX, as IIB are rare in cattle, recognizable by their extremely low density of mitochondria. (b) Oxydative fiber, I or IIA recognizable by its numerous mitochondria and its thicker Z lines than in IIX fibers.

predominantly rely on glucose as fuel, and they are innervated by high-frequency neurons. They have high myosin ATPase activity and are poor in myoglobin and mitochondria. Type-IIB fibers are the largest-sized fibers.

Contractile speed and proportion of glycolytic metabolism thus increase in the order I→IIA→IIX→IIB.

Muscle Fibers – Methods of Identifying Fiber Type

Various methods have been put forward for identifying fiber type, some relying on histological characterization, others on biochemical characterization.

Histochemistry

These methods are able to histologically characterize each individual fiber, making it possible to assess the in-tissue distribution of each fiber type. The proportions of each type are obtained by calculation. The use of computer algorithms allows semiautomated analysis of digital images, and this step is now faster and less arduous than before (Figure 2). Three main histological approaches are implemented to characterize the fibers based on their contraction speed, metabolic characteristics, or, using antigen/antibody reaction, to precisely identify the different myosin isoforms.

The first and most common method is based on the sensitivity differentials of actomyosin ATPase under different pH conditions (Table 1 and Figure 2).

The second method uses histochemical reactions for enzymes of aerobic oxidative metabolism reflecting mitochondrial content. It is often succinyl dehydrogenase activity that is highlighted, which distinguishes the glycolytic metabolism fibers from oxidative metabolism fibers.

Table 1 Histoenzymology staining properties of different fiber types

Stain	*Type I*	*Type IIA*	*Type IIB and IIX*
Adenosine triphosphatase (ATPase) pH 9.4	+	+++	+++
ATPase pH 4.6	+++	–	++
ATPase pH 4.3	+++	++	+
Nicotinamide adenine dinucleotide dehydrogenase	+++	++	+
Succinic dehydrogenase	+++	++	+

The third method is based on identifying the mysosin isoforms by immunohistochemistry using specific monoclonal antibodies. This highly accurate method is better for identifying hybrid fibers which contain multiple isoforms of myosin (Figure 2).

Biochemistry

MyHC isoforms have slightly different molecular weights, which means that they can be separated by electrophoresis. The density of each band is proportional to the quantity of the corresponding MyHC isoform. This technique, based on analysis of a muscle extract, is less arduous than histological methods and offers the added advantage of giving a global quantification of the proportion of each isoform.

The fact that monoclonal antibody specific to each isoform are now available has also opened up the possibility of using enzyme-linked immunosorbent assay to determine their respective proportions.

Finally, given that the different fiber types also vary in terms of metabolism, it is equally possible to evaluate their

respective proportions by assaying enzymes that reflect oxidative or glycolytic metabolism. This type of routine analysis is able to characterize type of muscle metabolism on a large number of samples, but the approach offers far less granularity than histology or electrophoresis, and it is unable to differentiate contractile type, which means it cannot differentiate the relative proportions of type-I and type-IIA fibers, both of which use oxidative metabolism.

Biochemical methods can fall down against histological methods because they cannot identify the distribution of each cell type and are unable to characterize hybrid fiber types.

The different histological techniques available are not strictly cross-compatible. The sharpest method available today uses immunohistochemistry to identify different MyHC isoforms. Coupling with histoenzymology techniques is time consuming but affords a more robust identification of cell types.

Muscle Fiber Types – Factors of Variation

The proportion of each fiber type in the muscle varies according to the muscle's function. Muscles that need to resist fatigue, like postural muscles, are generally dense in type-I and -IIA fibers. In contrast, muscles that need to be ready to deliver short bursts of intense work will mainly contain type-IIX and/or -IIB fibers. As stated earlier, fiber type can change from one type to another during the animal's lifetime.

Indeed, the characteristics of muscle fibers are shaped by muscle position and function, as well as species, breed, sex, age, physical exercise, environmental temperature, and even intake of growth hormones.

Genetic Factors

For any given muscle, fiber-type composition varies measurably between different species. Pigs, chicken, and turkey have pale muscles rich in glycolytic fibers (IIX and IIB) and less dense in oxidative fibers (I and IIA). The reverse pattern is found in cattle, sheep, horses, or ducks, which have red (therefore, myoglobin rich) and strongly oxidative muscle containing a majority of type-I and -IIA fibers (Table 2).

Fiber type composition is also shown to vary within the same species. Such variations are often the result of genetic selection efforts designed to increase carcass yield. This type of genetic selection policy tends to lead to an increase in the proportion of type-IIX and/or -IIB muscle fibers, which generally have a larger cross section. Wild boar, for example, has far redder oxidative muscling than the domestic pig. Furthermore, the domestic pig is sedentarized, whereas a wild boar will typically roam long distances on a daily basis, which promotes a more oxidative muscle metabolism (see Section Physical Activity).

Table 2 Percentage of fiber types in longissimus muscles of meat animals

Species	*Type I*	*Type IIA*	*Type IIB and/or IIX*
Cattle	22	33	45
Sheep	10	46	44
Pig	11	12	77
Rabbit	3	10	87

Physical Activity

Regular physical activity will progressively shift metabolic–contractile fiber properties to an oxidative muscle metabolism. Muscle from pigs experimentally subjected to several weeks of endurance training showed increased proportions of type-IIA and -I muscle fibers. To lesser extent, animals raised under free-range conditions generally present redder and more oxidative muscle than more sedentary animals raised indoors. Type-IIB and -IIX fibers give way to type-IIA and -I fibers, in a shift that always occurs in the order IIB→IIX→IIA→I via the transitional hybrid forms.

Age

The proportional composition of each fiber type changes with age of the meat animal. In cattle, it has been shown that rate of type I fibers increases with animal age, both in young cattle and older animals (cows and bulls), whereas rate of type IIB fibers decreases in young cattle and remains stable in older animals. This age-related increase in the proportion of type-I fibers is visibly transposable to other species.

Temperature

Animals adapt to temperature variations by adjusting their muscle metabolism and consequently by modifying the proportion of each main muscle cell type. Raising large white pigs in a cold environment will tend to increase the proportion of type-I fibers in their posture muscles.

Nutrition

As a general rule, feed and diet have little effect on muscle fiber type composition. However, extreme cases of diet restriction can trigger an increase in the quantity of type-I fibers in certain strongly oxidative postural muscles.

Influence of Fiber Type on Meat Quality Properties

Sensory and Technological Qualities

Tenderness

Tenderness is one of the decisive meat quality criteria. Fiber type has a paradoxical effect on meat tenderness. Calpain/calpastatin ratio is higher in fast-twitch glycolytic muscle (type IIX and IIB) than in redder slow-twitch muscle (type I). Furthermore, white muscle, dense in type-IIB and -IIX fibers, is more vulnerable to postmortem proteolysis than red, predominantly type-I muscle. Paradoxically, the increase in proportion of type-I fibers actually improves the tenderness of beef. It has been shown that muscle composed of fibers with a larger cross-sectional area yields tougher meat than muscle composed of lower diameter fibers. However, type-IIX and -IIB fibers are significantly bigger than type-I fibers. Taken together, these observations suggest that the mechanical

strength of type-IIX and -IIB muscle fibers could ultimately be due to their larger cross section. Meat tenderness is also tied to the connective tissue component. White muscles are designed to deliver ballistic-type power. The layering framework of connective tissue and probably extracellular matrix–muscle fiber interaction are adapted to these constraints, which could partly explain the higher mechanical strength of white muscle.

In certain cases, meat chilled too quickly will contract and lose much of its tenderness. This phenomenon, called cold shortening, only occurs in red meat that is dense in type-I fibers, i.e., mainly beef or sheepmeat. However, in contrast with type-IIB fibers, the Ca^{++} ion pump tasked with maintaining proper Ca^{++} homeostasis gets inhibited by cold stimulus in type-I fibers. Then, the muscles exposed to cold temperature soon after bleeding contract, yielding tough meat. The meat industry is well aware of the effects of cold shortening, and chilling process are generally adapted accordingly.

Flavor

Meat flavor stems largely from its lipid content. Oxidative muscle fibers packed with cellular organelles such as mitochondria are widely recognized as having higher phospholipid content than that by type-IIX and -IIB muscle fibers. Type-I fibers also contain intracellular lipid reserves in the form of lipid droplets that are only rarely observed in type-IIX and -IIB fibers. However, there are muscle lipid reserves stored in adipocytes that are usually found in the perimysium of all muscles, regardless of fiber type composition. Finally, the range of different results obtained to date is too wide to converge on a clear and specific relationship linking fiber type to total lipid content.

Given the range of compositional differences in phospholipids, glycogens, soluble proteins and so on, it is more likely that flavor is partly dependent on fiber type composition. Further targeted studies are needed in order to investigate the effect of fiber type on meat flavor.

Color and water-binding capacity (juiciness and product yield)

Meat color is dependent on the meat's myoglobin content. Myoglobin is the oxygen-binding protein that gives muscle cells a reddish pigmentation. Oxidative type-I and -IIA muscle fibers contain high amounts of myoglobin, and the higher the muscle's type-I and -IIA fiber content, the redder the meat. Heterogeneity in-muscle fiber type composition is sometimes the root cause of visible aspect defects. Two-shade coloring is a ham defect characterized by excessively darker red coloring in certain muscles tied to a higher proportion of myoglobin-rich type-I and -IIA fibers. Although without any obvious impact on flavor, patchy coloring prompts consumers to reject sliced cooked ham.

Myoglobin content is not the only factor of variation in meat color. Conditions of slaughter and slaughter technology affect the kinetics of postmortem muscle metabolism, with knock-on effects on rate and amplitude of pH drop in the meat.

In response to preslaughter stress, the added energy required from muscle cells is provided by a surge in muscle cell metabolism that is further accelerated by electrical stunning (pigs and poultry). This accelerated metabolism continues after exsanguination. When the bloodstream stops, metabolites like lactate and protons accumulate in the muscle cells, and this buildup leads to acidification of the meat muscle.

The glycogen degradation associated with this pH drop can be exceptionally quick in type-IIX and -IIB muscle fibers, especially in pork, and also, although to a lesser extent, in poultry.

A low pH combined with the fact that muscle temperature is still high causes partial denaturation of the meat proteins, which consequently lose some of their water-binding capacity. The meat end product will consequently be excessively pale, soft, and exudative meat, which loses a lot of juice during cooking and will ultimately turn out dry once cooked. This rapid change in postmortem metabolism, therefore, affects not only color but also juiciness and water-binding capacity as a whole. The proportion of intramuscular water lost through evaporation when the carcasses are chilled, then through exudation from the butchered meat cuts, and again during cooking, can vary substantially and measurably affect yield after cooking. Pig and turkey, unlike sheep and cattle, have the kind of highly glycolytic type-IIX and -IIB muscle that makes them particularly vulnerable to this phenomenon.

Salting – cooking

The agrifood industry has broadly adapted to consumer requirements by commercializing processed meat products. Although, beef and sheepmeat is still widely sold fresh, a large share of pork and turkey meat is industrially processed into ready-to-eat cold meats. Salting is an essential step in the cold meats chain. Not only does salting keep bacterial growth in check, it also reduces cooking losses and starts to breakdown the product structure, thereby improving end product juiciness, texture, and cooking yield.

Adding salt increases the ionic strength of the matrix, leading to partial denaturation of myofibrillar proteins. That said, a number of studies led on minced meat, myofibrillar protein extracts, and myosin converge to indicate that fast-twitch glycolytic muscle fibers (type IIX and type IIB) are more sensitive to the increased salt content than slow-twitch fibers (type I). However, Fourier transform infrared spectroscopy (FTIR) microspectroscopy studies have recently made it possible to characterize protein denaturation *in situ*, revealing changes in the secondary structure of myofibrillar proteins that were only weakly dependent on cell type.

Cooking, like salting, causes muscle protein denaturation, and myofibrillar protein extracts from glycolytic fibers are denatured earlier than myofibrillar protein extracts from type-I and -IIA fibers. As was the case with the denaturation tied to an increase in ionic strength, thermal denaturation as tracked by *in situ* FTIR microspectroscopy is practically independent of fiber type.

These findings suggest that myofibrillar protein denaturation is dependent on fiber type in meat that has been mechanically broken down, whereas the molecular interactions that are able to continue in the resulting meat pieces iron out the cell type-related differences in time course changes.

In the future, these characteristics could be capitalized in order to optimize meat salting processes and thereby reduce

NaCl content in meat products without losing out on sensory quality or technological properties. In today's industry chain, pork is by far the meat that goes through the most presale processing. Transposing these processing-induced transformations to meat from other species could enable significantly better value gains for certain muscles. Beef and sheepmeat is generally red muscle dominated by type-I and -IIA fibers. Process flows would need to be readapted in order to gain full process control over the sensory qualities and technological properties of any new product.

Nutritional Quality

Nutritional quality criteria on meat have traditionally revolved around lipid content and composition and essential amino acid profile in raw meat. Over the past few years, researchers have turned to look at the effects of meat structure and technological processes on the bioavailability of high nutritional value muscle proteins. The potential impacts of metabolic and contractile muscle type on nutritional qualities need to be investigated. Indeed, given the compositional differences between muscle fiber types, the nutritional qualities of meat can be expected to vary according to their proportions in the source muscle. Iron is an essential micronutrient, and heme iron, which is found mainly in type-I and -IIA fibers, is one of the nutrients that are easiest for the human body to assimilate. Vitamin B_3 is found in higher concentrations in muscle that has a higher proportion of glycolytic (type IIX and type IIB) fibers, whereas vitamin B_{12} is abundant in oxidative muscle (dense with type-I and -IIA fibers).

Carnosine is a bioactive dipeptide of β-alanine and histidine found in animal sources, where it is highly concentrated in muscle, especially glycolytic muscle (type-IIX and -IIB fibers). Its biological function is probably to buffer the intracellular pH variations induced by metabolic activity in glycolytic muscle. Carnosine has proven antioxidative properties and helps to protect against protein glycation and cross-linking. It may also play a vital preventive role against Alzheimer's disease.

The carnosine found in meat appears relatively unaffected by transformation processes. Although it is partially degraded in the small bowl, approximately one-fifth of all carnosine intakes is absorbed and released into the blood, where it can potentially be taken up into human body tissue.

Glutathione, which is also found in meat, is another bioactive peptide with antioxidant powers. However, no single specifically designed study to date has been able to establish a solid link between glutathione concentration and metabolic–contractile muscle class.

Food Safety Properties

There have been few attempts to evaluate the impact of muscle fiber type composition on the food safety properties of meat.

The microbiological properties of meat have been extensively documented but without finding evidence of fiber type composition-related variation in bacterial colonization. However, each muscle fiber type varies in composition and oxidative muscle generally has a higher ultimate pH than glycolytic muscle, and both these factors are known to modulate bacterial growth. Studies are underway to determine bacterial tropism toward the different fiber types.

Food safety properties are not driven by microorganism growth alone. For instance, it has been shown that certain cooking processes generate compounds that are potentially toxic for the end consumer, including heterocyclic aromatic amines, which are carcinogens that form at temperatures in the range 170–250 °C, typically reached when searing and panfrying. The production of these compounds varies according to muscle but is visibly independent of metabolic type and contractile class. This variability is thought to be tied to water transfer kinetics that differs according to muscle and depends on the muscle's structural organization.

Finally, various studies have shown that excessive consumption of red meat is associated with a moderate increase in the risk of colorectal cancer. Eating white meat (mainly poultry) is not associated with risk of cancer. These observations point out an effect of fiber type on cancer risk, as red meat contains high proportions of oxidative type-I and -IIA fibers. Recent research investigating the issue has singled out heme iron as the culprit, because it is thought to be a tumor promoter and is found in much higher concentrations in type-I and -IIA fibers than in type-IIX and -IIB fibers. However, protective nutrients like calcium or chlorophyll inhibit the heme's promoter effect. It would thus appear that simply getting calcium from dairy products significantly curbs the adverse effects of eating red meat. Other antioxidant nutrients from vegetables are equally more likely to possess similar protective properties to calcium and chlorophyll. Studies are underway to explore these issues further.

Conclusion

Muscle is composed of different types of fibers, all characterized in terms of contractile speed (fast or slow twitch), energy metabolism, and compositional profile of myosin isoforms. The in-muscle proportion of each fiber is essentially dependent on species, breed, and muscle function, but these proportions can change during the animal's life. Muscle fiber content and morphology vary according to metabolic type and contractile class and are responsible for variation in the sensory, technological, nutritional, and food safety properties of the meat.

Although genetic selection makes it possible to impel small-scale variations in the proportions of muscle fiber types, controlling muscle fiber composition and/or fiber type variability is not a conceivable option. Advanced characterization of the raw material combined with optimizations to its transformation processes should enable the food industry to gain greater process control over the quality properties of consumer end products. Nevertheless, further research is needed in order to gain deeper insight into the links between muscle fiber type and meat quality variability and the underlying molecular mechanisms involved.

See also: Connective Tissue: Structure, Function, and Influence on Meat Quality

Further Reading

Glade, M.J., 1999. Food nutrition and the prevention of cancer: A global perspective. Nutrition 15 (6), 523–526.
Kirchofer, K.S., Calkins, C.R., Gwartney, B.L., 2002. Fiber-type composition of muscles of the beef chuck and round. Journal of Animal Science 80, 2872–2878.
Klont, R.E., Brocks, L., Eikelenboom, G., 1998. Muscle fiber type and meat quality. Meat Science 49, 219–229.
Lee, S.H., Joo, S.T., Ryu, Y.C., 2010. Skeletal muscle fiber type and myofibrillar proteins in relation to meat quality. Meat Science 86, 166–170.
Lefaucheur, L., 2010. A second look into fiber typing. Relation to meat quality. Meat Science 84, 257–270.
Monin, G., Ouali, A., 1992. Muscle differentiation and meat quality. In: Lawrie, R. (Ed.), Developments in Meat Science – 5. London: Elsevier Applied Science, pp. 89–157.
Pette, D., Staron, R.S., 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. Reviews of Physiology, Biochemistry, and Pharmacology 116, 1–5.
Pette, D., Staron, R.S., 2000. Myosin isoforms, muscle fiber types, and transitions. Microscopy Research and Technique 50, 500–509.
Picard, B., Duris, M.P., Jurie, C., 1998. Classification of bovine fibers by different histochemical techniques. Histochemical Journal 30, 473–479.
Schreurs, N.M., Garcia, F., Jurie, C., *et al*., 2008. Meta-analysis of the effect of animal maturity on muscle characteristics in different muscles, breeds, and sexes of cattle. Journal of Animal Science 86, 2872–2887.
Totland, G.K., Kryvi, H., 1991. Distribution of muscle fiber types in major muscles of the bull (*Bos taurus*). Anatomy and Embryology 184, 441–450.
Xiong, Y.L., 1994. Myofibrillar protein from different muscle fiber types: Implications of biochemical and functional properties in meat processing. Critical Reviews in Food Science and Nutrition 34 (3), 293–320.

Relevant Websites

http://www.austincc.edu/apreview/PhysText/Muscle.html
Austin Community College District.
http://en.wikipedia.org/wiki/Skeletal_striated_muscle
Wikipedia.

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NUTRIENT CLAIMS ON PACKAGING

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Introduction

Food packages are one of the primary tools used to provide consumers with information about the nutrient content of the product. Throughout the past few decades, food packages have also been used to provide health-related claims or information to assist consumers with their purchasing decisions. Labels on the product package and point-of-purchase information, can influence consumers' purchasing patterns and overall knowledge of the foods they are buying. Labeling information can include the actual nutrient content, as well as information on the structure–function or specific health claims related to the food. The nutrient content is based on product analyses or databases containing nutritional data; however, many consumers do not completely understand how to use this information to make decisions, so they rely more on the labeling statements dealing with the structure–function of the food, such as 'low fat,' 'lean,' 'no trans fat,' or on specific health claim statements, such as 'a low-fat diet reduces risk of heart disease.'

The nutrient content data are typically designed to present information in a standard format that allows consumers to easily make comparisons among foods at the point of purchase to enable food choices that meet certain consumer expectations and contribute to food choices that prevent or manage chronic disease. For example, a nutrient label might include information on serving size, kilocalories per serving, and fat from serving. Nutritional claims are typically general statements that allow consumers to make informed dietary choices based on recognized health recommendations and scientific criteria.

Over the past few years, there has been a growing trend in fitness programs across the United States, and individuals seem to have an increased desire to improve overall health through exercise and diet. The food industry has responded to this trend with 'healthier options' to allow consumers to make better choices. Other consumers have health-related conditions, such as diabetes and cardiovascular diseases, that often contribute to their food selections.

In the United States, the contribution of red meat products to the incidence of cardiovascular disease, obesity, and certain types of cancer is often criticized, and nutritional guidance encourages consumption in moderation. Unfortunately, the positive nutrient aspects of meat's role in the diet are often overlooked. However, from a global perspective, it is important to understand the contribution of red meat to key micronutrients and protein intake, especially for individuals with limited food supplies and in developing countries. According to the Food and Agricultural Organization (FAO), it is estimated that more than 2 billion people in the world are deficient in key vitamins and minerals, particularly vitamin A, iodine, iron, and zinc, and that meat and meat products contain important levels of protein and some micronutrients that are essential for growth and development. So this article will explore some of the perceived concerns, as well as the positive attributes of red meat in the diet.

Concern Over Fat

Overall, meat remains an important component of the Western diet and is a major component in total food expenditures in most European countries. It has been reported that the Americas, Australia, and Europe consume more meat per capita than other parts of the world, while Africa, the Middle East, South and Southeast Asia consumes the least. These differences are attributed to multiple factors such as overall economic development, cultural and religious preferences, and supply. Regardless of the location, health factors due to changing work and lifestyles, environmental and welfare concerns, and the

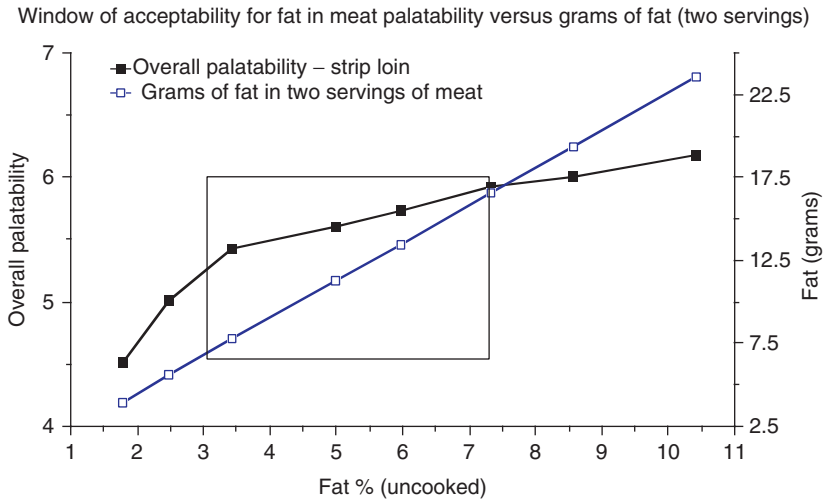

Figure 1 Reproduced from Savell, J.W., Cross, H.R., 1988. The Role of Fat in the Palatability of Beef, Pork and Lamb. Washington, DC: National Academy of Sciences.

availability of wider food choices have continued to influence eating habits and needs. These trends reflect the many factors influencing our modern food, nutrition, and health concerns. Diseases of the cardiovascular system account for half or more of the deaths in countries where death from infectious diseases can be prevented and treated.

The 2010 Dietary Guidelines Advisory Committee Report (USDA, 2010) advised healthy Americans to limit their intake of saturated fatty acids (SFA) to less than 10% of total energy intake and cholesterol to less than 300 mg per day. This and previous health-related nutritional recommendations have been automatically interpreted to mean they should change their diet to eliminate beef and other red meat. For example, in 2009, the International Food Information Council Foundation reported that 63% of consumers in the United States were trying to decrease the amount of animal fat in their diets. However, scientific data do not always support these nutritional recommendations.

A detailed review of 54 studies related to red meat consumption and coronary heart disease concluded that when fresh, lean red meat is low in saturated fat, and if consumed in a diet low in SFA, is associated with reductions in low-density lipoprotein (LDL)-cholesterol in both healthy and hypercholesterolemia subjects. Lean red meat provides key nutrients relative to the energy it provides that contribute to the fight against chronic diet-related noncommunicable diseases.

Changes in merchandising methods and livestock production practices have been credited with removing unwanted fat and improving the nutritional composition of beef, pork, and lamb, respectively. The role of fat on the characteristics of meat products is both diverse and complex. Fat itself is a mixture of a diverse group of substances consisting of triacylglycerols, phospholipids, sterols, and sterol esters. Fats' influence on meat product quality is profound. Fat level influences texture, flavor, binding properties, and color. Low-fat meat products often lack flavor, juiciness, and acceptable texture. The important parameters that influence consumer choices of purchase must be taken into consideration before a new meat product with a healthy image is marketed. A meat product perceived as being more 'healthy' must be similar in most other quality attributes to its 'less healthy' alternative. It is this challenge that is at the core of the technological problems associated with developing real healthy alternatives. This article examines the effects of fat reduction on the sensory and physicochemical properties of meat products, the use of fat replacers in meat products, and the legal aspects of fat reduction regarding labeling issues. The nutritional enhancement of meat products using functional ingredients is also discussed.

Role of Fat in Meat Products

Successful fat reduction in meat products requires a substantial knowledge of the functions of fat and functional food ingredients in meat products. The functional and sensory properties of fat as they relate to specific types of meat products are complex and somewhat product dependent. It is not a simple matter of using less fat in formulations because fat has significant effects on the physiochemical and sensory properties of the finished product. Humans have an inbuilt recognition for fat, which often plays a role in food acceptance. This has been ascribed to an innate human preference for a fat-associated volatile aroma, flavor, or textural feature. The 'Window of Acceptability' explains the relationship between fat and taste, and it demonstrates that consumers want a minimum of 3% fat for taste, but no more than 7.3% fat for diet health reasons (Figure 1).

Fat has a profound effect on the rheological and textural properties of meat products. It affects appearance, mouthfeel, texture, juiciness, flavor, and storage stability. It is not surprising, therefore, that fat removal adversely affects texture and results in a tough, rubbery, and dry product. As the fat content of processed meats is gradually reduced while the water content is increased, the product's water-binding capacity will replace its fat-binding capacity as the critical issue in production, thereby affecting the product's texture. In ground meat products, such as beef patties, fat imparts a soft texture, which is in contrast to the harder texture of the fibrous muscle proteins. Low-fat beef

patties are firmer, denser, and less cohesive during initial biting. In finely comminuted products, such as frankfurters, fat is present in the form of small oil droplets or particles surrounded by a protein film. When the fat level is reduced in these products, the products' structure is disrupted and the meat juices or added water are less effectively immobilized.

Fat is a major determinant of the sensory characteristics of products and it plays a key role in the flavor of meat products. Fat is a precursor to a large number of flavor compounds such as aldehydes, ketones, lactones, volatile fatty acids, and secondary alcohols. In addition, fat modifies the perception of existing or added flavor compounds by influencing the balance, intensity, and release of flavors, and by affecting their distribution and migration.

Prior to ingestion, binding of flavor compounds to ingredients within the food matrix occurs. During chewing, volatile aroma and flavor components are released. The amount of flavor released depends on the retention of flavor components in the food matrix and on the food type. In reduced fat foods, altered flavor–ingredient interactions result in different flavor release behavior and flavor tends to be intense but transient.

Fat also provides meat products with many functional properties during processing that will change when the fat level is changed. Fat is responsible for heat distribution during cooking, diluting protein content, and delaying protein extraction by physically separating muscle proteins, therefore allowing more tolerance in mixing procedures. For meat products to be viable on a commercial basis, it is necessary for water and fat losses that occur during processing to be kept to a minimum. Therefore, particular attention needs to be given to the water-holding capacity (WHC), cooking losses, and binding ability of meat systems produced by low-fat formulations. When fat is reduced in meat systems, either more lean meat (i.e., protein) is added or more water is added. In the former case, fat reduction combined with increased protein will increase the WHC. Alternatively, fat reduction by addition of water and no extra lean meat will result in a lower WHC. Because the WHC of a meat system derives from the protein component, any 'dilution' of this component will reduce its WHC.

Cooking losses not only affect the economics of producing processed meats, but can also result in compositional changes that can affect the palatability of finished products. Weight loss during cooking is due to the losses of both water (and water-soluble components) and lipids. The extent of loss depends on a number of factors including the extent of heating (i.e., time and temperature), shape and size of sample, composition, pH, physical properties of the fat, and cooking method.

Strategies for Fat Reduction

The main strategies for fat reduction in processed meat products generally follow two basic approaches: physical methods, such as the trimming of fat or using leaner materials, and the use of functional additives together with water. However, the addition of water alone adversely affects the quality of many low-fat meat products. Therefore, the most popular fat-reduction strategy today is the binding of added water with a protein or carbohydrate-based fat replacer.

Methods of reducing fat in meat products have been comprehensively reviewed; some reviews are listed in the 'Further Reading' section at the end of this article. It was not until the late 1980s and early 1990s that the development of ingredients specifically for fat replacement of all categories of food increased. The fact that there are so many ingredients now available for use in fat replacement means that this has been one of the strongest growth areas in the field of ingredient development for some time.

Many terms are used to describe ingredients that can be used for fat reduction, and these terms are often used interchangeably. Some authors give different descriptions for each. 'Fat replacer' is a blanket term used to describe any ingredient utilized in fat reduction. 'Fat mimetics' are defined as a partial replacement for fat by mimicking or imitating a particular function, but not all of the functions of fat in food. 'Fat substitutes' are ingredients whose physical and thermal properties resemble fat but have fewer calories than fat or have no calories. Most of the ingredients used in the reduction of fat can be classified under three headings: nonmeat proteins (soy, milk, wheat, blood, surimi, etc.); carbohydrates (hydrocolloids, gums, starches, fibers, and cellulose derivatives); and other products (functional blends, oils, and synthetic products). A list of some functional ingredients available and their suppliers is given in Table 1.

Nutritional Composition

For the most part, the United States has been leading the way in the commercialization of 'low-fat or lite' meat products, and the United States Department of Agriculture (USDA) nutritional database has a large number of 'lite' products from well-known US companies. For example, 'Fat-free' wieners have a 98% reduction in fat, are 76% lower in calories, and 46% lower in cholesterol; whereas 'light' wieners are 51%, 41%, and 13% lower in fat, calories, and cholesterol, respectively. In general, the 'normal' fat products have lower moisture contents than their low-fat or lite counterparts. It must also be noted that research using microscopy has shown that fat loss from meat products depends on two factors. The first is the instability of the fat itself (i.e., the fat droplet size and distribution, and the protective properties of the surrounding fat droplet membrane against coalescence). The other factor is the ability of fat to translocate from the inner to the outer parts of the product. The latter factor is enhanced when the probability of fat droplets to coalesce is increased, as in the case of high-fat coarse, comminuted meat products (e.g., hamburgers). In some cases, the solubilization of collagen can allow melted fat to diffuse along channels. Consequently, it is not surprising to find that the percent cooking loss increased as the fat level on the ground beef products increased from 5% to 25%.

Fat is more easily lost during cooking from high-fat burgers (30%) than low-fat burgers (10%) and researchers have found that the relationship between fat content of the beef burgers and fat loss is quadratic. This loss occurs due to expansion of fat droplets as they melt and the formation of pools and

Table 1 Some ingredients used in the fat reduction of meat products

Ingredient	*Product name*	*Manufacturer*
Soy protein	Supro	Dupont Protein Technologies (St. Louis, MO, USA)
Whey protein	Carbelac	Carbery Milk Products (Carbery, County Cork, Ireland)
	WPC30	Dairygold (Michelstown, County Cork, Ireland)
Blood protein	Plasma powder	Harimex (Loenen, The Netherlands)
Carrageenan	Gelcarin	FMC Biopolymer (Philadelphia, PA, USA)
	Genugel	Copenhagen Pectin (Lille Skensved, Denmark)
Starches		
Tapioca	Tapiocaline	Tipiak Ltd (Saint-Herblain, France)
	Instant N-oil	National Starch & Chemical (Bridgewater, NJ, USA)
	Instant Textra	National Starch & Chemical (Bridgewater, NJ, USA)
Waxy maize	Firm-tex	National Starch & Chemical (Bridgewater, NJ, USA)
Dietary fibers		
Potato	Potex	Lyckeby Stärkelsen (Kristianstad, Sweden)
Wheat	Vitacel	J. Rettenmaier & Söhne GmbH (Rosenberg, Germany)
Citrus	Herbacel	Herbafood Ingredients (Havel, Germany)
Oat	Advanced oat fiber	Food Ingredients International (Northants, UK)
Pea	Swelite	Consucra (Fontenay, Belgium)

Note: This table only shows a small number of ingredients used for fat reduction. There are various types of the above ingredients with numerous suppliers and manufacturers. The mention of an ingredient name or manufacturer does not constitute an endorsement by the author.

channels. The dense protein matrix of low-fat ground beef prevents fat migration by reducing the probability of fat droplets coalescing and expanding. When added water is used to replace fat, thereby increasing the amount of free water, cooking loss increases as fat decreases.

Nutritional Enhancement of Meat Products

In addition to health demands, consumers also want high-quality, convenient, and safe meat products. To satisfy their needs, food scientists are developing methods to enhance meat and meat products nutritionally. This is carried out in two ways: first, through the manipulation of the fatty acid profile of meat by altering the fatty composition of beef, for example, conjugated linoleic acid (CLA) and the n-3 fatty acid contents; second, functional ingredients, for example, dietary fibers, plant sterol esters, phytochemicals, vitamins/minerals, and proteins, can be added to existing meat products to fortify and improve their nutritive value.

CLA refers to a mixture of positional and geometric isomers of linoleic acid (18:2n-6) in which the double bonds are conjugated instead of existing in the typical methylene-interrupted configuration. CLA has been found to exhibit powerful anticarcinogenic effects at relatively low dietary levels. In addition, it has been shown to be antiatherogenic; to have antidiabetic properties; to enhance immune response; and to have beneficial effects on growth, health and body fat levels. Because there are potential health benefits from CLA consumption, there is considerable research effort directed to increasing the CLA content of ruminant-derived food. Research from Teagasc, Dublin, among others, has shown that an increase in the proportion of grass in the diet resulted in a linear increase in the CLA concentration, while a grass silage and concentrated diet resulted in a lower CLA concentration. CLA concentrations in Irish and Australian beef can be two or three times higher than those in US beef. This finding presumably reflects the greater consumption of polyunsaturated fatty acids (PUFA)-rich pasture throughout the year by cattle in these countries.

The fatty acid composition of beef can also be manipulated by including fatty acids in the diet of animals. Inclusion of bruised whole linseed, a rich-source of linolenic acid, resulted in 100% increase in the concentration of linolenic acid in muscle, while feed treated with a combination of linseed oil and fish oil increased the n-3 PUFA concentrations. Further research is in progress to improve the transfer of dietary PUFA to muscle as well as developing meat products, such as patties, which will contain higher amounts of n-3 fatty acids and CLA. Meat and fat for these products are being sourced from cattle raised on supplemented grass diets.

Although the meat industry has been slow to follow the functional trend and incorporate functional ingredients into products, some manufacturers have developed products in which they advertise the functional nature of the additives (Table 2). A meat company in Germany has developed a pork sausage and a turkey sausage with added calcium for the children's market. The company has also developed turkey salami with a probiotic culture, an additive normally seen in dairy products. Research carried out in the UK showed that the fortification of meat products with vitamins and minerals is technologically feasible. No adverse effect on color, texture, or flavor was found with any of the additives tested and, in most cases, there was no significant loss of added vitamins as a result of processing, storage, or cooking.

Dietary fiber from a number of vegetable and fruit sources, such as oat, wheat, soy, pea, potato, and citrus, have been added to the formulations of several meat products such as

Table 2 Examples of fortified and nutritionally enhanced meat products currently available

Product	*Company*	*Functional ingredient*
Sliced meats	Reinert (Germany)	Extra calcium
Sliced meats	Salumifico Fratelli Veroni (Italy)	n-3
Cured meat	Comprofio (Portugal)	Natural fiber
Sliced meats	Pouttu (Finland)	Plant sterols
Frankfurters	Atria Oyj (Finland)	Stanol ester
Chicken meatballs	Atria Oyj (Finland)	Stanol ester
Coated chicken	Glenhaven (Ireland)	Added vitamins
Coated chicken	Grampian (UK)	Added vitamins
Salami snack	R. Wilke (Germany)	Guarana, taurine, and ginseng

patties and sausages. In many instances, these fibers not only have beneficial physiological effects because they are more resistant to digestion in the human intestine, but they also have important technological properties that can offset the effect of fat reduction. Research at The National Food Centre has shown that the incorporation of pea and potato fiber in both reduced fat and normal fat beef patties reduced cooking losses and had no detrimental effect on flavor and texture. Preliminary studies have also shown that oat fiber delayed the release of flavor volatiles. Soy ingredients, in addition to being high-quality protein, have been identified as having beneficial health effects. A number of epidemiological studies have suggested that consumption of soybeans and soy foods is associated with lowered risks of several cancers and cardiovascular disease and improved bone health. Lycopene is the carotenoid in tomatoes that gives them the characteristic red color. This phytochemical antioxidant has been associated with reduced risks of cancer and coronary heart diseases. Lycopene has been used in the production of beef patties and has had significant antioxidative effects and considerably extended the shelf life of the products.

The invention of plant sterol ester led to the first commercial application of Benecol® margarine, in Finland in 1995. People have participated in nearly 40 published clinical trials that show the efficacy of plant stanol ester in lowering serum, total, and LDL-cholesterol concentration. These studies have shown that the use of 2–3 g of esterified stanol per day significantly reduces LDL-cholesterol levels by approximately 10–20%. Recent studies have shown that when stanol ester was added to low-fat meat products, LDL-cholesterol was lowered by approximately 14%. Since 1999, Benecol® foods enriched with stanol ester have been introduced in the United States and many European countries. To date, most of the products have been dairy based. However, chicken meatballs and frankfurters containing the stanol ester have recently been introduced to the Finnish market. Similarly, another Finnish company has introduced Multibene cold cuts, featuring a unique combination of cholesterol-lowering plant sterols, low-sodium PanSalt™, and beneficial minerals. The products will be among the first to be approved under the EU Novelty Food Act. According to the manufacturers of these 'healthier' meat products, the addition of the plant sterols has no detrimental effect on the flavor or texture of the products.

Labeling of Meat Products with Nutrient Claims

Regardless of type of meat product, it is important that information about foods and their nutritional value appearing on the label and used for their presentation, marketing, and advertising should be clear, accurate, and meaningful. The EU Regulation 1169/2011 on the provision of food information to consumers changes existing legislation on food labeling, and it requires mandatory nutrition information on process foods, as well as mandatory origin labeling for unprocessed meat from pigs, sheep, goats, and poultry. At an international level, Codex Alimentarius and FAO have developed guidelines (Table 3) for the most commonly used nutrition claims (such as 'low,' 'light,' etc.).

In the United States, The Nutrition Labeling and Education Act (1990) imposed new mandates for labeling of many food products and restricted how various nutrition claims may be made. In 1994, the Dietary Supplement Health and Education Act added even more complexity to the nutritional labeling program. The Food Safety and Inspection Service of the USDA regulates labeling of meat and poultry products. Nutritional labeling and nutrient claims requirements are codified in The Code of Federal Regulations in Sections 317.300–317.400 for red meat products and Sections 381.400–381.500 for poultry products. Most nutrient content claims are of two distinct types of statements: (1) implied nutrient claims, which are statements suggesting that a particular nutrient is absent or present at a certain level and (2) expressed nutrient claims, which is any direct statement about the level or range of nutrient in a food.

The US Food and Drug Administration (USFDA) and the US Department of Agriculture Food Safety and Inspection Service define 'lean' as a serving of meat, poultry, seafood, or game meat that contains fewer than 10 g of fat, fewer than 4 g of saturated fat, and fewer than 95 mg of cholesterol per 100 g. The USFDA's definition of 'extra lean' is a serving of meat, poultry, seafood, or game meat containing fewer than 5 g of fat, fewer than 2 g of saturated fat, and fewer than 95 mg of cholesterol per 100 g.

Additionally, simple, explicit statements that do not imply anything regarding low or high level or how the product compares to similar foods are allowed without any disclaimers. Statements indicating that a product is '___ free' or

Table 3 Table of conditions for nutrient contents

Component	*Claim*	*Conditions*
		Not more than
Energy	Low	40 kcal (170 kJ) per 100 g (solids) or 20 kcal (80 kJ) per 100 ml (liquids)
	Free	4 kcal per 100 ml (liquids)
Fat	Low	3 g per 100 g (solids) or 1.5 g per 100 ml (liquids)
	Free	0.5 g per 100 g (solids) or 100 ml (liquids)
Saturated Fat	Low[a]	1.5 g per 100 g (solids), 0.75 g per 100 ml (liquids), and 10% of energy
	Free	0.1 g per 100 g (solids) or 0.1 g per 100 ml (liquids)
Cholesterol	Low[b]	0.02 g per 100 g (solids) or 0.01 g per 100 ml (liquids)
	Free	0.005 g per 100 g (solids), 0.005 g per 100 ml (solids), and, for both claims, less than: 1.5 g saturated fat per 100 g (solids), 0.75 g saturated fat per 100 ml (liquids), and 10% of energy of saturated fat
Sugars	Free	0.5 g per 100 g (solids) or 0.5 g per 100 ml (liquids)
Sodium	Low	0.12 g per 100 g
	Very Low	0.04 g per 100 g
	Free	0.005 g per 100 g
		Not less than
Protein	Source	10% of NRV per 100 g (solids), 5% of NRV per 100 ml (liquids), or 5% of NRV per 100 kcal (12% of NRV per 1 MJ) or 10% of NRV per serving
	High	2 times the values for 'source'
Vitamins and minerals	Source	15% of NRV per 100 g (solids), 7.5% of NRV per 100 ml (liquids), or 5% of NRV per 100 kcal (12% of NRV per 1 MJ) or 15% of NRV per serving
	High	2 times the value for 'source'

[a]In the case of the claim 'low in saturated fat,' trans fatty acids should be taken into account where applicable. This provision consequentially applies to foods claimed to be 'low in cholesterol' and 'cholesterol free.'

[b]In the case of the claim 'low in saturated fat,' trans fatty acids should be taken into account where applicable. This provision consequentially applies to foods claimed to be 'low in cholesterol' and 'cholesterol free.'

Abbreviation: NRV, nutrient reference value.

'low' in a nutrient must comply with specific regulations for making a claim. Additional requirements apply to '_____ fat-free' claims. The rules for nutrition labeling and nutrient claims are extensive and complex, particularly in the United States, and care should be taken in designing labels for 'low-fat' or 'lite' products.

Summary

Many different factors influence consumer demand for meat products and, currently, health is a primary factor. Therefore, it is important that the industry produces great-tasting low-fat meat products that consumers enjoy as part of an ongoing healthier diet and lifestyle. This article provided an overview of health concerns related to meat consumers, strategies for reducing fat content in processed meat products, and key points related to nutritional labeling of meat.

Further Reading

Altman, T.A., 1999. FDA and USDA Nutrition Labeling Guide: Decision Diagrams, Checklists and Regulations. Lancaster, PA: Technomic Publishing Co.

Colmenero, F.J., 2000. Relevant factors in strategies for fat reduction in meat products. Trends in Food Science and Technology 11 (2), 56–66.

Colmenero, F.J., Carballo, J., Cofrades, S., 2001. Healthier meat and meat products: Their role as functional foods. Meat Science 59, 5–13.

Desmond, E.M., Troy, D.J., Buckley, D.J., 1998. Comparative studies of nonmeat adjuncts used in the manufacture of low-fat beef burgers. Journal of Muscle Foods 9 (3), 221–241.

Hafs, H.D., Zimbelman, R.G. (Eds.), 1994. Low-fat Meats: Design Strategies and Human Implications. San Diego, CA: Academic Press.

International Food Information Council Foundation, 2009. 2009 Food and Health Survey: Consumer Attitudes Toward Food, Nutrition and Heatlh. Washington, DC: International Food Information Council Foundation.

Keeton, J.T., 1994. Low-fat meat products: Technological problems with processing. Meat Science 36, 261–276.

Li, D., Siraiamornpun, S., Wahlqvist, M.L., Mann, M.J., Sinclair, A.J., 2005. Lean meat and heart health. Asia Pacific Journal of Clinical Nutrition 14, 113–119.

McNeill, S., Van Elswyk, M.E., 2012. Red meat in gobal nutrition. Meat Science 92, 166–173.

Moloney, A.P., 2002. The fat content of meat and meat products. In: Kerry, J.P., Kerry, J.F., Ledward, D.A. (Eds.), Meat Processing: Improving Quality. Cambridge: WoodHead Publishing, pp. 137–153.

Pearson, A.M., Dutson, T.R. (Eds.), 1997. Production and Processing of Healthy Meat, Poultry and Fish Products, Advances in Meat Research Series, vol. 11. London: Blackie Academic & Professional.

Pearson, A.M., Gillett, T.A., 1999. Reduced and low-fat meat products. Processed Meats. Gaithersburg, MD: Aspen Publications.

Pereltsvaig, A., 2013. Global Geography of Meat (and Fish) Consumption No. 2013.

Roller, S., Jones, S.A., 1996. Handbook of Fat Replacers. London: CRC Press.

Sandrou, D.K., Arvanitoyannis, 2000. Low-fat/calorie foods: Current state and perspectives. Critical Reviews in Food Science and Nutrition 40 (5), 427–447.

Savell, J.W., Cross, H.R., 1988. The Role of Fat in the Palatability of Beef, Pork and Lamb. Washington, DC: National Academy of Sciences.

USDA, 2010. Part D. Section 3. Fatty acids and cholesterol. Report of the 2010 Dietary Guidelines Advisory Committee. Washington, DC: USDA.

Wood, J.D., Fisher, A.V. (Eds.), 1990. Reducing Fat in Meat Animals. London: Elsevier Applied Science Publishers Ltd.

NUTRITION OF MEAT ANIMALS

Contents

Pigs

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Introduction

Pigs accumulate lean and fatty tissues through the retention of protein, lipid, water, and ash in the process of growth. The nutrition of pigs grown for meat is, therefore, a matter of satisfying the provision of the nutrient requirements for growth. Pigs may be weaned at a weight (and an age) depending on the farm system, but usually at more than 6 kg and less than 16 kg live body weight (3–8 weeks of age). They can be harvested at any age, but usually at live weights of more than 60 kg and less than 160 kg, depending on particular market requirements.

With respect to the production of lean meat, the growth of pigs is best considered in relation to the whole-body protein mass (Pt). The chemical components of the whole live body of growing meat pigs can be expressed as a function of Pt using the allometric form $a \times Pt^b$, where a and b are parameters that show biological variations:

$$\text{water} = 3.62 \times Pt^{0.938} \quad [1]$$

$$\text{ash} = 0.265 \times Pt^{0.928} \quad [2]$$

$$\text{lipid} = 0.50 \times Pt^{1.30} \quad [3]$$

According to these equations, pigs approaching 60 kg live weight with 10 kg of Pt will have 31 kg water, 2.2 kg ash, and 10 kg lipid. As the Pt increases with growth to maturity, there is a decrease in the content of water and ash relative to protein. The major part of the body water is held in association with protein in muscle. The water content of fatty tissue ranges from 10% to 20%, the higher values being found in younger and less fat animals. The major minerals are retained at rates (grams of mineral per kilogram Pt) of approximately: calcium, 90; phosphorus, 60; potassium, 15; sodium, 10; and magnesium, 2.5. Pigs also contain a small amount of liver and muscle glycogen, glucose, and microminerals.

For lipid, the exponent in eqn [3] will range from 1.1 to 1.4, showing fatness to increase with growth to maturity. The lower value is associated with lean pig types and controlled feeding regimens, and the higher with fatter pig types and *ad libitum* access to feed. Lipid accumulation with increasing pig mass is shown in Figure 1, which relates to an exponent of 1.3. The ratio of lipid to protein in tissue gains achieved in any particular circumstance is dependent on the quantity and quality of the dietary nutrient provisions; that is, the intake of energy and the balance of energy to protein in relation to need.

There is a significant gut content (kilogram per kilogram live weight) of approximately 0.06 that is related, among other things, to the level of feeding, feed digestibility (fiber content or water-holding capacity), and specific ingredient inclusions.

Retention of Protein and Lipid in Pigs

As pigs grow, they change in weight, size, and shape. For adequately fed pigs, a general relationship between weight (W, kg) and pig age (t, days) can be expressed as:.

$$W = A - \exp\{-\exp[-B(t - t_0)]\} \quad [4]$$

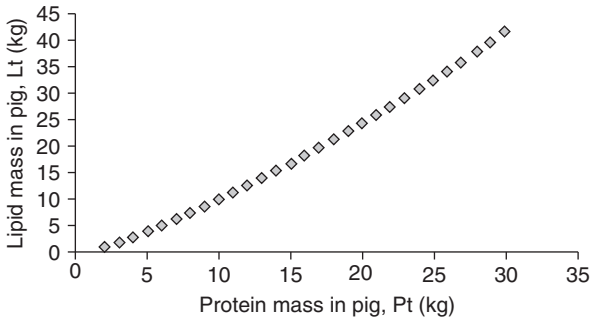

Figure 1 Lipid mass (Lt) in relation to Pt of pigs grown for meat.

The expression 'exp' denotes e^x (??). In the equation, A is the asymptote of W (mature weight) and B is a growth coefficient. The curve of weight against time is sigmoid (growth rate accelerating in early life and decelerating later), and t_0 is the point of inflection measured in terms of pig age and occurring at 1e (0.37) of the age at maturity. Meat pigs are usually harvested at less than 0.5 of their mature size. In the case of contemporary meat pigs, maturity may be attained at live weights of 200–300 kg. Typically, at the point of inflection, a maximum growth rate slightly in excess of 1 kg daily is achieved by healthy modern genotypes at approximately 100 kg live weight and 160 days of age.

It appears that the change in size may follow the same trajectory as weight, whereas shape may not. Width across the ham may increase more slowly than length and other breadth measurements. The area of the longissimus dorsi muscle increases at a lower rate than that of the whole carcass, the exponent of the allometric equation relating the two being approximately 0.73 for normal pig types, but greater for more muscular breeds.

To provide for nutrient requirements, an expectation for the daily rate of protein retention (PR, kilogram per day) is needed. This may be expressed as a function of pig live weight (W), or more dependably as a function of pig Pt:

$$\mathrm{PR}\ (\mathrm{kg\ day^{-1}}) = \mathrm{Pt} \times B \ln\left(\frac{\mathrm{Pt_{max}}}{\mathrm{Pt}}\right) \quad [5]$$

where, $\mathrm{Pt_{max}}$ is the asymptote (A) for Pt.

The peak value for the rate of growth, occurring at the point of inflection is ($A \times B/e$). Parameter values of 0.0110 for B and 30 kg for $\mathrm{Pt_{max}}$ may be used to describe the slower-growing pig types, and of 0.0125 and 50 kg to describe the faster growing types. Intermediate values are a reasonable expectation for high-quality commercial types, and these are used in Figure 2. In the figure, at approximately 30 kg live weight (Pt ~ 5 kg), potential rates for PR are approximately 0.1 kg daily, rising to a maximum of 0.16 kg or more between 60 and 110 kg live weight (Pt ~ 10–18 kg). The curve is quite flat topped, so some authorities are content to consider PR to be effectively constant for most of the 20–120 kg growth phase. Following the peak, the rate of PR declines (at a slower rate than it increased) to zero at attainment of mature weight.

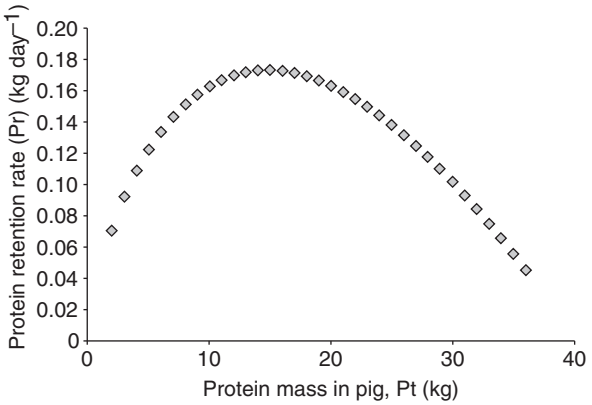

Figure 2 Daily rate of PR in relation to Pt of pigs grown for meat.

Obtaining numerical values for Pt and B from empirical data is more useful in defining growth expectations for Pt than for the lipid mass (Lt), as the latter is substantially more prone to environmental variation. However, expectations would be for the value of A in the case of lipid to be 2–3 times that of protein.

There appears to be a (minimum) level of fatness that pigs are genetically disposed to attain. To achieve this, they may forgo the use of dietary energy to fuel PR, the latter otherwise being the priority. This level can be represented as the minimum ratio of lipid to protein, $(\mathrm{Lt{:}Pt})_{\mathrm{min}}$, in the body. At fatnesses above this, pigs will catabolize body lipid to yield required energy should the dietary supply be limiting. The $(\mathrm{Lt{:}Pt})_{\mathrm{min}}$ is related to breed type and to sex (in the order male < female < castrate). Values of $(\mathrm{Lt{:}Pt})_{\mathrm{min}}$ appear to range between 0.5 and 1.5. The level of fatness above $(\mathrm{Lt{:}Pt})_{\mathrm{min}}$ actually attained will depend on the quantity and quality of the nutrient supply. Appetite limits will restrain daily lipid retention (Lr) to fatness levels at or near the minimum during the first quartile of growth to maturity. By the end of the second quartile, pigs given *ad libitum* access to food will have fattened to levels substantially beyond that normally required for optimum carcass lean (an Lt:Pt ratio of approximately, or less than 1:1; this seems like a high proportion of fat).

Growth promoters, such as hormones and beta-agonists, may increase PR and decrease Lr. This would influence the leanness of the carcass, the efficiency of growth, and the balance of protein to energy needed in the diet. However, presently available fast-growing and lean pig genotypes, together with modern production practice, indicate little need for the modification of pig growth by exogenous means.

Response to Nutrient Supply

Pigs respond linearly to reach a plateau when the nutrient supply is increased, according to the general rule of first limiting resource. Response in PR to increasing daily protein supply is limited by provision of essential amino acids, provision of energy, the production environment (particularly health), and ultimately the expectation for PR as described earlier. Given the absence of limitation by shortfalls in other resources, response to increasing energy supply is linear for both PR and LR (supporting $(\mathrm{Lt{:}Pt})_{\mathrm{min}}$) until the maximum expected daily protein retention ($\mathrm{PR_{max}}$) is attained. At this point, PR is maintained at the maximum daily rate (the plateau); further ingestion of energy goes to the retention of lipid and the animal fattens.

As $\mathrm{PR_{max}}$ varies not only according to pig type and production environment but also according to live weight (and Pt), it follows that the break point of the linear/plateau response alters as pigs grow. Changing live weight also brings a change in the balance of energy required for basic body functioning (maintenance) and energy required for protein and lipid retention. Thus, ideal nutrient allowances and balances are in a state of continuous and progressive change.

The overall efficiency with which feed is used for growth is approximately 2 kg of cereal-based balanced feed for 1 kg of body weight in young growing pigs, and approximately 3:1 in older pigs. This is because older pigs, being heavier, have a

higher maintenance requirement, and, eating more, are also more likely to be fattening.

Nutrition of pigs concerns both the quality and the quantity of the diet provided. The first addresses the balance of nutrients, whereas the second addresses the level of feed supply. In the first quartile of growth to maturity, PR_{max} is rarely attained, owing to appetite limitation. Pigs are, therefore, fed *ad libitum*, and indeed nutrient intake is encouraged by provision of nutrient-dense diets and the use of palatable food ingredients. If it is desired that fattening be prevented or constrained, the quantity of feed provided in subsequent quartiles may be appropriately restricted by imposition of a rationing regimen. Selection of the feeding level determines lean growth performance in the earlier phases of growth and carcass fatness in the later stages.

Control of feeding level is the most effective and most frequently used means of controlling both the rate and the composition of pig growth. Appetite encouragement will increase growth rate and the efficiency of conversion of ingested food into body tissues, provided that the growth increase is in terms of muscle and not fat growth. The water content of muscle (~70%) is much greater than that of fatty tissue (~15%). Pigs with a propensity to retain unwanted fatty tissue not only have carcasses of lower value but they also produce them less efficiently. Thus, reducing feeding level worsens the feed conversion efficiency of lean-growing pigs by slowing down growth, but improves the feed conversion efficiency of fat-growing pigs.

Feed Intake

Because the nutrients supplied daily to pigs are the products of diet nutrient density and level of intake, it is evident that formulation of the diet in terms of nutrient densities is contingent on knowledge of the amount of the diet that will be eaten. It may be supposed that, in the absence of any restraint, pigs will eat the amount of feed that will provide for requirements to maintain body functions (maintenance), and achieve PR_{max} and LR_{max}. One restraint is the capacity of the intestinal tract, which is related to the size of pigs. The utilization of the available capacity will further depend on the fiber content of the diet, the water-holding capacity of ingredients, and diet digestibility. Not only do feed ingredients interact with gut capacity, but also the prediction of food intake is further obfuscated by environmental effects, especially those of temperature, housing, and disease.

Empirical measurement of food intake (FI; kilogram per day) by pigs may be described as:

$$FI = a \times [1 - \exp(-b \times W)] \quad [6]$$

where, W is the live weight (kilogram) of the pig, and the coefficient a is indicative of the likely food intake limit. Expected values for a and the parameter b would normally be approximately 3.2 and 0.019, respectively (Figure 3). Food intake increases with the live weight of the pig, but at a diminishing rate. Both coefficient and exponent will vary according to pig type and production environment. In early life, feed intake is particularly influenced by the pig's health, whereas later it is influenced by the density of stocking and the method of feed provision.

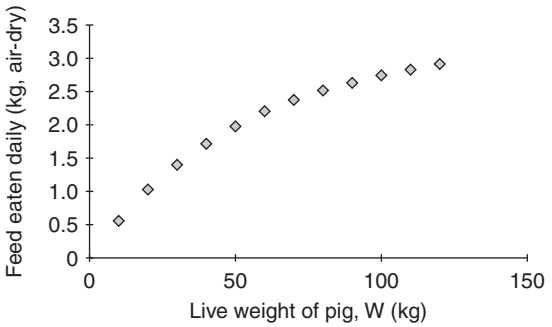

Figure 3 A food intake curve for pigs grown for meat.

Influence of Nutrition on the Eating Quality of Pig Meat

By far, the most important influence is through the direct effect of feed supply level on the absolute quantity of fat that is present in the carcass.

Eating quality comprises tenderness, juiciness, and flavor, usually assessed using a trained taste panel. Apart from influences through levels of fatness, nutrition of the pig affects these characteristics in only a limited way. Tenderness is the most important of the three and its level is raised after fast growth in the finishing stage, compared with slow or interrupted growth – at equal carcass leanness, faster growing pigs have more tender meat than those grown more slowly, but this effect is small.

In general, higher tenderness and juiciness are found in meat from fatter carcasses, the concentration of fat within the muscle (intramuscular or marbling fat) being the important determinant. The concentration of marbling fat is increased when diets with a high ratio of energy to essential amino acids are fed. In several studies, marbling fat has been increased by 30% or more using this strategy to the benefit of juiciness and tenderness in cooked pork.

The restriction of feed intake, the use of diets of low nutrient density, or the use of low-appetite pig types will improve carcass muscle percentage, and this characteristic is often taken as a substantive definition for the eating quality of meat from pigs. Increasing the feeding level (intake of energy) will, in contrast, directly enhance the fatness of the carcass. The attitude for or against fat depends on social custom, the market demand for lard as a commodity, human dietary requirements for energy, and dietary fashion. Approximately two-thirds of carcass fat is in the form of subcutaneous fat, which can readily be removed postmortem to varying degrees as required. Intramuscular fat (and, to a lesser extent, intermuscular fat) is associated with enhanced flavor, tenderness, and ease of cooking of meat.

The ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids in pig meat is generally favorable in relation to perceptions of human benefit in the Western diet. PUFA can be further enhanced by provision of unsaturates in the diet. The propensity for dietary fatty acids to be transferred unaltered to

carcass fat also facilitates the natural supplementation of pig meat with particular PUFA considered to be of benefit in the human diet. One approach is to include fish oil in the diet, a source of the key omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Another is to include linseed (flaxseed), a source of α-linolenic acid, which, once deposited in tissues, leads to the formation of EPA and DHA in the pig itself. High levels of PUFA (adversely) soften carcass fat because of their low melting point. Another potential problem is oxidation of PUFA during processing and storage of meat, leading to discoloration and off-flavors in cooked pork. These can be avoided by raising dietary levels of vitamin E, a powerful antioxidant. There has been a general increase in dietary vitamin E levels in recent years.

Apart from the effects of feeding level on carcass fatness (which is substantial), the influence of nutrition on the eating quality of pig meat is not particularly great. Of greater importance than nutrition are pig type, pre- and postslaughter treatment, and method of cooking. There may, however, be particular negative and positive dietary ingredient effects on meat flavors. This phenomenon, although appreciated by consumers of products from animals that range widely and have an eclectic choice of feed sources, is as yet largely unexamined by objective science.

In some countries, entire male pigs are reared for pig meat production, whereas in others the males are castrated at birth to reduce concentrations of skatole and androstenone, which cause off-odors and flavors after cooking. Feeding sources of fiber that are digested in the hind gut, such as chicory and the pulp of beet after sugar extraction, can reduce levels of skatole in carcass fat because the change in fermentation pattern causes less skatole to be absorbed from the gut. This appears to be associated with the nonstarch polysaccharides mitigating the effects of tryptophan degradation by gut bacteria. The concentration of skatole is closely linked to off-odor production, so this dietary change may remove the problem of boar taint. However, androstenone is not affected by dietary fiber, so its negative effects on taste may still remain.

Dietary Energy in Pig Feeds

The gross energy of the diet before ingestion (GE, measured in MJ) is of little value as a descriptor because some fractions contain energy that is unavailable to pigs due to their indigestibility. The digestible energy (DE) is that which is not voided in the feces. Energy digestibility is adversely affected by fiber content and positively affected by fat.

The DE content (MJ per kilogram DM) of a diet may be predicted from its chemical constituents. The following relation (eqn [7]) may be postulated from knowledge of the GE values of carbohydrate, oil, and protein, given that the energy of starch and oil is highly digestible and that of fiber is largely indigestible (each of the components is expressed in gram per kilogram).

$$\text{DE(MJ per kg DM)} = 0.016\ \text{Starch} + 0.035\ \text{Oil} + 0.019\ \text{Protein} + 0.001\ \text{Fiber} \quad [7]$$

This postulate is confirmed by empirical regression of chemical composition against DE determined in live pigs:

$$\text{DE (MJ per kg DM)} = 17.5 - 0.015\ \text{NDF} + 0.016\ \text{Oil} + 0.008\ \text{CP} - 0.033\ \text{Ash} \quad [8]$$

where, NDF is neutral detergent fiber and CP is crude protein. The constant of 17.5 reflects the starch contribution, whereas calculation of the contribution (MJ kg^{-1}) from dietary NDF ($17.5-[0.015 \times 1000]=2.5$) is low and that from dietary oil is large ($17.5+16.0=33.5$).

Because some of the DE is lost in the urine and in gases from the tract, and is, therefore, not available for metabolism, the term metabolizable energy (ME) may be used. The ME/DE ratio in pig diets is variable according to the rate of deamination, but is usually approximately 0.96.

Of the DE, the starch, lipid, and protein fractions are largely digested in the small intestine, yielding useful substrates for metabolism. Some dietary energy, particularly from the fibrous nonstarch polysaccharides, disappears in the large intestine as volatile fatty acids, and these are used less efficiently. Owing to the differing costs of metabolism of the different dietary components, DE may overvalue the energy contribution of proteins and nonstarch polysaccharides, and undervalue that of fats and starches. Further, older pigs use fiber more effectively than younger ones. These considerations suggest that foods would be more accurately valued according to their net energy (NE) yield. True NE is a combined function of the energy value of the feed and the use to which energy is put by the animal; that is, the body functions of maintenance, PR, and LR. Usually, for a growing pig diet, NE is approximately 0.71 of DE.

The laboratories of Institut National de la Recherche Agronomique in France have suggested eqn [9], where NE is MJ(kg DM)$^{-1}$ and the nutrient composition is in g kg^{-1}.

$$\text{NE} = 0.0113(\text{Digestible CP}) + 0.0350(\text{Oil}) + 0.0144(\text{Starch}) + 0.0121(\text{Digestible residue}) \quad [9]$$

The same laboratories have published tables of nutritive values of feeds containing, among other things, values for NE (see Further Reading).

Dietary Amino Acids in Pig Feeds

Of the amino acids that make up dietary protein, some 11 cannot be synthesized by pigs and are deemed 'essential': lysine, methionine + cysteine, threonine, tryptophan, isoleucine, leucine, histidine, phenylalanine + tyrosine, and valine. Lysine is often the first limiting resource in diets based on cereals, and it is common to express requirement in terms of lysine, with the other amino acids stated in terms of a ratio to lysine = 1, to give the appropriate balance. The balance of interest is that needed by pigs for the functions of maintenance, tissue turnover, and PR (Table 1). Of the total dietary provision, the amino acids not in balance will be deaminated and excreted. This is a natural consequence of pigs converting lower quality

Table 1 Balance of essential amino acids (in relation to lysine = 1.00) for growing pigs

Lysine	1.00
Methionine	0.30
Methionine + cysteine	0.59
Threonine	0.65
Tryptophan	0.19
Isoleucine	0.58
Leucine	1.00
Histidine	0.34
Phenylalanine	0.57
Phenylalanine + tyrosine	1.00
Valine	0.70

Source: Reproduced from Whittemore, C.T., Hazzledine, M.J., Close, W.H., 2003. Nutrient Requirement Standards for Pigs. Penicuik: British Society of Animal Science.

pig-food proteins into higher quality human-food proteins in the form of pig meat.

Most of the amino acids are digested anterior to the terminal ileum, from where they are absorbed. There is substantial endogenous secretion of amino acids into the small intestine. Some of these are not fully reabsorbed before the terminal ileum and are lost. Nitrogen compounds passing into the large intestine may appear to have been digested but are of no value to the pig.

There is a basal level of endogenous amino acid loss that will occur regardless of level of feeding or of feed-ingredient type, and this level is considered as a cost that should be attributed to the pig rather than to the feed. The other fraction of the endogenous losses is considered to be feed-related. When these losses are apportioned, the requirement for amino acids is expressed as 'standardized ileal digestible amino acids,' which is approximately 5% points higher than the empirically determined overall ileal digestibility. Consequently, the statement of pigs' requirements must be enhanced to cover the pig-related, unavoidable endogenous losses.

Diet Ingredients

The feeds that omnivorous and monogastric pigs may eat are diverse. Most diets are founded on carbohydrates from 'starchy' crops – maize corn, barley, wheat, rye, oats, sorghum, cassava, and rice. Also, widely employed in pig diets are the by-products of the use of these crops for the manufacture of human food, especially from the bread, bakery, brewing, and starch-based industries, and possibly also from new-technology energy-generating processes. Although feeds containing higher levels of nonstarch polysaccharide tend to be used for ruminant rather than monogastric diets, pigs do not abhor these. Roots are no longer frequently grown specifically for consumption by pigs, but by-products of potato and sugar beet processing are valuable.

Energy may also be supplied from vegetable and animal fats, such as soybean oil, oil-seed rape (canola) oil, and tallow. Approximately half of the amino acid requirement may be found in the 'starchy' food ingredients, the rest is provided in the form of protein supplements. In the middle and later stages of growth, these are usually of vegetable origin – soybean, oil-seed rape, sunflower, lupin, field peas, and beans. Oil extracted from the first three of these is a valued, volume, human-food commodity, and the extraction results in a by-product with a high concentration of protein.

In the earlier stages of growth, young pigs benefit from the inclusion in the diet of animal protein, such as fish meal, skimmed milk, meat and bone meal, and blood products. These are more expensive but are higher in concentration of essential amino acids and more readily digested. Nutritional values for some diet ingredients are given in Table 2.

Mineral and Vitamin Requirements of pigs

There are common and wide differences between estimates of pigs' requirement for minerals and vitamins determined on the basis of depletion experiments and the (higher) dietary inclusion levels found to be efficacious in practice. Furthermore, there are wide differences in efficacy depending on production circumstance. Table 3 gives guidelines for the diet content of macrominerals, and for the dietary additions of microminerals and vitamins. The provision of minerals and vitamins to pigs is not an exact science.

Water Requirement of Pigs

All growing pigs, including those given food that is mixed with water or is itself in liquid form, should have access to potable water. A separate watering point is needed for every 8–12 pigs. The variation in daily water consumption is large and depends on the health and welfare status of the pigs, the environment, and the feed type. It is suggested that under normal conditions, pigs will drink 4–6 times the weight of food that is eaten.

Energy Requirement of Pigs

The requirement for energy is the sum of the costs of maintenance, activity, PR, and LR. Energy is also expended in response to cold and disease, but thermoneutrality and reasonable health are assumed here.

Estimation of the DE requirement of growing pigs must include the metabolic costs of retention of new body tissues that are lost as heat:

$$\mathrm{DE}(\mathrm{MJ\ day^{-1}}) = [(0.444W^{0.75} \times 1.10) + (23.6\mathrm{Pr}/0.44) + (39.3\mathrm{Lr}/0.74)]/0.96 \quad [10]$$

The first term in eqn [10] estimates maintenance requirement, which is amplified by 10% to allow for activity. The second term states the energy content of the retained protein to be 23.6 MJ kg^{-1}, and estimates the efficiency of use of energy for PR to be 0.44. The third term states the energy content of the retained lipid to be 39.3 MJ kg^{-1}, and estimates the efficiency of use of energy for LR to be 0.74. These terms are expressed in ME, which is approximately 0.96 of DE.

Table 2 Guide to the composition of some diet ingredients (g kg^{-1} (air dry))

	DM (g kg^{-1})	*NE (MJ kg^{-1})*	*DE (MJ kg^{-1})*	*CP (g kg^{-1})*	*Standardized ileal digestible amino acids (g kg^{-1})*[a]				
					CP	*Lys*	*Met + Cys*	*Thr*	*Trp*
Barley	870	9.6	13.0	100	75	2.8	3.3	2.5	1.0
Wheat	870	10.5	14.2	105	92	2.5	3.6	2.6	1.1
Maize	870	11.1	14.6	78	68	1.8	2.9	2.3	0.4
Oats	870	8.0	11.4	98	72	3.0	3.7	2.3	1.0
Cassava	880	10.0	12.5	25	5	0.1	0.1	0.0	0.0
Wheat feed	880	7.7	11.4	156	112	4.6	4.4	3.6	1.7
Maize gluten feed	880	7.0	11.3	200	150	4.0	5.5	4.7	0.9
Sugar-beet pulp	890	6.6	10.8	85	45	2.9	1.0	1.0	0.3
Peas	860	9.7	14.0	205	167	12.5	3.7	5.9	1.4
Field beans	860	8.6	13.4	255	205	12.8	3.7	6.9	1.6
Lupin (white)	875	8.8	14.5	330	280	13.3	6.0	9.1	1.8
Sunflower (ext)	890	5.4	9.5	330	272	9.6	11.5	9.8	3.5
Rapeseed meal (ext)	900	6.5	11.9	340	262	14.0	12.8	11.0	3.4
Soya (ext)	875	8.1	14.8	435	382	24.0	11.3	14.5	5.2
Soya (full fat)	880	11.4	16.8	350	298	18.9	8.9	11.9	3.7
Fish meal	920	10.0	17.0	698	620	50.0	23.4	27.0	6.5
Soya oil	990	31.0	36.0						
Skimmed milk	950	11.1	16.5	340	315	26.2	11.4	14.0	4.4

[a]Supplemental free L-lysine, DL-methionine, L-threonine, L-tryptophan, and DL-tryptophan contain standardized ileal digestible amino acid of 790, 980, 980, 980, and 800 g kg^{-1} of product, respectively.

Abbreviations: CP, crude protein; Cys, cysteine; DE, digestible energy; DM, dry matter; ext, fat extracted; Lys, lysine; Met, methionine; NE, net energy; Thr, threonine; Trp, tryptophan.

Source: Reproduced from Whittemore, C.T., Hazzledine, M.J., Close, W.H., 2003. Nutrient Requirement Standards for Pigs. Penicuik: British Society of Animal Science.

Table 3 Range of dietary additions of minerals and vitamins commonly found in compounded feed offered to growing pigs

	Weaned pigs of up to 15 kg live weight	*Growing pigs of 15–50 kg live weight*	*Growing pigs of 50–150 kg live weight*
Macrominerals (per kg final feed)			
Calcium (g)	7.5–9.2	6.0–8.2	5.5–7.2
Phosphorus (g)	6.5–7.0	5.0–6.4	5.0–6.0
Digestible phosphorus (g)	3.0–3.4	2.4–2.7	2.0–2.3
Sodium (g)	1.5–2.5	1.5–2.0	1.4–2.2
Microminerals (added to each kg final feed)			
Zinc (mg)	100–200	100–200	60–150
Manganese (mg)	30–50	30–50	25–45
Iron (mg)	80–175	80–150	65–112
Cobalt (mg)	0.2–0.5	0.2–0.5	0.2–0.5
Iodine (mg)	0.2–1.0	0.2–1.0	0.2–1.0
Selenium (mg)	0.2–0.3	0.15–0.3	0.15–0.3
Copper (mg)	6–20	6–15	6–15
Vitamins (added to each kg final feed)			
Retinol (mg)[a]	1.5–4.8	1.2–3.6	1.2–2.7
Cholecalciferol (mg)[b]	0.02–0.05	0.02–0.05	0.015–0.037
DL-α-Tocopherol acetate (mg)[c]	40–100	35–60	20–60
Menaphthone (mg)	2–5	2–4	1–2
Thiamin (mg)	1.5–3	1–2	1–2
Riboflavin (mg)	4–10	4–6	2–4
Nicotinic acid (mg)	20–40	15–25	10–25
Pantothenic acid (mg)	8–20	10–15	8–15
Pyridoxine (mg)	2.5–5	1–3	1–3
Cyanocobalamin (mg)	0.03–0.05	0.02–0.03	0.015–0.022
Biotin (mg)	0.125–0.20	0–0.15	0–0.05
Folic acid (mg)	0.5–1.5	0–1.0	0–1.0
Choline (mg)	200–500	100–300	0–200

[a]1 mg retinol is 3333 IU vitamin A.

[b]1 mg cholecalciferol is 40 000 IU vitamin D3.

[c]1 mg DL-α-Tocopherol acetate is 1 IU vitamin E.

Note: Essential fatty acids. The dietary requirement for essential fatty acids for all classes of pigs will be likely met from a dietary inclusion level of 5–50 g linoleic acid per kilogram.

Source: Adapted from Whittemore, C.T., Hazzledine, M.J., Close, W.H., 2003. Nutrient Requirement Standards for Pigs. Penicuik: British Society of Animal Science.

Table 4 Energy and lysine requirements of growing pigs expressed as concentration of nutrient per kilogram air-dry diet. Feed intake was as presented in **Figure 3**

Live weight (kg)	*Net energy (MJ NE per kg diet)*	*Digestible energy (MJ DE per kg diet)*	*Standardized ileal digestible lysine (g kg^{-1} diet)*	*Total lysine (g kg^{-1} diet)*
10–30	10.4	14.7	9.7	11.5
30–60	9.3	13.6	8.1	9.6
60–90	9.0	13.2	7.1	8.4
90–120	8.9	13.0	6.2	7.3

Source: Reproduced from Whittemore, C.T., Hazzledine, M.J., Close, W.H., 2003. Nutrient Requirement Standards for Pigs. Penicuik: British Society of Animal Science.

Estimation of the NE requirement of growing pigs is by summation of the energy required for basal metabolism (maintenance of body functions when no feed is being consumed), and the energy value of PR (23.6 MJ kg^{-1}) and LR (39.3 MJ kg^{-1}). To the basal allowance of 0.750 MJ kg$^{-1}W^{0.60}$, a further 10% may be added to cover energy expended in activity. It is important that the estimation for maintenance used is that derived in accordance with the determination of the NE value of feeds. Energy requirements determined in this way are given in **Table 4**.

A statement of the requirement for lysine, when combined with **Table 1**, sets the requirement for all the essential amino acids. The requirement for lysine is estimated as that needed for maintenance, PR, and basal endogenous losses. Lysine requirements are given in **Table 4**. The lysine in protein used for maintenance is estimated as 0.058 kg lysine per kilogram protein. The lysine in PR is estimated as 0.070 kg kg^{-1}. Ileal digested lysine, even when supplied in limiting amounts, is used with less than 100% efficiency because of amino acid cycling in the body of pigs. The maximum efficiency appears to be 0.82, and lower values are often experienced. The requirement for amino acids is higher in the presence of disease; these estimates assume reasonable health. In **Table 4**, where the maintenance requirement is estimated as 0.9 g protein per kilogram $W^{0.79}$, and the basal endogenous losses are estimated at approximately 5%, the standardized ileal digestible lysine requirement (kilogram per day) is given as:

$$[((0.0009 \times W^{0.75} \times 0.058) + (\mathrm{Pr} \times 0.07))/0.82] \times 1.05 \quad [11]$$

Protein Requirement of Pigs

Protein is comprised of essential and nonessential amino acids. Minimum provision of the combination of the two may be estimated as 2.5 times the total of the essential amino acids, or 15.25 times the requirement for lysine. This minimum will usually be exceeded as the economic purpose of pigs is to convert feeds of lower quality into those of higher quality, and this will not be best served by the provision of perfectly balanced diets. The availability of industrially manufactured free amino acids, and the imperative to minimize (or to utilize effectively) releases of nitrogen into the environment does, however, raise awareness of the need for optimization of dietary provision of nitrogenous compounds. There is particular interest in the possibility of developing diets of significantly lower total protein (crude protein, or $N \times 6.25$) content, but adequately providing for the requirement of essential amino acids. Presently, this implies supplementation with manufactured amino acids. However, the possibility arises that specific pig genotypes might respond satisfactorily to dietary levels of protein (and amino acids) below those norms suggested presently here. Conventional wisdom suggests that such reductions in protein: energy ratios will cause a concomitant increase in carcass fatness; but that wisdom depends on the assumption that all pig genotypes will, inevitably, convert excess energy consumption into body fat; whereas this might not always be the case. Besides, there is a view that many pigs are already too lean for optimization of eating quality, and that some additional lipid, particularly within the muscle mass, might be beneficial.

Conclusion

The nutrition of growing pigs is a matter of process control. Energy supply, essential amino acid supply, and diet ingredient inclusion are all subject to management choice. By manipulation of the quantity and quality of the feed presented to pigs, the rate of meat production and the composition of that meat may be closely controlled. Such control is seminal to both the profitability of pig farming and consumer acceptability of the product.

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See also: Bacon Production: Bacon; Wiltshire Sides. Boar Taint: Biological Causes and Practical Means to Alleviate It. Chemical Analysis: Raw Material Composition Analysis. Chemical and Physical Characteristics of Meat: Adipose Tissue; Color and Pigment; Palatability; pH Measurement; Protein Functionality. Classification of Carcasses: Pig Carcass Classification. Growth of Meat Animals: Adipose Tissue Development; Growth Patterns; Muscle. Ham Production: Cooked Ham; Dry-Cured Ham. Modeling in Meat Science: Meat Quality. On-Line Measurement of Meat Composition. Quality Management: Farm Level: Pork Quality. Sensory and Meat Quality, Optimization of. Species of Meat Animals: Pigs

Further Reading

INRA, 2003. Tables de composition et de valeur nutritive de matières premières destinées aux animaux d'élevage Paris: INRA Editions and AFZ. Sauvant, D., Perez, J.-M., Tran, G. (coordinators).

Kyriazakis, I., 1999. A Quantitative Biology of the Pig. Wallingford: CAB International.

National Research Council, 1998. Nutrient Requirements of Swine, tenth ed. Washington, DC: National Academy Press.

Noblet, J., Fortune, H., Shi, X.S., Dubois, S., 1994. Prediction of net energy value of feeds for growing pigs. Journal of Animal Science 72, 344–354.

Seve, B., Hess, V., 2000. Amino acid digestibility in formulation of diets for pigs: Present interest and limitations, future prospects. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition 2000. Nottingham: Nottingham University Press, pp. 167–181.

Whittemore, C.T., 1998. The Science and Practice of Pig Production. Oxford: Blackwell Science.

Whittemore, C.T., Close, W.H., Hazzledine, M.J., 2002. The need for nutrient requirement standards for pigs, a report of the British Society of Animal Science nutritional Standards Working Group: Pigs. Pig News and Information 23, 67N–74N.

Whittemore, C.T., Hazzledine, M.J., Close, W.H., 2003. Nutrient Requirement Standards for Pigs. Penicuik: British Society of Animal Science.

Wood, J.D., 2009. Factors affecting pork quality. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent Advances in Animal Nutrition 2009. Nottingham: Nottingham University Press, pp. 311–326.

Poultry

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Introduction

Internationally, poultry meat production has consistently increased over the years and it is expected that this trend will continue. The major factor that has contributed to the consistent growth in world production of poultry meat is the genetic progress in poultry strains for meat production. In meat chickens, the age of male birds to reach the market weight of 2 kg has steadily decreased from 63 days in 1976 to 28–30 days in 2010 and the efficiency of converting feed into meat continues to improve. Rates of genetic change for growth and feed efficiency have also changed the physiology of the birds. Consequently, nutrient requirements and nutritional management have been changing to satisfy the genetic potential of the new strains. Poultry is unique among domestic animals in that any change in nutrition is reflected in bird performance in almost a real-time manner. This phenomenon has been successfully exploited by the commercial poultry meat industry to improve growth and carcass yield of birds.

Poultry is an omnivore, which can be classified nutritionally as having a simple digestive system with nonfunctional caeca. The digestive tract of poultry has more organs, but is shorter than in other domestic animals. In fast-growing meat chickens, the time taken for the feed to pass from mouth to cloaca (and to digest and absorb the nutrients) is less than 3 h. To compensate for the relatively short digestive tract and digesta transit time, poultry needs to be offered concentrated, easily digestible form of feeds that contain not only adequate concentrations, but also a good balance of nutrients.

The term 'poultry' encompasses a range of domesticated species including chickens, turkeys, ducks, geese, game birds (such as quails, pheasants, and partridges), and ratites (emus and ostriches). A discussion of the nutrition of all these species is beyond the scope of this article so the focus will be on meat chickens, which constitute more than 90% of the poultry meat market. However, in general, principles of nutritional management of meat chickens are applicable to other poultry species grown for meat. The overall aim of poultry feed formulations is to lower costs and maximize economic efficiency. In the past, there had been a tendency to overformulate diets when doubts existed on the availability of critical nutrients (especially amino acids and phosphorus) or if the nutrient requirement was uncertain. This practice is no longer acceptable because this is not only wasteful, but also excess nutrients are excreted in the manure and are ultimately a source of pollution. Future directions in poultry nutrition will be driven not only by the need to maximize biological and economic performance of birds, but also by societal issues (environment, antibiotic growth promoters, welfare, traceability, and the use of genetically modified ingredients).

Nutrient Requirements

For maximum growth and good health, the intensively reared poultry needs a balanced array of nutrients from its diet. In the main, poultry needs a steady supply of energy, protein, essential amino acids, essential fatty acids, minerals, vitamins, and, more importantly, water. Birds obtain the energy and the required nutrients following the digestion of natural feedstuffs, though trace minerals, vitamins, and some essential amino acids (lysine, methionine, threonine, and tryptophan) are offered as synthetic supplements.

Energy

In the real sense, energy is not a nutrient but the property of energy-yielding nutrients, primarily carbohydrates and fats, and also protein. These nutrients, when oxidized during metabolism, release adenosine triphosphate that serves as a precursor for the synthesis of chemical body components forming the muscle tissues. The energy value of a feedstuff for poultry is usually expressed as apparent metabolizable energy (AME) or true metabolizable energy (TME). The TME values are widely used in feed formulation in North America and some parts of Europe, whereas the AME concept is popular in the other parts of the world. The AME is defined as the gross energy of the feed consumed minus the gross energy contained in the excreta. In TME calculations, energy losses from endogenous sources are accounted for and it is defined as the gross energy of the feed consumed minus the gross energy of the undigested feed in the excreta.

It has been long known that the birds eat to satisfy their energy needs, provided that the diet is adequate in all other essential nutrients. The energy concentration in the diet is therefore a major determinant of feed intake in poultry. With changes in dietary energy concentration, the feed intake will change and specifications for other nutrients must be altered to maintain the required intake of nutrients. For this reason, the dietary energy concentration is often used as the starting point in the formulation of practical diets for poultry.

To sustain the faster growth rate, modern meat chicken strains are typically fed high-energy diets. Historically, the industry has used nutrient requirements recommended in the publication by National research Council (NRC). Recommended minimum nutrient concentrations for meat chickens at different ages are presented in Table 1. These recommendations, however, should only be considered as guidelines

Table 1 Nutrient requirements of meat chickens as percentages or units per kilogram of diet (90% dry matter)

Nutrient	*Unit*	*1–21 days*	*22–42 days*	*43–56 days*
Metabolizable energy	Kcal/kg	3200	3200	3200
	MJ/kg	13.4	13.4	13.4
Crude protein	%	23	20	18
Amino acids				
Arginine	%	1.25	1.10	1.00
Glycine+serine	%	1.25	1.14	0.97
Histidine	%	0.35	0.32	0.27
Isoleucine	%	0.80	0.73	0.62
Leucine	%	1.20	1.09	0.93
Lysine	%	1.10	1.00	0.85
Methionine	%	0.50	0.38	0.32
Methionine+cysteine	%	0.90	0.72	0.60
Phenylalanine	%	0.72	0.65	0.56
Phenylalanine+tyrosine	%	1.34	1.22	1.04
Proline	%	0.60	0.55	0.46
Threonine	%	0.80	0.74	0.68
Tryptophan	%	0.20	0.18	0.16
Valine	%	0.90	0.82	0.70
Fatty acid				
Linoleic acid	%	1.00	1.00	1.00
Major minerals				
Calcium	%	1.00	0.90	0.80
Chlorine	%	0.20	0.15	0.12
Magnesium	%	0.06	0.06	0.06
Nonphytate phosphorus	%	0.45	0.35	0.30
Potassium	%	0.30	0.30	0.30
Sodium	%	0.20	0.15	0.12
Trace minerals				
Copper	mg	8	8	8
Iodine	mg	0.35	0.35	0.35
Iron	mg	80	80	80
Manganese	mg	60	60	60
Selenium	mg	0.15	0.15	0.15
Zinc	mg	40	40	40
Fat soluble vitamins				
A	IU	1500	1500	1500
D_3	ICU	200	200	200
E	IU	10	10	10
K	mg	0.5	0.5	0.5
Water soluble vitamins				
B_{12}	mg	0.01	0.01	0.007
Biotin	mg	0.15	0.15	0.12
Choline	mg	1300	1000	750
Folacin	mg	0.55	0.55	0.5
Niacin	mg	35	30	25
Panthothenic acid	mg	10	10	10
Pyridoxine	mg	3.5	3.5	3.0
Riboflavin	mg	3.6	3.6	3.0
Thiamin	mg	1.8	1.8	1.8

Source: Adapted with permission from National Research Council, 1994. Nutrient Requirements of Poultry, ninth ed. Washington, DC: National Academy of Sciences, pp. 26–27.

and as the basis for setting dietary nutrient concentrations in practical diets. The most recent publication on poultry was in 1994 and is now 20-years-old, which is a long period given the genetic advances that have been made in both broilers and layers over this period. Currently, the recommendations suggested by commercial breeding companies provide guidelines that more closely match the requirements of modern bird strains than those recommended by NRC (1994).

The dietary energy concentrations employed in a given situation are largely dictated by the availability and cost of energy-rich feedstuffs. Because of the high cost of cereals, particularly of maize, the use of low-energy diets (as low as

2850 Kcal kg^{-1} or 11.9 MJ kg^{-1}) for broiler feeding is a common practice in many countries.

Protein and Amino Acids

The function of dietary protein is to supply amino acids for muscle growth and maintenance. The synthesis of muscle protein requires the supply of 20 amino acids, all of which are physiologically essential. Ten of these cannot be synthesized at all or rapidly enough to meet the metabolic requirements and are therefore designated as essential elements of the diet. The balance can be synthesized from other amino acids and referred to as nonessential elements of the diet. The essential amino acids for poultry are: lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, valine, phenylalanine, and arginine. In addition, glycine is considered by some to be essential for young birds. Cysteine and tyrosine are considered as semi-essential amino acids because they can be synthesized from methionine and phenylalanine, respectively. Lysine, methionine, and threonine are the most limiting amino acids in practical poultry diets.

Poultry does not have a requirement for protein *per se*. However, to synthesize nonessential amino acids, an adequate dietary supply of nitrogen from protein is essential. This ensures that the essential amino acids are not degraded to supply the nitrogen for the synthesis of nonessential amino acids. Satisfying the recommended requirements for both protein and essential amino acids will therefore ensure that all amino acids are provided to meet the physiological needs.

Amino acid requirements of meat birds are influenced by an array of factors, including genotype, sex, physiological status, environment, and health status. However, most changes in these amino acid requirements do not necessarily lead to changes in the relative proportion of all of the different amino acids and the required changes in amino acids can be expressed in relation to a balanced protein or 'ideal protein' for the meat chicken. In the ideal protein concept, lysine is used as the reference amino acid and requirements for the other essential amino acids set as a percentage (or ratio) of the lysine requirement (Table 2). The advantage of this system is that once the lysine requirements under a variety of conditions are determined, the needs of all other essential amino acids can be calculated. This approach has now become an accepted practice in the industry to set the amino acid specifications in feed formulations.

A recent development has been the wider use of digestible amino acid concentrations, rather than total amino acid concentrations, in feed formulations. The use of digestible amino acid content is particularly relevant in regions where diets are formulated using poorly digestible ingredients such as canola meal, cottonseed meal, sunflower meal, meat meal, and feather meal. In the USA, where the highly digestible maize and soybean meal contribute more than 95% of the protein requirement, the use of digestible amino acid system is less relevant. Formulating diets based on digestible amino acids makes it possible to increase the range and inclusion levels of alternative ingredients in poultry diets. In effect, this approach improves the precision of formulation, reduces the need for safety margin, may lower feed cost, ensures more predictable bird performance, and reduces nitrogen excretion into the environment.

Table 2 Ideal amino acid ratios of meat chickens at three growth periods

Amino acid	*1–21 days*	*22–42 days*	*43–56 days*
Lysine[a]	100	100	100
Arginine	105	108	108
Histidine	35	35	35
Isoleucine	67	69	69
Leucine	109	109	109
Methionine+cysteine	72	72	72
Phenylalanine+tyrosine	105	105	105
Threonine	67	68.5	68.5
Tryptophan	16	17	17
Valine	77	80	80

[a]Recommended digestible lysine requirements for meat chickens during 1–21 days, 22–42 days, and 43–56 days are 1.070%, 0.865%, and 0.745%, respectively.

Source: Adapted with permission from Dudley-Cash WA, 1996. Bottom line nutrition. Feedstuffs (USA) July 6, p.11 & 15.

Fats and Fatty Acids

Because of the greater energy density of fat compared with carbohydrates and protein, fats are usually included in poultry diets to achieve dietary energy concentrations. In most practical diets, fat is added in the range of 3% to no more than 5%. Other benefits of using fats include better dust control in feed mills and poultry houses and improved palatability of diets. Poultry does not have a requirement for fats as a source of energy, but requirement for linoleic acid (18:2, n-6) has been demonstrated. Linoleic acid is the only essential fatty acid needed by poultry, but its deficiency has rarely been observed in birds fed practical diets.

Minerals

Minerals are needed for the formation of skeletal system, for general health, as components of general metabolic activity, and for the maintenance of acid–base balance in the body. Calcium and phosphorus are the most abundant mineral elements in the body and are classified as macrominerals along with sodium, potassium, chloride, sulfur, and magnesium. Macrominerals are elements required in the diet at concentrations of more than 100 mg kg^{-1}.

Calcium and phosphorus are necessary for the formation and maintenance of the skeletal structures. In general, 60–80% of total phosphorus present in plant-derived ingredients is in the form of phytate phosphorus. Under normal dietary conditions, phytate phosphorus is poorly utilized by poultry due to insufficient quantities of endogenous phytase. It is generally assumed that approximately one-third of the phosphorus in plant feedstuffs is nonphytate and is biologically available to poultry. For this reason, phosphorus requirement for poultry is expressed as nonphytate phosphorus, rather than total phosphorus. A ratio of 2:1 must be maintained between calcium and nonphytate phosphorus in poultry diets to optimize the absorption of these two minerals.

Dietary proportions of sodium, potassium, and chloride largely determine the acid–base balance in the body so that the

physiological pH is maintained. If a shift occurs toward acid or base conditions, metabolic processes are altered to maintain the pH with the likely result of depressed performance. The dietary electrolyte balance is described by the simple formula ($Na^+ + K^+ - Cl^-$) and expressed as mEq kg^{-1} diet. Prevention of electrolyte imbalance needs careful consideration. Under most conditions, a balance of approximately 250 mEq kg^{-1} diet appears satisfactory for optimum growth. It must be emphasized that overall balance between these three minerals as well as their individual concentrations is important. To be effective, their dietary concentrations must each be within respective acceptable ranges, not deficient and not excessive. Birds exposed to heat stress consume increased amounts of water and are better able to withstand heat when the water contains electrolytes. The replacement of part of the sodium chloride in the diet formulation with sodium bicarbonate has proven useful under these conditions.

Trace minerals, such as copper, iodine, iron, manganese, selenium, zinc, and cobalt, function as components of larger molecules and as cofactors of enzymes in various metabolic reactions. These elements are required only in very small concentrations in the diet (Table 1). Practical poultry diets should be supplemented with major and trace minerals because typical cereal-only based diets are deficient in these minerals. Organic forms of trace minerals are generally considered to have a higher biological availability compared to inorganic forms.

Vitamins

Vitamins are generally classified as fat-soluble (vitamins A, D, E, and K) and water-soluble (vitamin B-complex and vitamin C). All vitamins, except for vitamin C, must be provided in the diet. Vitamin C is not generally classified as a dietary essential because it can be synthesized by the bird. However, under adverse circumstances, such as heat stress, dietary supplementation of vitamin C may be beneficial. The metabolic roles of the vitamins are more complex than those of other nutrients. Vitamins are not simple body-building units or energy sources but are mediators of, or participants of, all biochemical pathways in the body.

Water

Water is the most important nutrient in poultry nutrition. Supply of clean water is essential at all times and deprivation even for a short period can irreversibly depress growth rates. Both feed intake and growth rate are highly correlated with water intake. Precise requirements for water are difficult to state and are influenced by a number of factors including ambient conditions, age, and physiological status of birds. Under most conditions, water intake is assumed to be twice the amount of feed intake.

Ingredients Used in Poultry Feed Formulations

Feed represents the major cost of poultry production, constituting up to 60% of the total cost. Of the total feed cost, approximately 95% is used for meeting the energy and protein requirements, approximately 3–4% for the major mineral, trace mineral, and vitamin requirements, and 1–2% for various feed additives. Poultry diets are formulated using a mixture of several ingredients, including cereal grains, cereal by-products, fats, plant protein sources, animal by-products, vitamin and mineral supplements, synthetic amino acids, and feed additives. These are invariably assembled on a least-cost basis using ingredient prices together with their nutrient contents. A classification and examples of ingredients used in poultry diets are given in Table 3.

Energy sources constitute the largest component of poultry diets, followed by plant protein sources and animal protein sources. Plant protein sources are included to supply the major portion of dietary protein (or nitrogen) requirements. Plant protein sources, with the exception of soybean meal, are generally imbalanced with regard to essential amino acids. Unless supplemented with animal protein sources or crystalline amino acids, diets based on plant-derived ingredients will not meet the requirements for certain essential amino acids.

Feed Additives Used in Poultry Feed Formulations

In addition to the ingredients discussed above, poultry formulations contain an array of substances known as 'feed additives.' These are nonnutritive substances usually added in amounts of less than 0.05% to maintain health status, uniformity, and production efficiency in intensive production systems. These additives have now become vital components in practical diets. A list of commonly used feed additives is presented in Table 4.

Special mention needs to be made of the ban in the European Union and different degrees of voluntary withdrawal in other parts of the world on the use of in-feed antibiotics in animal feeds. Antibiotics have been used in poultry diets for more than 60 years as growth promotant and as a prophylactic measure against necrotic enteritis, a fatal bacterial disease caused by *Clostridium perfringens*, in meat chickens. The withdrawal of these preventive measures has serious implications for the poultry industry, including disease outbreaks. At present, there is increasing focus on alternatives to sustain good gut flora and gut health, and potential alternatives include enzymes, probiotics, prebiotics, essential oils, botanicals, and organic acids (Table 4). During the past 10 years, these products have been widely tested and the evaluation will continue in the future. Most of these alternatives have been shown to 'mimic' the working effects of in-feed antibiotics on gut flora, but none of the current generation of alternatives, on their own, are capable of fully replacing the in-feed antibiotics.

Feeding Programs

Broilers are usually fed on an *ad libitum* basis to ensure fast growth to market age. However, in recent years, intermittent feeding programs are becoming popular, especially during the first 2–3 weeks of life, as a means of lowering mortality levels caused by metabolic diseases, such as sudden death syndrome

Table 3 Classification and examples of ingredients used in poultry feed formulations

1. Energy sources
   a. Cereals: maize, wheat, barley, sorghum, rice, triticale, and millets
   b. Cereal by-products: milling by-products from rice and wheat, maize gluten meal
   c. Root and tuber meals: cassava root meal and sweet potato tuber meal
   d. Fruit and fruit by-products: banana meal and date by-products
   e. Fats and oils: tallow and vegetable oils
   e. Miscellaneous: cane molasses
2. Plant protein sources
   a. Oilseed meals: soya bean meal, canola meal, cottonseed meal, sunflower meal, safflower meal, coconut meal, palm kernel meal, groundnut meal, and sesame meal
   b. Grain legumes: field peas and lupins
   c. Green leaf meals: alfalfa leaf meal, sweet potato vine meal, and cassava leaf meal
   d. Miscellaneous: dried brewers' spent grain and dried distillers grain
3. Animal protein sources
   a. Fish products: fish meal
   b. Animal by-products: meat meal, blood meal and poutry by-product meal
   c. Milk by-products: skim milk powder
4. Mineral supplements
   a. Calcium supplements: limestone and shell grit
   b. Calcium and phosphorus supplements: dicalcium phosphate, monocalcium phosphate, defluorinated phosphate, and bone meal
   c. Trace minerals: trace mineral premixes
   d. Sodium sources: salt and sodium bicarbonate
5. Others
   a. Vitamin supplements: vitamin premixes
   b. Nonnutritive feed additives (see **Table 4**)

Table 4 Feed additives commonly used in poultry feed formulations

Additive	*Examples*	*Reasons for use*
Enzymes	Xylanases, β-glucanases, and phytase	To overcome the antinutritional effects of arabinoxylans (in wheat and triticale), β-glucans (in barley), or phytate (in all plant feedstuffs) and to improve the overall nutrient availability and feed value.
In-feed antibiotics[a]	Avilamycin,virginiamycin, zinc bacitracin, avoparcin, tylosin, and spiramycin	To control gram positive, harmful bacterial species in the gut and to improve production efficiency; as a prophylactic measure against necrotic enteritis
Coccidiostats	Monensin, salinomycin, and narasin	To prevent and control the clinical symptoms of coccidiosis
Pigmentants	Xanthophyll (natural and synthetic)	To improve the skin color and appearance of carcasses
Antioxidants	Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and ethoxyquin	To prevent autooxidation of fats and oils in the diets
Antifungals		To control mold growth in feed; to bind and mitigate the negative effects of fungal toxins
Antibiotic replacers[b]		
1. Direct-fed microbials	Probiotics	Provision of beneficial species such as *Lactobacilli* and *Streptococci*
2. Prebiotics	Fructo oligosaccharides and mannan oligosaccharides	Binding of harmful bacteria
3. Organic acids	Propionic acid and diformate	Lowering of gut pH and prevention of the growth of harmful bacteria
4. Botanicals	Herbs, spices, plant extracts, and essential oils	Prevention of the growth of harmful bacteria
5. Antimicrobial proteins/peptides	Lysozyme, lactacin F, lactoferrin, and α-lactalbumin	Prevention of the growth of harmful bacteria

[a]The use of avoparcin, zinc bacitracin, spiramycin, virginiamycin, and tylosin phosphate as animal feed additives was banned in 1998 in the European Union.
[b]Envisaging a total ban on in-feed antibiotic use, a multitude of compounds (individually and in combination) is being currently tested.

and a disease ascites, which are associated with fast-growing meat chicken strains.

Broiler diets are offered either as crumbles or pellets. Broiler starter diets are usually offered as crumbles, whereas grower and finisher diets are given as pellets. Compared to mash-type diets, crumbles and pellets reduce bulk density, improve feed intake, reduce feed wastage, and enhance the feed efficiency. This, in particular, has more effect on the growth rate of meat chickens fed low-energy diets where volumetric density has an adverse effect on feed intake.

Continuous genetic improvement has resulted in meat chickens that grow faster every year on less feed. Despite the dynamic nature of the industry, nutrient requirement recommendations have remained somewhat static. Current nutrient recommendations (Table 1) list the nutrient requirements only for three time periods, namely, 0–3 weeks, 3–6 weeks, and 6–8 weeks. In practice, however, the grow-out periods can range from 4 to 10 weeks of age, depending on local market needs. Recognizing that changes in nutrient needs are more dynamic than these general recommendations, phase-feeding systems are widely used by the commercial industry to maximize performance and increase profit margins. Dietary protein and amino acid specifications are usually decreased in a progression of feeds from start to finish in a manner that satisfies the requirements and economics. Typical feeding programs over a 5–7 week production cycle now include four to five feeds such as prestarter, starter, grower, and finisher; or prestarter, starter, grower, finisher, and withdrawal. Withdrawal diets are usually fed during the last 5 days of growth and involve the removal of pharmaceutical additives and the reduction of protein/amino acids. In recent years, this has been extended to the reduction of vitamin and trace minerals, and also to energy.

'Sex separate' growing is practised in some situations where the uniformity of the final product is important and where uniformity has been hard to attain. This practice requires specific formulations for male and female broilers and can lower feed costs because considerable evidence indicates that females require 1–2% less protein and amino acids than the males. Males and females have similar nutrient requirements during the first 2 weeks of life, after which the females, due to their slower growth rate, tend to respond differently to dietary amino acid levels. Sex separate feeding also allows the marketing of male birds at an earlier age than females.

A recent development has been the feeding of whole grains (wheat or barley) along with a balanced concentrate feed. The benefits of whole grain feeding include reduced feed processing costs and improved flock health. These benefits appear to arise from a combination of two physiological actions: physical benefits of gizzard development and increased proventriculus secretions, and better matching of daily requirements through self-selection by the bird. The usual method of whole grain feeding has been to blend 10–25% of the grain on top of the feed in the delivery trucks or at the poultry house.

Feeding for Leaner Carcass Production

A major reason for the popularity of chicken meat has been the consumer perception of a healthy product. In chickens and turkeys, the white meat from the breast is very lean. This image of low-fat meat has contributed much to enhancing the consumption of poultry at the expense of red meats. Nevertheless, there are opportunities to further improve the image of chicken meat and to lower fat levels in the whole carcass through nutritional manipulations.

Defining the most desirable amount of fat in meat chickens is complex. Fat deposition is an integral part of broiler growth and its extent and distribution in the carcass is an important aspect of meat quality. Although the skin fat is essential for acceptable eating qualities, visceral and abdominal fats are considered as waste materials. Excessive fat deposition is not only energetically expensive, but also lowers saleable carcass yield and increases cooking losses.

Restriction of Energy Intake

It is well established that when birds are fed energy in excess of metabolic needs, fat deposition increases largely in the area of abdomen and viscera. Several nutritional approaches are possible to restrict energy intake and these include feed restriction and narrower energy to protein ratios.

Novel Nutritional Approaches

A number of novel and potentially useful approaches have also been attempted in recent years. These include the use of β-agonists, conjugated linoleic acid (CLA), organic chromium, and polyunsaturated fatty acids (PUFA).

β-agonists

β-agonists have been studied as repartitioning agents in livestock species for nearly two decades. Although the exact mechanism of the action of β-agonists is unclear, a cascade of events is believed to occur following binding of the compound to the β_2-receptor, leading to tissue responses and partitioning of energy away from fat synthesis and into protein accretion. The interest in the use of β-agonists declined during early 1990s following an outbreak of food poisoning after eating livers from cattle treated with the β-agonist, clenbuterol. However, there is renewed interest in the use of β-agonists since the recent approval of ractopamine for use in pig diets by the Federal Drug Administration (FDA). The available data indicate that avian species do respond to β-agonists. In addition to performance responses, large decreases have been reported in the abdominal fat and carcass fat, with females generally responding more than the males.

Conjugated linoleic acid

CLA is a mixture of positional and geometrical isomers of linoleic acid (18:2); the predominant isomer being rumenic acid, a *cis*-9 *trans*-11 isomer. Ruminant meat products contain relatively high concentrations of CLA (0.5–1.5% of total fatty acids), whereas meats from simple-stomached animals are poor sources (0.1–0.2% of total fatty acids). CLA has been shown to regulate energy metabolism and repartition energy in a range of animals so that body fat is significantly reduced while lean body mass is increased. Studies in chickens have reported up to 35% decrease in abdominal fat with the dietary inclusion of 1% CLA.

Chromium supplementation

The beneficial effect of chromium in human health is well documented for its role as an integral component of the glucose tolerance factor, which potentiates the action of insulin. Insulin regulates energy production, muscle protein accretion, fat metabolism, and cholesterol utilization. Plant

feed ingredients commonly used in diet formulations contain low levels of chromium and additions of this mineral may therefore become a common micronutrient for animals in the future. Supplementation of broiler diets with 300–400 μg kg^{-1} organic chromium has been shown to markedly lower the carcass fat content and abdominal fat pad weight.

Concentration and source of dietary fats

Vegetable oils and animal fats are commonly used to achieve necessary levels of metabolizable energy in poultry diets. The type and concentration of fat used can contribute to excess fat deposition in fast-growing broiler chickens that are fed *ad libitum*. Of interest are the fats that contain high levels of PUFA. High dietary concentration of PUFA is known to inhibit lipogenesis and lower fat deposition in broilers. It is well documented that feeding diets containing sunflower oil (rich in PUFA of omega-6 series) and flax oil or fish oil (rich in PUFA of omega-3 series) lower abdominal fat pad mass in broilers compared to fats containing tallow (rich in saturated fatty acids).

Nutrition and Carcass Quality

Carcass quality in poultry may be considered in relation to two components. On the positive side, quality relates to physical appearance, including meat color, skin color, and finish, and the absence of off-flavors. On the negative side, presence of blemishes, in the skin and muscles, can downgrade the quality.

Skin Color

Skin color still influences the consumer attitudes toward fresh poultry. Pronounced regional differences in consumer preferences have been shown for fresh whole carcasses based solely on skin color, ranging from white to pale yellow to deeply pigmented. The deposition of pigments in the skin is determined by the dietary concentrations of the carotenoids, and can be easily manipulated by inclusion of ingredients with pigments (such as maize, maize gluten meal, and green leaf meals) or by the addition of synthetic xanthophyll sources.

Meat Color

Color of raw poultry meat is critical for consumer selection. Colors that differ from the expected pale tan to pink raw meat or from the tan to gray cooked meat will result in consumer rejection. Poultry meat color may be influenced by preslaughter and processing conditions, and nutritional factors have no effect on the color.

Carcass Finish/Downgrading

A good finish relates to acceptable physical appearance with no skin tearing or lesions. With the recent trend toward processing and the focus on special carcass parts rather than the whole carcass, the relative importance of carcass finish has declined during the past decade. Nutrition, provided that the feed has been properly supplemented with vitamins and minerals, is not usually associated with the physical downgrading of carcass.

Oily bird syndrome, a condition observed only in warmer climates, is associated with oily or greasy skin and increased skin tears. Because of the oily nature of the carcass, dietary concentration and source of fat have come under close scrutiny. Although there does not appear to be any simple relationship with fat concentrations, there is some evidence that the problem is more frequent in birds fed diets containing saturated fats such as tallow. A major quality problem encountered in turkeys, and more recently in meat chickens grown to heavy weights, is the incidence of pale, soft, exudative meat, but nutrition is not a factor in the development of this problem.

Skin strength is highly correlated with its collagen content and skin with less collagen is more prone to tearing. Zinc, copper, and vitamin C all play a role in collagen synthesis and, provided that processing conditions are not responsible, increased dietary additions of these may be useful in lowering skin tearing.

Maintenance of litter quality, in terms of moisture levels, is a critical component in producing meat birds of good carcass quality. Wet litter will not only lead to accumulation of ammonia and increase incidence of disease conditions, but also cause breast and hip lesions and lower the carcass quality. Several nutritional factors can increase the moisture content of the excreta, resulting in wet litter. Dietary electrolyte imbalance is a major cause of wet droppings. Electrolyte minerals, sodium, potassium, and chloride show independent effects for increasing water intake of poultry, when concentrations are increased above requirements. Dietary concentrations above 0.25% sodium, 1.15% potassium in young broilers (and 0.80% in older broilers), and/or 0.30% chloride increase water intake and wet litter problems. Feeding of diets based on cereals that contain significant amounts of nonstarch polysaccharides, such as wheat, barley, triticale, and rye, can also cause wet litter problems. But this can easily be mitigated by the use of appropriate feed enzyme supplementation.

Off-Flavors

The fatty acid composition of carcass lipid is the major determinant of the flavor and shelf-life stability of fresh and cooked meats. Large changes can be made to the diet without any effect on meat flavor, but the use of some ingredients is known to cause fish taints or rancid odors. These include fish meal, oils containing high levels of PUFA such as fish oil and flaxseed oil, and restaurant greases. The formation of off-flavors, however, can be largely suppressed by the provision of antioxidants, both natural and synthetic, in the diets.

See also: Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Human Nutrition: Macronutrients in Meat; Micronutrients in Meat. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Beta-Agonists; Poultry. Muscle Fiber Types and Meat Quality.

Nutrition of Meat Animals: Pigs. Slaughter-Line Operation: Poultry

Further Reading

Blair, R., Daghir, N.J., Morimoto, H., Peter, V., Taylor, T.G., 1983. International nutrition standards for poultry. Nutrition Abstracts and Reviews – Series B 53, 669–713.

Ensminger, M.E., Oldfield, J.E., Heinemann, W.W., 1990. Feeds and Nutrition. Clovis, CA: The Ensminger Publishing Company.

Farrell, D.J., Stapelton, P. (Eds.), 1986. Duck Production Science and World Practice. Armidale, Australia: The University of New England.

Fisher, C., Boorman, K.N. (Eds.), 1986. Nutrient Requirements of Poultry and Nutritional Research. Poultry Science Symposium 19. London, UK: Butterworths.

Hunton, P. (Ed.), 1995. Poultry Production. World Animal Science. Amsterdam, The Netherlands: Elsevier.

Leeson, S., Summers, J.D., 1997. Commercial Poultry Nutrition, second ed. Guelph, Canada: University Books.

Leeson, S., Summers, J.D., 2001. Scott's Nutrition of the Chicken, fourth ed. Nottingham, UK: Nottingham University Press.

Lemme, A., Ravindran, V., Bryden, W.L., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poultry Science Journal 60, 421–435.

National Research Council, 1994. Nutrient Requirements of Poultry, ninth revised ed. Washington, DC: National Academy Press.

Ravindran, V., Blair, R., 1991. Feed resources for poultry production in Asia and the Pacific. I. Energy sources. World's Poultry Science Journal 47, 213–231.

Ravindran, V., Blair, R., 1992. Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. World's Poultry Science Journal 48, 205–231.

Ravindran, V., Blair, R., 1993. Feed resources for poultry production in Asia and the Pacific. III. Animal protein sources. World's Poultry Science Journal 49, 219–235.

Ravindran, V., Bryden, W.L., 1999. Amino acid availability in poultry – *in vitro* and *in vivo* measurements. Australian Journal of Agricultural Research 50, 889–908.

Richardson, R.I. and Mead, G.C. (Eds.),1999. *Poultry Meat Science*. Poultry Science Symposium 35, Wallingford, UK: CAB International.

Scott, M.L., 1987. Nutrition of the Turkey. Ithaca, NY: M.L. Scott & Associates.

Scott, M.L., Dean, W.F., 1991. Nutrition and Management of Ducks. Ithaca, NY: M. L. Scott & Associates.

Ruminants

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Glossary

Adenosine triphosphate (ATP) The most common energy-carrying molecule used in cellular energy metabolism.
Conjugated linoleic acid (CLA) A long-chain fatty acid with anticancer properties.
FAMACHA A method of identifying parasitized sheep and goats by visually evaluating the color of their mucous membranes for anemia.
Squamous cells Flat, tightly packed cells which form a single layer of epithelium.
Volatile fatty acids (VFAs) Short-chain fatty acids which are the primary product of ruminal carbohydrate fermentation and they are the primary source of energy within the ruminant animal.

Introduction

Ruminants are mammals of the suborder Ruminantia and include animals in the families of Giraffidae, Cervidae, Antilocapridae, Ovidae, Bovidae, and Camelidae that chew their cud; respective examples are giraffes, deer, antelope, sheep, cattle, and camels. Although meat from most of these animals is consumed, only a few have been specifically domesticated for meat production. The principal and recurring cost of ruminant production is feed costs, and it is therefore economically important to understand the anatomical differences relative to other livestock; the manner in which the feed consumed is digested and the nutritional requirements of meat-producing ruminants. Thus, the nutritional management of various classes of the domesticated species is studied and documented scientifically.

Anatomical Distinction

Nutritional requirements of ruminants are different from those of nonruminants because animals in this suborder have a modified alimentary tract. Ruminant animals have four distinct chambers posterior to the esophagus and anterior to the small intestine. These chambers are the reticulum, rumen, omasum, and abomasum (Figure 1), with the first three being the 'forestomachs'; in the fourth chamber, or abomasum, acid secretion and peptic digestion occur, as in nonruminants. Following the abomasum is the small intestine and colon. In adult ruminants, these four chambers can occupy 75% of the abdominal cavity, with the rumen being the largest. The reticulum is the most cranial of the chambers and dorsally is continuous with the rumen. There are two, heavy muscular folds (sulcus ruminoreticularis and esophageal groove) extending from the cardia at the top of the reticulum to the omasum, which can contract to restrict the flow of partially digested feed or digesta into the rumen. The rumen itself is subdivided or saculated by muscular pillars. The contents of the rumen exit via the reticulo-omasal orifice into the omasum, a spherical chamber containing many muscular laminae with water- and nutrient-absorptive capabilities. Digesta exit the chamber into the abomasum via the omaso-abomasal orifice.

The interior of the forestomachs is covered with stratified squamous epithelium, which is organized into different structures in each of the three chambers (Figures 2–4). Unlike other stratified epithelium, that of the reticulum, rumen and omasum are vascularized, and the products of fermentation (volatile fatty acids (VFAs) and ammonia) are absorbed across the ruminal epithelium.

The rumen is proportionately smaller at birth than in adult animals and increases in size as it becomes progressively colonized by microflora. It is capable of fermenting feed within 4 weeks after birth and reaches adult proportions by 100 days after birth. The weight of ruminal contents in adult sheep and cattle varies with the type of feed provided, ranging between 4 and 6 kg in sheep and between 30 and 60 kg in cattle, and contains 10–15% dry matter (DM). The rumen harbors many different species of anaerobic bacteria, protozoa, and fungi (Table 1), which are involved in the proteolysis of proteins, hydrolysis and biohydrogenation of fats, and fermentation of carbohydrates in feed. These feed-digestive processes are aided

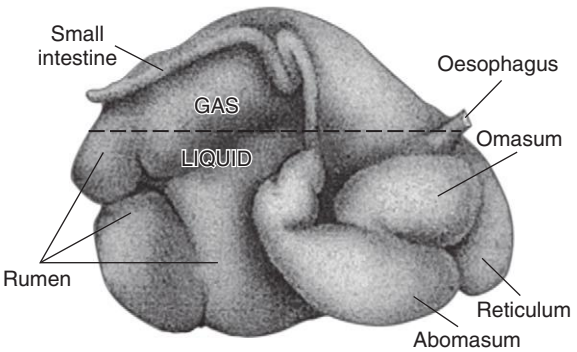

Figure 1 The chambers of the ruminant stomach (viewed from the right side). Reproduced from Bowen, R.A., 1997. Digestive Anatomy in Ruminants. Fort Collins: Colorado State University.

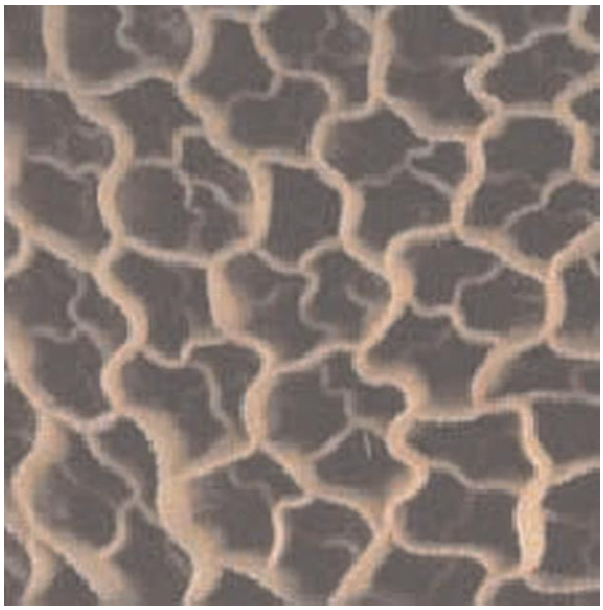

Figure 2 In the reticulum, the epithelium has folds that form a polygonal or reticular (honeycomb) structure. Reproduced from Bowen, R.A., 1997. Digestive Anatomy in Ruminants. Fort Collins: Colorado State University.

Figure 4 In the omasum, the epithelium is organized into broad longitudinal folds, which appear like leaves. Reproduced from Bowen, R.A., 1997. Digestive Anatomy in Ruminants. Fort Collins: Colorado State University.

Figure 3 In the rumen, the epithelium forms numerous papillae that vary in shape and size, depending on the feed provided to the animal. Reproduced from Bowen, R.A., 1997. Digestive Anatomy in Ruminants. Fort Collins: Colorado State University.

by the rhythmic contractions of the muscular pillars in the rumen wall, which mixes the digesta. These muscular pillars participate in the rumination of feed and eructation of gases produced during fermentation of feed components.

Rumination, or chewing the cud, is the process by which the animal is able to regurgitate part of the consumed feed, remasticate it, and swallow the digesta. This process aids in rupturing the cell walls of the feed and reinoculating the digesta with fresh microflora, facilitating the continuation of fermentation. Accumulation of gases produced during fermentation of feed can cause distension of the rumen; these gases are eliminated by the process of eructation or belching, a reflex event occurring once or twice a minute. The eructed gases contain methane and carbon dioxide and are of concern for the environment and for animal production, because they contribute to global warming and represent waste of feed energy. The estimated annual contribution of carbon dioxide and methane to the environment is 2741×10^6 and 82×10^6 metric tonnes, respectively, from domestic and wild ruminants.

Feed Digestion in Ruminants

The proteolysis of feed proteins results in the release of amino acids. These amino acids can either be incorporated into microbial protein or deaminated. The ammonia released is absorbed across the rumen epithelium and converted to urea in the liver, and the carbon skeletons of the amino acids are fermented. The urea produced in the liver is either excreted in urine or incorporated into saliva to be secreted into the mouth, mixed with the feed, and used for synthesis of microbial protein (Figure 5). Similarly, the ruminal microbes can utilize nonprotein nitrogen in feed for the synthesis of microbial protein. The rumen is a continuous culture system and the outflow from the rumen contains undegraded feed and products of fermentation, such as microbial biomass and water-soluble vitamins.

Fat in ruminant diets is very small in comparison to diets of nonruminants and rarely exceeds 6% of total diet DM. Feed

Table 1 Relative number and mass of microbial organisms in the rumen

Group	*Number per ml*	*Net mass (mg per 100 ml)*	*Percentage of total microbial mass*
Small bacteria	1×10^{10}	1600	60–90
Selenomonads	1×10^{8}	300	
Oscillospira flagellates	1×10^{6}	25	
Ciliated protozoa			10–40
Entodinia	3×10^{5}	300	
Dasytricha+Diplodinia	3×10^{4}	300	
Isotricha+Epidinia	1×10^{4}	1100	
Fungi	1×10^{4}	–	5–10

Source: Adapted from Van Soest (1994). © Cornell University Press.

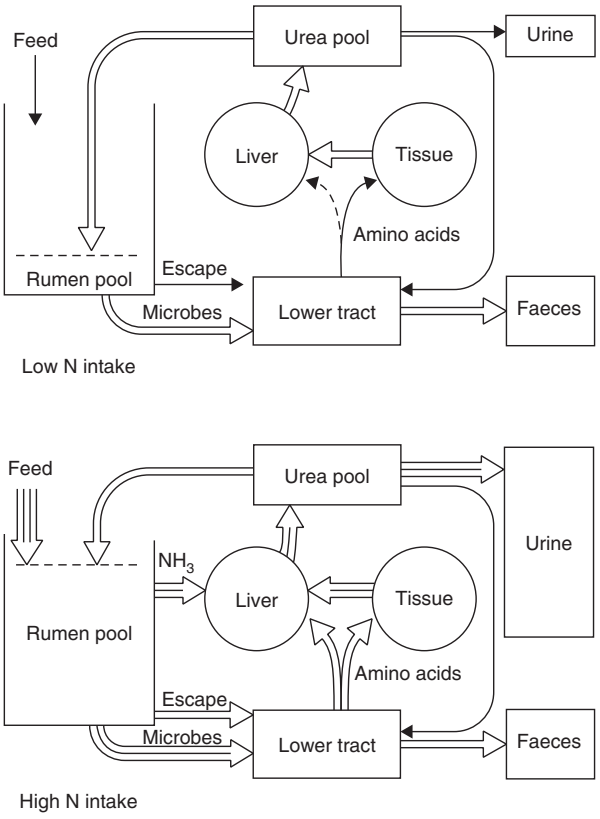

Figure 5 Nitrogen utilization in ruminants at low and high nitrogen intake. Reproduced from Van Soest (1994). © Cornell University Press.

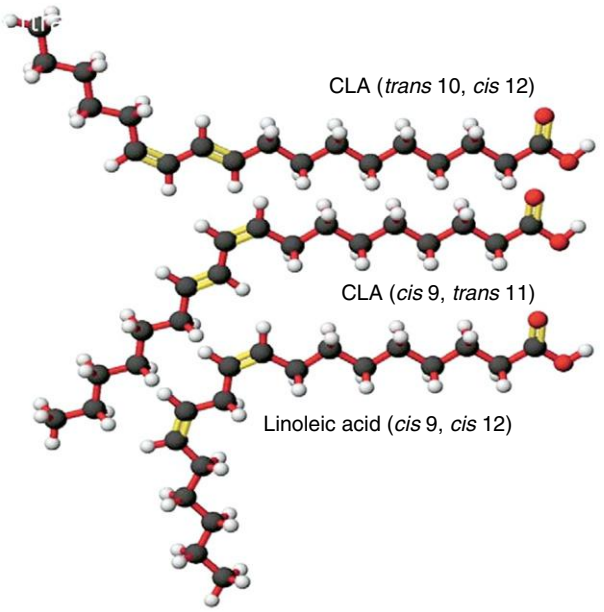

Figure 6 Naturally occurring and conjugated linoleic acids (CLA). Fatty acid at the bottom is the natural linoleic acid; the fatty acid in the middle is a CLA (no methylene group between double bonds and *trans* bond between carbons 11 and 12); the fatty acid at the top is also a CLA (*trans* bond between carbons 10 and 11). Reproduced from the Journal of Chemical Education 73 (12): A302–A303, 1996. © Division of Chemical Education Inc.

fats, which are galactolipids, are dismembered into their component galactose and triacylglycerols. The triacylglycerols, which are esters of fatty acids and glycerol, are hydrolyzed to their component fatty acids and glycerol. The alcohol and galactose are fermented to VFAs. The fatty acids are neutralized in the rumen by calcium salts forming insoluble particles that adhere to feed and microbes. Unsaturated oils in the diet can alter the microbial composition in the rumen by decreasing the protozoal population and the intimately associated methanogenic bacteria, altering the composition of the VFAs produced. The unsaturated fatty acids are extensively biohydrogenated to saturated fatty acids, usually to stearic acid, and in the process trap small, nonsignificant amounts of hydrogen, a byproduct of fermentation. During the process of biohydrogenation, some fatty acids can be converted into *trans* and conjugated fatty acids. When the double bonds in a fatty acid occur without being separated by a methylene group band ($-CH_2-$), conjugated fatty acids are formed (Figure 6). Conjugated linoleic acid (CLA) has demonstrated anticarcinogenic properties in many animal models. Thus, there is interest in producing products from ruminants that have elevated levels of CLA in the hope of preventing the onset of tumors in a wide section of the human population. Some of the unsaturated fatty acids in feed can also be deposited without being biohydrogenated when animals are supplied with high levels of oil in the diet as high-oil corn or flaxseed. Under these feeding conditions, the unsaturated fatty acids deposited in the meat can lead to formation of oxidative products, which can impart off-flavors to the meat.

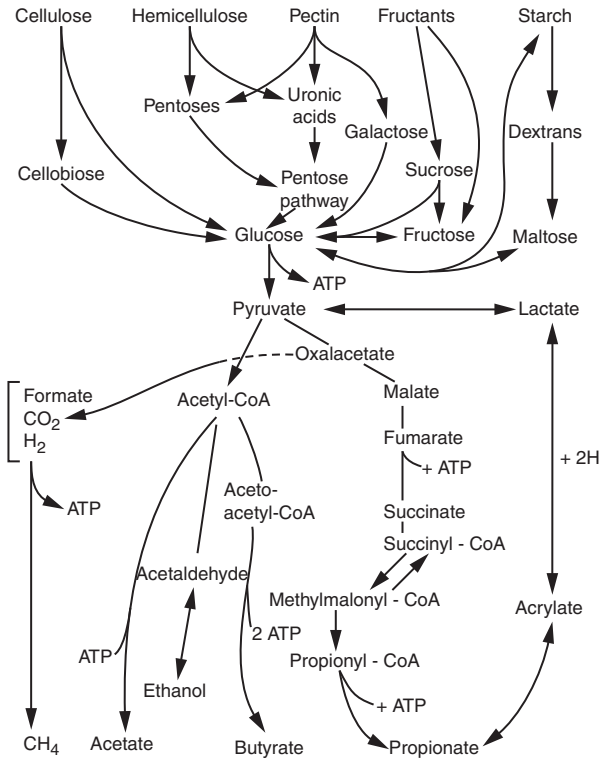

Figure 7 Pathways of carbohydrate metabolism in the rumen. Reproduced from Van Soest (1994). © Cornell University Press.

All carbohydrates in the feed and the carbon skeletons released from amino acids and lipids are fermented in the rumen to produce VFAs, namely, acetate, propionate, and butyrate, and the gases methane, hydrogen, and carbon dioxide (Figure 7). Some intermediary compounds of fermentation, such as lactate and succinate, can accumulate under certain feeding conditions. The rumen microbes produce VFAs, carbon dioxide, and methane to derive energy as adenosine triphosphate (ATP) for their life activities and growth. The fermentation of 1 mol of hexose (containing 2812 kJ of energy) completely to acetate, propionate, or butyrate will provide the animal 1758, 3072, or 2193 kJ, respectively. The amount of ATP generated and the yield of microbial biomass per mole of ATP are related to the outflow rate from the rumen (Table 2). When mixed feeds are fermented to varying proportions of fermentation products, the amount of bacterial protein nitrogen formed is approximately 27 or 36 g kg^{-1} of feed that is truly or apparently digested. The ATP available to the microbes for growth varies with the proportion of each of the VFAs produced. The proportions of each of the main VFAs and their production rate vary with the diet and the relative resistance of the feed components to fermentation.

Carbohydrates are the main repository of photosynthetic energy in plants, and in feeds constitute 50–80% of the DM of forages and cereals. Feeds contain cellular and cell wall carbohydrates. The cellular carbohydrates are generally storage carbohydrates, such as starches and compounds in intermediary metabolism as soluble disaccharides (two hexose units), hexoses, and pentoses and are very rapidly fermented in the rumen to VFAs. The starches are glucose units linked by α1–4 and α1–6 glycosidic bonds into straight and branching chains, respectively. These α1–4 and α1–6 glycosidic bonds of starch and the β1–4 glycosidic bond of lactose can be hydrolyzed by mammalian amylases and disaccharidases if these carbohydrates are not fermented in the rumen by the microbes. The amylases elaborated by the mouth and the pancreas hydrolyze starch and the disaccharidases in the brush border membrane in the small intestine hydrolyze the disaccharides to their component hexoses in preparation for absorption.

Table 2 Effect of dilution rate on fermentation products in a continuous fermenter with mixed rumen population and using glucose as an energy source

Product	*Dilution (turnover) rate (h)*		
	0.02	*0.06*	*0.12*
Acetate[a]	1.18	1.11	1.13
Propionate[a]	0.16	0.22	0.26
Butyrate[a]	0.23	0.18	0.15
Methane[a]	1.67	1.34	1.04
ATP[a]	5.59	5.19	5.03
$Yield_{ATP}$[b]	7.50	11.6	16.70
$Yield_{glucose}$[c]	42.40	60.20	83.90
Nitrogen in cells (%)	9.90	–	12.00
Microbial crude protein (g per mole glucose)	26.10	–	61.20

[a]Moles produced per mole glucose fermented.
[b]g cells per mole ATP.
[c]g cells per mole glucose.
Source: Adapted from Van Soest (1994). © Cornell University Press.

The cell wall polysaccharides are composed of pectin, cellulose, and hemicellulose and provide structural support to the plant. They are more resistant to microbial degradation than are the cellular carbohydrates. Cellulose is comprised of β1–4-linked glucose chains, which are hydrolyzable by microbial cellulases but not by mammalian enzymes. Furthermore, the cellulose chains can twist around each other to enhance the resistance to hydrolysis. Resistance to degradation can be further increased with advancing maturity of the plant by the deposition of hemicellulose, which consists of polymers of arabinose, xylose, mannose, galactose, and glucuronic acids linked by either α or β bonds. Although pure hemicellulose is soluble, the hemicellulose in plants is intermolecularly and covalently associated with cellulose and lignin (phenolic compounds). This covalent bonding increases with plant maturity and renders the hemicellulose resistant to mammalian and microbial enzymes. Thus, fermentative degradability of cell wall carbohydrates in total is decreased with advancing plant maturity.

The fermentation of feed is unavoidable in ruminants. The advantage is that ruminants can consume and derive energy from cellulosic, fibrous feeds, and utilize nonprotein nitrogen to produce microbial biomass. Rumination is an added advantage for digestion of poor-quality roughage. Because the fermentation chamber precedes the main site of digestion, the fermentation products, which are microbial protein and water-soluble B vitamins, receive the most efficient use, unlike lower-tract fermentation in horses where the fermentation chamber is distal to the main site of digestion. Microbial proteins, being

a combination of bacterial and protozoal proteins, can be composed of a better balance of amino acids than some feed proteins and may meet the amino acid requirements of the host animal better than some feed proteins. In such situations, the synthesis of microbial protein is advantageous to the animal; but when higher-quality proteins, such as casein or soybean protein are extensively degraded, then pregastric fermentation is a disadvantage.

Extensive pregastric fermentation of dietary proteins, starches, and soluble carbohydrates is partially offset for the host animal by the opportunity to digest the fermented products, microbial protein, and B vitamins. Some loss of dietary energy must be expected because eructated gases are natural products of fermentation. Furthermore, only 50–70% of the microbial nitrogen is available protein, whereas the remainder is bound in cell wall structures and nucleic acids. Proteins, especially most soluble proteins, are extensively deaminated during fermentation and, although there is an opportunity to recapture this nitrogen as urea recycled via the saliva, the production of urea is energetically expensive to the animal and loss of urea in urine is inevitable. Thus, some valuable amino acids in high-quality proteins may be better used if they are not fermented because feed nutrients can be wasted during fermentation, reducing the efficiency of production.

The efficiency of production is largely related to the type of feed provided to the animals. Although feed-to-gain ratios in ruminants can be as low as 6.0, that cannot rival those in nonruminants such as swine (2.5). It is the ability of the ruminant to utilize fiber that places the domesticated ruminant in its unique position in the world economy. Owing to this ability, production of meat from ruminants can eliminate competition for the same foods between humans and livestock and yet provide humans in many developing nations with excellent quality protein. In developed nations, it is an opportunity to utilize the vast photosynthetic energy captured as forage because, even though ruminants can consume concentrate feeds, they have a requirement for fibrous feeds for maintenance of the functionality of the rumen.

Classes of Meat-Producing Ruminants

All animals can be harvested as meat, but there is a need to maintain a part of the herds to produce progeny that can be used for meat production. In developed countries, specific breeds of cattle, sheep, and goats have been developed for the purpose of producing high-quality beef, lamb, and goat meat and this is the focus here. In developing countries, livestock agriculture tends to be less intensive and the quality of product is not an important consideration. Animals have multiple uses and the ability to harvest meat is an additional opportunity. The discussion relating to nutritional requirements of different classes of meat-producing ruminants pertains to practices employed in developed countries.

Sheep and Goat Production

Sheep and goats are similar and many aspects are discussed elsewhere. Goats are raised extensively, for meat, milk, and fiber production, in nearly every country of the world. Although breeds differ greatly in productivity (milk, meat, and fiber production) and in their ability to adapt to terrain, climate, feed availability, and feed quality, the small size of goats relative to that of cattle often makes goats more cost-effective and adaptable for protein production than cattle, especially where high-quality feed resources are limited. Another unique aspect of goats is that, relative to cattle, they are highly selective browsers, and seek out leaves of woody shrubs and trees as a primary feed source. This makes goats ideal for areas where woody plants are abundant relative to grasses. In addition, goats can be used as a complementary species in conjunction with sheep, cattle, or horses, which primarily choose to graze grasses. When grazing pressure is managed properly, especially using a multispecies, complementary grazing program, goats can effectively control the spread of invasive woody plants in pasture or range environments.

Early age at puberty and frequent twinning make rapid establishment of a goat herd possible. Because of a five-month gestation period and early maturity, offspring can be ready for market one year after breeding. Goat meat is preferred to beef by members of many ethnic and religious backgrounds, and US consumption of goat meat has increased greater than 10-fold since 1989.

Productivity of goats, such as that of cattle, is highly dependent on availability of nutrients. Supplementation of poor-quality forage (mature grasses or cereal straws) with higher-quality feeds containing greater percentages of protein and energy (legumes, some tree leaves, and oilseed meals) will increase digestibility and consumption of the poor-quality forage and improve productivity. After the protein needs of both the animal and the rumen microbes have been met, supplementation of the diet with additional energy, in the form of cereal grains, grain byproducts, citrus pulp, fruits, or tubers, can increase productivity.

Management and control of internal parasites is the primary health concern in goat production, and the parasite species of primary interest differs widely depending on geographical region. Internal parasites primarily reside on pasture but complete their life cycle within the goat. Larvae are consumed by grazing goats; larvae mature into adults within the host; adult nematodes lay eggs in the digestive tract (either the abomasum or the small intestine) of the host; the eggs are passed in the feces and hatch into infective larvae on pasture. High animal density in confined pasture areas contribute to increased parasite exposure and increased parasite burdens in infected animals. Heavy parasite burden, particularly when animals are infected with *Haemonchus contortus*, causes anemia in goats, which can be diagnosed by observing differential color of mucous membranes around the eye, the gums, and inside the vulva. Edema or swelling below the jaw is also an indication of a heavy parasite load. A FAMACHA eye color chart, using a 5-point color scale applied to the inside of the lower eyelid, can be used to estimate the level of parasitism (**Table 3**). The level of redness is inversely proportional to the level of anemia and, hence, the level of parasitism (**Figures 8** and **9**).

Although internal parasites can be controlled using anthelmintics, cograzing of goats in conjunction with cattle permits each host species to safely consume the larvae of parasites specific to the other species, resulting in fewer

infective larvae consumed by the target host species. In addition, grazing forages high in condensed tannins, such as sericea lespedeza, birdsfoot trefoil, sainfoin, lotus, and sulla, as well as leaves from some shrubs, trees, and cassava forage, may reduce parasite infection. The condensed tannins suppress egg-hatching and larvae viability of nematodes. Overgrazing pastures contributes to the consumption of infective larvae as animals graze closer to the ground. To reduce level and prevalence of internal parasitism, grazing should be discontinued as forage height is reduced below 10 cm.

Table 3 Association between color of inside of lower eyelid and level of anemia

FAMACHA Scale	*Color*	*Anemia level*
1	Red	Nonanemic
2	Reddish-pink	Nonanemic
3	Pink	Mild anemia
4	Pinkish-white	Anemic
5	White	Severe anemia

Source: Reproduced from Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Veterinary Research 33, 509–529.

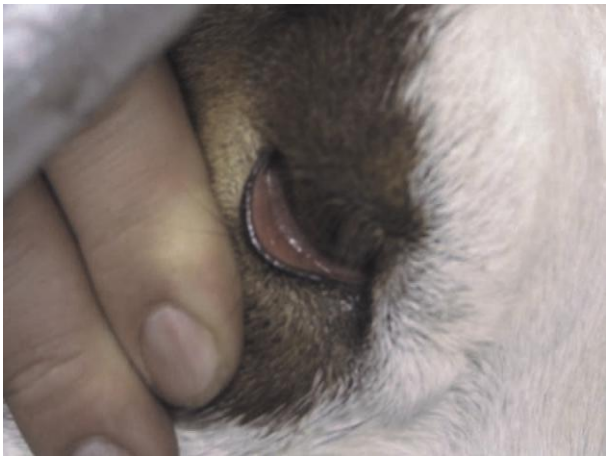

Figure 8 Normal, healthy, color of membrane tissue of lower eyelid. Photo courtesy of Dr. B. Faris.

Scrapie is a transmissible spongiform encephalopathy (TSE), which affects the central nervous system in goats and sheep, and is characterized by extensive scraping of the hide and, ultimately, death. Although rarely found in goats, scrapie is nearly 100% fatal and most often transmitted vertically from doe to kid early after birth, primarily through contact with placenta and birthing fluids. Quarantine and slaughter of infected animals is essential to eradication of scrapie from the herd.

Great caution must be taken whenever importing goats developed in regions with disparate climatic normalcy. Imported goats may have little or no resistance to local parasites and diseases. In addition, whereas goat breeds indigenous to equatorial and tropical regions are tolerant to high temperature and humidity, goats developed for high productivity under temperate climatic conditions will likely suffer greatly and have poor productivity and reproductive performance under tropical or heat-stress conditions.

Male goats are subject to development of crystals, termed urinary calculi, which obstruct the flow of urine from the bladder. Urinary calculi are rare in grazing goats and are primarily a risk in goats fed a grain-based diet. The risk can be dramatically reduced by (1) maintaining a 2:1 ratio of calcium-to-phosphorus and (2) including an ingredient, such as ammonium chloride or ammonium sulfate at 0.6% of the diet on a DM basis, to acidify the urine. Additional preventative measures include providing continuous, free-choice, access to salt and fresh, clean water. Intact male goats grow faster and have leaner carcasses compared to male castrates. However, with adequate nutrition, both male and female kids can reach reproductive maturity at 6 months of age. So to prevent early, unplanned, breeding, bucks should either be separated from does after weaning, or should be castrated if they cannot be separated after 4 months of age. There is anecdotal evidence

Figure 9 (left) Normal, healthy color of membranes of upper and lower gums and (right) anemic color of membranes of upper and lower gums. Photo courtesy of Dr. B. Faris.

that castration before 60 days of age may further exacerbate the risk of urinary calculi, so it is not recommended that goats intended for confined feeding be castrated before 60 days of age.

Beef Production

Beef production is on the rise worldwide and world annual per capita beef consumption is expected to rise from 5.6 kg in 1994 to 9.7 kg of retail beef by the year 2019. There is immense disparity in per capita beef and veal consumption among the nations, ranging from 1.5 kg in India to 70.2 kg in Argentina, with the United States being third at 45.3 kg in 2010. Trade in beef is expected to expand in the world as consumption in population-dense countries such as China increases, pressuring beef producers to improve efficiency. A part of this efficiency is achieved through improved nutritional management of beef cattle.

Traditionally in North America, calves are born in spring (February to May) and dam and calf are turned out into pasture, where they grow over the summer months. Bull calves other than those designated to be kept for reproduction are castrated. In the event that natural insemination is practised, the bulls are also put on pasture. Cows not with calf at the end of summer are culled, often fattened and processed. In late September or October, the calves are expected to be less dependent on the dam's milk for nourishment because they should have a fully functioning rumen and can be weaned without significant consequences. Calves weighing approximately 250 kg are separated into heifers and steers, and grazed or backgrounded in feedlots on growing diets until they are approximately 350 kg. At this weight, the cattle are adjusted to diets containing ever-increasing levels of concentrate (cereal grain) to produce animals weighing 525–600 kg and ready for processing by the age of 15–20 months.

Although extensive focus is placed on cattle in feedlots, there is substantial literature on balancing forage production in pastures to effectively manage maintenance of pasture for future years and to obtain the best feed for the cattle in the current year. The carrying capacity of pasture represents the number of animal units (dam and calf, a steer or heifer) that can be supported on a pasture for a month and is affected by many factors (Table 4).

The majority of the cattle to be harvested are placed in feedlots for fattening before processing. To achieve efficient growth performance, cattle in most North American feedlots are implanted with pelleted androgenic or estrogenic steroidal hormones in the ear, or heifers are fed melengestrol acetate (MGA; specifically for heifers to suppress ovarian follicle stimulation), and provided with beta adrenergic agonists, ionophores, and antibiotics. Beta agonists approved for use in feedlot cattle include ractopamine hydrochloride and zilpaterol hydrochloride. Beta agonists increase the amount of lean muscle deposited on a beef carcass by upregulating gene transcription resulting in increased protein production and reduced protein degradation in the muscle cell. The ionophores licensed for use in growing and fattening cattle are monensin, lasalocid, laidomycin propionate, and salinomycin. The ionophores control the microbial fermentation in the rumen and shift the VFAs produced in the rumen toward more (49–76%) of the higher energy-yielding propionate, with corresponding reductions in acetate or butyrate for significant diets. This shift in the rumen metabolism translates to a 5–8% increase in feed conversion efficiency due to decreased feed intake (4–10%) with small increases in weight gain. The implants appear to increase weight gain in animals by influencing the use of energy and amino acids absorbed from the alimentary tract toward protein accretion. Antibiotics such as tylosin are provided to fattening cattle when they are fed in excess of 80% grain on DM basis. Under such conditions, the fermentation in the rumen is brisk, with high rates of VFA production, and the ruminal pH is reduced (<5). This situation leads to erosion of the rumen papillae and can aid the passage of pathogenic bacteria, which can lodge in the liver and cause abscesses. Provision of low doses of tylosin in fattening cattle diets can decrease or eliminate the abscesses. The use of implants and nontherapeutic, feed-grade antibiotics is banned in the European Union because it is suspected that there is transfer of the steroidal compounds in meat to humans and because of development of antibiotic-resistant pathogens.

Because the beef industry is of immense economic importance, the romance of cattle ranching has given way to practical considerations of meeting cattle needs. At slow rates of gain, an animal takes longer to arrive at target weight, so that more nutrients and energy are expended in maintaining an animal than if rates of gain are high. To achieve the required efficiency, the nutritional requirements of cattle are continuously being assessed and published periodically by the National Research Council of the Agriculture Research Service, United States Department of Agriculture. Similar publications are available in the United Kingdom, the European Union, and Australia that outline the requirements of beef cattle for their particular circumstances. These publications include discussion of the factors (Table 5) that affect nutritional requirements of beef cows and bulls through various stages of life and those of heifers and steers maintained exclusively for the production of beef.

The rate of production in feedlot-fed cattle is related largely to the frame size and the amount of feed they can consume. Cattle have a requirement for fiber and cannot be provided complete concentrated diets without negative consequences, which can limit the amount of feed they can consume. This limitation is especially observed when young weaned calves are brought into the feedlot. In these calves, the rumen capacity is limited and they cannot consume high levels of concentrate because the microbes in the rumen are not adapted to high levels of grain. It is preferable not to feed high levels of grain quickly to young calves, as this will make them gain fat rapidly and animals will be ready for slaughter at lower final

Table 4 Factors affecting carrying capacity of a pasture

- Cultivated or native – forage production
- Fertilization
- The species mix and percentage of each forage
- Climate – temperature, precipitation, and hours of daylight
- Type of terrain
- Distance to water source
- Type of animal unit – cow and calf or steer

Table 5 Factors affecting energy and nutrient requirements

- Intended use of the animal
- Sex of animal
- Age of animal
- Frame size of animal
- Surface area of the animal (area affected by the weather)
- Physiological state of animal (young, pregnant, lactating, etc.)
- Previous nutritional state (animals with poor nutritional status gain at faster rates when adequate nutrients are fed as they are compensating for previous status)
- Expected rate of production, and whether animals are being grazed or feedlot-fed (for grazed cattle, the terrain they have to traverse to obtain food will affect their requirement)
- Type of diet provided – high-forage or high-grain
- Relative digestibility of the feed
- Intake potential of the animal
- Anabolic implants or growth promotants
- Ambient temperatures (animals placed outside the zone of thermoneutrality on either side will be affected)

weights with lower yields of edible meat. Therefore, lower-energy diets with adequate protein are provided for the animals to grow in frame size until they weigh approximately 350–400 kg. Once this is achieved, animals must be adapted to higher-grain diets in 5% grain increments per week until they receive 85–95% grain on DM basis to capture the residual growth and deposit the appropriate amount of external and internal fat in readiness for processing. Byproducts from the grain processing industries (for production of grain-based ethanol and extraction of starch for food-grade, corn-based sweeteners) are often substituted for 5–60% of the grain portion of the finishing diet. The ingredients most commonly used as supplements in cattle growing or fattening diets include distillers' grains (either wet or dried, mostly from corn, but also from sorghum, wheat, and barley), corn gluten feed, and wet or dried citrus pulp. Although these byproduct ingredients are high in neutral detergent fiber, the fiber is nonstructural in function and is highly fermentable. Although these byproducts are often relatively high in crude protein, their abundance in cattle-producing areas, their energy content, and their competitive price result in their inclusion as an energy source rather than strictly as a protein supplement. The overall digestibility of such high-grain diets does not exceed 76% and, if economical, lower-grain diets can be used successfully.

Beef cattle can benefit from higher-quality protein, such as soybean protein, that is protected from degradation in the rumen when they are younger and there is an opportunity for the animal to absorb the amino acids to support the growth acceleration that is possible in these animals. But at the 400 kg weight, the rumen is large enough that the microbial protein produced will meet the reduced demand adequately. The concern is to meet the requirements of the rumen microflora, which is approximately 12% crude protein. Although rumen microflora can propagate with a nonprotein source of nitrogen such as urea, their growth rates are improved when true protein is provided in the diet.

The production of sheep and goats is less extensive in North America, than in the United Kingdom, Australia, and New Zealand. However, sheep and goat production is less intensive than beef production and is based on grazing cultivated or native pasture. As in the case of beef production, the specific nutritional requirements of sheep and goats have been studied and publications detailing their requirements are available.

Veal Production

Veal is usually produced from culled dairy bull calves and is a minor part of the industry. Traditionally, veal is pale, lean beef from very young animals. After the calves have received colostrum, they are separated from the cow and maintained on a liquid diet of whole milk or milk replacer until they are approximately 154 kg or 3 months of age. These calves are susceptible to digestive disorders and therefore have to be limit-fed cautiously until they are approximately 4-weeks old, after which they can be provided whole milk or milk replacer ad libitum. At ad libitum intake, calves can consume 1–1.6 kg of DM per day or 1.5% of body weight without any detrimental effects. Feed conversion efficiencies ranging between 1.3 and 1.5 kg of milk DM per kilogram gain have been observed. Such high feed efficiencies are possible because whole milk or equivalent quality milk replacer is fed to the calves, because the calves are maintained on fluid feed and the development of the rumen is discouraged, and because the provision of fluid diets up to slaughter maintains the esophageal groove functionality and, as a result, fluid milk passes directly from the reticulum to the omasum. Since the components of the diet are not subjected to fermentation in the rumen, the calves reap the benefit of the high quality of the milk proteins and other components at a time when they have a high growth potential.

Veal is produced with high-quality feed that could otherwise be used for human consumption. Although milk proteins can be substituted by vegetable proteins, such as soy protein in milk replacers, calves can develop allergic reactions to the proteins, which thus require extensive processing to render them safe for use in milk replacers. The incorporation of adequate amounts of vitamins and minerals into milk replacers is essential to produce superior-quality veal. Often, insufficient iron is provided in the diet in order to maintain the pale color; however, this can affect the calves' growth rate and the elimination of supplementary iron is a practice that is not recommended by many national animal welfare councils.

General Aspects of Feeding Ruminants

Cattle, sheep, and goats need supplemental fat-soluble vitamins A, D, E, and K, which pass to the lower alimentary tract for absorption. Ruminants also require various elements, and especially cobalt to facilitate the synthesis of B_{12} for the host animal. The requirements of all the mineral elements for each class of cattle along with the signs of deficiency and toxicity for each mineral element are also detailed in numerous livestock management publications.

The capture of energy in the feed can vary substantially, depending on the extent of processing applied to feed. Hay is

Table 6 Equation for water requirement of cattle

Water intake (liters per day) = −019.76+[0.4202×Maximum temperature °F]+[0.1329×DM intake (kg/d)]−[6.5966×precipitation (cm per day)] −[1.1739×dietary salt (% DM)]

Source: Equation from National Research Council, 1996. Nutrient Requirements of Beef Cattle, seventh revised ed. Washington, DC: National Academy Press.

better used than silage, but if climate at a location is such that hay or pasture is not available year round, then feed must be harvested and stored for later use. Forage can be harvested for hay, if weather permits, or as silage. Cereal crops can be grown and harvested for grain or forage, such as barley or corn silage. Many grains need to be processed to crack the exterior hull to facilitate attachment and degradation by ruminal microbes. However, overprocessing of the grain can lead to very high fermentation rates that cause overproduction of VFA, leading to acidity in the rumen and in the blood. If fermentation rates are very high, the rate of gas production is enhanced to such extents that the animal is unable to eructate the gas and bloat will follow. Bloat can lead to pressure increases and distension of the rumen, which can block the air passage, causing asphyxiation without warning. Similarly, bloat can occur when ruminants are fed hay from or are pastured on certain immature succulent legumes such as clovers or lucerne (alfalfa). Therefore, if animals are grazing legume pastures they must be watched. Pastures must contain an adequate percentage of grasses to reduce the occurrence of bloat-related hazards; alternatively, 'bloat guard' additive can be provided to the animals.

Above all, animals require water, and the requirement relates to the ambient temperature, to exercise, to animal size, to feeds provided to the animals, and the amount consumed. The requirement for water is less if the pasture is immature, as in early spring, but as the pasture matures the DM content increases and so does the need for water. Similarly, water need increases with the grain content of the diet. The water requirement for feedlot cattle can be calculated (Table 6) and can double between 4 and 32 °C for any class of animal. Just as the DM intake influences water requirement, the water availability affects DM intake. To achieve profitable production efficiencies, it is necessary to pay close attention to provision of adequate clean water along with other nutrients.

See also: Growth of Meat Animals: Metabolic Modifiers; Physiology. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists; Disease Control and Specific Pathogen Free Pig Production; Red Meat Animals. Nutrition of Meat Animals: Pigs; Poultry. Species of Meat Animals: Cattle; Meat Animals, Origin and Domestication; Sheep and Goats

Further Reading

Agriculture and Food Research Council (AFRC), 1993. Energy and Protein Requirements of Ruminants. Wallingford, Oxon: CAB International. Compiled by Alderman G and Cottrill BR. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients.

Bowen, R.A., 1997. Digestive Anatomy in Ruminants. Fort Collins: Colorado State University. Available at: http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/rumen_anat.html (accessed December 2013).

Church, D.C. (Ed.), 1988. The Ruminant Animal: Digestive Physiology and Nutrition. Englewood Cliffs, NJ: Prentice Hall.

FASonline, 2002. Per Capita Beef and Veal Consumption. Washington, DC: USDA Foreign Agricultural Service. http://www.fas.usda.gov/dlp2/circular/1997/97-03/beefpcap.htm (accessed December 2013).

Gipson, T.A., Merkel R.C., Williams K., Sahlu T., 2005. Meat Goat Production Handbook. USDA/FSIS/OPHS project #FSIS-C-10-2005. Langston: Langston University.

National Research Council, 1996. Nutrient Requirements of Beef Cattle, seventh revised ed. Washington, DC: National Academy Press.

Van Soest, P.J., 1994a. Microbes of the Gut, Nutritional Ecology of the Ruminant, second ed. Ithaca, NY: Comstock Publishing Associates, A Division of Cornell University Press, pp. 253–280.

Van Soest, P.J., 1994b. Nitrogen Metabolism, Nutritional Ecology of the Ruminant, second ed. Ithaca, NY: Comstock Publishing Associates, A Division of Cornell University Press, pp. 290–311.

Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., Nelson, G.J., 1999. Advances in Conjugated Linoleic Acid Research. Champaign, IL: AOCS Press. vol. 1.

Relevant Websites

http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/rumen_anat.html
Colorado State University.

http://www.fas.usda.gov/dlp2/circular/1997/97-03/beefpcap.htm
USDA Foreign Agricultural Service.

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ON-LINE MEASUREMENT OF MEAT COMPOSITION

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Glossary

At-line Refers to a measurement device that is installed beside a processing line, but requires a product to be either partially diverted or sampled in order to take a measurement due to the measurement device requiring more time to take a measurement than the process throughput permits.

Chemical lean 1 minus the fat content, as measured by chemical analysis, commonly expressed as a percentage (e.g., 80CL = 80% lean by chemical composition).

Hyperspectral imaging Hyperspectral imaging is the technique by which spectral cameras collect information as a set of 'images.' Each image represents a range of the electromagnetic spectrum and is also known as a spectral band. These 'images' are then combined and form a three-dimensional data set for processing and analysis.

In-line Refers to a measurement device that is installed as part of a processing line, operating at the same throughput and measuring product in a continuous manner without the need for product sampling, diversion or intervention.

Spiral CT CT scanner whereby the object being imaged moves linearly through the CT aperture as the object is scanned, creating a spiral scanning path through the object.

Introduction

Meat in its fundamental form is generally modeled by way of a three-compartmental model comprising fat, water, and protein. Of these three, fat content is generally the most commonly measured attribute. For example, boneless beef (termed manufacturing meat) is commonly traded on the basis of chemical lean (CL). CL is equivalent to 1 minus the fat content and is commonly expressed as a percentage (e.g., manufacturing meat with 20% fat content is referred to as 80CL). A number of techniques exist for measuring the various qualities and compositional attributes of meat. This article outlines some of the near- and on-line techniques for measuring the compositional nature of meat within a meat-processing environment. Consideration of the methods to evaluate a technique against reference methods is also discussed.

Performance of In-Line Methods

Official reference methods for determining meat composition in terms of fat, water, and protein (herein referred to as base tests) are the accepted benchmark against which in-line or near-line measurement technologies are assessed. The performance of the base test is critical because any uncertainty in the base test will contribute to, and fundamentally limit, the performance of the new technology.

The performance of a base test is usually expressed as repeatability, reproducibility, and bias. Repeatability is the standard deviation of repeated measures by the same laboratory, reproducibility is the standard deviation of repeated measures by different laboratories, and bias is the consistently high or low values produced by a method relative to known values. These figures are expressed from a perspective of the performance of the base test to the known or actual value of the sample. However, when comparing different on-line methods for measurement, it is important to express the performance of the measurement technique in terms of its ongoing predictive ability to produce a measure relative to the accepted value for the sample.

From a production perspective, the two most important indicators of performance of any near-line or on-line measurement technique is its *accuracy* and its *repeatability*. These terms are often confused and rarely explained. Manufacturers

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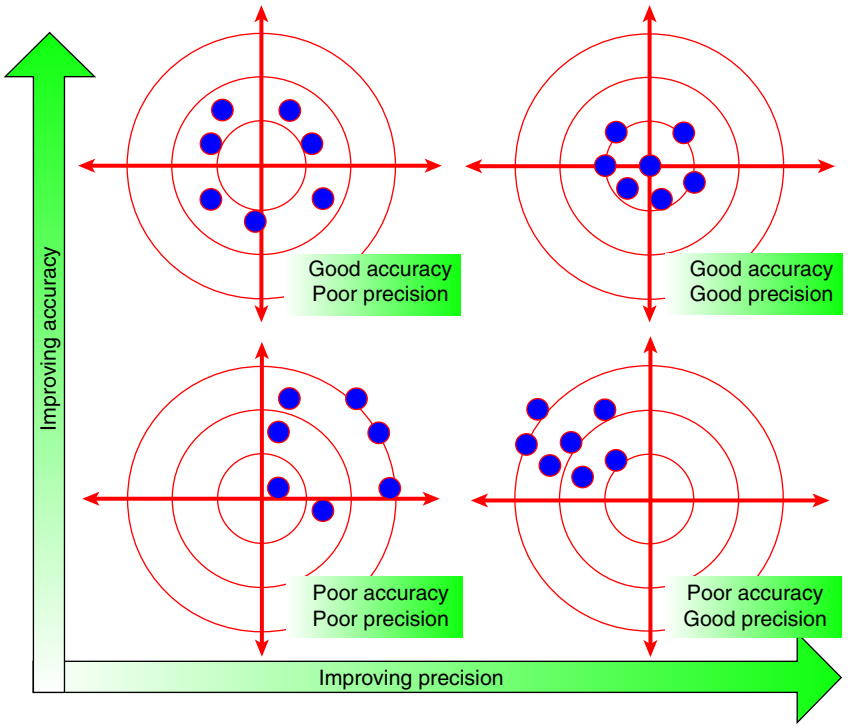

Figure 1 Example of accuracy and precision.

often quote performance figures that present a measurement device in the most favorable light. This often leads to disappointment with the actual performance.

Accuracy refers to how close an average measurement is to the accepted value of the sample. People discussing the accuracy of a measurement device should state which base test has been used to quantify the accuracy of that device. Otherwise, the concept has little meaning.

An easy way to understand the concept of device accuracy is to think of its measurements as being shots at a target, as in Figure 1. The center of the bull's-eye represents the best estimate of the actual value as determined by the base test.

Good accuracy is achieved when the average of many measurements (i.e., the center of the grouping of shots) lies close to the center of the bull's-eye, as in the top two targets.

Precision refers to the *repeatability of many measurements on the same sample*. Accuracy includes the effects of precision, because variations in individual measurements due to poor precision will generally reduce the individual *accuracy* of each measurement compared to the base test, even though the average of many test results may be close to the bull's-eye.

A measurement device with high precision will produce virtually identical measurements of the same sample (right two targets in Figure 1). However, if that device has poor accuracy, all these measurements may be 'off target' (bottom right target in Figure 1).

A measurement device can have excellent precision but poor accuracy and vice versa. The ideal measurement device will have both good accuracy and good precision, as in the top right target in Figure 1.

The figure for accuracy will always be greater than the figure for precision and is the performance figure that is most often quoted when comparing techniques and technologies. Where possible, performance figures of the measurement techniques discussed here are accuracy figures, expressed as the standard deviation of the error in prediction of the measurement technique as compared to the actual value as determined by way of the accepted base test.

Precision and accuracy are reported using a variety of units. Although Pearson's correlation coefficient (R^2) is often cited as a measure of accuracy, it indicates only the strength of the correlation between two variables after performing some form of fitting. Similarly, the root mean squared error (RMSE) indicates the degree of closeness of a fit. More useful measures of accuracy include metrics such as the standard error of prediction (SEP) or standard error of estimate (SEE), generated using data from independent validation trials.

Optical Systems

Optical Sorters

Humans, with their inherent ability to discriminate on the basis of color, shape, and size, are able to discriminate quite accurately between similar cuts of meat on the basis of (visible) fat content. Because humans primarily use their sense of sight for inspection tasks, it is not surprising that significant effort has gone into utilizing technology that mimics this sense to automate inspection tasks. There are numerous companies that manufacture and market optical-based natural product inspection systems for a variety of applications. Key Technology Inc. (Walla Walla, WA, USA, www.keyww.com) is one of the leading manufacturers and suppliers of product inspection,

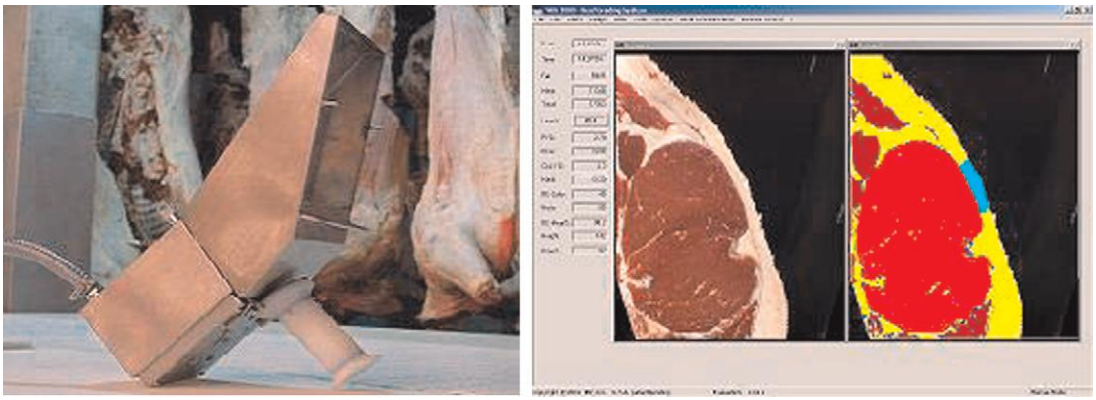

Figure 2 e+v's VBG beef camera and output screen. Reproduced with permission from e+v (http://www.eplusv.com/start_E.htm).

sorting, and control systems, for a wide range of food products, including meat and meat products. Their Prism@2 sorter, for example, uses controlled illumination and a variety of camera configurations to detect and discriminate between acceptable and unacceptable (including contaminants) product at high throughput rates. The use of monochrome, color, and visible/infrared (Vis/IR) cameras further enables vision technology to be optimized for a wide range of natural product inspection applications. Complex product transportation and conveyor mechanisms enable full 360 degree inspection of the surface of the product.

This technology has been proposed for the measurement of CL in diced (or finely portioned) meat products by using color cameras to discriminate between meat and fat, then using this difference to estimate the CL of a diced meat stream-based on the relative amounts of visible meat and fat. Although a correlation between the visible level of fat and the overall CL of a meat product stream does exist, this correlation depends on, and is proportional to, the individual portion shape and size within the product stream and the author is not aware of any published trials or operational systems currently in operation. Although potentially suited to continuous flow of diced meat product, they are not particularly well suited to inspection of discrete portions such as meat boxes, because the visible fat-to-lean ratio will be highly dependent on species, cut, orientation, shape, and even seasonal variations.

Video Image Analysis

Video Image Analysis (VIA) systems use electronic camera technology, along with computer-based digital image analysis techniques, to extract key features from one or more images of carcasses and derive estimates of various carcass attributes including carcass yield and composition. Because VIA only images the visible surface of the carcass, a variety of approaches are used to infer internal attributes such as fat depth and muscle size and dimension. Color is commonly used to infer fat depth from carcass images, whereas multiple VIA images taken from different viewpoints (including views of the inner surface in situations where the carcass is split along the backbone before imaging) are used to assist in estimating meat yield and distribution. Some systems also use a series of lines

Figure 3 e+v's VSS 2000 lamb VIA system. Reproduced with permission from e+v (http://www.eplusv.com/start_E.htm).

of light to extract additional three-dimensional information about the outer shape of the carcass. In applications where the carcass or side is cut at right angles to the spinal column (e.g., beef sides quartered in the chiller), VIA can be used to obtain direct measures of fat cover, eye muscle size, and dimension to infer composition and yield (Figure 2).

VIA systems such as ViaScan (Cedar Creek Company, Brisbane, Australia) and VSS 2000 (e+v Technology GbH, Oranienburg, Germany) are commercially deployed in Europe, North America, and Australasia, primarily for beef and lamb carcass assessment as well as for pork and poultry (Figure 3). However, because they only measure surface topography, they are not as accurate as measurement technologies that are able to perform tomographical measurements of internal structure.

Ultrasound

Ultrasonic scanners use ultrasound to determine the boundaries between meat and fat using ultrasound signals that are reflected off these boundaries. These are noninvasive but require the ultrasound transducers to be in contact with the surface of the carcass or live animal to ensure reliable ultrasonic coupling. Ultrasound is primarily used as a technique for inferring fat

Figure 4 Carometec AutoFOM. Reproduced with permission from Carometec (http://www.carometec.com).

depth and (occasionally) key muscle dimensions as a means of estimating carcass composition and yield. Although the majority of these are hand-held probes, Carometec (Carometec A/S, Herlev, Denmark) sell an automated ultrasound carcass assessment system that estimates total lean meat percentage, predicting the yield to an RMSE of 2% (Figure 4).

Because these scanners require direct contact with the surface to prevent air pockets blocking the signal, they are largely deployed in pork assessment where the skin is left intact on the carcass. With beef and lamb, the pelt-removal process results in air gaps within the surface fat due to bubbles or tears, resulting in very poor ultrasonic signal transmission. As a result, ultrasonic scanners have proved commercially unreliable when applied to assessing beef and lamb carcasses post hide or pelt removal.

Near Infrared Spectroscopy

Other chapters within this publication cover near infrared spectroscopy (NIR) as a noninvasive technology capable of inferring a variety of meat-related attributes on carcasses, cuts, and end products (Listed in See also section). NIR refers to the range within the electromagnetic spectrum with wavelengths from approximately 760–2500 nm, slightly longer than visible light (approximately 400–760 nm). In combination with midinfrared (approximately 2500–30 000 nm), NIR is often referred to as vibrational spectroscopy, because the spectra measured is the result of intermolecule vibrations due primarily to the covalent bonds within molecules. The sample is illuminated with a broad-band visible/NIR illuminant (usually extending down into the visible range) and the resulting (reflected/transmitted/transflected) NIR light detected and separated into its constituent frequency components over the NIR range of the spectrum.

The discriminative power of NIR lies in the very high signal-to-noise ratio (SNR; typically many orders of magnitude, with some of the newer systems claiming 10^6 or greater), which enables subtle differences between signals, invisible to the naked eye, to be reliably used to discriminate between samples. This precision of NIR instrumentation means these subtle changes can be measured even in the presence of gross spectral changes unrelated to the target parameter(s). Any spectral changes caused by one difference in sample composition may be independently extracted, enabling NIR to measure an enormous range of tests from a scan of a single sample.

Generally, NIR requires relatively little sample preparation, is nondestructive and very fast, compared to the traditional base tests. The test may take only a matter of seconds per sample. Equipment may be deployed much closer to the sample itself (field, conveyor, etc.) than would be possible for the normal complex, laboratory-only equipment used to perform the base test, which generally requires more complex sample preparation. It is possible to measure a wide range of chemical elements and physical characteristics of a material simultaneously. NIR has been implemented on-line to measure composition in homogenous product streams (e.g., ground or boneless meat streams) but for whole cuts or carcasses it still requires either a sample to be taken or the instrument applied to the product at a predetermined location, in order to perform a measure. Therefore, it is still currently considered an 'at-line' rather than 'on-line' technology.

Fundamental to NIR is the calibration of the instrument. The calibration must be prepared in advance using statistical methods to match subtle features of the NIR spectra to the effect of the various components of the samples. The accuracy of this calibration is, therefore, limited by the accuracy and precision of the base test used to calibrate.

There are over 10 000 published papers on NIR and a high proportion of these are directly associated with agriculture. NIR has been used to predict protein in fresh beef and chicken carcasses and chicken breast with standard error of performance (SEP) of 0.809, 2.03, and 2.04, respectively. Good predictions of fat, moisture, protein, collagen, starch, collagen-free protein, iron, and iodine have also been reported, and NIR is an accepted and established rapid technique for the basic chemical composition of red meat and pork, with calibrations delivering $R^2 > 0.95$ and prediction errors of less than 2% by mass, respectively.

A recent trend has been the use of NIR to determine the fatty acid content and composition in raw meat as a technique for inferring quality parameters to complement compositional analysis. Although published results indicate that NIR is generally less accurate for predicting different attributes of meat quality, it nonetheless is promising when categorizing meat into quality classes on the basis of quality traits. The ability to develop calibrations for quality as well as compositional attributes from a single spectral measure and instrument offers significant potential to develop on-line solutions, limited only by the reliability of the method of calibration.

The majority of NIR instruments are designed as laboratory instruments and are not particularly suited to on-line implementation, due to issues around sample size, presentation, scan time, sample preparation, and the delicate nature of the fundamental mechanisms. However, on-line instrumentation and applications are becoming more prevalent. NDC Infrared Engineering (Irwindale, CA, USA) currently market the *MM55plus*, an NIR food sensor for a variety of on-line food analysis applications. This filter-based reflectance NIR device has been installed at the outlet of a meat grinder and is used to collect measurements continuously. Industrial scale meat batches of beef and pork, with chemical compositions of 7–26% fat,

58–75% water, and 15–21% protein, were measured in an industrial environment. Prediction errors (SEP) for these sample sets were in the ranges of 0.8–1.5% for fat, 0.9–1.5% for water, and 0.4–0.7% for protein (dependent on sample set and species), respectively. This system has been implemented for regular use in a Norwegian meat manufacturing plant.

FOSS and Wolfking (Slagelse, Denmark) have developed a continuous fat analyzer (CFA) to control fat content in ground meat products on-line in real time. The CFA samples meat directly from the blender every three seconds, measures the sample for fat, moisture, and protein, and returns the sample to the blender. It uses NIR and neural-network technology to standardize lean-to-fat ratios, controlling the in-feed conveyors so as to maintain the desired fat content. Wolfking claim accuracies of 0.3%.

Modern diode-array NIR instrumentation is becoming more prevalent in off-the-shelf commercial equipment, with diode-array instruments from manufacturers such as FOSS (Hillerod, Denmark) and Perten (Huddinge, Sweden). Although generally not as accurate as more established fixed grating and scanning monochromator instrumentation, their robust nature means they are more suited to on-line industrial applications where sampling is a major challenge. Installed over a continuous meat product streams moving at linear rates in the order of 1 ms^{-1}, SEPs of 2.15–2.28% have been reported, with predictions for fat content of standard 27 kg blocks of 0.7–1.05%. This work clearly shows the potential of this technology to on-line large-scale measurement using NIR that more modern and robust diode-array NIR technology offers.

A recent development is the application of hyperspectral instrumentation to the measurement of meat composition and quality by NIR. Hyperspectral NIR instruments produce an individual spectrum for each picture element within an image, thereby adding an additional dimension of spatial resolution to the data acquired. Using hyperspectral NIR imaging with a spatial resolution of 0.38 mm per pixel, good calibrations for saturated and unsaturated fatty acids have been reported for intact raw beef cuts ($R^2 = 0.87$ and 0.89, SEP of 1.7% and 3.4%, respectively) of Wagyu beef.

Total Body Electromagnetic Conductivity

Total body electromagnetic conductivity (TOBEC) utilizes the fact that lean meat conducts electricity better than fat. This technique and technology is more than 30 years old. The EMME Company (EMME standing for electronic meat measuring equipment) originally patented a 'method and apparatus for measuring fat content in animal tissue either in vivo or in slaughtered and prepared form' in 1973 (US patent number US3735247).

TOBEC uses a varying electromagnetic field, generated by applying a radio frequency signal to a solenoidal coil, through which the animal or meat product passes longitudinally. Because of difference of electroconductivity and dielectric properties between various body components, the load observed by the source that drives the solenoidal coil takes on a different value from that of the empty sample zone. By utilizing other predetermined parameters of the sample, the load difference may be utilized to infer the fat-to-lean ratio to a commercially acceptable standard. Because water content is highly correlated to lean content of meat, either attribute can be inferred.

TOBEC technology has been developed for both meat processing and medical applications. Numerous publications document the investigations of TOBEC for meat-related applications. These include the evaluation of carcass composition in pig and lamb carcasses, the prediction of commercial yield and lean in beef hindquarters and pig carcasses, the assessment of live lamb chemical composition, and the measurement of compositional differences in hams, loins, and bellies in pigs.

A number of TOBEC machines, specifically targeting the measurement of CL in boxed manufacturing meat, have been commercialized. Much of the published market assessment of these devices has been undertaken by the Meat Research Laboratory of the CSIRO (Cannon Hill, Australia), reflecting Australia's position as the world's largest exporter of manufacturing meat (predominantly beef). One of the first systems developed was the EMME Model M60. In an industrial trial in 1977, the device demonstrated an accuracy of ±7.68% against the manufacturer's claimed accuracy of ±2% of the value determined chemically 'with appropriate temperature control' due to the technology being very sensitive to the operating environment.

In 1991, the EMME-M60 was compared with a second generation TOBEC machine, the MQ-25 (Meat Quality Incorporated (formerly Agmed Incorporated), Springfield, IL, USA) was assessed for measuring cold-boned beef. The MQ-25 operated at throughput rates in excess of 16 cartons per minute and demonstrated SEE for single cartons ranging from 1.34 to 1.91 dependent on meat piece size. A further evaluation in 1994 of a more recent model (MQ-27) for monitoring lean meat content of hot-boned beef concluded that results (SEE of 1.71) were comparable with results previously obtained for cold-boned beef. Chemical evaluation of CL was implemented by core sampling frozen cartons, taking two 20 g subsamples from each core and testing for fat content using the microwave drying procedure described by CSIRO. The MQ-27 is marketed on the basis of an achievable accuracy (SEE) per carton of between 1.5% and 2%.

An important tennet of TOBEC is the direct relationship between the temperature of the meat product and its electroconductivity. The vast majority of published prediction equations include product temperature as an independent variable. In the 1991 trial, the temperature of the meat near the geometric center and at four other points was measured using a digital probe thermometer for each carton. The arithmetic mean of these values was used to correct the mean electrical conductivity measurement for the temperature of the meat according to a formula provided by the manufacturer of the equipment.

The hot-boning trial in 1994 found that temperature did not play a significant role in the performance of the machine. Note, however, that this trial was on hot-boned meat, which would have been a temperature somewhere between 10 and 20 degrees warmer than the equivalent cold-boning operation. Ongoing anecdotal evidence from numerous installations within Australasian meat plants (predominantly cold-boning plants) indicates that the temperatures both of the product

and the operating environment are critical to maintaining accurate and consistent operation of TOBEC equipment.

Contaminant Detection

The fundamental principle of TOBEC has also been utilized to detect metallic contaminants. There is a wealth of manufacturers providing this technology, with an example in place in the majority of meat-processing plants around the world.

Radioactive Isotopes

In the early 1990s, a radioactive isotope-based approach was developed for measuring the percentage, by weight, of fat and boneless meat. Using the radioactive isotope Californium-252, the Institute of Geological and Nuclear Sciences Ltd. (Wellington, New Zealand), in conjunction with the Meat Industry Research Institute of New Zealand Inc. (MIRINZ, Hamilton, New Zealand) developed a system called NEUGAT that used the relative transmission of neutrons and gamma rays emitted by the isotope to measure meat composition.

The system demonstrated an accuracy of 1.1% CL in validation trials over trial periods of at least 1 h duration at throughput rates of 15 ton h^{-1}. However, due to the advent of electronic X-ray generation technology, this technology was not commercialized because of the technical complexity and safety issues regarding permanent radiation isotopes.

X-Ray Systems

Single-Energy X-Ray

Single-energy X-ray systems for measuring meat and meat product composition have been available for many years. These systems operate on the principle of the differential X-ray absorption between lean and fat due to their elemental composition. Devices such as the Anyl-Ray (Packaging Technologies, KartidgPak, Davenport, ID, USA) shine a collimated beam of broad energy X-rays onto the product and detect the amount of X-rays that penetrate completely through to the other side. The use of a conveyor enables two-dimensional images of meat products to be created using a single row of X-ray detectors aligned at right angles to the direction of travel. The intensity of the signal from each detector is proportional to both the density and the thickness of the product between the detector and the X-ray source.

The Anyl-ray is not an on-line device. It requires a specific weight sample to be taken from the product stream, minced to create a degree of homogeneity, then packed into the sample presentation tray and analyzed. Standard deviations have been reported to be between 0.6% and 0.8%. In spite of quite high acceptance in the industry, anecdotal evidence from operational sites often indicate much higher error levels, highlighting potential issues regarding sampling, sample preparation, calibration development, and the effects of operator experience, or lack thereof.

More recently, Robotics Technology Limited (RTL), a joint venture between Scott Automation Limited (Dunedin, New Zealand) and Silver Fern Farms Limited (Dunedin, New Zealand), have developed a proprietary single-energy X-ray scanning system. The first of its kind in the world, this system takes two separate single-energy X-ray scans of each carcass as it enters the boning room and determines cut planes for primal cutting to maximize the saleable yield of each carcass. Although the system is commercially available in conjunction with RTL's primal cutting technology, there is no published data on its ability to estimate carcass composition or yield. The RTL X-ray system is also used as a stand-alone grading system to predict primal weight proportions.

X-Ray based Contaminant Detection

A similar principle is used in systems developed for identifying contaminants in food products. Examples include the Eagle range of X-ray based food product inspection systems (Eagle Product Inspection, Tampa, FL, USA) that can detect and reject foreign body contaminants such as metals, glass, stone, bone, PVC, teflon, and stainless steel in pumped and packaged meat product streams.

Applied Sorting Technologies, Pty. Ltd. (Melbourne, Australia) have developed the XR-2000 and XR-3000 series of food inspection machines. Production machines have now been supplied to 20 leading Australian food producers, chiefly meat works in Queensland and NSW inspecting carton meat automatically at the rate of approximately 40 cartons per minute. Cartons with detected contaminants such as lead shot and other embedded foreign objects are automatically rejected. These machines have been tested by Meat and Livestock Australia to successfully detect and reject 27 kg meat cartons with 2 mm diameter lead shot in the meat at high production rates.

X-ray systems have also been developed to detect and reject bone in poultry, fish, and red meats. Companies such as Marel (Marel, Gardabaer, Iceland) and Meyn (Meyn Food Processing Technology B.V., Oostzaan, The Netherlands) produce systems for bone detection with stated accuracies of up to 98% detection rate with a 3% false detection rate.

Dual-Energy X-Ray

Single-energy X-ray systems have proven effective for at-line meat composition measurement but the inherent dependence on product thickness has limited their adaption to on-line measurement applications. Advances in dual-energy X-ray (DXA) systems, both in the security and medical areas, have recently been investigated for meat measurement applications.

In DXA systems, two sets of X-ray detectors are used, each with a sensitivity distribution centered around a different X-ray energy (approximately 50–70 keV and 100–120 keV). This effectively produces two images of the product – one for the higher-energy X-rays and another for the low-energy X-rays. Each image is effectively a density map of the product scanned at the X-ray energy level determined by the X-ray response of the detector. Because the relative absorption of X-rays by different materials (having fundamentally different elemental composition) varies with X-ray energy, the effect of thickness can be eliminated as a dependent variable. However, these two X-ray measures are still highly correlated with each other (as

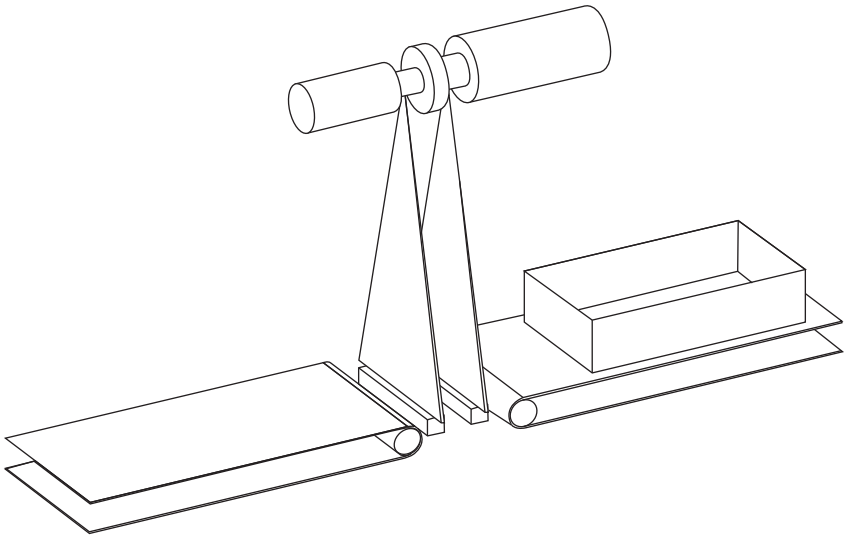

Figure 5 Setup of the FOSS MeatMaster™ instrument, illustrating the two X-ray sources and corresponding detector arrays. Reproduced from Hansen, P.W., Tholl, I., Christensen, C., *et al.*, 2003. Batch accuracy of on-line fat determination. Meat Science 64 (2), 141–147, with permission of Elsevier Science Publishers.

with spectral data points within NIR spectra). The advent of complex data analysis techniques, such as those used in the area of NIR spectrum analysis, has enabled DXA systems to provide rapid and accurate assessment of meat and meat product composition.

The Anyl-Ray2 (Packaging Technologies, KartidgPak, Davenport, ID, USA) utilizes DXA technology. Using two levels of X-rays has reduced the necessary sample size and the sensitivity to packing density, air pockets, precise sample weight, and surface (thickness) uniformity. However, the fundamental operational mode of the device has not changed from its predecessor and it is still an at-line instrument.

FOSS (MeatMaster™, FOSS Electric A/S, Denmark) and EAGLE (Eagle-FA, EAGLE Product Inspection, Tampa, FL, USA) produce DXA-based systems for measuring the fat content of fresh, frozen, boxed, and loose meat on-line. The MeatMaster™ system incorporates two X-ray generators whereas the Eagle-FA uses a single broad-spectrum X-ray generator with high- and low- X-ray detector arrays superimposed over each other (Figure 5).

The Foss MeatMaster™ can measure meat in cartons or boxes as well as continuous product flow of meat loose on the conveyor at rates of up to 38 ton h^{-1}, and comes in standard and compact machine footprints (Figure 3). The MeatMaster™ is capable of repeatabilities (one standard deviation) of $\leq$0.5% for fat and weight and accuracies (RMSEP, one standard deviation) of between 1% and 1.5% absolute for fat and 1% and 2% relative for weight, dependent on product format, over a fat range of 2–85% (Figure 6).

Mettler Toledo acquired the Smiths Detection X ray-based food inspection business in 2011 and rebranded it as EAGLE Product Inspection. The EAGLE-FA System can measure CL to ±1% accuracy at-line speeds of up to 30 cartons per minute or 108 ton h^{-1} (Figure 7).

The DXA technology also enables metal contaminant detection down to diameters of 4 mm and bone detection down to 10 mm to be performed simultaneously.

Figure 6 FOSS MeatMaster II. Courtesy of FOSS Analytical, Denmark (http://www.foss.dk).

More recently, DXA has also become readily accepted as a technique for determining carcass composition in sheep and pigs and carcasses. A wide range of commercial medical full body DXA scanners have been used to measure carcass attributes such as total, lean, and fat tissue mass with very high correlations in sheep and pigs ($r2 \gg 0.9$). However, it is important to note that DXA estimates do inherently differ from chemically determined values, due to fundamental difference in the measurement systems. In particular, DXA

Figure 7 Eagle-FA720. Reproduced with permission from Eagle Product Inspection (http://www.eaglepi.com).

estimates of bone mass do not correlate as well with chemical ash, because DXA measures bone mineral content. This has been addressed by researchers by applying corrections to the algorithms built in to medical DXA scanners. Further, meat and fat content are estimated in regions where bone is not present and extrapolated to produce full carcass assessment.

In 2012, five new X-ray systems have been built by Robotics Technology Limited for installation in existing lamb plants to undertake lamb yield analysis, as part of FarmIQ (a 7-year partnership between the New Zealand Primary Growth Partnership Funding program and New Zealand's largest meat exporter, Silver Fern Farms Ltd.). These systems are likely to incorporate DXA technology to further enhance their ability to measure carcass composition as part of estimating carcass yield and meat/fat/bone ratios.

Computed Tomography

The use of CT as a virtual dissection tool and its correlation to manual dissection has been well documented in the literature and has been shown to be more precise and reliable than manual dissection. Although the scanning aperture of commercial CT scanners is increasing, largely driven by the need to cater for ever-increasing human body sizes, cost and technological complexity mean that it has yet to reach a size where on-line carcass scanning is commercially practicable. When compared with the European Union's EUROP carcass classification system, CT analysis of rib cuts generated a higher predictive value in estimation of carcass tissue composition.

More recently, spiral CT has been investigated as a potential technology for assessing carcass tissue volume and weights of sheep, pigs, and cattle as well as fatty acid and meat quality characteristics. With the ongoing advancement of CT technology, the Danish Meat Research Institute (now part of the Danish Technological Institute) have recently announced a development program that aims to implement a prototype CT scanner in a pork deboning line capable of measuring 700 middles per hour.

Summary

Although a number of technologies have been investigated for measuring meat composition, and in particular fat, on-line, no one technology has proved to be comprehensive in providing a complete solution. Of the technologies currently in application within the industry, DXA provides significant advantages of 100% inspection and CL measurement combined with metal contaminant detection. NIR offers significant advantages in accuracy and in more specific compositional measures such as protein, water, collagen, and iron, but current off-the-shelf technology still requires sampling and must be thus considered an at-line technology at best. Recent developments in fast, wide-area scanning, diode-array NIR technology and spiral CT may well offer opportunities in the future for on-line inspection of continuous product with a high level of accuracy.

See also: Automation in the Meat Industry: Cutting and Boning. Environmental Contaminants. Growth of Meat Animals: Muscle. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. On-Line Measurement of Meat Quality. Prediction of Meat Attributes From Intact Muscle Using Near-Infrared Spectroscopy

Further Reading

Bill, B.A., Eustace, I.J., Gibbons, R.A., Jones, P.N., 1983. Development of a microwave oven method for the estimation of water and fat in meat and water in meat products. Meat Research Record, *CSIRO*.

Frisullo, P., Marino, R., *et al*., 2010. Assessment of intramuscular fat level and distribution in beef muscles using X-ray microcomputed tomography. Meat Science 85 (2), 250–255.

Hansen, P.W., Tholl, I., Christensen, C., *et al*., 2003. Batch accuracy of on-line fat determination. Meat Science 64 (2), 141–147.

Hollo, G., *et al*., 2007. Application of X-ray computer tomography (CT) in cattle production. Asian-Australasian Journal of Animal Sciences 20 (12), 1901–1908.

Hunter, T.E., Suster, D., *et al*., 2011. Dual energy X-ray absorptiometry (DXA) can be used to predict live animal and whole carcass composition of sheep. Small Ruminant Research 100 (2/3), 143–152.

Kobayashi, K., Matsui, Y., *et al*., 2010. Near infrared spectroscopy and hyperspectral imaging for prediction and visualisation of fat and fatty acid content in intact raw beef cuts. Journal of Near Infrared Spectroscopy 18 (5), 301–315.

Lin, R.-S., Forrest, J.C., Judge, M.D., Lemenager, R.P., Grant, A.L., 2001. Electromagnetic scanning of beef hindquarters for estimating beef carcass composition. Journal of the Chinese Society of Animal Science 30 (2), 115–127.

Madsen, N.T., 2011. Deboning Solutions. *Proc. iMAC, A 2011, International Meat Automation Conference*, Australia, Feb 9–11.

Mlcek, J., Sustova, K., *et al*., 2006. Application of FT NIR spectroscopy in the determination of basic chemical composition of pork and beef. Czech Journal of Animal Science 51 (8), 361–368.

O'Farrell, M., Wold, J.P., *et al*., 2010. On-line fat content classification of inhomogeneous pork trimmings using multispectral near infrared interactance imaging. Journal of Near Infrared Spectroscopy 18 (2), 135–143.
Purchas, R.W., Archibald, R., West, J.G., Bartle, C.M., 2007. An evaluation of the Eagle™ FA DEXA (dual-energy X-ray absorptiometry) scanner as a method of estimating the chemical lean in cartons of boneless beef. Food New Zealand 7 (1), 24–29.
Ribeiro, F.R.B., Tedeschi, L.O., *et al*., 2011. Evaluating the application of dual X-ray energy absorptiometry to assess dissectible and chemical fat and muscle from the 9th-to-11th rib section of beef cattle. Professional Animal Scientist 27 (5), 472–476.
Weeranantanaphan, J., Downey, G., *et al*., 2011. A review of near infrared spectroscopy in muscle food analysis: 2005–2010. Journal of Near Infrared Spectroscopy 19 (2), 61–104.

Relevant Websites

http://www.carometec.com
Carometec A/S.
http://www.cedarcc.com/product-solutions/viascan.aspx
Cedar Creek Company.
http://eplusv.com
e+v Technology GmbH.
http://www.eaglepi.com/
Eagle Product Inspection.
http://www.foss.dk/industry-solution/meat
FOSS.

ON-LINE MEASUREMENT OF MEAT QUALITY

C Borggaard, Danish Meat Research Institute, Maglegaardsvej, Roskilde, Denmark

Glossary

At-line A site for obtaining measurements from samples removed from the processing line.
Boar taint An unpleasant odor that can be experienced during cooking of pork from some (not all) uncastrated pigs.
Dark, firm, and dry (DFD) A condition in meat with an elevated ultimate pH (from preslaughter stress resulting in low glycogen).
Electrical impedance The measure of opposition that a circuit presents to a current when a voltage is applied; it extends the concept of resistance to AC circuits.
Electronic nose A device intended to detect odor or flavor (electronic tongue) using sensor arrays and pattern recognition systems.
Hyperspectral imaging Hyperspectral imaging uses cameras with carbonate compensation depth or complementary metal-oxide semiconductor area sensors to acquire images at a number of specific wavelengths (usually between 350 and 1000 nm). These systems can give information on both structure and chemical composition.
In-line A site for obtaining measurements on the processing line.
Marbling Marbled meat contains various amounts of intramuscular fat, giving it an appearance similar to a marble pattern.
Microwave A form of electromagnetic radiation (frequencies between 300 MHz (0.3 GHz) and 300 GHz).
Optical measurements A collective term, used here, covering spectroscopic techniques involving the wavelength regions from the ultraviolet through the visible and the near-infrared parts of the electromagnetic spectrum using one or more wavelengths.
pH A measure of acidity or basicity of an aqueous solution.
Pale, soft, and exudative (PSE) A condition in meat, especially pork.
Tenderness A sensory panel evaluation (a subjective measure) of one of the sensory properties of meat in contrast to a shear value (an objective measure).

Introduction

Meat quality assessment is highly subjective and what is considered 'good quality' is dependent on the consumer and may vary according to culture. There are, however, a number of important traits that industry and consumers can agree on as being important indicators of meat quality.

These include:

- tenderness and juiciness,
- appearance (color and structure),
- fat content, including subcutaneous fat (if it has not been trimmed) and intramuscular fat,
- drip loss/weep/cooking loss caused by pale, soft, and exudative (PSE) or red, soft, and exudative meat,
- fat quality (problems with soft fat related to the degree of saturation of the fatty acids and oxidative stability of fat),
- protein content (composition can be measured online by various instruments), and
- off-odors (e.g., rancidity due to fat oxidation or boar taint).

For a long time, there has been a desire to be able to measure some, if not all, of these traits very early in the slaughter process and preferably at-line speed. If such assessments were feasible, sorting into quality classes would be possible and the individual carcasses could be designated to products in an optimal fashion.

This article focuses on instrumental measurements of meat quality that can be made in-line or at-line at industrial speeds in an abattoir or in a meat processing plant and will be limited to instrumental methods that in most cases are nondestructive, although not necessarily noninvasive. An at-line measurement is characterized by the sample being physically removed from the process line before measurement. The removal can be made either automatically or manually. At-line measurements are often only used as spot wise tests and results are usually not available in real time. In contrast, in-line measurements are made by instruments that are placed on the line or that have been built into the process line. In general, this enables measurements to be taken in real time, thus obtaining data on the entire production flow.

In the following, the quality measurement methods are grouped according to measurement principle. It should be noted that, because of the inhomogeneous nature of meat and the speed of operation required, many useful laboratory methods for determining meat quality have proved extremely difficult to implement as in-line applications.

Optical Measurements

Optical measurements, including fiber optic probes, are used for measuring the reflectance either on the surface or inside an intact muscle. Either the measurements can be made at a single wavelength or an entire wavelength region can be scanned. In this context, the term optical will be limited to refer to measurements that can be made within the wavelength regions 200–1700 nm of the electromagnetic spectrum. These regions cover the long-wavelength ultraviolet (UV) (200–400 nm), the visible (VIS) (400–700 nm), and the short-wavelength (700–1700 nm) near infrared (NIR). The UV part of the spectrum can typically be used to make various contaminants or certain chemical constituents fluoresce. This property can be used as an aid for highlighting organic contaminants on the surface of entire carcasses (e.g., the VerifEYE Solo Inspection System for finding fecal remains on beef carcasses) or for nonquantitatively detecting the presence of lipid peroxidation in fat samples.

The short-wavelength part of the infrared spectrum is used extensively for analytical purposes in the field of NIR spectroscopy. With this technique, it is possible to quantify the macroconstituents (e.g., protein, fat, and water) of food products. The short-wavelength infrared range is also utilized in thermographic or infrared cameras. In the slaughter industry, these cameras are being used as a research tool for specialists working with animal welfare. The heat-sensitive cameras make it possible to measure slightly elevated body temperatures of the pigs that are in lairage at the slaughter house, thereby quantifying possible stress conditions among the animals.

The visible part of the electromagnetic spectrum can be used for measuring color and surface texture, which are especially valuable for assessing meat quality. Measurements on raw meat with visible light and short-wavelength infrared can be made in both reflection and transmission modes. However, due to the strong light-scattering abilities of meat, it is not possible to make transmission measurements with path lengths exceeding a few millimeters except in the wavelength region from 850 to 1000 nm, where transmission is measureable even through 2–3 cm of meat.

If spectra are to be acquired from inside a muscle, it is necessary to use an insertion probe-type arrangement. With probes, it is possible to acquire images of the meat only in one-dimension along the line of insertion. When using probe-type instruments that are based on a single wavelength, it is also difficult to distinguish meat that is PSE or dark, firm, and dry (DFD) from meat that is either pale or dark due to low or high pigment content. If structural properties of the meat are to be separated from differences in chemistry, more than one wavelength will be required. Most VIS–NIR spectroscopic techniques are applied as reflectance measurements of a cut surface. The advantage is that a large surface area can be viewed. The disadvantage is that the reflected image will be strongly influenced by specular reflection, which does not contain chemical information about the object being observed.

Measuring Pale, Soft, and Exudative

The first probe-type instrument for measuring meat quality to emerge was the TBL Mark II fiber optic probe, made by MacDougall in the late 1970s. Although it only used a single wavelength, it was designed to measure the paleness of meat, and because PSE meat is notoriously pale, muscles with this defect could be detected using the instrument. The instrument was handheld and self-contained with a numeric display for the operator. The light source was a light-emitting diode (LED) operating at a single wavelength in the visible region. A needle-shaped steel tube containing optical fibers brought light from the LED to a point inside a muscle. Another optical fiber guided reflected light back to a detector. The registered intensity of the reflected light could then be related to the paleness of the meat at the point of measurement. With this type of instrument where the reflectance of the meat is only measured at a single wavelength, one must be aware that high brightness can be due to several effects: the presence of scattering centers (as in PSE meat) and by low pigment concentration. In dark-cutting beef, the meat is very translucent, that is, the light from the transmitting fiber is not reflected back to the receiving fiber. This gives the meat a dark appearance. However, a dark appearance may also be due to a high myoglobin concentration (deoxymyoglobin), which absorbs light in the wavelength region 520–590 nm before it can be registered at the receiving fiber. Arrangements of in- and outgoing fibers as in the TBL Mark II are commonly used in present day visual and NIR spectroscopic applications.

A major problem with the probe measuring at a single point is that that the sampling area inside the muscle is very small. As previously mentioned, an insertion probe can at most acquire data only in one dimension. This is a serious problem in nonhomogeneous media, such as meat.

The Canadian Colormet handheld probe used a xenon flash as light source and had a photodiode array as detector. For the first time, this permitted full spectra (400–700 nm) to be obtained. It was tested successfully for color measurements on veal and pork as well as for PSE occurrence in pork. Even though the Colormet and TBL Mark II had excellent potential as a tool for sorting, they gained only sporadic acceptance by the industry.

Measuring Pale, Soft, and Exudative and Marbling in Pork

The next logical step after the development of the TBL Mark II was to select an LED light source that operated in the NIR region and to select a wavelength in the NIR region that was reasonably independent of meat color, in order to measure marbling or lean meat percent. In the Danish optical probes, an LED with a wavelength of 950 nm is used (corresponding to a strong water absorption band). The system developed at the Danish Meat Research Institute and named the MK probe (manual classification) was originally designed for measuring lean meat percentage in pork on the killing line. It was equipped with a depth measurement device that enabled the reflection to be measured continuously along an insertion line while the probe was inserted into the muscle. From the resulting reflection profile, the thickness of subcutaneous fat could easily be measured and related to carcass composition. The choice of an NIR light source also meant that the instrument (with a modified software) was well suited for detecting PSE meat; introduction of the depth-measuring device also offered a tremendous improvement in sampling area

compared with the TBL fiber optic probe. When configured as a meat quality-measuring device, the Danish manual system was called meat quality and marbling (MQM). The hardware of this system is basically equivalent to the present day Fat-O-Meater II for carcass grading from the company Carometec Food Technology®.

In profiles from the MQM instrument, marbling is seen as small spikes (reflection peaks) on an underlying curve representing the reflection from the meat. Results obtained with the MQM instrument demonstrated that the system could be used to sort porcine carcasses in two groups with high or low intramuscular fat in the loin muscle. The main limiting factor on the achievable accuracy is once again the limited sampling volume that is measured.

A probe similar to the Danish was developed in parallel by a New Zealand company called Hennesey Grading Systems. This instrument contains all the same features as that of the MQM, the differences lying mainly in the choice of LED wavelength and software. The Hennesey probe uses a green light (550 nm) LED and is, therefore, optimized especially for distinguishing between fat and meat for grading purposes (meat is very dark in green light, whereas fat is very bright).

Near-infrared Spectroscopy for Measuring Connective Tissue, Protein, Myoglobin, and Tenderness and its Future Applications

Spectroscopic techniques have been used for the direct and indirect quantification of collagen in carcasses or cuts. Published work includes measurement of connective tissue in beef by UV fluorescence and NIR reflectance. Also, measurement of myoglobin in both pork and beef by VIS-wavelength spectroscopy and measurement of protein content in intact pork muscles by NIR spectroscopy have attracted some attention. However, apart from color-measuring systems using non-spectroscopic techniques, these procedures have never been commercialized for online use at abattoirs due to the inhomogeneity of the muscle. For ground meat where the sampled volume is representative of the entire batch, spectroscopic methods working in reflectance or transmission mode have proven to be both reliable and fast and are used routinely by many suppliers of ground meat and for measuring fat and protein content of diced meat samples on a conveyor or in pipeline systems. These applications of NIR spectroscopy are typically used in-line on mixers and grinders and as at-line measurements.

Work has been published on the application of NIR spectroscopy for measuring hydroxyproline in pork sausages under laboratory conditions. In chorizo-type sausages with hydroxyproline concentrations varying from 0.2% to 0.8%, a standard error of 0.05% and an R^2 of 0.8 were achieved. However, it is unlikely that NIR spectroscopy will be able to be used online for measuring connective tissue due to lack of homogeneity in meat, and because of the fact that NIR spectroscopy lacks specificity, the method in general will not be able to distinguish between different types of animal protein.

In the future, research and development within spectroscopic methods should be directed toward imaging spectroscopic techniques called multiwavelength imaging or hyperspectral imaging. The difference between these techniques and classical spectroscopy is that with hyperspectral imaging it is possible to acquire an image of a large area of the sample that contains multiwavelength spectral information in each pixel as opposed to the classical spectroscopy, where a spectrum is acquired at a single point or is the average over a larger area. The links in the 'See also' section gives an idea of the types of instrumentation that are available. The main advantage of these types of systems is that classical image analysis and spectroscopy can be combined, giving much more detailed information about the product being studied than either of the two techniques can give separately.

Research has been conducted on using NIR reflectance spectroscopy for the online measurement of tenderness, as defined by shear force measurements or a sensory panel. Several promising results have been reported, but the main obstacles are the lack of reproducibility of the reference methods (e.g., Warner–Bratzler shear force and sensory panel measurements) and the sampling being representative of the muscle or carcass being evaluated. Work at MIRINZ and AgResearch in New Zealand demonstrated the possibility for using NIR for measuring tenderness (shear force) with a reported R^2 in excess of 0.6. The results of the NIR method are limited by the inaccuracies of the reference method that in this case is notorious for its lack of reproducibility. However, the result will be sufficient for sorting (e.g., the tenderer one-third cuts for a select quality) but will be improved with better standardization.

Measuring Surface Meat Color

Surface measurements of color are difficult to make in a well-defined manner unless working under strict laboratory conditions. However, if conditions at the slaughter facility or processing plant can be reasonably standardized, one can choose to use the semiobjective color comparison blocks (such as the Japanese or Australian systems).

When using these color blocks, it is important that measurements are made under exactly the same lighting conditions every time, because the color blocks have spectra in the visible region that look nothing like meat spectra.

The handheld Minolta systems for color measurements constitute an objective way of measuring color of meat surfaces and have been used at line speed in the pork industry. They have gained considerably in popularity because of their small size, low cost, and ease of operation. However, a standardized measurement procedure taking the blooming process into consideration is of utmost importance for reliable results.

Finally, color vision sorting systems that rely on images acquired by ordinary color cameras and image analysis are to be mentioned. These are marketed by companies such as e+v Technology® from Germany (VPS 2000) and do a fair job in assessing the color of, for example, freshly cut surfaces of primal cuts. These systems are being used at meat processing plants, especially in southern Europe for sorting hams of high-quality products according to color uniformity.

A couple of instruments based on vision technology have been developed for assessing marbling, meat and fat color,

area of the eye muscle, and saleable meat yield on quartered beef carcasses. These include the VIAscan® Chiller Assessment System made by the Cedar Creek Company in Australia and the VBG 2000 made by e+v Technology. Both systems are widely accepted for grading purposes in larger beef-producing countries such as the US and Australia. Both instruments are handheld and are placed on the freshly cut surface of the loin muscle, where important information on carcass value can be gained.

Measuring Fat Softness in Pork

In the past years, pork farmers have seen a considerable rise in feed prices, especially high-protein feeds like soy beans. For this reason, producers have increased the content of maize in the feed used for rearing pork. The downside of this increased use of maize is that the back fat becomes more unsaturated, which is undesirable for producers of high-price products like Parma- and Serrano-type hams. The back fat is also used in the production of salami sausages, where unsaturated fat causes problems with rancidity. In the production of bacon, the sliceability of the product is degraded with increased degree of unsaturation in the fat due to the fat becoming softer.

Unsuccessful attempts have been made to measure the softness of fat using mechanical and ultrasonic devices. The propagation of ultrasound through subcutaneous fat is dependent on the fatty acid composition. The speed of sound is slightly larger for hard fat than for soft fat. However, this method is difficult to use online at the slaughter line, as the fat thickness at the point of measurement also has to be known with good accuracy.

Another approach to measuring fat softness was made at the Institute of Food Research in Bristol, UK, in the 1980s. The instrument consisted of a handheld device with two needles that, when inserted into the subcutaneous fat of the carcass, could determine the softness by measuring the force necessary to change the distance between the needles. However, this type of measurement was not reliable as the measured force also was dependent on fat thickness and the amount of connective tissue present.

Fat softness is strongly correlated to the relative amount of unsaturated fatty acids in the back fat; therefore, this trait should be measurable by using spectroscopic techniques, especially NIR spectroscopy. The reference methods for unsaturation of fatty acids to measure the iodine number for the fat sample either by titration or by the more elaborate method using gas chromatographic techniques to determine the fatty acid composition. NIR spectroscopy has been used for online determination of the softness of the back fat on pork carcasses. It should be noted that NIR cannot, with any accuracy, assess the fatty acid composition. What it actually does is predict the iodine value, which correlates well with softness.

In the mid-1990s, an online method based on the use of NIR spectroscopy was developed in a joint effort by Mitsubishi Rayon Engineering in Japan and the Danish Meat Research Institute. This handheld device was capable of sorting the warm pig carcasses 40 min after slaughter into three groups of softness judged by an expert classifier providing the 'ground truth' reference values. Reflection readings were acquired at 12 wavelengths provided by narrow band pass filters almost evenly spaced in the wavelength region 450–1215 nm. For illumination, a broadband filament lamp was used. The instrument was tested on 580 carcasses, of which 160 were labeled as unacceptably soft by the classifier. The instrument misclassified 10 of these as being firm. Four out of 124 carcasses with firm fat were misclassified by the instrument as soft. The measurements were made by placing the instrument directly on the surface of the fat. The main problems with this type of surface measurement are again the variations in fatty acid composition between the various fat layers of the subcutaneous fat, making it difficult to achieve a representative assessment for the entire carcass. The VIS–NIR measurements only penetrate 1–3 mm, and with a probe area of roughly 3 cm^2, the sampling volume was rather small.

The way to overcome the problem of variability in fatty acid composition as one moves through the layers of subcutaneous fat is to combine a multiwavelength transmission measurement in the NIR part of the spectrum (1100–2200 nm), with a depth-measuring system similar to the one used in the Fat-O-Meter system. Using two thin probes spaced in parallel by only a few millimeters, it is possible to acquire a full NIR spectrum for every 0.25 mm. Light is sent, via optical fibers, through one of the probes illuminating the meat around its tip. The other probe collects the transmitted light and returns it to a monochromator via other fibers. One example of such a system is the NitFom developed by the company Carometec Food Technology. However, a slight drawback of the NIR transmission technology is that the transmitting and receiving probes cannot be more than 1–2 mm apart due to the strong absorptions of light by water in the wavelength regions above 1400 nm. This small distance between probes can make it difficult for the operator to use, as the force needed to penetrate the carcass rind is substantial. This system has now become available in a fully automated version to be used on the slaughter line.

Electrical Measurements

pH

pH is measured routinely at many meat processing plants. Manufacturers of sausages and cooked and uncooked hams usually test the pH of the incoming raw materials. Measuring pH gives important information about color and water-holding capacity of the meat, and in beef it can give information on problems with dark, firm, and dry (DFD) meat. pH measurements are also an important parameter in the fermentation processes of meat products, where pH is measured to establish whether the fermentation is proceeding as expected.

Many abattoirs, meat processors, and meat researchers routinely use standard laboratory combination electrodes combined with portable pH meters for performing the measurements. This type of equipment works well, but extreme care must be taken due to the risk of electrode breakage and glass splinters ending up in products. In recent years, manufacturers of these combination electrodes have developed them to ensure that they are more robust and easier to insert into meat. However, for routine online use at production plants, one

must consider using electrodes that are more robust than the standard laboratory glass combination electrodes. The pH meter/electrode pH-K21 from NWK®-Technology GmbH, Germany is a good solution for industry. With its metal casing, it is as close as one can get to a truly shock-resistant and watertight system. A steel shell encases the measuring probe, which is specially developed for measuring pH values in meat, with telescopic protection reducing the risk of the tip of the electrode breaking during operation. The manufacturer claims that the electrode enables up to 600 measurements per hour in a measuring range of pH 2–14 and 0–80 °C and it can be operated on batteries for up to 10 h. Although 600 measurements per hour seem unrealistic, as it only allows 3 s for response time per measurement, the pHK21, in fact, has the fastest response time of any pH meter/electrode systems tested. Furthermore, generally meat pH measurement of carcasses involves a subsampling of carcasses, such as 20%, for quality control measures.

From the mid-1980s, several companies started to manufacture pH electrodes based on ion-selective field-effect transistor (ISFET) technology. One example of this type of instrument is made by the company DeltaTrack®. The biggest advantages of the ISFET electrodes are that there is no glass in the electrodes, making them almost unbreakable, and contrary to glass combination electrodes, they can be stored in a dry state. Two main disadvantages are that the ISFET electrodes cannot be used with standard pH meters and also that they have major problems with proteins clogging the ion-sensitive part of the transistor in the tip. Although the electrode tip can be cleaned with a toothbrush, it appears to suffer from permanent and irremovable clogging after approximately 800 measurements, in the author's experience. Thus, although the technology was initially seen as an attractive alternative to standard electrodes, it is not seen as reliable as the combination electrodes, due to the clogging of the electrodes by proteins, which results in inaccurate measurements.

Impedance

Handheld electrical impedance probes were in use as early as in the 1970s for measuring the lean meat content in porcine carcasses. Instruments of this type consisted of a probe that at the tip had a number of small electrodes in the shape of rings. Each ring was electrically insulated from the others. By measuring the electrical impedance (resistance to alternating current (AC)) between electrodes while the probe is inserted into the carcass, one is able to tell when the tip of the probe is moving from a fat layer with high impedance into muscle with low impedance. This probe system was then combined with a depth-measuring device like the one used in the Fat-O-Meater-type systems, thereby enabling simultaneous measurements of impedance and depth.

Pale, Soft, and Exudative and Water-Holding Capacity

In the early 1980s, a system called the Testron MST Impedance Tester was developed in Germany for sorting porcine carcasses according to their condition as PSE or normal. The instrument, which is handheld, consisted of two parallel blades at the tip that can be inserted into the muscle to be studied. An alternating potential difference with a frequency of 1500 Hz was applied between the two blades and the resulting current monitored. From this, the impedance could be calculated and used as a means of sorting meat according to water-holding capacity. The main reasoning is that meat with a low water-holding capacity also exhibits lower impedance toward an applied electric current, due to a loss in membrane integrity.

A problem with this instrument is that a large part of the drop in potential between the two electrode blades takes place not in the meat between the blades but in the transition between blade and meat. Thus, a large part of what the instrument is measuring is due to the buildup of a shielding layer of ions in the transition layer between the instrument and the meat. The logical way to overcome this contact problem is to use four electrodes. Two of the electrodes are used to apply an AC (approximately 0.01 A) to the meat while the resulting potential difference between the other two electrodes is measured. From the size of the potential and the phase shift between applied AC current and measured AC potential difference, the impedance of the meat can be calculated. This technique has two major advantages. First, as the measurement of the potential difference can be made without drawing any current (at least less than 1 nanoampere (10^{-9} A)), it is ensured that there is no measurable drop in potential at the meat–electrode interface. Second, use of constant current amplitude instead of constant potential amplitude ensures that the buildup of ions around the electrodes does not influence the measurement.

Scientists at Purdue University developed a so-called tetrapolar electrical impedance-measuring device based on the above principles. This instrument was configured with four electrodes placed as rings on a needle-shaped probe in much the same way as in the grading systems from the 1970s. The probe was equipped with a thermometer, so that impedance recordings could be temperature corrected. This instrument gave some very promising results in measuring PSE in pork.

In both of the above-mentioned systems, measurements were made using only a single frequency. This technique warrants further investigation as it is used by researchers to identify PSE and low water-holding capacity carcasses and shows a lot of potential.

Marbling in Beef

In the late 1970s, a method named electrical impedance tomography (EIT) was developed as a medical imaging technique. This method uses many electrodes to measure the electrical impedance in a patient for acquiring a three-dimensional image of, for example, the patient's lungs. In terms of resolution, it is vastly surpassed by more sophisticated methods, such as CT-scanners, and has, therefore, been of little use as a diagnostic tool. However, it is inexpensive and robust and thus has some potential as a means of measuring intramuscular fat in beef loin muscles.

At the Danish Meat Research Institute, EIT was tested as a handheld method to predict marbling. Twelve electrodes (thin needles) were arranged on the periphery of a circle 70 mm in diameter and an AC with constant amplitude could be applied between any two of the 12 needles and the resulting phase shift and potential difference between any other needle combinations measured. Simultaneously, it is possible to scan the

entire frequency range from 10 Hz to 150 kHz. This electrode arrangement was calibrated on fillet muscles from 100 animals with intramuscular fat ranging from 1% to 10%. Measurements were made on the hot carcass approximately 40 min postmortem. The reference method was the intramuscular fat determination by the Schmid–Bondzynski–Ratzlaff method. An independent test of this instrument showed that the intramuscular fat content in the fillet muscle could be estimated with accuracy better than 1% (standard error of estimation).

Microwaves

Microwaves are a part of the electromagnetic spectrum and are situated in the wavelength region 1–300 mm (300 GHz down to 1 GHz) between the so-called terahertz region (used at air ports in the security check) and radio waves.

For spectroscopic applications, microwaves are used in rotational spectroscopy. In this part of the electromagnetic spectrum, the photons do not have enough energy to cause vibrational transitions within molecules as do NIR or infrared lights, but the energy in microwaves is enough to make molecules rotate. The only requirement for rotation to occur is that the molecule has a dipole moment. In foods and especially meat, water is abundant and the water molecule has an extremely strong dipole moment. This strong dipole moment is the reason why water completely dominates the spectra of meat all the way from the NIR part of the spectrum over the mid-infrared to microwave region.

These strong water absorptions, which usually are a nuisance in NIR and IR spectroscopy, can be utilized in the microwave regions for measuring the water content of a product. Luckily, in the case of meat, for each animal species, there is an almost fixed ratio between protein content and water content (roughly 0.3) and the rest is fat. If the water content of a meat sample can be measured, then the fat content can be estimated. The greatest advantage of microwave spectroscopy over NIR and IR is its ability to penetrate a sample in much greater depths. The sampling volume with microwaves can thus be many liters at a time. The limitations are that the measurements are strongly affected by variations in salt content and whether the meat samples are frozen or not.

Microwaves for Measuring Fat

In-line systems for measuring fat in meat are commercially available. One system is from Thermo Scientific applies guided microwave spectroscopy. In the meat industry, this system can be placed on the outlet from a mixer or a grinder where the product is pushed through a steel compartment where a microwave spectrum is acquired. From this, the water content can be calculated on the basis of a calibration made for this type of product. Accuracies for 800 kg batches of meat are of the order of 1% fat under routine use of the instrument.

Another instrument based on microwaves, which may be used by smaller butcher shops for measuring fat, is from the Scottish company Distell and is named the Fish Fatmeter. It is handheld and was originally developed for measuring fat content in, for example, Salmon. However, calibrations have been made for ground pork, beef, and lamb. The operator presses the flat sampling head of the instrument onto the surface of the sample of ground meat and a fat percentage in the underlying product is measured. Owing to the nonhomogeneity of the ground meat, up to eight repeated measurements (subsamples) have to be made in order to get a reliable result on a 500-g sample. The main problem with this instrument is the presence of air pockets in the sample, which will strongly influence the reading. Also, the product thickness over the point of measurement must be at least 5 cm, otherwise the measurement is influenced by the container/tray. If proper care is taken during measurements to get good contact with the product, accuracies of approximately 1% can be achieved on 500-g trays of ground meat.

Dual-Energy X-Rays

In the meat industry, X-ray systems are being used extensively for detecting the presence of foreign bodies. Usually, X-ray images are acquired using only a single X-ray tube operating at a fixed electron acceleration voltage. In recent years, dual-energy X-ray systems have become available. Here, images are acquired with either two X-ray tubes operating at different voltages or with two detector systems, one sensitive to high-energy photons and the other to low-energy photons. The technology is comparable to the dual-energy X-ray analysis systems used for many years at hospitals for measuring bone density and quantifying obesity in patients. A number of companies that have been active in marketing foreign body detectors have switched to using X-rays at two energies. The advantage of using dual energies is that it makes it easier to find certain low-density foreign bodies in the products. At the same time, the systems can be used to find the fat content in boxes of meat or in bulk products on a conveyor. Some examples of these systems are the MeatMaster™, from the company Foss, and the Eagle FA. These instruments can analyze 100–150 ton h^{-1} of fat content and are able to measure through boxes of meat with thicknesses up to 20 cm. During routine operation, accuracies of 0.8–1% fat are achievable. A few of the Japanese suppliers of X-ray inspection systems will be presenting similar dual X-ray energy machines in the very near future.

Mechanical Measurements of Toughness in Beef and Lamb

Researchers have investigated the measurement of meat tenderness using a system that measures the force necessary for a blunt rod to penetrate a muscle. Such 'penetrometer' systems are made by combining a depth measurement system (such as the ones used by Fat-O-Meater-type instruments) with a force-measuring device (strain gauge) on the rod, achieving simultaneous measurements of force and distance. These two quantities can be directly related to the work necessary to push the rod through the muscle. It was postulated that the penetrometer system could be used online as a (reasonably) nondestructive version of the well-known shear force measurements (e.g., Warner–Bratzler or Meat Industry Research

(MIRINZ) tenderometer) used in the laboratory. The most prominent of these is the Tendertec developed in Australia in the early 1990s. With this instrument, correlations to meat toughness of $R^2 = 0.78$ have been reported, but this is on beef from a very wide range in age, which is hardly relevant in most countries. Newer experiments have shown that the method is unable to detect differences in tenderness in loin muscles from younger beef carcasses.

Another system has been developed by MIRINZ in New Zealand for testing lamb, which is different from the shear force instrument mentioned above. It is also called a tenderometer and consists of two concentric rings of needles, with the inner ring able to turn relative to the outer ring. When inserted into meat, the instrument measures the torque necessary to turn one of the rings a given number of degrees relative to the other.

Recently, the viscoelastic (or biomechanical) measurements of meat have been investigated for their ability to assist in online measurement of tenderness. Viscoelasticity is a property of deformable materials, like meat, that can behave both as a liquid and as an elastic material. The measurements can be performed by applying a periodic force over a relatively small surface area of the meat. The difference between this approach and the standard shear force measurement is that the deformation force can be applied at varying frequencies typically in the interval 0–10 Hz. This enables the measurement of the amount of energy that is disseminated in the meat sample during a compression/relaxation cycle as a function of frequency. For predicting sensory tenderness of beef loin muscles, viscoelastic measurements have been reported as being superior to ordinary shear force measurements. However, it remains to be seen whether or not Viscoelastic measurements have potential for being commercialized in a handheld, noninvasive, online instrument.

Chemical Analysis of Odors

Among the positive-quality characteristics of meat is the absence of any off-odor and this is normally considered a given property of unspoiled meat or meat products. Off-odors due to feed ingredients can be controlled via advice to farmers and feedstuff companies. However, the situation is somewhat more complicated when slaughtering uncastrated male pigs, older sheep or goats, or sheep grazing some types of forages/pastures.

Boar Taint

In the European Union, concerns about animal welfare have resulted in an agreement with farmers to stop surgical castration of male pigs before the year 2020. In some countries, such as Australia, castration of male pigs has not occurred for a number of years, due to increased growth rates, higher percentage of lean in the carcass, and healthier status of entire males, which all provide economic advantages. It is undisputed that meat from a small percentage of uncastrated male pigs exhibits an unpleasant odor when heated, and this so-called boar taint has until now prevented large-scale production of 'entire males' in many countries, in spite of the growth advantages. Consequently, the economic incentive to avoid castration is strong, and so, of course, is the animal welfare aspect. Australia has focused heavily on reducing boar taint in entire males for a number of years, through strategies such as immunocastration (Improvac) and specialist feeds such as inulin. Certainly, it will be increasingly important to be able to identify carcasses with boar taint in pig-producing countries around the world.

A mixture of chemical compounds probably causes boar taint, and much effort has been devoted to elucidating their individual structures. The two main candidates are skatole and androstenone. Skatole is a metabolite of the essential amino acid tryptophan, and androstenone is a metabolite of a male sex hormone. In some parts of the world, most attention has been focused on skatole, whereas experience gathered elsewhere seems to indicate that androstenone is equally important. This is perhaps because of differences in slaughter weight, breeds, feed, tradition, etc. Interestingly, skatole can occur in the fat of female pigs and strategies to reduce skatole taint include keeping pens clean. This emphasizes the importance of an online method for taint, for both entire male and female pigs.

Some years ago, Danish scientists developed a high-capacity (180 determinations per hour) automated analytical procedure based on spectrophotometric determination of skatole in back fat. Fat samples (approximately 1 g from each pig) are collected on the slaughter line and analyzed in an on-site laboratory. Turnaround from the time the sample is extracted from the carcass until the result is received is approximately 12 min, which is more than adequate for sorting tainted carcasses after the chilling tunnel under Danish conditions; however, serious logistic problems may arise at higher line speeds. This system is still in operation at a large Danish abattoir.

At the moment, there is no at-line sampling for an androstenone assay able to keep up with line speeds at a normal slaughter house (up to 600 male pigs per hour). However, a number of promising instrumental techniques are available. Potential methods should be able to measure the amount of skatole and androstenone in the headspace over a heated fat sample. Androstenone has a molecular mass approximately three times greater than skatole and it is much more lipophilic, thus its concentration in the headspace over a heated fat sample is very low. An instrumental method must, therefore, be extremely sensitive.

Sheepmeat Taints

The characteristic mutton odor, associated with the cooked meat of older sheep, can be problematic for some consumers who find the odor disagreeable. Branched chain fatty acids (BCFAs), particularly 4-methyloctanoic 4-ethyloctanoic acid and 4-methylnonanoic acids, have been implicated as the main compounds responsible for this aroma. The concentrations of these compounds increase in sheep fat as an animal ages but can also be influenced by breed and diet. Another odor associated with sheepmeat is 'pastoral' odor, and this odor becomes evident as a result of cooking the meat of pasture-fed ruminants. 3-Methylindole, also involved with 'boar'

taint in pigs, and to a lesser extent 4-methylphenol (p-cresol) are the main compounds implicated as contributors to 'pastoral' flavor. The odor is unacceptable to some consumers, although those habituated find it acceptable.

The availability of instrumentation to measure these odiferous compounds would allow segregation of unacceptable and acceptable carcasses. All of the instrumental measures in the next section have shown potential for measuring both the 'mutton' and 'pastoral' odors in sheepmeat. Furthermore, as illegal substitution of mutton (older sheep) for lamb (young sheep) occurs in some markets, such as Australia, online tests for BCFAs have potential for eliminating this from occurring.

Potential Instrumentation for Odor Compounds

Electronic noses have been proposed as a candidate for at-line sampling and delivery of samples to instrumentation and measurement. However, electronic noses all suffer from the fact that it takes up to several minutes to purge the detector before a new measurement can be made. Another possible candidate is a time-of-flight mass spectrometer. These systems are already used to monitor airborne pollutants (including toxic gasses) in the field and have a very high sensitivity. One version of these instruments known as the proton transfer reaction – time-of-flight mass spectrometer (PTR-TOF-MS) has been tested on fat samples for measuring boar taint components with encouraging results. Another possibility is rapid methods using solid phase micro extraction (SPME), and these methods also show some promise. The SPME is usually implemented as a small fiber coated with one of a range of available polymers. The idea is to place a SPME fiber in the headspace over the fat sample to be analyzed for odor components for a well-defined length of time. The polymer coating can be selected in such a way that it will preferably adsorb molecules that are polar or nonpolar or volatile or nonvolatile. The amount of material adsorbed on the fiber coating will be proportional to the concentration of the substances in the fat sample if equilibrium has been reached. The process can be made to work within minutes. When the fiber is inserted into the injection system of, for example, the mass spectrometer (or GC–MS) and heated, the adsorbed materials will be released and made available for measurement. With the PTR-TOF-MS gas analyzer, the time from insertion of the SPME fiber to a result is given can be done within 2 s. Sensitivity of such an arrangement can be as low as a few parts per trillion. The advantage of the SPME technique is that if the headspace from a heated sample was to be measured directly, fat and water vapor would be drawn into the PTR-TOF-MS, where it over time will cause problems. However, much work remains to be done if such a system was to be implemented as an automated at-line method.

See also: Boar Taint: Biological Causes and Practical Means to Alleviate It. Chemical and Physical Characteristics of Meat: Adipose Tissue; Color and Pigment; pH Measurement; Water-Holding Capacity. Foreign Bodies. Laboratory Accreditation. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Microbiological Analysis: Standard Methods. Modeling in Meat Science: Meat Quality. On-Line Measurement of Meat Composition. Prediction of Meat Attributes From Intact Muscle Using Near-Infrared Spectroscopy. Tenderness Measurement

Further Reading

Andersen, J.R., Borggaard, C., Barton-Gade, P., 1989. On-line system for measuring the intrinsic colour of pork. In: Warriss, P.D. (Ed.), Proceedings of the 35th International Congress on Meat Science and Technology, vol. 1, pp. 208–211. Copenhagen, Denmark: CABI Publishing.

Andersen, J.R., Borggaard, C., Nielsen, T., Barton-Gade, P., 1993. Early detection of meat quality characteristics: The Danish situation. Proceedings of the 39th International Congress on Meat Science and Technology, pp. 153–164. Calgary, Canada: Danish Meat Research Institute.

Andersen, J.R., Borggaard, C., Nishida, J., Rasmussen, A.J., 1999. Development of a NIR filter based instrument for on-line measurements of fat quality in pork. Proceedings of the 45th International Congress on Meat Science and Technology, pp. 372–373. Yokohama, Japan.

Forrest, J.C., Morgan, M.T., Borggaard, C., *et al.*, 2000. Development of technology for the early post mortem prediction of water holding capacity and drip loss in fresh pork. Meat Science 55, 115–122.

Gariépy, C., Jones, S.D.M., Tong, A.K.W., Rodrigue, N., 1994. Assessment of the Colormet TM fibre optic probe for the evaluation of dark cutting beef. Food Research International 27 (1), 1–6.

Geesink, G.H., Schreutelkamp, F.H., Frankhuizen, R., *et al.*, 2003. Prediction of pork quality attributes from near infrared reflectance spectra. Meat Science 65, 661–668.

George, M.H., Tatum, J.D., Dolezal, H.G., *et al.*, 1997. Comparison of USDA quality grade with Tendertec for the assessment of beef palatability. Journal of Animal Science 75, 1538–1546.

György, V., Károly, V., 2004. Solid-phase microextraction: A powerful sample preparation tool prior to mass spectrometric analysis. Journal of Mass Spectrometry 39, 233–254.

Hoving-Bolink, A.H., *et al.*, 2005. Perspective of NIRS measurements early post mortem for prediction of pork quality. Meat Science 69 (3), 417–423.

Hugo, A., Roodt, E., 2007. Significance of porcine fat quality in meat technology: A review. Food Reviews International 23 (2), 175–198.

Kress-Rogers, E., Brimelow, C.J.B., 2001. Instrumentation and Sensors for the Food Industry, second ed. Boston: CRC Press, Cambridge: Woodhead Publishing Limited.

MacDougall, D.B., 1980. Use of a fiber optic probe for segregating pale, soft, exudative and dark, firm, dry carcasses (abstract). Journal of the Science of Food and Agriculture 31, 1371–1375.

Madsen, N.T., Borggaard, C., Rasmussen, A.J., Christensen, L.B., 1999. On-line measurement of intramuscular fat/marbling in beef carcasses. 45th International Congress on Meat Science and Technology, pp. 378–379. Yokohama, Japan.

Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Science 71 (1), 100–121.

Miles, C.A., Fursey, G.A.J., Jones, R.C.D., 1985. Ultrasonic estimation of solid/liquid ratios in fats, oils and adipose tissue. Journal of the Science of Food and Agriculture 36, 215–228.

Morgan, M.T., Forrest, J.C., 1997. Color vision system and tetrapolar electrodes for assessing pork quality. Proceedings NPPC Quality Summit, Des Moines, IA.

Phillips, D.M., Loeffen, M.P.F., 1993. A new approach to an old problem, measuring meat tenderness. Meat Focus International 2, 16.

Rosenvold, K., Micklander, E., Hansen, P.W., *et al.*, 2009. Temporal, biochemical and structural factors that influence meat quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.

Swatland, H.J., 1986. Color measurements on pork and veal carcasses by fiber optic spectrophotometry. Canadian Institute of Food Science and Technology Journal 19, 170–173.

Swatland, H.J., 1995. On-Line Evaluation of Meat. Lancaster, PA: Technomic.

Utzinger, U.r.s., Richards-Kortum Rebecca, R., 2003. Fiber optic probes for biomedical optical spectroscopy. Journal of Biomedical Optics 8 (1), 121–147.

Watkins, P., Clifford, D., Rose, G., *et al.*, 2010. Sheep category can be classified using machine learning techniques applied to fatty acid profiles derivatised as trimethylsilyl esters. Animal Production Science 50, 782–791.

Watkins, P.J., Kearney, G.A., Rose, G., *et al*., 2012. Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat. Meat Science. Available at: http://dx.doi.org/10.1016/j.meatsci (accessed 11.08.12).
Watkins, P.J., Rose, G., Warner, R.D., Dunshea, F.R., Pethick, D.W., 2012. A comparison of solid-phase microextraction (SPME) with simultaneous distillation-extraction (SDE) for the analysis of volatile compounds in heated beef and sheep fat. Meat Science 91, 99–107.
Winstanley, M., 1987. Soft fat and the consumer. Institute of Meat Bulletin 10–13.
Wold, J.P., Lundby, F., Egelandsdal, B., 2006. Quantification of connective tissue (Hydroxyproline) in ground beef by autofluorescence spectroscopy. Journal of Food Science 64 (3),

Relevant Websites

http://www.carometec.com/
Carometec Food Technology®.
http://www.cedarcc.com/product-solutions/viascan/viascan%C2%AE-chiller-assessment-system.aspx
Cedar Creek Company.
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ENCYCLOPEDIA OF MEAT SCIENCES

SECOND EDITION

EDITED BY

MICHAEL DIKEMAN
& CARRICK DEVINE

AP

ENCYCLOPEDIA OF
MEAT SCIENCES

SECOND EDITION

VOLUME 3

ENCYCLOPEDIA OF
MEAT SCIENCES

SECOND EDITION

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VOLUME 3

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GUIDE TO USING THE ENCYCLOPEDIA

Structure of the Encyclopedia

The material in the encyclopedia is not arranged by ordinary alphabetical order, but by alphabetical order according to 97 principal topic areas taken to allow all papers belonging to each principal topic to appear together in the same volume. Within each principal subject, article headings are also arranged alphabetically, except where logic dictates otherwise.

There are four features that help you find the topic in which you are interested:

1. The contents list.
2. Cross-references to other relevant articles within each article.
3. A full subject index.
4. Contributors list.

1 Alphabetical Contents List

The alphabetical contents list, which appears at the front of each volume, lists the entries in the order that they appear in the encyclopedia. It includes both the volume number and the page number of each entry.

2 Cross-References

All of the entries in the encyclopedia have been cross-referenced. The cross-references, which appear at the end of an entry as a See also list, serve four different functions:

i. To draw the reader's attention to related material in other entries.
ii. To indicate material that broadens and extends the scope of the article.
iii. To indicate material that covers a topic in more depth.
iv. To direct readers to other articles by the same author(s).

Example

The following list of cross-references appears at the end of the entry Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants.

> *See also*: Chemical Analysis: Sampling and Statistical Requirements; Standard Methods. Foodborne Zoonoses. Growth of Meat Animals: Metabolic Modifiers. Microorganisms and Resistance to Antibiotics, the Ubiquity of: Antibiotic Resistance by Microorganisms. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production

3 Index

The index includes page numbers for quick reference to the information you are looking for. The index entries differentiate between references to a whole entry, a part of an entry, and a table or figure.

4 Contributors

At the start of each volume there is list of the authors who contributed to that volume.

PREFACE

The Encyclopedia of Meat Sciences, second edition, an extensive revision of the first edition published in 2004, covers all the essential meat topics, ranging from animal production, processing, analytical procedures, and food safety, to final consumption including health issues and nutritional aspects. There are more than 230 articles and these provide a greater breadth of coverage than any existing work on meat science. In addition to publication in print, the Encyclopedia is also available for licensing online that can allow regular updating. The articles are designed to bring a nonexpert up to a level of understanding the interactions among the various disciplines covered in the articles. Most articles are 3000–4000 words long and include a list of Further reading and Websites to expand the content beyond the immediate scope of this work. The Encyclopedia is, therefore, a valuable resource for several levels of education and experience.

The Editors gratefully acknowledge the contributions of the authors of the articles and the Editorial Advisory Board. The board not only proposed subjects to be covered, but also found contributors and then reviewed the articles. The work involved in an Encyclopedia such as this requires an extensive interactive cooperation among the Editors, the Editorial Advisory Board, the contributors, and the publishers, particularly the staff of the Major Reference Works division of Elsevier. The staff included Nancy Maragioglio, Donna de Weerd-Wilson, Anna Gebicka, Cari Owen, Will Bowden-Green, Sam Mahfoudh, Zoey Ayres, and Marise Willis.

The Editors are particularly grateful to Cari, Will, and Sam, who worked very closely with us and who diligently pursued all avenues to obtain contacts with contributors, maneuvered around obstacles, facilitated the day-to-day management, and linked everyone together to meet the deadlines.

Michael Dikeman and Carrick Devine
Editors, August 2014

INTRODUCTION

Meat consumption by hunter–gatherers predated the agricultural revolution. Consumption of meat and fish runs in parallel with human development that is still in process. Humans and animals have now coexisted for thousands of years for their mutual benefit, even though their relationship is changing. Meat does not come from a single, or even a few, animal species, but is derived from a wide variety of species ranging from poultry to pigs, cattle, sheep, goats, and wild game to thousands of species of fish. While many of these species are now intensively farmed, some still coexist with nomadic tribes, whereas, others are raised by families in small village communities, or are even hunted by remnants of hunter–gatherer communities. The second edition of the Encyclopedia of Meat Sciences discusses how the domesticated species evolved; the wide range of harvesting methods for animals, poultry and fish; the historical changes in production, processing and nutritional value, including the beneficial effects of optimum amounts of meat in a diet.

The meat industry is based on obtaining animals, poultry, and fish from pastures, feedlots and specialized intensive production systems, and from extractive industries such as fishing. It is understandable, therefore, that the genetics and management of animals and production systems are prominent in the Encyclopedia. However, the broad field of meat science is much more than harvesting animals and processing meat from them. It includes issues such as preslaughter stress and its effects on meat quality; religious issues; animal welfare; and humane slaughter techniques, all of which are extremely important to ensure that meat quality, cultural issues, and market requirements are harmonized.

Processing methods for the various species are different, but they have all historically developed to ensure, either by conscious design or by experience, that the underlying principles of physiology and biochemistry in the conversion of muscle to meat are optimized. Biochemistry and physiology are extremely important and fundamental disciplines, because they explain how unfortunate, undesirable processing defects such as PSE or cold shortening and toughening can occur and can be avoided. Progress in this area has also enabled significant changes in production and subsequent quality since the first edition of the Encyclopedia of Meat Sciences in 2004.

Understanding these changes requires an appreciation of the structure of carcass tissues, from gross carcass attributes to consideration and understanding of changes at the ultrastructural level. The form and function of muscle tissues, how they change through growth, how they impinge on meat quality, and the way that connective tissue and fat can be major contributors to the final product quality are all covered in these pages. Topics such as cold shortening that can cause meat toughening or inhibition of tenderisation are explained, as well as how procedures such as electrical stimulation evolved to prevent these problems. Assessment of meat quality from measurements such as muscle pH, tenderness prediction through spectral measurements on uncooked meat, color changes on display and storage, and reduction of microbial contamination are critical for many aspects of the meat industry and are also discussed.

There have been many and significant advances in meat animal production based on genetic, nutrition, growth biology, and metabolic modifier research. In regard to meat processing, advances in refrigeration and freezing technology, which is the foundation of perhaps the most important changes ever encountered for food is discussed. Even so, such advances also depend on the way in which microbiology and packaging are integrated to ensure wholesome products with a long shelf life, minimal spoilage, and desirable sensory attributes. However, there are many other ways to preserve food that are also important. Of ever-increasing importance is the topic of food safety, which must receive extensive attention because meat is a perishable product and is critical for a high quality of living and even for human survival. Meat marketing and pricing in all its forms, from wet markets to hotel, restaurant and institutional trade, and transportation are also important. Whole-tissue meat is usually cooked, so, many of the desirable attributes such as flavor development relate to the temperature interactions with various proteins and sugars during cooking. Other cuts are processed in various ways, from smoking to mincing to sausages and the technologies involved are covered.

Not all muscles or cuts of meat are suitable for the same cooking and preparation methods. Therefore, out of necessity, a vast range of highly desirable products has evolved with variations from one ethnic background to another. Other products are merchandized through fast-food restaurants. One can now consume a hamburger in China that is almost identical to that in Chile or in the United States owing to a consistency of product specifications that has become universal. Meat is not only a major source of quality protein and some vitamins and minerals; it often forms the central part of a meal, and is desirable to have the appropriate flavors, aromas, and appearance to conform to the expectations and the way meat is used in various cultures.

This second edition of the Encyclopedia of Meat Sciences also covers controversial health-related aspects of meat consumption and this aspect needs considerably more research. In recent years, the ready availability of meat and other foods has given rise to some health concerns. However, the issues are not always what they seem. The positive and potential negative health-related aspects of meat eating are addressed by experts in dietary and health aspects of meat consumption, but the effect of a single food item should not be considered in isolation.

The wide coverage of topics will ensure that this second edition of the Encyclopedia of Meat Sciences will be an important resource for students or professionals with an interest in meat science or those engaged in the livestock and meat industries. Most of the articles in the second edition are not only a revision of those in first edition but there are additional areas covered. The relatively short nature of the articles makes the Encyclopedia easy and interesting to read.

Michael Dikeman and Carrick Devine
Editors, August 2014

CONTENTS TO VOLUME 3

T

P

PACKAGING

Contents

Equipment

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This article is a revision of the previous edition article by N Penney, volume 3, pp 969–976, © 2004, Elsevier Ltd.

Glossary

Barrier property A measure of a packaging material's resistance to a permeant, such as a gas, moisture vapor, or a sensory trait like aroma.

Blooming Exposing fresh meat surfaces to air to allow the oxygenation of myoglobin on the surface of meat cuts, which changes the color of the meat from purple to bright red or pink; cuts are usually allowed to bloom for 30 min in order to obtain the desirable color.

Case ready Retail-packaged meat products that are cut and packaged in a centralized facility and shipped to stores, where they are ready to be placed directly in retail cases.

Controlled-atmosphere packaging The gaseous environment surrounding the product is altered and maintained at a specified composition. Oxygen present is completely removed and subsequent oxygen produced through respiration is removed using oxygen scavengers.

Modified-atmosphere packaging Retail packaging with an oxygen-impermeable package and a headspace that allows introduction of a specific atmospheric environment to control spoilage. It is sometimes termed controlled atmosphere.

Mother bags Large oxygen-impermeable packages in which several case ready, oxygen-permeable packages can be placed to allow a controlled atmosphere during shipping and storage.

Myoglobin Protein pigment found in muscle that gives meat its color. It contains an iron (Fe) held within a heme ring. The chemical state of the iron largely dictates the color of the meat. Myoglobin may be present in the following forms: Oxymyoglobin, oxygen is attached to the iron, which is in the reduced state (Fe^{2+}). It is red or pink in color; Deoxymyoglobin, iron is deoxygenated and in the reduced state (Fe^{2+}). It is purple in color; Metmyoglobin, iron is in the oxidized state (Fe^{3+}). It is brown in color; Carboxymyoglobin, carbon monoxide is attached to the iron, which is in the reduced state (Fe^{2+}). It is bright cherry red in color.

Overwrap packaging Retail cuts are placed in foam trays with a soaker pad and the product and the tray are incased in a clear, flexible, and oxygen-permeable film allowing exposure to ambient air.

Oxidation The loss of an electron from an iron molecule (resulting in Fe^{3+}) of myoglobin. It results in the brown metmyoglobin pigment.

Oxygenation The attachment of oxygen to the iron in the heme ring of myoglobin.
Oxygen permeability The amount of oxygen allowed through a packaging film, usually expressed as ml per m^2 per 24 h at a specific temperature.
Oxygen scavenger A material that actively removes oxygen from an environment.
Preformed trays Rigid trays, of specific size and shape, commonly used in modified-atmosphere and controlled-atmosphere packaging systems. Trays will incase the product in its entirety, allowing a headspace of gas around the cut. Rigid trays are typically impermeable to oxygen.
Reduction The gain of an electron by an iron molecule (resulting in Fe^{2+}) of myoglobin. It results in the red or pink oxymyoglobin or the purple deoxymyoglobin.
Shrink tunnel or bath A steam tunnel or water bath within the production line that exposes the packaged product to a high temperature for a short time. This highly controlled heating shrinks the polymer package tightly around the meat product.
Skin packaging A process of packaging in which a flexible barrier film is rolled onto a meat product, which is then heated and vacuum sealed, forcing the softened film to mold itself into the shape of the meat.
Thermoformed packaging A process of packaging in which products are placed into heat-softened films, formed into a desired shape, and sealed with a nonforming layer over the top of the formed film. The intrapackage environment may be vacuum or gas flushed.
Two-phase packaging The gaseous environment surrounding the product is changed from anaerobic, for transport and long-term storage, to aerobic, for short-term storage and display.
Vacuum packaging A process of packaging in which retail or wholesale cuts are placed in oxygen-impenetrable bags, all air is removed from the bag, and the bag is sealed.

Introduction

Although fresh meat is highly perishable, its packaging has, until recently, been a matter of only minor concern to meat traders, health officials, and consumers. Unwrapped fresh or frozen meat has been the currency of the wholesale and retail meat trade. Small meat species, such as rabbits and poultry, are, in many parts of the world, still traded commercially in their natural packaging, that is, their own skin.

Packaging is not just the materials immediately surrounding a product but rather is the synthesis of the product, its processing, and labor and machines required in order to address specific functional and marketing requirements. These functions relate to all aspects of distribution, storage, and merchandizing along with containment, protection, preservation, portioning, unitization, convenience, and communication.

For meat and meat products, packaging should provide the customer with an appropriately portioned product in a safe and wholesome condition. Meat packaging has to protect the product from a physical environment that threatens product safety, product damage, and loss of pack integrity.

The functional requirements of meat packaging systems are dictated by the required marketing performance. For example, the packaging requirements for international trade in chilled meat differ from those for domestic supply. The overriding performance requirement is, however, the same in both cases – adequate storage life insuring product resilience to meet customer expectations. Briefly, packs for retail display should facilitate contact of meat with oxygen to allow blooming, whereas those for transport and extended storage should preclude such contact.

The packaging technologies required to meet the varied customer expectations are numerous and the machinery commonly used are discussed below.

Nonpreservative Packaging

This type of packaging contains the product and protects it from contamination and moisture loss without creating in-pack conditions very different from the ambient conditions. Consequently, unless microbial growth is prevented by freezing, addition of preservatives, or is retarded by chilling, the product in such a pack is highly perishable with a very short shelf life and would only be appropriate for domestic supply.

Wrapping

The simplest form of flexible packaging – wrapping – does not require machinery at all. A meat product is simply placed on a sheet of material, often greaseproof paper or plastic cling film, which is wrapped around the product to protect it from the environment and vice versa.

Tray Overwrap

Overwrapped trays are widely used in supermarkets for fresh meats, processed meats, and poultry. Fresh meats are placed on a semirigid tray, often incorporating some drip-containing device, and overwrapped with a plastic film of high-oxygen permeability. As with general wrapping, overwrapped trays are not sealed and, because of the high-oxygen permeability of the films used, provide ambient aerobic conditions around the product, giving the product a limited shelf life. The machinery available for overwrapping trays varies from hand wrapping as it ranges from a roll of cling film, through wrapping aids, to fully automated wrapping machines (Figure 1).

Preservative Packaging

Packaging in this group is characterized by its ability to extend product life over that achieved by simple overwrap technology. This is achieved by creating and maintaining in-pack conditions that differ markedly from those of the ambient environment, thus, creating conditions that modify or restrict microbial growth.

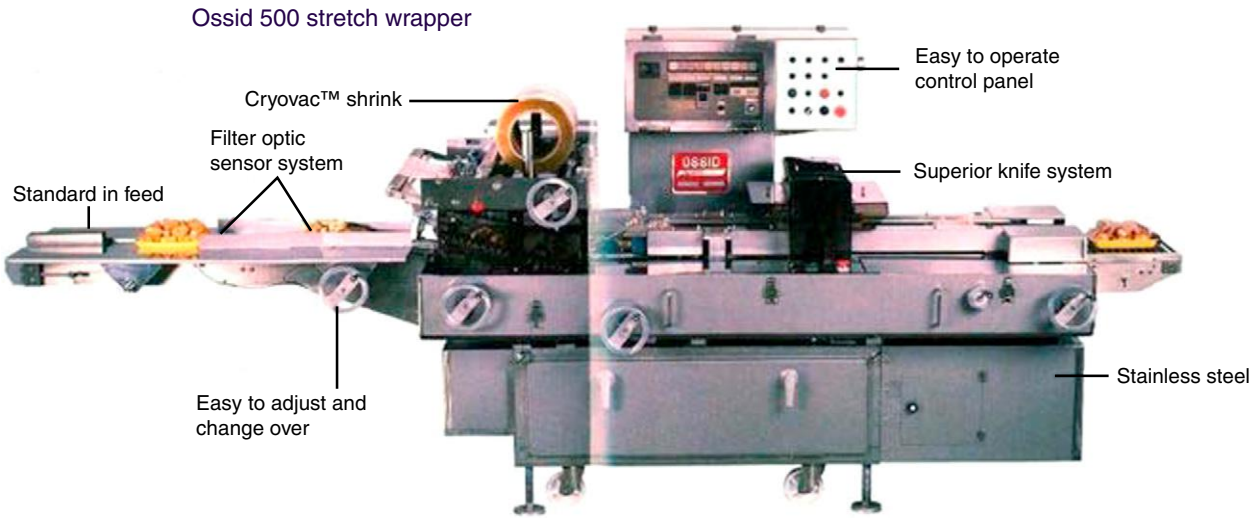


Figure 1 Ossid 500 stretch wrapper. Fresh meat on semirigid trays is feed into the wrapper, on the left, where it is wrapped in oxygen-permeable stretch film, weighed, labeled, and priced, emerging on the right ready for the display case. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

Vacuum Packaging

The basic principle of vacuum packaging is quite simple and has been successfully used commercially for approximately 40 years. The preservative effect in vacuum packs is achieved by removing air from within the pack and maintaining an oxygen-deficient environment around the product by sealing the product in a flexible film of low oxygen permeability. This oxygen-deficient in-pack environment extends the product's storage life by selecting a slower growing anaerobic spoilage microflora and preventing the proliferation of the fast-growing aerobic spoilage organisms. The technologies and machinery used to achieve this are in large amount and varied.

Vacuum clip

A vacuum pack is achieved, using this technology, by placing a piece of fresh meat or meat product into a tube of oxygen-barrier film that has been sealed at one end with a metal or plastic clip (Figure 2). A vacuum nozzle is placed at the open end of the tube and a vacuum is applied to remove air from within the pack. While the pack is under vacuum, the packaging material is gathered around the nozzle and sealed with another metal or plastic clip. Fresh meat packages prepared in this way can then be heat shrunk to give a tight pack of attractive appearance. Processed meat, such as luncheon meat, could be cooked with this type of packaging.

The machinery available for vacuum clip ranges from a simple hand-operated model to fully automated models mounted in-line for 'chub' packed sausage manufacture.

Vacuum chamber

The most common technology used in the fresh meat industry is the vacuum chamber. Fresh or processed meat is placed into a flexible pouch made of an oxygen-barrier film, which is then placed into a chamber with the open end of the pouch positioned over a seal bar. The chamber is closed and a vacuum

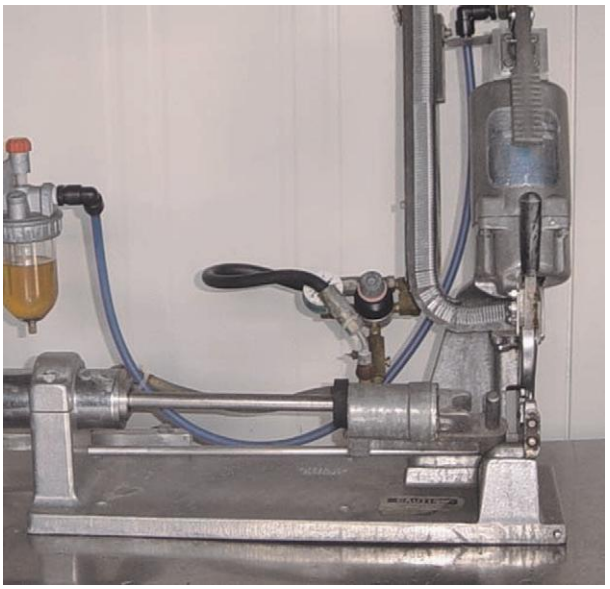


Figure 2 Hand-operated Clippertie vacuum clip system. Meat product packaged in oxygen barrier is sealed with a metal or plastic clip (contained in the reverse-J-shaped formation) around the gathered packaging material.

applied. The chamber is then evacuated to a level where water vaporizes from the meat surface, insuring that an oxygen-free atmosphere is maintained around the product. At this stage, the open end of the pouch is hermetically sealed and the vacuum released, which results in the film collapsing tightly around the product (Figure 3).

Close contact between the meat and the packaging film can be enhanced by heat shrinking the packaging on the product after heat sealing. Packaged product is passed through a shrink tunnel or bath that subjects the packaging material to temperatures between 80 and 85 °C, depending on film formula,

Figure 3 Twin vacuum chamber machine. Meat in oxygen-barrier bags is placed on one platform. The chamber hood is lowered, a vacuum is applied within the chamber, and the vacuum-barrier bag is sealed and the camber is vented. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

Figure 4 Rotary vacuum chamber machine. Meat in oxygen-barrier bags is placed on conveyer at the left and fed into one of many vacuum chambers for evacuation and sealing. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

for 1–2 s. This treatment causes the packaging material to shrink in two dimensions.

There is a vast range of vacuum chamber machinery available, from small single-chamber machines to handle 1–2 kg cuts for the corner butcher, through twin chamber (Figure 3), to multichamber rotary machines that can process 100 tons of cuts over a day's production (Figure 4).

In large high-throughput meat plants, modern vacuum chamber machines form the central part of an integrated meat packaging system, where the meat cut is measured, a barrier bag is constructed to size from a roll of barrier tubing, and the product is automatically loaded at the packaging station before normal chamber evacuation and sealing.

Figure 5 Flow wrap. Flexible-barrier film is passed through a former (on the left of figure), producing a tube around the meat (center) before vacuuming and sealing. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

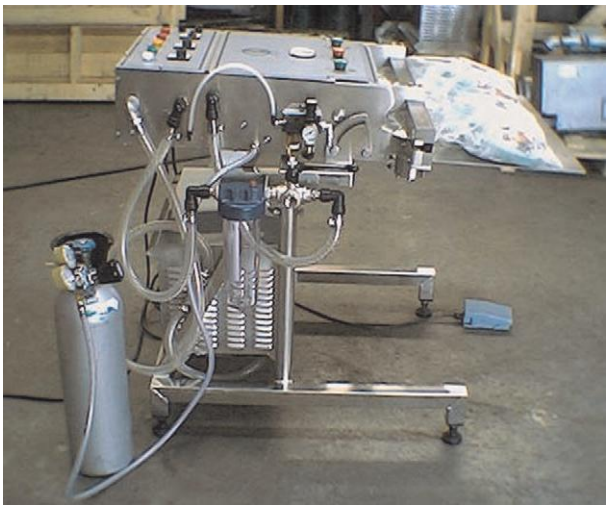


Figure 6 Snorkel machine. Barrier bags of meat are clamped over snorkels (on the right of figure). Air is evacuated through the snorkels before sealing. Reproduced with permission from Oakham Industries, Auckland, New Zealand.

An alternative, recently introduced, in-line system uses a vertical form-fill and seal principle that employs a single roll of flexible-barrier film passed through a forming tool, which forms the material into a tube around the meat cut. The two edges and one end of the formed tube are hermetically sealed to enclose the product in a custom-made barrier pouch. The enclosed product is then conveyed to a vacuum chamber and vacuum sealed or gas flushed automatically (Figure 5).

Snorkel

An improvement on the vacuum clip is snorkel technology (Figure 6), in which the meat is placed in a flexible-barrier pouch and the open end of the pouch is placed over two evacuating snorkels and clamped to make the pack airtight. As the vacuum is drawn, the packaging material collapses around

the product and, when all air has been removed, the snorkels retract and the pouch is hermetically sealed.

Vacuum skin pack

Vacuum skin packaging is a variant of vacuum chamber packaging (Figure 7). Meat cuts or sliced processed meats are placed on trays or flat sheets of flexible-barrier material before being introduced manually or automatically into a vacuum chamber. A flexible-barrier film is fed from a stock rolled over the trayed product. The upper film material is softened by heating; the skin is formed by drawing a high vacuum on the inner and outer sides of a barrier film and subsequently venting the upper side to atmosphere, forcing the softened film down so that the soft film molds itself to the shape of the product and adheres to the lower film or tray to produce a skintight leakproof package. Vacuum skin packaging is particularly useful for irregularly shaped product when the product shape needs to be maintained.

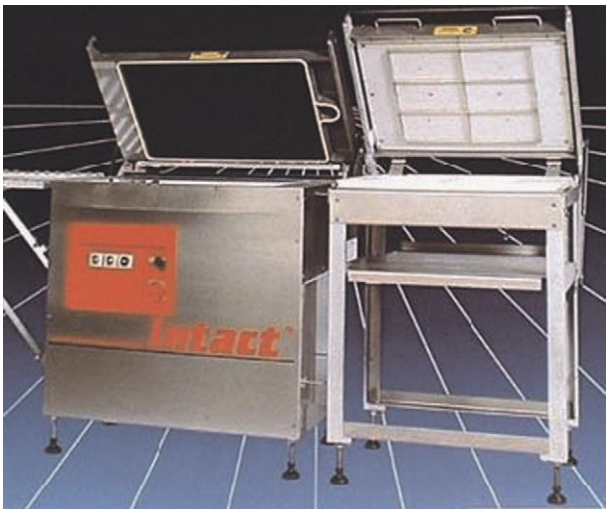


Figure 7 Vacuum skin packaging machine. Trays of meat are placed on magazine (right) before being placed in chamber. Barrier film is softened by a heating element in lid before being molded to trays below. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

Vacuum skin packaging machinery ranges from small bench-top models, allowing small butchers to package one or two trays at a time, to fully automated continuous-flow models, packing many hundreds of packs per hour.

Pi-Vac

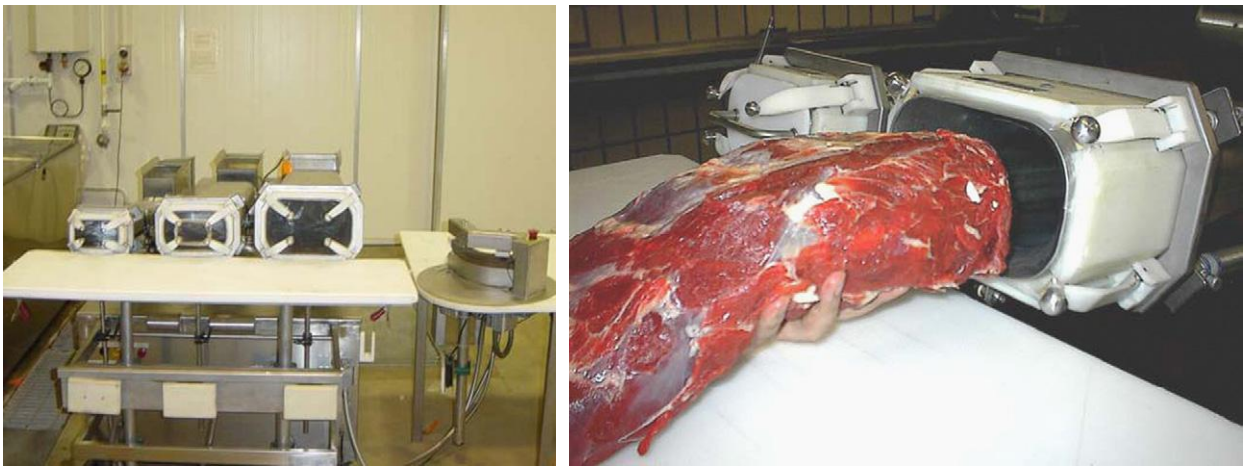
Pi-Vac is a development that extends the vacuum clip technology, which has recently been marketed in Europe. This technology claims to be a 'vacuum-free vacuum pack'. The film is fed into tubes (Figure 8(a)) and the film edge is stretched over fingers in such a way that it allows the open tube to have meat placed in it (Figure 8(b)).

The oxygen-free atmosphere around the meat product is achieved by using the specially constructed barrier tube of film that is stretched before the meat is introduced into the tube. The stretched film contracts over the meat to its prestretch dimensions, forcing air out and bringing the film into close contact with the meat product. The open end of the tube is clipped or heat sealed, producing an air-free meat pack.

Controlled- and modified-atmosphere packaging

In modified-atmosphere packaging (MAP), the gaseous environment around the meat is modified before heat sealing, and then gradually changes as a result of the interaction between the product and the packaging. For meat, the in-pack environment is usually altered by evacuation followed by backflushing with the desired gas mixture.

For fresh meat, oxygen mixtures are used for retail display packs, whereas oxygen-free mixtures are used for transport/storage packs. Subsequently, changes in the composition of the in-pack atmosphere are determined by the gas barrier properties of the packaging material; the metabolic activities of the product; its microflora; and the solubility of components of the gas mix in the product. For processed meats,



(a) (b)

Figure 8 (a) A Pi-vac machine with three tubes. Barrier film is prestretched inside one of the three tubes with the edges placed over 'fingers' (middle tube) that are stretched to enlarge the opening before introduction of meat. (b) Meat is placed in a tube of prestretched film pulled open by the fingers. Reproduced with permission from Pi-Patente GmbH, Wettenberg, Germany.

where meat bloom is not an issue, oxygen-free mixtures are used.

As in MAP, in controlled-atmosphere packaging (CAP) the gaseous atmosphere around the meat is altered, but it is then maintained at a specified composition – effectively zero oxygen – regardless of product or microbial respiration or any other environmental changes. To maintain complete anaerobic environment, oxygen scavengers are used. In a mother bag/overwrap system, the oxygen scavenger is added to the mother bag and never comes in contact with the consumer. Some processed meats, such as jerky or pepperoni, may contain an oxygen-scavenging sachet in the package labeled 'DO NOT EAT.' Other packaging systems may use an active oxygen-scavenging layer in a multilayer film.

Thermoforming

Thermoforming involves the use of a semirigid base material that is drawn from a feeder reel into a heating station where the material is softened. The softened material is drawn into a mold by application of a vacuum to form the base of a meat tray. In some machine models, the tray formation is mechanically aided by the insertion of a tray-shaped die.

The formed trays are part of a continuous sheet and move onto the product-loading station, as the next cycle commences, with product loaded either manually or, in some cases, automatically (Figure 9). Forming film can be rigid, allowing minimal flexibility, semiflexible, allowing some flexibility, or flexible. These films can be used in combination with colored or clear nonforming films for customized packaging options.

The filled web of trays is indexed to the vacuum, flushing, and sealing chamber, where a barrier lidding material is simultaneously fed into the chamber above the trayed product. A vacuum is applied in the chamber and the ambient atmosphere is removed. The machines can be configured to produce either vacuum packages or MAP trays depending on the product requirement. The sealed web of MAP packs then passes through a series of knives that separate the individual packs before cartoning and distribution to retail. Many products packed in a thermoform pouch require gas flushing; however, many fresh meat products are packed in vacuum using thermoform equipment. Thermoforming equipment allows processors to reduce labor and increase throughput when packaging meat products.

Preformed trays

MAP machines that use preformed trays have many similarities with the thermoforming machines without tray material reel feed and heating and forming station. The use of preformed trays allows a greater variety in tray sizes, designs, colors, and materials to suit changing market demands.

The trays can be filled with product and placed into the vacuum, flushing, and sealing chamber. There can also be an attached tray denester, from which the trays are placed automatically into an indexed in-feed system, fed to the loading station, and then into the vacuum, flushing, and sealing stage, similar to that of the thermoforming machines. The excess lidding film is trimmed from the tray flange before cartoning and dispatch. An example of a machine using preformed trays is shown in Figure 10.

CAPTECH chamber snorkel machines

This technology extends the use of CAP and is known as the CAPTECH system. It is used for very low oxygen/carbon dioxide CAP for extended shelf life where very low oxygen levels are required to be maintained over a long storage period. Meat cuts

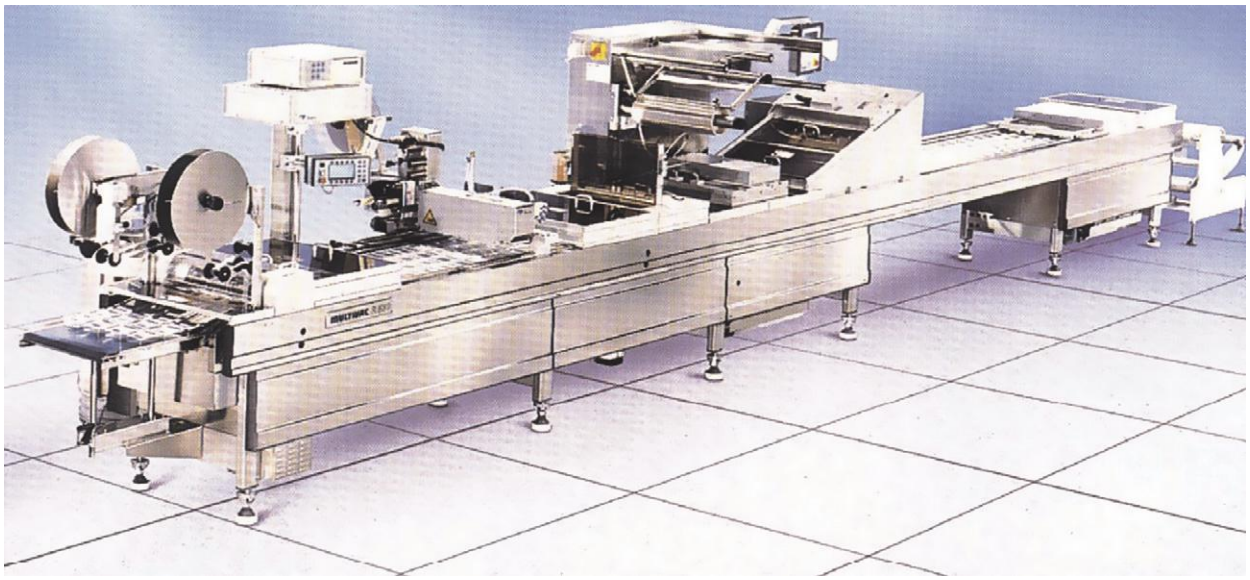


Figure 9 Thermoforming machine. Semirigid base material (far right) is drawn into the heating station (right), where the material is softened and a tray is drawn. The product is loaded into the trays and moved onto the gassing and lidding station (middle), where the pack atmosphere is modified and the pack is lidded. The packaged product is trimmed (far left) for dispatch. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

Figure 10 Preformed trays MAP machine. Meat in preformed trays is placed in the drawer in the front of the machine where it is flushed with a MAP gas and sealed. Reproduced with permission from Cryovac Sealedair Corporation, Hamilton, New Zealand.

or display packs are placed into high-oxygen barrier bags and the open end of the pouch is placed over the internal snorkel before the chamber lid is lowered. A vacuum is applied to both the chamber and the snorkel at the same time to maintain equal pressure within the chamber and the bag, thereby preventing any distortion of the packing materials or the product. When the vacuum reaches the predetermined level, the chamber is slowly vented and the pack gas is injected into the meat pack through the evacuation snorkel at a rate that prevents pack damage. When the preset level of gas is reached, the pouch opening is hermetically sealed and the chamber is fully vented and opened. The equipment is shown in Figure 11.

Mother bag snorkel machines

This technology does not rely on chamber evacuation to remove air from the package and can be used for products that may be damaged by vacuum; however, the amount of air removed from the pack may vary and may not be suitable for packs that require accurate control of pack gas atmosphere.

Meat cuts or display packs are placed in an oxygen-barrier pouch. The open mouth of the barrier pouch is stretched over the flat snorkels and is clamped to make an airtight seal. The clamping action initiates a vacuum in the snorkels and the air within the pack is removed, collapsing the packaging material around the product. After a preset time, the evacuation is stopped and the pack is backflushed with the required MAP gas.

If more accurate control of the gas composition in the pack is required, the vacuum and backflush cycle can be repeated, further diluting ambient air remaining within the pack. In very low oxygen mother bag packaging, oxygen scavengers are typically used to sequester residual oxygen that may be present in the package. The machinery is similar to that shown in Figure 6.

Figure 11 CAPTECH machine. Meat is placed in very high-barrier bags. The open end of the bags is placed over one of the three internal snorkels. The chamber is lowered, a vacuum is applied, and the storage atmosphere containing carbon dioxide is injected into the pack before sealing. Reproduced with permission from Securefresh Pacific Ltd., Auckland, New Zealand.

Mother bag flow wrap

Mother bag flow wrap uses a vertical form-fill and seal principle that employs a single roll of flexible-barrier film passed through a forming tool to form the material into a tube. A number of case-ready trays are transported into the tube as it is formed. The two edges, and one end of the formed tube, are hermetically sealed to enclose the product in a custom-made barrier pouch. The pouch with the enclosed product is then flushed with the required MAP gas mix and sealed automatically. The machinery is similar to that shown in Figure 5.

Two-phase packaging

The general principle of two-phase packaging is to change the gaseous environment surrounding the meat from anaerobic to aerobic between the transport/storage and the storage/display phases.

One system uses a vacuum chamber to replace the ambient air in a ridged-barrier tray with low oxygen, carbon dioxide, or nitrogen, or a mixture of both, and seal a ridged, domed lid onto the ridged tray to enclose the CAP atmosphere. After transport and storage, the dome is removed to allow air into the pack, which will effectively 'bloom' the meat for an attractive retail display.

Another system uses the same principle to produce an anoxic atmosphere around the product but seals the pack with a multilayer peelable-barrier film. After transport and storage, the top barrier layer of the lidding film is removed to expose the oxygen-permeable layer, which allows the anoxic transport atmosphere to be replaced with ambient air, resulting in meat 'bloom' for retail display.

In a third variation, the 'windjammer,' meat is placed in a preformed tray. The ambient atmosphere in the tray in the chamber is replaced with a nitrogen–carbon dioxide mix in a vacuum chamber where the 'transport atmosphere' is replaced with a 'display atmosphere' and sealed with a high-barrier film.

When the pack is required for retail display, a septum is placed on the lidding film and a needle is inserted through it. The meat pack is then transferred to a vacuum chamber where the anoxic transport atmosphere is replaced, through the needle, with a high-oxygen display atmosphere. The needle is then removed and the product is ready for retail display.

See also: Packaging: Modified and Controlled Atmosphere; Overwrapping; Technology and Films; Vacuum

Further Reading

Bell, R.G., 2001. Meat packaging: Protection, preservation and presentation. In: Hui, Y.H., Nip, W.-K., Rogers, R.W., Young, O.A. (Eds.), Meat Science and Applications. New York, NY: Marcel Dekker, pp. 463–490.

Blakistone, B.A., 1998. Principles and Applications of Modified Atmosphere Packaging of Foods, second ed. London: Blackie Academic and Professional.

Ooraikul, B., Stiles, M.E., 1991. Modified Atmosphere Packaging of Food. New York, NY: Ellis Horwood.

Modified and Controlled Atmosphere

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Glossary

Carboxymyoglobin A form of myoglobin that results in red color due to carbon monoxide.
Controlled atmosphere packaging (CAP) A package with an atmosphere that is intentionally controlled to remain approximately the same during the package life.
Deoxymyoglobin A form of myoglobin responsible for purple color due to absence of oxygen ligand.
High-oxygen packaging A gas mixture of up to 80% oxygen and 20% carbon dioxide.
Low-oxygen packaging A gas mixture of up to 80% nitrogen and 20% carbon dioxide.
Methemoglobin A form of hemoglobin in which the iron is in the ferric state, not the ferrous of normal hemoglobin, and is responsible for marrow color.
Metmyoglobin A form of myoglobin responsible for brown discoloration due to ferric iron.
Modified atmosphere packaging (MAP) A barrier package where the initial atmosphere is removed, then the package is refilled with a single gas or a combination of gases.
Oxymyoglobin A form of myoglobin redox responsible for red color due to effects of oxygen ligand.
Peelable films An oxygen-permeable package contained within an outer oxygen impermeable barrier that is removed (peeled) at the retail market to enable oxygenation.

Introduction

Both modified atmosphere (MAP) and controlled atmosphere packaging (CAP) systems have gained popularity because of centralized preparation of retail meat cuts and distribution over increasingly longer distances. These factors require meat in packages to have a shelf life that is greater than that provided by overwrapping and require packaging to provide benefits different from those offered by vacuum packaging. Case-ready meat programs have been in wide use in Europe for several decades but are a relatively recent part of meat retailing in the US. Nevertheless, case ready is experiencing phenomenal growth in the US, accounting for nearly 65% of fresh meat retail cases.

Modified Atmosphere Packaging

MAP involves placing product in a barrier package, removing the existing initial atmosphere with a vacuum pump or by gas flushing, and then refilling the package with a single gas or a combination of gases. Although the gas composition within the package after sealing is typically not altered, oxygen scavengers as well as muscle and microbial metabolism may reduce residual levels of oxygen in some types of MAP.

Controlled Atmosphere Packaging

CAP implies that the package atmosphere after air evacuation is intentionally controlled to remain approximately the same during the package life. Another term sometimes used is 'intelligent' packaging, which may include monitoring the package and possibly making adjustments based on this information. These package adjustments could deal with oxygen scavenging or control, moisture and gas permeability, ethylene control, temperature control, odor removal, aroma emission from plastics, microbial growth, product freshness or spoilage, and package integrity. To meet this very demanding requirement, a film that is completely impermeable to gases must be used.

Proponents of CAP advocate the use of

1. A film with a layer of nonplastic material, such as aluminum;
2. Heat seals on all package perimeters; and
3. Complete evacuation of air because product color is significantly affected by even minute amounts of oxygen in the package. Antimicrobials and antioxidants also can be incorporated into films to improve shelf life.

Purposes of Modified Atmosphere Packaging and Controlled Atmosphere Packaging

Both MAP and CAP have the ability to prolong product shelf life and thus are very useful when meat must be transported to long distances. This technology is primarily used for the distribution of fully prepared case-ready retail packages either as individual packages or as several retail packages in a master or mother package. In addition, distribution of carcasses or larger cuts to the location where retail cuts are prepared and retail packaged is possible. For example, lamb carcasses can be shipped in CAP and larger pork wholesale products (loins, shoulders, or spare ribs) packaged in MAP can be shipped to retail locations for further processing. In addition to improving color life, modifying the within-package atmosphere can influence the time before microbial growth and lipid oxidation cause spoilage.

Color Properties

Packaging systems need to promote both the development and the stability of an attractive, bright, fresh saleable appearance when meat products are cut into retail cuts and placed on display. This should provide sufficient display life by allowing products to be sold and used by the consumer with minimal loss. Desirable appearance relates not only to muscle color but also to color of fat, bone marrow, and liquid purge, which all can influence consumer purchasing decisions. As a result, discoloration can decrease shelf life more quickly than microbial deterioration or spoilage. Packaging should also provide a barrier to water vapor transmission in order to control surface dehydration of the meat.

Advantages and Disadvantages of Case-Ready Meat and Central Processing

MAP systems have been essential to the development of case-ready meat programs. The benefits of the case-ready method include better control of sanitation and use of Hazard Analysis and Critical Control Points principles at a centralized processing facility; improved product safety resulting from less handling; a lower requirement for skilled labor at individual retail stores; more consistent and high-quality products; greater marketing flexibility (the retail manager can order product to fit anticipated individual retail store needs); the ability to rapidly replenish case items that are out of stock or in short supply; and more accurate records of sales, purchasing patterns, and inventory.

The case-ready programs that are made possible primarily by MAP have some disadvantages and challenges, such as a higher per-package cost because more specialized films and packages are needed to maintain desired quality and safety for the longer time from initial fabrication to purchase by the customer. Although MAP can reduce food loss and waste, the impact of packaging material waste, such as trays and film, on the environment can be a concern. As a result, environmentally friendly packaging materials that are biodegradable have received interest. Other disadvantages to case-ready systems using MAP can include the effect of oxygen level on bone marrow discoloration and cooked color development.

Role of Gases in Packaging

Modified Atmosphere Packaging

The initial gas atmosphere for MAP is obtained by removing air from the package followed by refilling the package with a desired gas blend mix such as high oxygen, low oxygen, ultralow oxygen, and carbon monoxide at a low level (0.4%). High-oxygen MAP involves a gas mixture of up to 80% oxygen (65–80% is most common) and 15–30% carbon dioxide. Complete air evacuation is not as critical for high-oxygen as for low- and ultralow-oxygen MAP because of the final oxygen level. Low- and ultralow-oxygen MAP uses a gas mixture of carbon dioxide and nitrogen. The purpose of this system is to minimize oxygen exposure during storage and distribution. This will slow the onset of microbial spoilage and lipid oxidation while promoting myoglobin oxygenation (bloom) before display. Merely assuming that packages have the proper gas composition may lead to serious consequences, even if the deviations in composition are small. In addition, leak detection is extremely important.

Peelable packaging includes a retail package with an inner film that is oxygen permeable and contained within a second outer layer of barrier film that maintains a low-oxygen condition. This outer oxygen-impermeable barrier is removed (peeled) at the retail market to enable oxygenation (blooming) of the meat to a desired bright color. Alternatively, individual oxygen permeable retail packages can be sealed in an individual outer 'master' or 'mother' bag that is oxygen impermeable. The inner package is then removed at the retail location to facilitate product oxygenation. Minimizing both storage time and oxymyoglobin formation before packaging in peelable films is critical because the conversion of oxy- to deoxymyoglobin is not direct but through metmyoglobin. More specifically, deoxymyoglobin regeneration requires both oxygen consumption accompanied by subsequent metmyoglobin reduction. This reaction tends to decrease with storage time, suggesting that oxygenation, or bloom, after peelable films are removed at retail and the product is exposed to air will be greater for product that has spent less time in the oxymyoglobin redox state before initial packaging.

Oxygen

Oxygen is essential in fresh meat packaging to both develop and maintain bright red meat color. Its use at 65–80% of in-package gas helps to drive the oxymyoglobin pigment layer deeper into meat. This extends the time until metmyoglobin approaches the surface of the meat, which results in discoloration. Although color life is dependent on numerous factors, 80% oxygen:20% carbon dioxide can increase color stability up to three times of that expected with aerobic packaging. However, the primary disadvantage of the high-oxygen packaging is lipid oxidation and the development of oxidized off-odors. To counteract this, product enhancement with a solution containing an antioxidant may be useful. High-oxygen packaging can also predispose product to marrow discoloration and cooked color defects such as premature browning.

Residual Oxygen Effects

Sufficiently low levels of oxygen are necessary for the success of ultralow-oxygen packages because partial oxygen pressure as low as 1.4 mmHg will favor metmyoglobin formation. Nevertheless, low levels are difficult to achieve with initial package evacuation of air and thus a low level of oxygen may persist in the in-package atmosphere until it is used by muscle or microbial metabolism or is removed with an oxygen scavenger. For example, simple gas displacement may result in residual oxygen of up to 10%, whereas chamber evacuation can achieve 0.1–1% oxygen, depending on evacuation cycle time. As a result, residual oxygen in low- and ultralow-oxygen packages is typically present shortly after package closure and is often used for both muscle and microbial metabolism. This produces carbon dioxide, which can be an asset in lessening

aerobic microbial growth and may favor a more desired microbial population. However, at low oxygen levels, the formation of metmyoglobin is favored, resulting in surface discoloration. With time, oxygen may be used up by the meat and metmyoglobin may return to purplish-red deoxymyoglobin via metmyoglobin reducing activity (MRA). Oxygen scavengers can also be used to minimize the amount of muscle metabolism necessary to result in suitably low oxygen levels and MRA.

Oxygen Scavenging

To rapidly attain and then maintain zero oxygen in MAP, oxygen absorbers or scavengers are often needed to remove residual oxygen. The most common oxygen scavengers are reduced iron powders mixed with acids, salts, or both that can be oxidized in the presence of oxygen, usually on the addition of a wetted humectant. Active packaging may use a polymeric oxygen scavenging system that both absorbs oxygen within the package and serves as a barrier film. The oxygen scavenging layer consists of three components including an oxidizable polymer that binds oxygen molecules, a photoinitiator, and a catalyst. After the package is sealed, it passes under an ultraviolet light, whereupon the photoinitiator provides energy to start the reaction and the catalyst speeds up the oxygen scavenging. Another important consideration is immediate packaging after fabrication to minimize the uptake of oxygen by meat. Increased time before packaging will more likely result in oxygen release into the within-package atmosphere as well as slow metmyoglobin reduction and bloom.

Carbon Dioxide

Carbon dioxide is a component typically used in modified atmosphere packaging to slow bacterial growth; thereby extending the time until spoilage. Carbon dioxide is more effective with low levels of initial contamination and also at cold temperatures. Its effects include altered cell membrane function, nutrient uptake and absorption, and enzyme functions.

Carbon dioxide is very soluble in both the muscle and fat components of meat, especially at cold temperatures and increased pH. This can diminish the volume of gas within the package headspace, resulting in package collapse because of a loss of gas volume. As a result, enough carbon dioxide must be added to the gas mix to account for absorption into meat and its consequent loss from the within-package atmosphere. Adding nitrogen to the gas blend lessens the possibility of package collapse.

Nitrogen

Nitrogen is essentially inert in a meat package and is frequently used as a filler gas to dilute oxygen. It is especially used with cured product to provide conditions under which cured color does not fade very rapidly and where nitrite is an ingredient used for microbial control.

Carbon Monoxide

The use of carbon monoxide (CO) in modified atmosphere packaging results in a stable bright red color via the formation of carboxymyoglobin. There was early concern that this stable color would mask microbial spoilage, but at the 0.4% use permitted in the US, this was not found to be a problem when retail packages are removed from the master pack before display. Nevertheless, the use of expiration dates on labels has been recommended. Although carbon monoxide can result in cooked color defects, such as persistent pink color, consumer apprehension toward CO is more likely the limited factor preventing its use in modified atmosphere packaging.

Gas Effects on Bone Marrow

In addition to muscle color, packaging can influence the color of bone marrow because hemoglobin oxidation results in bone marrow discoloration, sometimes referred to as bone darkening. This has been particularly problematic in high-oxygen packages as the 80% oxygen tends to increase methemoglobin formation. Low-oxygen and CO packaging can minimize marrow discoloration by maintaining hemoglobin in a ferrous redox state.

Gas Effects on Cooked Color

Packaging atmosphere can influence cooked color in addition to raw color. In particular, premature browning of cooked product before pasteurization temperatures has been associated with high-oxygen packaging. Before cooking, myoglobin redox form influences the protein's stability toward heat induced denaturation. Oxy- and metmyoglobin are less heat stable than deoxymyoglobin. As a result, the increased depth of oxygen penetration in high-oxygen packaging can predispose beef to premature browning. Vacuum packaging, which maintains deoxymyoglobin within the interior of steak/patties, can prevent premature browning. Meat packaged in CO will often remain red/pink after cooking because carboxymyoglobin is relatively stable against heat denaturation.

Film Composition and Gas Permeability

To adequately maintain the desired gas composition during storage, distribution, and display, packages must consist of materials that are effective barriers to the transmission of gases and water vapor. Gas transmission rates are affected by film material(s) and thickness, environmental temperature, and the difference in partial pressures of the appropriate gas between the inside and the outside of the package. Most barrier packages are constructed of three layers of film. The outer layer is designed to be scuff and abrasion resistant; the middle layer provides barrier properties; and the inside layer is the sealant layer. More details on film materials, how they are manufactured, and their functions and limitations are given in another article.

Enhancement of Meat

Injection enhancement of meat includes the incorporation of ingredients such as water, salt, phosphate, lactate, and

antioxidants via injection and/or physical manipulation. This process can increase yield of saleable product, create a more uniform tenderness, and improve color stability. Ingredient-based effects on color can be packaging dependent. For example, lactate tends to improve color life in high-oxygen packaging but not in CO or vacuum. Other ingredients added via injection enhancement can negate lipid oxidation associated with extended storage of MAP.

Key Factors for Success in Product Quality Using Modified Atmosphere Packaging and Controlled Atmosphere Packaging

The essential keys to success of both MAP and CAP operations include:

- Using clean, acceptable quality product.
- Rapid completion of packaging after meat fabrication.
- Temperature control during storage and display.
- Complete evacuation of air before filling the package with the desired atmosphere.
- Use of packaging materials with appropriate gas and moisture transmission rates.
- Packaging that remains sealed until opened at the point of use.
- Other considerations are package cost, package reliability, the storage and display life provided, acceptability of the retail package to the customer and to the meat marketing manager, packaging speed (productivity), equipment efficiency, and the required headspace gas.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment. Packaging: Equipment; Overwrapping; Vacuum. Quality Management: Abattoirs and Processing Plants

Further Reading

Brody, A.L., 1989. Controlled/Modified Atmosphere/Vacuum Packaging of Foods. Lancaster, PA: Technomic.

Brody, A.L., Strupinsky, E.R., Kline, L.R., 2001. Active Packaging for Food Applications. Boca Raton, FL: CRC Press.

Church, N., 1994. Developments in modified-atmosphere packaging and related technologies. Trends in Food Science and Technology 5, 345–352.

Eilert, S.J., 2005. New packaging technologies for the 21st century. Meat Science 71, 122–127.

Gill, C.O., 1996. Extending the storage life of raw chilled meats. Meat Science 43, S99–S109.

Hermansen, P., 1983. Comparison of modified atmosphere versus vacuum packaging to extend the shelf life of retail fresh meat cuts. Proceedings of the 36th Reciprocal Meat Conference, pp. 60–64. Savoy, IL: American Meat Science Association.

Holland, G.C., 1980. Modified atmospheres for fresh meat distribution. Proceedings of the Meat Industry Research Conference, pp. 21–39. Savoy, IL: American Meat Science Association.

Hotchkiss, J.H., 1994. Packaging muscle foods. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods: Meat, Poultry and Seafood Technology. New York: Chapman and Hall, pp. 475–496.

Jeremiah, L.E., 2001. Packaging alternatives to deliver fresh meats using short- or long-term distribution. Food Research International 34, 747–772.

Jeyamkondan, S., Jayas, D.S., Holley, R.A., 2000. Review of centralized packaging systems for distribution of retail ready meat. Journal of Food Protection 63, 796–804.

Kropf, D.H., 1980. Effects of retail display conditions on meat color. Proceedings of the 33rd Reciprocal Meat Conference, pp. 1–32. Savoy, IL: American Meat Science Association.

Kropf, D.H., 2000. Meat, modified atmosphere packaging. In: Hui, Y. (Ed.), Encyclopedia of Food Science and Technology. New York: Wiley, pp. 1561–1566.

McMillin, K.W., 2008. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Science 80, 43–65.

Renerre, M., Labadie, J., 1993. Fresh meat packaging and meat quality. Proceedings of the 39th International Congress of Meat Science and Technology, Session 8, pp. 361–387. Calgary, Alberta, Canada.

Rooney, M.L., 1995. Active Food Packaging. Glasgow: Blackie Academic and Professional.

Singh, P., Wani, A., Saengerlaub, S., Langowski, H., 2011. Understanding critical factors for the quality and shelf-life of MAP fresh meat. Critical Reviews in Food Science and Nutrition 51, 146–177.

Sorheim, O., Nissen, H., Aune, T., *et al.*, 2001. Use of carbon monoxide in retail meat packaging. Proceedings of the 54th Reciprocal Meat Conference, pp. 47–51. Savoy, IL: American Meat Science Association.

Taylor, A.A., 1982. Retail packaging systems for fresh meat. In: Lincoln, N.E. (Ed.), Proceedings of the International Symposium of Meat Science and Technology, pp. 353–365. Savoy, IL: American Meat Science Association.

Zhao, Y., Wells, J.H., McMillen, K.W., 1994. Application of dynamic modified atmosphere packaging systems for fresh red meats: Review. Journal of Muscle Foods 5, 299–328.

Zhou, G.H., Xu, X.L., Liu, Y., 2010. Preservation technologies for fresh meat. Meat Science 86, 119–128.

Relevant Websites

http://modifiedatmospherepackaging.com/
Dan Sensor.

http://www.modifiedatmospherepackaging.com/Applications/Modified-atmosphere-packaging-fresh-meat.aspx
Dan Sensor.

http://www.foodsmart.govt.nz/food-safety/high-risk-foods/modified-atmosphere-foods/
New Zealand Ministry for Primary Industries.

http://en.wikipedia.org/wiki/Controlled_atmosphere
Wikipedia.

http://en.wikipedia.org/wiki/Modified_atmosphere
Wikipedia.

Overwrapping

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Glossary

Blooming Exposing fresh meat surfaces to air to allow the oxygenation of myoglobin on the surface of meat cuts, changing the color from purple to bright red or pink, cuts are commonly allowed to bloom for 30 min to obtain the desirable color.

Carboxymyoglobin Carbon monoxide is attached to the iron and it is in the reduced state (Fe^{2+}), bright cherry-red in color.

Case pulls The packages removed from retail case due to discoloration, package failure, or being past the 'sell-by' date.

Case-ready The retail packaged meat products that are cut and packaged in a centralized facility and shipped to stores, ready to be placed directly in retail cases.

Modified atmosphere packaging Retail packaging with an oxygen-impermeable package and head-space that allows for the introduction of a specific atmospheric environment to control spoilage, sometimes termed controlled atmosphere.

Mother bags Large oxygen-impermeable packages in which several case-ready, oxygen-permeable packages can be placed to allow for a controlled atmosphere during shipping and storage.

Myoglobin A protein pigment found in muscle that gives meat its color, contains an iron (Fe) held within a heme ring. The chemical state of the iron largely dictates the color of the meat. Myoglobin may be present in the following forms: cholemyoglobin, hydrogen peroxide is attached to the iron and it is in the oxidized state (Fe^{3+}), brownish-green in color; cyanmetmyoglobin, cyanide is attached to the iron and it is in the oxidized state (Fe^{3+}), red or pink in color; deoxymyoglobin, iron is deoxygenated and in the reduced state (Fe^{2+}), purple in color; metmyoglobin, iron is in the oxidized state (Fe^{3+}), brown in color; oxymyoglobin, oxygen is attached to the iron and it is in the reduced state (Fe^{2+}), red or pink in color; and sulfmyoglobin, sulfide is attached to the iron and it is in the oxidized state (Fe^{3+}), brownish-green in color.

Oxidation The loss of an electron from a molecule, in myoglobin, from the iron (resulting in Fe^{3+}), which results in the brown, metmyoglobin pigment.

Oxygenation The attachment of oxygen to the iron in the heme ring in myoglobin.

Oxygen permeability The amount of oxygen allowed through a packaging film, usually expressed as ml per m^2 per 24 h at a specific temperature.

Psychrotrophs The bacteria that prefer to grow in cold environments.

Reduction The gain of an electron to a molecule, in myoglobin, to the iron (resulting in Fe^{2+}), which results in the red or pink oxymyoglobin or the purple deoxymyoglobin.

Retail cuts The cuts of meat produced from wholesale/primal/subprimal cuts, that are small enough for one serving or one meal. Consumers purchase retail cuts for preparation at home.

Shrink The value of meat lost in the retail case. This may be due to repackaging, lower the price, further processing (trimming, flavoring, or grinding), or completely discarding.

Wholesale/primal/subprimal cuts Large cuts of meat removed from the carcass in a specified manner. Several retail cuts will be produced from each wholesale/primal/subprimal cut. The terms wholesale and primal are synonymous and subprimals are cut from primal. Examples would be a ribeye roll, or a lamb loin.

Introduction

In the US and in many other countries, the majority of retail food stores changed from butcher service to largely self-service meat departments after World War II. Customers selecting individual meat packages from an array of product offerings by meat retailers led to the passing of perceptions from generation to generation about various meat quality attributes. In particular this 'knowledge' meant that bright red color for beef and bright pink for pork became the most important quality factors in choosing fresh-chilled or frozen meat at the self-service counter. Ensuring that the customer gets this desired meat color became a prime objective in presenting meat to the public, and led to overwrapping of meat with plastic film that allowed sufficient oxygen penetration but prevented moisture loss. Presentation of meat with the most acceptable color became all-important in meat merchandising.

This article presents an explanation of the chemical forms of myoglobin, the major pigment of meat, and its change from one form to another, as well as those factors that influence pigment change. Controlling the chemistry of myoglobin, achieved by proper use of overwraps, has a critical influence on acceptable meat color life. Requirements for functional overwrapped packages are also dealt with to help readers understand how to meet customer expectations, as well as the requirements of special plastic films to meet the needs of marketing, including the effects of type of packaging on microbial flora and, thus, its impact on spoilage and safety. Also discussed are management practices that will minimize 'case pulls' and discards. Case pulls are packages that are withdrawn

from the retail case for reworking and repackaging or are discounted in price. These steps impact the financial contribution to overhead and net profits of the meat segment of retail business. Systems to lengthen product life and provide a reasonable display life are also presented.

Meat Pigment Chemistry

A brief explanation of myoglobin chemistry, the major chemical forms of myoglobin and related pigments, and a description of pigment layers will be helpful in understanding the impact of the packaging on the color of fresh, chilled meat. An illustration of the meat color (myoglobin) triangle is provided elsewhere in the encyclopedia. The three major chemical forms of myoglobin in fresh meat include deoxymyoglobin, which contains iron in the reduced or ferrous state (Fe^{2+}) and has a darker purple-red color. Its formation is favored at extremely low oxygen partial pressures (1.4 mm Hg or less) and, when exposed to air or another source of oxygen, it combines very quickly with oxygen to form the bright red or pink color of oxymyoglobin, which also has iron in the ferrous (Fe^{2+}) state but has oxygen attached. Oxymyoglobin is stable at high partial pressures of oxygen but, if relatively low oxygen partial pressure develops, it is vulnerable to oxidation with conversion to brown metmyoglobin and resultant discoloration.

Oxygenation or Blooming

Because of the anaerobic condition of muscle before cutting, the fresh cut surface of meat will briefly present the purplish-red (beef) or purplish-pink (pork) color of deoxymyoglobin. Immediately after cutting, myoglobin at the newly cut surface will begin to react with oxygen of the air to form oxymyoglobin. This reaction is called oxygenation or blooming and proceeds for at least 20–30 min to a point where the meat may be considered sufficiently bright to be placed in display. This surface layer of oxymyoglobin gradually becomes thicker and thus extends deeper from the surface. This change occurs more rapidly at colder chilled temperatures. Although oxygen diffusion into muscle should theoretically be faster at warmer temperatures, such as 4.4 °C (40 °F) versus 0 °C (32 °F), the competition of meat enzymes for oxygen is greater at the higher temperature, which works against oxygen penetration at the warmer temperature; thus, oxygen penetration is greater at the colder temperature. Relatively fresh muscle (that with a good supply of reducing-capacity enzymes) will have two pigment layers: oxymyoglobin toward the surface and deoxymyoglobin at deeper locations. For intact muscles, oxygen diffuses more deeply into the muscle with longer time after initial exposure to air. This increasingly deeper penetration may continue for several days, depending on the microenvironment surrounding the muscle and the status of the reducing capacity of the muscle.

Discoloration

When the reducing mechanisms of the muscle are approaching depletion and the oxygen supply favors neither deoxymyoglobin nor oxymyoglobin, a third layer of pigment, brown metmyoglobin, begins to form between the other two pigment layers. This brown layer becomes thicker with time and moves toward the muscle surface. This brown layer is frequently not uniform in its closeness to the surface, so that spotty, nonuniform discoloration may be apparent at the surface. At some point, both the human eye and reflectance instruments begin to 'see' the brown discoloration. The perception of surface color is not only due to the chemical form of myoglobin immediately at the surface, but is also influenced to a limited degree by subsurface chemical state of myoglobin. Meat with a high degree of light scattering, such as pale, soft, exudative meat, allows less subsurface contribution to perceived color than does meat with less light scattering, such as dark, firm, dry (DFD) muscle.

Consumer Reaction

Some studies report that consumer discrimination begins when 20% of the detected pigment is metmyoglobin. A distinctly brown color is perceived with 40% metmyoglobin, and a brown to gray-greenish color is related to 60% of myoglobin being in the metmyoglobin form. Meat with 20% metmyoglobin is accepted about equally with bright red meat, according to one study; yet, other studies report a linear increase in consumer rejection as the percentage of metmyoglobin increases. Meat cubes, such as used for stew meat, present a greater opportunity for oxygen penetration from the much greater surface area of these small pieces.

Effects of Cutting/Mincing on Meat Color

Intact muscle that has been treated to improve tenderness with mechanical needles, knives, or the rotating knives of a cubing machine also has multiple entry points for oxygen penetration. Although the pigment layers are not clearly defined in these situations, the same principles seem to be involved.

Meat that has been minced, flaked, or made into small particles presents a more complicated case of multiple loci of different chemical forms of myoglobin, because multiple surfaces have been exposed to oxygen. In the extreme case of minced meat, all particles are susceptible to oxygenation during any exposure to oxygen, such as during mincing, but when they are packed into a solid mass for packaging the chemical form of myoglobin at any location in the mass depends on the continuing pattern of oxygen level, which is affected by muscle metabolism, consumption of oxygen, and the availability or depletion of reducing capacity. Often the center of a minced meat mass, given sufficient time, will revert to deoxymyoglobin while the surface continues to be in an oxymyoglobin state. Because so much of meat mass has been exposed to oxygen, and because of the small particle size and the enormous surface area, the process of converting oxymyoglobin to metmyoglobin to deoxymyoglobin may use up enzymatic resources of reducing capacity. A condition is quickly reached in which the metmyoglobin reducing ability is depleted, and some of the ground meat will revert to the oxidized metmyoglobin state. This leads to brown pigmentation becoming evident at locations where conditions favor the oxidized chemical state of myoglobin.

Other Color Effects

When fresh meat surfaces are exposed to carbon monoxide (CO), a very stable, bright cherry-red pigment is formed, known as carboxymyglobin. Even with very low concentrations of CO, the carboxymyoglobin pigment will form and is much more stable than oxymyoglobin. Green discoloration in fresh meat has been attributed to altered heme structure, possibly from choleglobin or sulfmyoglobin forms of myoglobin. Choleglobin results from the reaction of myoglobin with hydrogen peroxide, which may have been produced by microbial metabolism or chemical reactions. Sulfmyoglobin results from the reaction between hydrogen sulfide and myoglobin. Hydrogen sulfide can result from microbial metabolism or sometimes from chemical residues from certain treatments of carcasses or meat cuts.

Requirements for Overwrapped Packages

Customers have expectations of safety and quality both for the package and for the meat within the package. The package should have a solid backing so that it can withstand the frequent and occasionally abusive handling by customers. On at least one side it should be transparent to provide for a viewing of the product. Antifog or other anticondensation properties are also desirable. A leaky, messy package is very undesirable, as customers will not accept meat juice dripping on their clothes, in their automobile, or in their kitchens or refrigerators. Bacteria associated with raw meat may contaminate kitchen surfaces and cause infection or illness. Packages should also be resistant to puncture, whether from sharp edges of bone inside the package or from any sharp or pointed object outside the package, as puncture can lead to leaking or to dehydration of meat in the package. The integrity of the seal is also important.

The package must provide the proper internal microenvironment to ensure a muscle color that is acceptable to the consumer, and also acceptable color of fat and bone that may be part of a cut. Providing meat with expected color is critical, as this is used by the customer to determine freshness and acceptable quality. For overwrapped meat cuts, this requires an acceptably bright red (beef or lamb) or pink (pork) muscle color and an absence of brown, green, or dehydrated appearance on the meat surface. Fat color is expected to be white with perhaps a slight red or pinkish cast, whereas yellow, green, tan, or brown fat colors are undesirable. Bone color is expected to be white to gray, with possibly a reddish cast; yet, tan to black or green are undesirable bone colors.

Because much of the overwrapping packaging is done by machine, important factors in film selection are its suitability for use on machines, as well as optical clarity, sealability, and cost.

Types of Trays

Meat is customarily presented for retail sale on trays of rigid or expanded plastic, frequently with a soaker pad to absorb liquid (absorbent area of the pad not against the meat), and overwrapped with a clear film that is usually sealed by heat. Paper pulp trays were used at one time but have been replaced by expanded polystyrene, oriented polypropylene, or clear polystyrene, which have the advantages of not sticking to meat and not absorbing moisture; thereby becoming weak and less sanitary. The drip pad is likely to contain absorbent cellulose, with the side toward the meat being the nonabsorbent layer that keeps the pad from sticking to the meat. Recently, companies have been developing renewable foam trays for meat packaging, such as the NatureTRAY™ from Cryovac, which is made from a biopolymer that is compostable. Absorbent pads are also available consisting of renewable and biodegradable materials. The properties of these trays and pads are similar to their polystyrene counterparts, but the use of these more environmentally-friendly packaging materials is limited due to their higher cost.

Films Used

The first film used was cellophane, which was coated with nitrocellulose on one side for permeability to oxygen but impermeability to moisture. Pliofilm was next introduced, but its use has given way to plasticized polyvinyl chloride, which is the major overwrap film used in the US. In a stretch form, it may be approximately 0.017 mm (0.000 7 in.) thick and the high degree of plasticization provides excellent oxygen permeability, very good conformation to the outer contour of meat pieces, and superior gloss and clarity. Polyvinyl chloride should have a low moisture transmission rate and an oxygen permeability of at least 5000 ml m^{-2} per 24 h at 23 °C, but many films have oxygen transmission of up to 10 000. Low-density polyethylene is used in many countries at thicknesses of 0.025 4 mm (0.001 in.) and has sufficient oxygen permeability if no thicker than this. Modifying it with vinyl acetate increases oxygen permeability. It is a suitable barrier to moisture but needs treatment to overcome surface condensation. It shows somewhat marginal clarity and gloss.

Duration of Acceptable Retail Display

The major disadvantage of packaging by overwrapping is the relatively short time before meat becomes unsalable. With suitably low temperatures, overwrapped meat may be microbiologically acceptable for a week, but its display life is limited by appearance to perhaps 3–5 days at the very longest for intact muscle cuts. Many stores withdraw discolored or otherwise unsalable cuts, but may also have a 'case pull' policy at 2 or 3 days. Ground meat has a much shorter display life, and some stores may have a 1 day or even shorter 'pull' policy. Many stores prepare ground meat several times a day. Stores can also have a set time at which packages are price discounted, with the purpose of selling at a lower price rather than discarding them.

Problems Encountered with Overwrap

Overwrapped packages are not well suited for taking home and placing in a freezer, at least for not more than a few days. The meat and fat are vulnerable to oxidation, with rapid development of rancid aroma and taste. Meat in these packages may also develop 'freezer burn,' a brown dried-out condition,

because of the suboptimal protection provided by overwrap films in freezer conditions, especially with temperature fluctuations in the freezer.

Other Purchase Considerations

When purchasing meat, customers have numerous other considerations that go beyond the influence of the packaging on the perceived quality of the meat. These might include the number of chops or steaks per package or the size of a roast. Closeness of fat trim is important at a time when many customers are 'fat conscious.' The cost of packages is definitely considered. Market managers are faced with the challenge of meeting these varied customer expectations, but are also limited in the number of options that can be presented to the customer.

Microbial Effects

Microorganisms that cause spoilage or foodborne diseases are those that grow best under the conditions of packaging and the microenvironment surrounding the packaged product. With overwrapping, the high-oxygen permeability of the film strongly favors aerobic bacteria and, because meat is usually kept reasonably cold, psychrotrophs are also favored. Thus, the predominant spoilage organisms are the strictly aerobic pseudomonads, which can utilize the highly available glucose but, because the amount of glucose is limited, can also use amino acids for their nutrition. Metabolism of amino acids leads to putrid odors and flavors typical of this kind of spoilage. For DFD product, metabolism of amino acids occurs earlier and there is thus an early indication of spoilage. These organisms compete very aggressively, so that foodborne pathogens are less likely to grow in such an environment.

Management Practices and Systems to Enhance Overwrapping

Three keys words of advice for meat retailing have long been the three 'keeps': keep it clean, keep it cold, and keep it moving. Keeping the product clean should mean that there will be fewer microorganisms to multiply and cause discoloration or other deterioration and pose food safety problems. Keeping the product cold is critical in slowing microbial growth and maintaining acceptable quality. Keeping it moving reflects the importance of products being sold and consumed before the occurrence of any discoloration, other spoilage, or foodborne disease problems. Inventory control, or selling product in the order it was received at the store (first-in and first-out), is an important principle in managing a meat retail operation. The first two 'keeps' contribute to the strategy of delaying the onset of discoloration so that the product may be sold at full value instead of at a discounted price or being reworked, trimmed and repackaged, or perhaps discarded.

Retail Losses

Loss of saleable product is called shrink, and it has a definite impact on the net profit of the meat operation. In a 2008 estimate, 5% of value was lost to waste at the retail level. Retail loss (shrink) estimates are scarce in the literature and difficult to obtain, but one study reported 3.1% and 4.6% case pulls for beef retail packages in two different stores. It further cited the disposition of pulled packages from three stores as follows: converted (probably ground) and repackaged (4.1%), trimmed and repackaged (2.9%), rewrapped (2.9%), price reduced for quick sale (0.6%), and discarded (0.2%). Each of these steps results in an added cost or a loss in product value. In a survey of 10 large supermarkets in the US, another study reported shrink, defined as losses due to reduced value, to be 6% for beef cuts compared to 4% for overall market operations. However, when summarized, these losses were as high as 23% for one high-value beef cut, and 19 retail beef cuts exceeded 10% loss. The market manager would like to display a good selection of high-priced cuts for customer consideration, but these cuts also have a higher risk of shrink.

Historically, the lowest shrink at local retail stores over a number of years in this author's experience was represented by a retail company that, surprisingly, had a central retail cutting plant that delivered overwrapped meat to stores located as far as 128 km from the central cutting plant. This company developed and maintained an outstanding program of sanitation and cold temperatures and the bottom line was a shrink (loss of value) less than one-half that of other stores in the area. A temperature of −2.2 to −1.1 °C (28–30 °F) was consistently maintained in the holding coolers, the cutting and packaging room, the shipment assembly area, and on the delivery trucks. On delivery to local stores, the first priority was to immediately place the containers of retail packages in the chill room, without lingering on the loading dock. The company also operated another management practice extremely well, as they kept detailed computerized records of packages delivered to stores on each delivery and thus, had excellent information about likely anticipated needs for future deliveries. These practices are a good example for any store or meat market to follow.

Large processing companies adopted this practice in the late 1990s and early 2000s with the case-ready retail products packaged in high-oxygen, modified atmosphere packages, delivered directly to the store. Quality issues with the high-oxygen packaging caused processors and retailers to move back to a more traditional, aerobic overwrap package. This product was still cut and packaged at a central, case-ready facility, but the packages are stored and transported to retail stores in mother bags containing a mixture of nitrogen (N), carbon dioxide (CO_2), and a small percentage of CO. The growth of spoilage and pathogenic bacteria is controlled by the high levels of CO_2, whereas the carboxymyoglobin formed due to the exposure of the cut surfaces to the CO will allow for a stable, bright cherry-red meat color. According to the 2007 National Meat Case study, 64% of the total fresh meat case was made up of case-ready products and it is likely that those percentages have increased in the years since.

Cutting and Display in Local Outlets

Individual stores that prepare their overwrapped retail cuts in their back room can also use records to improve their

efficiency, but they need to recognize that the sale of any retail cut can be influenced by price, by whether a 'special' is being offered on that cut, and by a holiday period or time of year that can affect sales of any particular cut. Certain times of year lend themselves to promotion of certain cuts, such as the summer outdoor grilling season. Good market management involves the use of good sales records, but it also involves a perceptive sense of judgment about customer demand. If a cut is not available in the display case when a customer would like to buy it, it takes several minutes to unwrap a subprimal or wholesale cut, make retail cuts and package them in an overwrap package. A market would always like to have available on display a selection of cuts that meets the needs of its customers and would not like to have a costly 'out-of-stock' situation or make customers wait until their choice is available. Good records can help in anticipating sales and providing a reasonable assortment of packages without 'overcutting the case' (having too much of a given product on display) and perhaps having packages become reduced in value or unsalable.

An analogy regarding product life is that the 'alarm clock' which begins counting down to the end of product life immediately postmortem, or at the time the animal source of meat is stunned and bled. This clock can be slowed by cold temperature and good handling practices and sped up by warm temperature and inappropriate handling at critical points of product preparation and distribution. When a wholesale, or subprimal, cut is fabricated into retail cuts, the clock runs faster. When retail cuts are placed under display lighting, the clock runs much faster owing to the photochemical and sometimes warming effects of lighting. This adds up to the importance of planning the cutting, packaging, and display so that meat cuts will have a minimum time in the conditions in which they are most vulnerable to developing discoloration and other deterioration. In other words, the clock is always ticking toward a point at which product history adds up to discoloration and the alarm goes off. With good management, deterioration can be slowed so that the meat can be sold and consumed well before that point is reached. The case-ready system allows meat to be processed and cut in the highly-controlled and sanitized conditions of a processing plant. Furthermore, the mother-bag packaging and controlled atmosphere inhibits the growth of microorganisms and allows for the formation of a very stable, red color that consumers will choose to purchase.

Improving Overwrap Systems

Some very effective systems are in place that will make the sale of overwrapped cuts more profitable and will reduce shrink. Early practices, such as wrapping pork loins or other fresh uncured cuts in parchment paper and putting them in a box with exposure to air, have been partially replaced by bulk gas flushing, with perhaps five pork loins placed in a large barrier bag inside a box, with air withdrawn and replaced, usually with a mixture of CO_2 and N, before the bag is sealed. These bulk gas-packed loins maintain quality and food safety requirements much better and longer than those wrapped in parchment. As an example, stores promoting a 'red hot' special on pork loins might get part of their loin shipment packaged aerobically and cut that first, but have gas-packed loins as a hedge when sale of loin cuts might be unpredictable. The fact that gas packaging will maintain acceptable quality longer allows the product to be kept for the possible business the following week.

Commercial Packaging Situations

Some processors are experimenting with cutting bone-in and boneless pork loins in a central processing facility, reassembling and packaging these loins in a vacuum bag, and shipping them to a retail facility. At retail, the chops are removed and repackaged in an overwrap system. This system reduces the need for knives and saws at the retail store and improves the safety for store workers. Furthermore, the sanitary conditions at the cutting facility would be improved, greatly reducing bacterial contamination.

One rather comprehensive study done under commercial cutting and packaging conditions used a single gas flush of 0.61 l of CO_2 per kilogram of meat and storage for up to 19 days before cutting pork loin chops and overwrapping them for display. The initial CO_2 concentration of 78.5% of the within-bag atmosphere decreased to 55.1% by day 3 and to 41% by day 19, partially due to CO_2 absorption into the meat. Oxygen, which was 3.1% initially, increased to 7.1% during the storage. The increase of oxygen was partially due to oxygen that had been absorbed by the meat before packaging later coming out of the meat into the within-package atmosphere and partially due to ingress of oxygen through the large area of the bag. Although microbial counts increased from day 3 to day 19, they were still less than 5 $\log_{10}$ colony-forming units per square centimeter surface area. With longer storage, loin weight loss and discoloration increased. At day 19, off-odor was acceptable but discoloration at some locations was the limiting factor in shelf life. Loin eye (longissimus) color for chops cut and overwrapped in polyvinyl chloride and placed on a soaker pad on a styrofoam tray was acceptable through 3 days of display after the 19-day storage time. The psoas major (tenderloin) still had acceptable visual color for two display days when it had been stored in the gas pack for 10 days, and muscles from the sirloin end of the loin had acceptable color for 2 days of display if the loins had been stored in gas-pack for up to 11 days. The color stability of the psoas major was the limiting factor of pork chops in this study.

Another pork loin study compared gas mixes of 100% CO_2, 75% CO_2–25% N, 50% each of CO_2 and N, 25% CO_2–65% N–10% oxygen, and another sample stored in a vacuum package. Gas packages were triple-flushed using a Corr-Vac Mark III packager (M-TEK Inc., Elgin, IL, USA). Loin sections were stored at 1.1 °C (34 °F) for either 14 or 22 days, after which chops were cut, placed on soaker pads on polystyrene trays and overwrapped with polyvinyl chloride film, and displayed at 2.7 °C (37 °F) under 1076 lx (100 foot-candles) of warm white fluorescent (3000 K) display lighting. Oxygen concentration in bags flushed with CO_2 and N was very low (0.1%–0.4 %). The gas mix containing 10% oxygen resulted in chops with more graying and greening, stronger off-odors, and higher psychrotrophic microbial counts by over 1 $\log_{10}$. Drip loss from loins stored in the 100% CO_2 was higher than from loins receiving other treatments. The display life of chops was similar for all treatments, except shorter for those stored in

the gas mix containing 10% oxygen. Another study found no advantage for triple-flush over single-flush gas-pack for display life of overwrapped chops. This study also compared gas-to-meat ratios of 0.7:1, 1.3:1, and 3.0:1 for gas-pack pork loins that were cut into chops and displayed, and found no advantage for any particular ratio.

Other Packaging Systems

Other gas-pack systems are used to deliver wholesale cuts to retail stores where retail cuts are prepared and overwrapped. These include the CAP-TECH system used to deliver lamb carcasses and wholesale cuts, packaged in high CO_2 atmospheres, many thousands of miles to their destination. Compared to vacuum packaging, the CAP-TECH system extended the shelf life of fresh lamb from 12 to 30 weeks.

Vacuum-packaged distribution is used less for pork, perhaps because the pressure results in large amounts of purge. Vacuum packaging is widely used for beef subprimal cuts that are placed in a box, and hence called 'boxed beef', for distribution to retail stores where retail cuts are prepared and overwrapped for display. Boxed beef has been enormously successful as a part of the system that markets overwrapped retail cuts to the final consumers. Vacuum packaging is discussed in another part of this encyclopedia.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment. Packaging: Equipment; Modified and Controlled Atmosphere; Technology and Films; Vacuum

Further Reading

Gill, C.O., 1996. Extending the storage life of raw chilled meats. Meat Science 43, S99–S109.

Harte, B.R., 1987. Packaging of restructured meats. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research, vol. 3. New York: Van Nostrand Reinhold, pp. 433–458.

Hotchkiss, J.H., 1994. Packaging muscle foods. In: Kinsman, D.M., Kolula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods: Meat, Poultry and Seafood Technology. New York: Chapman and Hall, pp. 475–485 (chapter 18).

Kropf, D.H., 1980. Effects of retail display conditions on meat color. Proceedings of the 33rd Reciprocal Meat Conference, pp. 15–32. Savoy, IL: American Meat Science Associations.

Kropf, D.H., 1993. Colour stability: Factor affecting the colour of fresh meat. Meat Focus International 269–275.

Lundquist, B.R., 1987. Protective packaging of meat and meat products. In: Price, J. F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products. Westport, CT: Food and Nutrition Press, pp. 487–505.

Renerre, M., Labadie, J., 1993. Fresh red meat packaging and meat quality. Proceedings of the 39th International Congress of Meat Science and Technology, pp. 361–387, Session 8.1. Calgary.

Rizvi, S.S.H., 1984. Flexible packaging material properties that influence shelf life. Proceedings of the Meat Industry Research Conference, pp. 125–141. Savoy, IL: American Meat Science Association.

Sacharow, S., 1974. A functional review of fresh meat wraps. Food Engineering March, 87–90.

Sebranek, J.G., 1985. Stabilizing the properties of meat products with packaging systems. Proceedings of the Meat Industry Research Conference, pp. 150–167. Savoy, IL: American Meat Science Association.

Sorheim, O., Kropf, D.H., Hunt, M.C., Karwoski, M.T., Warren, K.E., 1996. Effects of modified gas atmosphere packaging on pork loin colour, display life and drip loss. Meat Science 43, 203–212.

Taylor, A.A., 1982. Retail packaging systems for fresh meat. Proceedings of the International Symposium on Meat Science and Technology, pp. 353–366. Savoy, IL: American Meat Science Association.

Warren, K.E., Hunt, M.C., Marksberry, C.L., *et al.*, 1992. Modified atmosphere packaging of bone-in pork loins. Journal of Muscle Foods 3, 283–300.

Technology and Films

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Glossary

Active packaging Systems that actively alter the meat products' environment to improve the shelf life of the product. Examples include gas scavengers, temperature control and monitoring, or antimicrobial properties.
Barrier property A measure of a packaging material's permeability, whether a gas, moisture vapor, or a sensory trait such as aroma.
Blooming Exposing fresh meat surfaces to air to allow the oxygenation of myoglobin on the surface of meat cuts, changing the color from purple to bright red or pink; cuts are commonly allowed to bloom for 30 min to obtain the desirable color.
Modified-atmosphere packaging (MAP) Retail packaging with an oxygen-impermeable package and a head-space that allows for the introduction of a specific atmospheric environment to control spoilage; sometimes termed controlled atmosphere.
Myoglobin The protein pigment found in muscle that gives meat its color, contains an iron (Fe) held within a heme ring. The chemical state of the iron largely dictates the color of the meat. Myoglobin may be present in the following forms: Oxymyoglobin, oxygen is attached to the iron and it is in the reduced state (Fe^{2+}), red or pink in color; Deoxymyoglobin, iron is deoxygenated and in the reduced state (Fe^{2+}), purple in color; and Metmyoglobin, iron is in the oxidized state (Fe^{3+}), brown in color.
Overwrap packaging Retail cuts are placed in foam trays with a soaker pad and the product and tray are incased in a clear, flexible, oxygen-permeable film allowing exposure to ambient air.
Oxidation The loss of an electron from a molecule, in myoglobin, from the iron (resulting in Fe^{3+}), which results in the brown, metmyoglobin pigment.
Oxygenation The attachment of oxygen to the iron in the heme ring in myoglobin.
Oxygen permeability The amount of oxygen allowed through a packaging film, usually expressed as ml m^{-2} per 24 h at a specific temperature.
Polymers A material containing a large number of repeating units (monomers) bonded together to make up a large part of the molecule. Polymers may be one of three types: homopolymers, made with one type of monomer (e.g., polyethylene and polyvinylchloride); copolymer, made with more than one type of monomer (e.g., polyvinylidene dichloride (saran)); and polyblend, made with two or more different types of polymer chains.
Reduction The gain of an electron to a molecule, in myoglobin, to the iron (resulting in Fe^{2+}), which results in the red or pink oxymyoglobin or the purple deoxymyoglobin.
Thermoformed packaging Products are placed into heat-softened films, formed into a desired shape, and sealed with a nonforming layer over the top of the formed film. The intra-package environment may be vacuum or gas-flushed.
Vacuum packaging Retail or wholesale cuts are placed in oxygen-impenetrable bags, all air is removed from the bag, and the bag is sealed.

Introduction

Packaging can be defined as the enclosure of products or items in any containers, such as bags, bottles, boxes, cans, pouches, trays, tubes or wraps. Packages contain the product and assemble it into units for storage, distribution, and offering for sale; and, in an increasing number of cases, they are used as a cooking utensil. Package sizes vary from single-serving units to very large units for distribution or transport. The primary goal of packaging is to maintain the product at a suitable quality level, frequently defined as freshness, as well as assure the safety of the product to the consumer at the point of ultimate consumption.

This article deals with functions of packages, including special properties and factors that influence these properties. It also deals with the chemistry of films and how this influences the many characteristics of packages, as well as discuss traits of individual polymers and deal with multilayer films. However, applications of packaging for meat products, including overwrapping, vacuum packaging, and modified-atmosphere packaging (MAP) will be covered in detail in other articles. Steps of the packaging process will be presented for thermoformed meat packages, and some elements of active packaging will also be discussed.

Functions of the Package

Protective Functions and Labels

A major function of the package is to serve as protection from environmental variables, which include temperature, moisture or humidity, oxygen, airborne particles, and light. It must also protect against biological contamination, such as that from humans, microorganisms, rodents, insects, and other pests. A package further serves as a marketing tool because it must provide space for a label that carries information, such as product identification, ingredients, nutritional information, net weight, verification of inspection, cooking instructions,

promotional material, company name and location, and instructions on how to contact the processor (such as a 'hot line' contact number). Labels today frequently display the ubiquitous universal product code, which is critical to laser scanning at the check-out counter in virtually all stores and also plays an important role in inventory management.

The package must protect against loss or gain of moisture and regulate permeation of gases such as oxygen, carbon dioxide, and nitrogen. This allows the package to function in managing meat color and appearance by minimizing oxidation, which can influence color, odor, flavor, and safety, and also provide the environment to discourage microbial growth, which in turn delays the occurrence of spoilage and lessens the impact of foodborne pathogens.

A critical consideration in designing a packaging system is to quantify the rate of change of the most limiting quality or safety factor, and develop requirements for the package so as to minimize change in this most critical characteristic so that the product's shelf life might be extended. The package cannot improve or completely stabilize the product quality, but it can only slow down quality loss. Chemists or packaging engineers can design and build films with marvelous properties, but some alternatives are costly and may not be economically justified. A major consideration is product's shelf life, especially in the light of long distances for distribution of product to the ultimate consumers. These economies must be weighed against the costs of improved packages.

Other Package Requirements

Considerations in selecting and designing a package include strength factors, which include tensile, elongation and tear (both initial and subsequent) strength, and resistance to puncture, all of which should be considered at all temperature and humidity conditions expected. The shelf life of the film itself is important. Permeability to gas, moisture, grease, and odor are important. Machinability factors include stiffness (flowability), static accumulation, and slip (which affects the ability of people to open bags); and merchandising factors not only include stiffness and static accumulation but also clarity or transparency, gloss (sparkle), absence of an undesired film color, and antifogging characteristics. The most widely considered factor, or the bottom-line trait, is the cost! Obviously, cost is much more than film cost and includes equipment costs and labor involvement or savings. Losses due to the consequences for the product of package failure must also be strongly considered in determining cost.

Barrier Properties

The barrier property of a packaging material is a measure of the resistance offered to a permeant, irrespective of it being gas like oxygen, carbon dioxide, nitrogen, or carbon monoxide; or moisture vapor; or a sensory trait such as aroma. This information is a very important part of a film or package specification. Oxygen permeability is usually expressed as cm^3 per 24 h m^{-2} at 0% relative humidity. Sometimes the film area used in the specification is 100 $in.^2$: an area of 1 m^2 is 15.5 times as large as 100 $in.^2$; so, one must be very careful in reading specifications. For some purposes, it can be critical if the permeability given as 3 cm^3 for the smaller area of 100 $in.^2$ is mistakenly taken to refer to 1 m^2; 3 cm^3 per 100 $in.^2$ corresponds to 46 cm^3 m^{-2}. Oxygen and moisture transmission values for some films are given in Table 1.

Factors Affecting Permeability

Permeation decreases as the thickness and density of the film and cross-linking and crystallinity of the polymer increase, whereas it increases as the permeant solubility in the polymer, pressure of the permeant, temperature, amount of plasticizers and fillers, and affinity for water decrease. Depending on their affinity for water, the relative humidity of the environment affects polymeric materials differently. Hydrophilic films, such as nylon and cellophane, have increased permeability at higher humidity, whereas hydrophobic films, such as polyethylene and polyvinylidene chloride, are not affected by humidity. The permeability to carbon dioxide may be 2–10 times that to oxygen for some polymers.

Other Important Properties

Other important properties of films include flowability, machinability, printability, stability over a wide temperature

Table 1 Typical oxygen and water vapor permeability transmission values for some film

Film	*Oxygen permeability (cm^2 per 24 h, 22.8 °C (73 °F), 0% RH)*		*Water vapor transmission (g per 24 h, 37.8 °C (100 °F), 90% RH)*	
	m^{-2}	*100 $in.^{-2}$*	*m^{-2}*	*100 $in.^{-2}$*
Ethylene vinyl alcohol	1.55	0.1	–	–
Polyvinylidene dichloride (Saran) coating	7.75–15.5	0.5–1.0	13.95	0.9
Nylon (0.0254 mm; 0.001 in.)	31–46.5	2–3	–	High
Polyester (0.0254 mm; 0.001 in.)	139.5	9	43.4	2.8
Oriented polypropylene (0.0190 mm; 0.00075 in.)	3100	200	–	0.4
Surlyn (0.0508 mm; 0.002 in.)	4650	300	10.85–17.05	0.7–1.1
Polyethylene (EVA) (0.0508 mm; 0.002 in.)	–	–	7.75–10.85	0.5–0.7

Abbreviation: RH, relative humidity.

Source: Modified from Gehrke, W.H., 1983. Film properties required for thermoformed and thermal processed meat packages. Proceedings of the 36th Reciprocal Meat Conference, pp. 55–59. Savoy, IL: American Meat Science Association.

range, shrinkability, thermoformability, hot tack, and seal strength. Visual traits, such as clarity, sparkle, absence of color, and antifog (minimizing of condensation on the inside of the package), can be very desirable. Some marketing options include packages with zip openers and resealable packages, such as luncheon meat packaging, with resticking properties that allow it to be reopened and sealed multiple times.

Film Chemistry

Polymer Chemistry

Plastic film materials are made up of a large number of small repeating units (monomers) bonded together to form a polymer, usually making up a large part of the molecule. Polymers may be classified as (1) homopolymers, which are made up of only one type of monomer in the chain; (2) copolymers, which contain more than one type of monomer in the chain; and (3) polyblends, which contain two or more different types of polymer chains. These materials may be further classified according to the backbone architecture as linear (containing a single backbone chain), branched (with regularly occurring branches), and cross-linked or networked (where the backbone chains are interconnected to form a single molecule). Polymers formed by addition reactions, in which one monomer is added to another without the formation of any by-product, include vinyl and diene compounds, whereas some important polymers, such as polyesters, polyamides (nylon), and polyurethanes, are formed by condensation reactions, which means that they are built from monomers with functional groups (such as –COOH, C–OH, and $–NH_2$), with small molecule by-products also being formed in these reactions.

Polyethylenes and Copolymers

Polyethylenes, the most abundantly produced plastics, are polymerized from the monomer ethylene (C_2H_4) and can be produced as low-, intermediate-, or high-density polyethylenes. The higher density forms have more crystalline regions and exhibit greater tensile strength, stiffness, and barrier capabilities, but decreased impact and tear resistance. High density also contributes better heat and grease resistance and improved compatibility with acids and alkalis; however, lower density confers greater clarity and low-density forms can form a strong seal at lower temperatures. These are relatively inexpensive plastic materials. An additional modification that results in linear low-density polyethylene imparts greater tensile and impact strength and elongation and puncture resistance than that found in other polyethylene films.

'Polyethylene copolymer with ethylene vinyl acetate' is polyethylene copolymerized with 5–20% ethylene vinyl acetate, which lowers the sealing temperature and improves seal performance, especially when lower temperatures are used in sealing; thus, it is often used in the food-contact layer. With a higher proportion of vinyl acetate hot tack, impact resistance, adhesion, low-temperature toughness, and stress crack resistance are improved, whereas stiffness, seal temperature, chemical resistance, and barrier properties are decreased.

Ethylene Vinyl Alcohol

Ethylene vinyl alcohol, a crystalline copolymer produced by saponification of vinyl acetate, vinyl alcohol, and ethylene, has high barrier characteristics but is moisture sensitive. It has greater oxygen permeability at high humidity and is frequently used in multilayer films, where it can be protected from moisture influences by the other film layers or by drying agents added to the laminate structure. This film can also be produced with a biaxial orientation to reduce moisture sensitivity. This plastic works well for packages for hot-fill products and is widely used for meat packages needing a good oxygen barrier.

Polyvinyl Chloride

Polyvinyl chloride (PVC), polymerized from vinyl chloride monomer, has very high oxygen permeability and excellent transparency and, if plasticized, is very flexible and a good heat sealer. This is the film of choice in the US for overwrapping of chilled fresh meat cuts that are sold in the bright red (beef) or pink (pork) condition, but product packaged in this film has relatively short display life.

Saran

Polyvinylidene dichloride (Saran) is produced by polymerization of polyvinyl chloride and polyvinylidene chloride, and is a dense, highly crystalline material that is a very good barrier to oxygen and moisture, is resistant to grease, and is a tough, abrasion-resistant film with good clarity. It is frequently used in meat packaging as a layer in multilayer pouches, bags, chubs, and thermoformed packages. It is most often used for modified-atmosphere applications.

Ionomers

Ionomers are sodium or zinc salts of ethylene/acrylic acid polymers and contain low levels of covalently bound ions. Ionomers are frequently used in laminate structures to give adhesion to aluminum foil. They are often used as the inside or meat-contact layer because of their wide heat-sealing temperature range and excellent hot tack. Ionomers work well in high-speed operations and provide good seal integrity under these conditions. They also have good grease and chemical resistance.

Polyesters

Polyesters are produced by the condensation of ethylene glycol and teraphthalic acid. They are widely used in food packaging because of their superior mechanical properties, good barrier to gases, resistance to high temperatures, and maintenance of excellent tensile strength over a wide temperature range, as well as having good clarity and resistance to many chemicals. Polyesters accept ink well for printing and also adhesives for lamination. They work in heat-shrinkable films, but are not readily heat-sealable if uncoated. Some processors have started using polyesters for thermo-formed cook-in-bag retail items.

Nylons

As films, nylon polymers, manufactured as a condensation product of a diamine and a diacid, possess good oxygen and aroma barrier properties. They are tough and abrasion-resistant, easily thermoformed, heat-resistant, and resistant to most chemicals, except for acids. They are usually part of a laminate structure with polyolefins to give moisture resistance and heat sealability. Nylons are widely used for vacuum packages for beef subprimal cuts, blister packs, and 'cook-in-the-bag' products because of their toughness and heat resistance.

Polypropylenes

Polypropylenes, polymerized from propylene monomer, have a wide range of uses, as they can be copolymerized, coextruded, surface-coated, and modified to give heat sealability. Their properties of good moisture barrier, resistance to high temperature, grease compatibility, stiffness, and clarity are appropriate for use in preparing cook-in steam-processed products.

Acrylonitriles

Acrylonitriles, when converted into film, have good gas barrier properties, fairly high tensile properties, and good chemical resistance. The major use is in thermoformed plastic containers because of their rigidity.

Polystyrene

Polystyrene is used primarily to make thermoformed trays, both clear and foam, for overwrapped meat products. Clear, thermoformed polystyrene trays have high oxygen permeability, excellent clarity, good stiffness and tensile strength, and are resistant to both acids and alkalis. Their special property is adequate melt strength for processing into foam trays.

Special Film Properties

Plasticizers are added to film resins to make the final package more flexible at the temperature of use. Stabilizers are used to prevent degradation of plastics when they are exposed to heat and light, either during processing or during the commercial life of the film. Light degradation can lead to a rapid loss of mechanical properties. Antioxidants can minimize these light effects and also rancidity of fat, which may be absorbed from the product into the film. Release agents help to overcome the sticky or tacky nature of films and make them easier to work with. Pigments are sometimes added directly to film resins and inks are used in printing directly onto packages.

Multilayer Films

Very few polymers have all the properties desired in a packaging film, regardless of the product or packaging system. However, combinations that have appropriate properties can be built using layers of different films that can be produced in thicknesses commonly ranging from 0.0254 mm (0.001 in.) to 0.3054 mm (0.012 in.). Film materials may be reinforced, coated, coextruded, laminated, and printed before they are formed into packages.

Coating

Coating may be done with a variety of materials, such as hot melt, waxes, emulsions, or solvent coatings, which improve one or more of the properties of barrier, scuff resistance, printability, adhesion, or sealability. The term coating implies applying a relatively thin layer to a much thicker base structure.

Lamination

Lamination is often preferred to achieve functional traits for many purposes. When base structures of different polymers or materials are combined, barrier or mechanical properties, sealability, printability, and machinability can be improved. An adhesive material may be placed between two base structures to form a single material. Additional layers may be added using adhesive for each new layer. Adhesive lamination, especially with solvent-borne adhesives, is decreasing in use because of health and pollution concerns. However, water-based and high-solids adhesives are increasing in use. In extrusion lamination, a polymeric resin is melted and extruded as a thin film between two base materials. This process uses less solvent material.

Coextrusion

In coextrusion, multiple extruders are connected to a single die. Molten resin from each extruder is forced through the die simultaneously with material from one or more extruders, sometimes as many as seven. The heat of the process causes the surfaces of the individual layers to interact and bond on cooling. Coextrusion is preferred for combining layers, provided they bond with each other.

Film Layer Arrangement

The arrangement of layers from the outside to the inside (next to the product) should use selection criteria of scuff/abrasion resistance and printability traits for the outer layer, barrier properties and mechanical structure in the middle, and heat sealability and compatibility with the layer adjacent to the product. Bags can be manufactured by extruding as a tube, cutting into appropriate lengths, and heat sealing one end of each bag. Pouches and bags are very similar, with a three-side seal sometimes being used. Adaptability to a high-speed operation must be considered, with filling and sealing being critical steps.

Sealing

Sealing is important, both in forming the empty package and in sealing with the product inside. The goal is a hermetic seal, which consists of a fusion weld of compatible thermoplastic materials produced by thermal heat sealing using hot bars or

hot wires or by thermal impulse sealing. Thermal impulse sealing controls thermal input by pulsing electric current. With impulse sealers, the materials can be cooled while still under jaw pressure, but this also means that impulse sealing is normally slower. The success of sealing is affected by the melt temperature of the material to be sealed, by the jaw pressure that forces the sealing layers into close contact, and by the dwell time (the time the material is heated under pressure). Too much jaw pressure can result in undesired thinning of films at the seal.

Three materials often used as the heat-sealing layer are polyethylenes (all density levels), ethylene vinyl acetate, and ionomers. More dense polyethylene has a higher melt temperature and, thus, requires more heat for sealing. Ethylene vinyl acetate requires less sealing time and energy than polyethylenes to produce an acceptable seal. Ionomers weld together well, even in the presence of contaminating or interfering materials, and also form strong seals at somewhat reduced sealing temperatures. Their exceptional hot tack allows packages to be filled and sealed at high speeds.

Thermoforming

Thermoforming is the forming of heat-softened films into a desired shape into which product may be placed before sealing a nonforming film layer over the top of the formed film. The process is done by equipment that performs the following steps: (1) heats the forming film by a top heater, single or double preheating, or sandwich (both sides) heating, or a combination of heating methods, (2) forms into the cavity of a die into the desired size and shape, (3) provides a loading area where the product can be placed into cavities either mechanically or by operatives, (4) brings the top layer (usually nonforming film, although it could be another formed film) in place over the formed film, (5) removes air from the inside the package either by pulling a vacuum or by gas flush, (6) introduces a gas or gas mixture into the package, (7) hermetically seals the package with a heat-seal bar that welds the films together under pressure, and (8) cuts or trims the excess film.

Forming can be negative, which means the heated film is pulled into the mold by vacuum or pressed-in by compressed air. Forming to too great a depth may result in overthinning of the film so that the desired barrier properties may be lost. Positive forming, used only for rigid films, uses a male-forming plug, and thickness is precisely controlled and detailed shapes can be formed on the bottom or walls.

Gases introduced into the package are often mixtures. These can be purchased premixed, or proper proportions from individual gas tanks can be accurately controlled with a gas mixer. Special problems may be encountered in evacuation of product with a high liquid content or a partly liquid product, which may bubble up. A longer pouch may be the solution to this problem, or steam flushing (an evacuation system) can solve the problem of boiling or bubbles associated with packaging of ready meals or other hot precooked products in either vacuum or modified-atmosphere packages.

Printing and labeling are important 'late in the process' steps in the packaging process. Personnel who assemble and pack boxes are responsible for detecting leaker packages.

Forming films often consist of nylon/barrier (often Saran)/sealant from outside to inside. The thickness of film layers must be adjusted for depth of the cavity because a greater drawdown may result in thinning of film, which can alter film effectiveness or even result in a package 'blowout.' In summary, the degree of thinning and also the uniformity of the formed film are affected by the type and original thickness of the forming film, the size of the cavity area, the depth and contour of the cavity, and the procedure used to heat and to draw or push heated film into the cavity.

Anaerobic Packaging with Blooming Agents

The Curwood packaging company offers meat suppliers a best-of-both-worlds package known as FreshCase®, which allows fresh meat to be vacuum packaged in a centralized facility in a thermoformed package but maintains a bright-red oxymyoglobin fresh meat color. According to their patent, this package is made up of a multilayer film in which the meat-contact layer contains a myoglobin-blooming agent. The myoglobin-blooming agent may be any agent or precursor that binds to undenatured myoglobin producing a desirable fresh meat color. These myoglobin-blooming agents may include a nitric oxide (NO)-donating compound, such as a nitrate or nitrodisulfide; an inorganic cyanide (MCN); a nitrogen heterocycle, such as pyridines or purines; a carbon monoxide (CO)-donating compound; or niacin. Additionally, the package would contain an oxygen-barrier layer to allow for vacuum sealing. The vacuum seal increases the refrigerated shelf life of retail-ready fresh meat products from a few days to several weeks. The thermofilm packaging reduces packaging waste compared to modified-atmosphere packaging and foam tray/PVC overwrap packaging. This system minimizes labor and potential for mishandling in the retail store, and the vacuum seal eliminates purge leakage in the retail case, the shopping bag, and the consumer's home, which would reduce the possibility for cross contamination.

Cook-in Packaging

As consumers demand more convenience from their food, processors must become more creative with their offerings available to consumers. Consumers demand minimal preparation time for 'home cooked' meals that they can simply fix and forget. Furthermore, research has shown that today's consumers prefer not to touch raw meat with their bare hands, and eliminating the handling of raw meat products in consumers' kitchens will greatly reduce cross contamination.

Perhaps the most demanding requirement for forming films is for applications in which thermal processing (cooking) is done in the same package that is used for distribution and merchandising. This film must shrink down onto shrinking product as the product is cooked and cooled, otherwise purge and cookout liquid will be evident. This film must also have good visual properties and be tough enough to withstand the rigors of shipping and abusive handling. Ham may be cooked in stainless steel molds that protect the film during cooking. A film construction of nylon/sealant or nylon/barrier/sealant is often used. An ionomer (Surlyn) sealant is commonly used and this gives a slight bond to the product, which minimizes purge of cooking. Packaging for poultry breasts is more

complicated, especially if skin is still attached. Surlyn sealant, as used for hams, adheres too tightly to poultry skin, so that skin comes off with the film when packaging is removed. The forming film for poultry breasts may use a nylon that is less sensitive to moisture or use a polypropylene/nylon/polyethylene, where the polypropylene protects the nylon layer from steam used for cooking. For poultry products, a special polyethylene may be used for sealant.

Retort Pouch

The retort pouch is a very demanding package that consists of a three-layer laminate that will be processed like a can, will be shelf-stable, and allows the convenience of frozen boil-in-the-bag products. It may be composed of an outer layer of polyester film, a middle layer of aluminum foil, and an inner layer of modified polypropylene. Material for retort pouches must provide superior barrier properties over a long shelf life, seal integrity and toughness/puncture resistance, and must withstand the thermal rigors of a canning process, which might have temperatures up to 135 °C (275 °F). The advantage of the retort pouch over a can is its flatter shape compared to the round shape of a can; thus, a more uniform heat and less heat is required to bring the temperature in the product center to the safe level.

Ovenable Films

Packaging companies are now offering vacuum bags and pouches made from films that allow the product to be packaged in a vacuum-sealed bag or pouch, and the subsequently packaged products can then be placed directly in the oven for cooking or used in sous vide cooking. These packages allow the product to be marinated, tumbled, seasoned, and packaged at a highly sanitized meat processing facility, and then the product may be shipped, stored, frozen, and cooked at a later date either in a restaurant or in the consumer's home. When processing steps, such as cutting, marinating, and seasoning, are limited to a processing facility, and consumers do not have to handle the uncooked product, the likelihood of mishandling or contamination is greatly decreased. During cooking, the packaging expands away from the meat, allowing for browning and reducing meat adhesion that makes the product easier to remove from the package after cooking. Cooking in bag allows for a juicier product and greater cook yields than when done in a typical oven cookery.

Quality Assurance in Film Production and in Packaging

Film Quality Assurance

Even though the processes in film manufacture seem highly automated and immune to process errors, considerable checking should be done to assure that specification requirements, such as film thickness and absence of defects, are met. Each step in the production of resins and films requires careful monitoring, so that the undesired by-products are washed away or separated from the film. Occasionally, a distinctive and undesirable odor may be transmitted from the film to the product, which can be costly both to film supplier and the meat processor.

Package Quality Assurance

Quality assurance regarding packages of product should include an inspection by the individuals placing packages in boxes, as this will allow detection of obviously defective packages that are fast leakers. Pulling samples at intervals for close inspection by quality assurance personnel for proper seals and absence of damage to packages can be useful and allow for adjustments or repairs to the packaging machine to minimize problem packages. In addition, good quality assurance programs include a next-day random checking of the product within boxes on pallets to detect slower leakers, and determine the level of compliance with company-stated goals regarding absence of bad packages.

Flexible Film Package Defects

Some packaging manufacturers have published charts of flexible package defects that are very helpful to companies using their film products and packaging equipment. Class 1 defects (the most serious defects) include a patch of nonbonding across the width of the seal, creating a leak; a mechanical slash into the package, resulting in the loss of hermetic integrity; a fracture or break through the packaging material; unsealing of the pouch or bag; failure of two sealant films to combine; mechanical piercing into the package; a leak at a manufactured notch that had been placed for easy opening; any other break through the package material; and a swollen package, indicative of gas formation within the package, usually a sign of serious microbial problems. Each of these leaks should lead to careful analysis and correction of the cause of the problem as well as rejection of the failed package. Discovery of Class 2 problems should also be quickly addressed. These may include a scratch or abrasion through part of the film; a blister that is not in the seal; a contaminated seal that might easily lead to a leaker; film delamination in the seal area; a compressed seal resulting from too much jaw pressure; a misaligned seal that might lead to the loss of hermetic integrity; seal creep, which is a partial opening of inner border of the seal; or a wrinkle that is greater than half-way through the seal. These can result in hermetic failure quickly or too early in the journey of the package to the ultimate consumer.

Active Packaging

Active packaging systems include oxygen scavengers, carbon dioxide scavengers or emitters, chemical preservative films, ethanol or ethylene emitters, temperature control and monitoring systems, and time–temperature integrated monitors. Oxygen and carbon dioxide levels are important in MAP (systems that affect their level are discussed in another part of this encyclopedia). Some processed meats have the potential for very long shelf-lives, especially when packaged in completely anaerobic conditions. Oxygen-scavenging films have

the potential to eliminate oxygen from a package without the use of a sachet labeled "DO NOT EAT." These triple-layer films have a middle layer that scavenges oxygen and plastic layers on either side for product contact and consumer handling. In some cases, the oxygen-scavenging properties are activated with a ultraviolet light during the packaging process.

Antimicrobial packaging is receiving much interest; a long list of possible ingredients for this purpose include anhydrides, bacteriocins, antimicrobial enzymes, radiation emitters, silver ions, zeolite products, sorbic acids, and interesting combinations. New innovations using essential oils, such as thyme, in a packaging system have shown promise as antimicrobials.

New packaging innovations have also resulted in the development of intelligent packaging. These products may feature time and temperature indicators to detect mishandling or temperature abuse of a product or to indicate when a cook-in bag product has reached a proper temperature. Other possibilities include freshness indicators in packaging that may detect levels of volatiles in a product. Other 'active' possibilities are odor removers or odor emitters.

Conclusion

Any packaging process involves a careful consideration of the package–product–process interaction. An excellent example of this is consideration of packaging for irradiated meat products, where migration of certain compounds or the possible effects of irradiation on the film may be important.

Consideration of raw materials for producing films will be more important in the future as one thinks about renewable resources versus petroleum-based raw materials. Considerable research is being focused on this, with some of the effort dealing with edible films and the challenges in their production and use. The issues of whether a packaging material can be recycled or whether it is biodegradable will be even more important in coming years.

See also: Additives: Functional. Chemical and Physical Characteristics of Meat: Color and Pigment. Curing: Physiology of Nitric Oxide. Packaging: Equipment; Modified and Controlled Atmosphere; Overwrapping; Vacuum

Further Reading

Brody, A.L., 1989. Controlled/Modified Atmosphere/Vacuum Packaging of Foods. Lancaster, PA: Technomic.

Brody, A.L., Strupinsky, E.R., Kline, L.R., 2001. Active Packaging for Food Applications. Boca Raton, FL: CRC Press.

Gehrke, W.H., 1983. Film properties required for thermoformed and thermal processed meat packages. Proceedings of the 36th Reciprocal Meat Conference, pp. 55–59. Savoy, IL: American Meat Science Association.

Hanlon, J.F., Kelsey, R.J., Forcinio, H.E., 1998. Handbook of Package Engineering, third edn. Timonium, MD: CTI Publications.

Harte, B.R., 1987. Packaging of restructured meats. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research 3. New York, NY: Van Nostrand Reinhold, pp. 433–468.

Hotchkiss, J.H., 1994. Packaging muscle foods in muscle foods. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Meat, Poultry and Seafood Technology. New York, NY: Chapman and Hall, pp. 475–496.

Jenkins, W.A., Harrington, J.P., 1991. Packaging Foods With Plastics. Lancaster, PA: Technomic.

Kropf, D.H., 2000. Meat, modified atmosphere packaging. In: Hui, Y. (Ed.), Encyclopedia of Food Science and Technology. New York, NY: Wiley, pp. 1561–1566.

Lawrie, R.A., 1998. Lawrie's Meat Science, sixth ed. Lancaster, PA: Technomic.

Lundquist, B.R., 1987. Protective packaging of meat and meat products. In: Price, J. F., Schweiger, B.S. (Eds.), The Science of Meat and Meat Products. Westport, CT: Food and Nutrition Press, pp. 487–505.

National Food Processors Association/Flexible Package Integrity Committee, 1984. Classification of Visible Exterior Flexible Package Defects. Arlington, VA: AOAC International.

National Research Council, 1958. Food-Packaging Materials: Their Composition and Uses, Publication 645. Washington, DC: National Academy Press.

Rizvi, S.S.H., 1984. Flexible packaging material properties that influence shelf life. Proceedings of the Meat Industry Research Conference, Chicago, IL, pp. 125–141. Savoy, IL: American Meat Science Association.

Rooney, M.L., 1995. Active Packaging. London: Blackie Academic and Professional.

Taylor, A.A., 1982. Retail packaging systems for fresh meat. Proceedings of the International Symposium on Meat Science and Technology, Lincoln, NE, pp. 353–366. Savoy, IL: American Meat Science Association.

Zhao, Y., Wells, J.H., McMillin, K.W., 1994. Application of dynamic modified atmosphere packaging systems for fresh red meats: Review. Journal of Muscle Foods 5, 299–328.

Relevant Website

http://www.fsis.usda.gov/Regulations_&_Policies/Packaging_Materials_for_Meat_&_Poultry_Products/index.asp
United States Department of Agriculture.

Vacuum

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Glossary

Blooming Oxygenation of deoxymyoglobin to form oxymyoglobin.
Coextruding Extruding multiple layers of plastic material simultaneously; the individual layers typically have specific properties of interest, such as O_2 impermeability or puncture resistance.
Deoxymyoglobin Myoglobin form resulting from H_2O bound to reduced or ferrous iron; it is of visual purple color.
Metmyoglobin Myoglobin form resulting from –OH bound to oxidized or ferric iron; it is of visual gray or brown color.
Oxymyoglobin Myoglobin form resulting from O_2 bound to reduced or ferrous iron; it is of visual red color.
Permeability The ability of a gas (i.e., O_2) to seep through a film.
Shrink packaging Polymer thermoplastic films designed to shrink on application of heat at approximately 80–90 °C.
Skintight packaging A specialized form of shrink packaging that uses thinner films to create a package that contours very closely to the product, so tightly that the texture of the product can often be detected through the package.

Vacuum/pressure

- Inches and mm Hg: Units of pressure; 29.9 in. or 760 mm Hg represent a pressure that is equivalent to 1 standard atmosphere or 760 Torr and can be related to other units;
- mbar: Unit of pressure; 1013 mbar is equivalent to 1 standard atmosphere or 760 Torr;
- mm Hg: Unit of pressure; 760 mm Hg pressure is equivalent to 1 standard atmosphere or 760 Torr;
- Pascal (pa): Unit of pressure; 101.3257 Pa is equivalent to 1 standard atmosphere or 760 Torr;
- Torr: Unit of pressure; 760 Torr is equivalent to 1 standard atmosphere or 760 mm Hg;
- Vacuum: Gaseous pressure within a space that is less than atmospheric pressure; 0% vacuum is equivalent to 1 standard atmosphere or 760 Torr; 99.9% vacuum is equivalent to 1 Torr.

Introduction

Vacuum packaging is usually described as packaging in a container, either rigid or flexible, from which substantially all air has been removed before sealing of the package. It is a good system for distribution and long-term storage of fresh meat cuts, providing longer product life and, if chilled and handled properly, allowing meat color to return to a highly desirable bright red or bright pink form in retail offerings, where retail cuts are packaged with oxygen-permeable film. With good vacuum packaging, the very low oxygen level obtained coupled with production of carbon dioxide in the bag discourages growth of aerobic microorganisms that cause spoilage odors and flavors. These processes also encourage more favorable conditions for anaerobes with production of lactic acid. Vacuum packaging is also very useful for many cured meat products in providing an environment that prolongs the cured meat color before fading occurs and also maintains other quality factors at an acceptable level.

This article covers what constitutes vacuum and the basics of myoglobin chemistry that influence color in vacuum-packaged products. It deals with the major uses of vacuum to deliver high-quality, safe meat products to the consumer. The less than hoped for success in retailing vacuum-packaged chilled or frozen meat is also discussed. Films and important film characteristics for successful use of vacuum packaging are described. The essentials of a good vacuum package are delineated, along with key aspects of management to optimize success in vacuum packaging. Systems for achieving desired results with vacuum are also discussed.

Measurement of Vacuum

The degree of vacuum or pressure can be expressed in a number of ways (Table 1). From the table, 100 Torr (or 100 mm Hg pressure) is equivalent to 26.0 in. of mercury, 87.0% vacuum, and 13 332 Pa, which can be expressed as 133.3 mbar. The importance of this table is that research studies often indicate that vacuum packaging is used, with no further indication of the degree of vacuum or pressure. This is a serious omission, which is indicated in the Section Myoglobin Chemistry. A vacuum/pressure of 50 Torr or 93.5% vacuum allows 6.5% residual air within the package that can represent an oxygen partial pressure, which is very favorable to the formation of metmyoglobin and to discoloration. Thus, an unknown vacuum level may allow unfavorable conditions in some research studies or commercial situations. The capability of a vacuum packager can be measured by the use of a device to measure vacuum in a bag, such as a Kennedy gauge, often used in the United States.

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Table 1 Comparison of vacuum/pressure measurements

mm Hg (Torr)	*Inches Hg vacuum*	*% vacuum*	*mbar*	*Inches Hg absolute*	*Pascal (Pa)*
760	0	0	1013	29.99	101 357
700	2.4	8	934	27.60	93 326
600	6.4	21	800	23.60	79 993
500	10.3	34	667	19.70	66 661
400	14.3	47	533	15.70	53 329
300	18.3	61	400	11.80	39 997
200	22.1	74	267	7.85	26 664
100	26.0	87	133.3	3.94	13 332
90	26.5	88	120	3.54	11 999
80	26.8	89.5	107	3.15	10 666
70	27.2	90.8	93	2.76	9 333
60	27.6	92.1	80	2.36	7 999
50	28.0	93.5	67	1.97	6 666
40	28.4	94.8	53	1.57	5 333
30	28.8	96.1	40	1.18	4 000
20	29.2	97.4	27	0.78	2 666
10	29.6	98.7	13.3	0.39	1 333
5	29.7	99.0	6.6	0.03	666.6
1	29.95	99.9	1.33	0.039	133.3

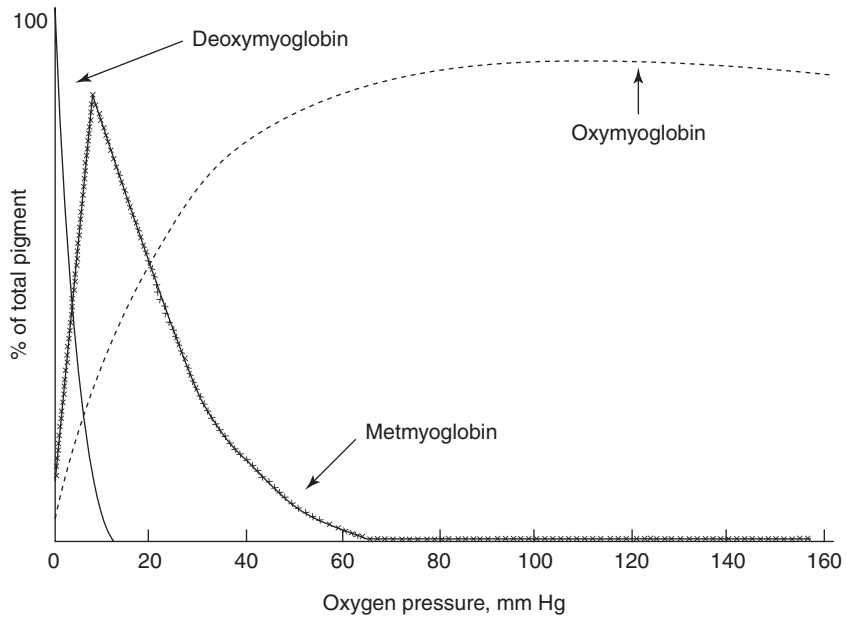

Figure 1 Forms of myoglobin at different oxygen partial pressures.

Myoglobin Chemistry

An understanding of the three major chemical forms of myoglobin is important to making decisions and determining major points of emphasis essential to achieving successful results with vacuum packaging. The three major chemical forms of myoglobin in fresh meat, irrespective of whether it is chilled or frozen, include deoxymyoglobin, which has iron in the reduced or ferrous state (Fe^{2+}) and exhibits a somewhat darker, purplish red color. Vacuum-packaged meat frequently has a preponderance of myoglobin in this form owing to the natural scavenging of oxygen within the vacuum package, provided that the film sufficiently limits the ingress of oxygen into the package. This is because deoxymyoglobin formation is favored at extremely low oxygen partial pressures (1.4 mm Hg or less). Figure 1 illustrates the relationship of oxygen partial pressure to the relative proportions of myoglobin forms. Early in the development of vacuum packaging, users apparently did not realize that very low partial oxygen pressures were needed to assure long product life and also to have fresh meat cuts, especially beef, retain the ability to bloom.

Blooming and Fresh Meat Color

On exposure of meat to air or another source of oxygen, deoxymyoglobin combines with oxygen to form the bright red

or pink oxymyoglobin, which also has iron in the ferrous state (Fe^{3+}) but with oxygen attached. This reaction is called oxygenation or 'blooming.' Oxymyoglobin formation is favored with high partial pressures of oxygen (Figure 1). There is no valid reason to have meat in the oxymyoglobin state until it is presented in a retail package for display. Even though some degree of blooming quickly occurs on the surfaces before freshly cut meat is vacuum packaged, this time of exposure and the amount of brightening should be kept to a minimum, because more brightening at this time can limit ability to brighten at a later time, preferably as cuts are being prepared for display. As illustrated in Figure 2, which depicts the fresh meat color (myoglobin) triangle, myoglobin in meat in the oxymyoglobin state must go through the oxidized state of metmyoglobin to get back to the deoxymyoglobin form. This is expensive in terms of reducing capacity, and the ability to achieve this change is limited and should be conserved. Otherwise, a point may be reached where the reducing capacity of the muscle is exhausted and the change to deoxymyoglobin may no longer be possible. When this happens at any location in the muscle, the formation of the brown metmyoglobin is inevitable and the ability to oxygenate is lessened. As illustrated in Figure 1, metmyoglobin formation is maximally favored at an oxygen partial pressure of 4 mm Hg, but it occurs at pressures as low as 1.4 mm Hg and can also occur at up to 25 mm Hg. An important point to be emphasized is that a very good vacuum must be achieved and maintained for products, such as beef subprimal cuts, not only to maximize product life but also to retain a high ability for the meat to bloom.

Pigment Layers in Meat

The three myoglobin pigment layers discussed in the article on overwrapping also occur in vacuum-packaged cuts. In relatively fresh meat that has not been mishandled, only a small proportion of oxymyoglobin occurs initially, but primarily deoxymyoglobin will be observed at a later time interval after vacuum packaging. During the time delay before this happens, small amounts of residual oxygen within the bag create areas where the partial pressure of oxygen favors formation of metmyoglobin. The surface of vacuum-packaged ground or whole-muscle product may become totally brown for a while. With sufficient time, the natural oxygen-scavenging ability of the muscle should return oxygen partial pressures to the very low levels that favor formation of deoxymyoglobin. This metabolic activity of the muscle also produces carbon dioxide inside the bag. Drawing an insufficient vacuum or placing highly oxygenated meat in the bag means that more oxygen has to be scavenged, which will delay appearance of the desired purplish red color. If a film bridges (over a space) to produce a void, these voids may have an atmosphere that favors oxidation to metmyoglobin and also a space for purge to accumulate. With good oxygen scavenging by the muscle, the surface oxymyoglobin layer will largely revert to the deoxy form and the vacuum-packaged cut will assume the purplish red color of this pigment. This color is good evidence of a successful vacuum package for fresh meat.

Discoloration of Vacuum-Packaged Meat

The occurrence of brown metmyoglobin on the surface of a vacuum-packaged cut too early in the package life may indicate that the degree of vacuum achieved by the vacuum pump/packager combination was not sufficient or that the film is not a sufficient barrier to ingress of oxygen (<30 ml per 24 h per m^2, or less, is the target for domestically marketed beef in countries such as the United States). This coloration also occurs when meat is exposed to air for too long after cutting and before packaging and absorbs too much oxygen, which can later be released into the within-package environment. The brown color might also indicate that the meat has been held in vacuum too long and has used up all of its metmyoglobin-reducing capacity, thereby losing its ability to return to the reduced deoxymyoglobin form. It could also be a consequence of a leak in

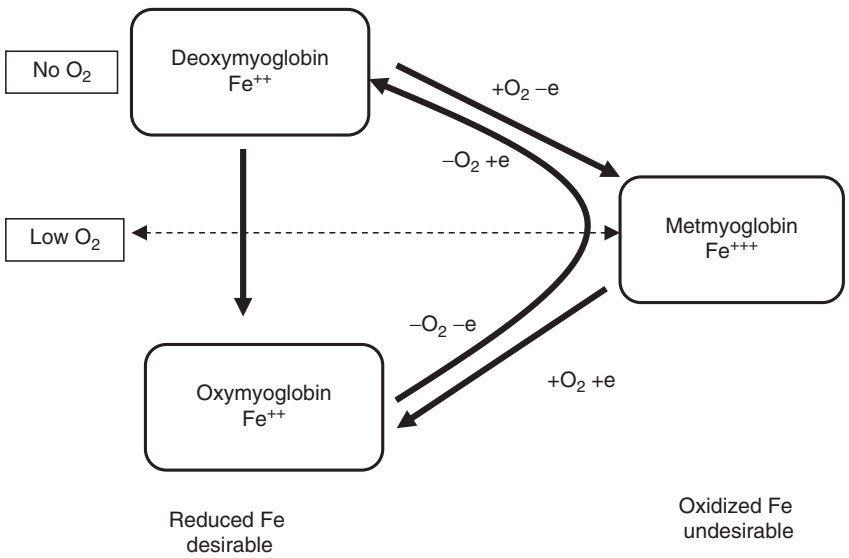

Figure 2 Fresh meat color (myoglobin) triangle.

the package. The results of a poor vacuum or a leak in the package can frequently be observed when these newly vacuum-packaged cuts are placed in the box; however, a slow leaker may not be noticeable for several hours. Some company's quality assurance programs for packaging include next-day random sampling of a predetermined number of boxes on so many pallets as a check for unsatisfactory packages, usually leakers. This procedure is used more for vacuum-packaged cured products than for vacuum-packaged fresh meat.

Increased meat surface area, such as six sides in small meat cubes cut for stew beef or with meat that has been tenderized by penetrating needles or knives or with the rotating knives of a meat cuber, may allow a great deal of oxygen to diffuse into the structure of meat to react with myoglobin. This oxygen is included with meat placed in the package and can later be released from the meat into the sealed package, where it alters the partial oxygen pressure enough to favor metmyoglobin formation.

Delay before Packaging

A classic study was reported, in 1970, in which beef semimembranosus muscle samples were exposed to air for 2, 6, 24, or 48 h at 3.3 °C before vacuum (anoxic) packaging. Samples held aerobically for 2 h and then vacuum packaged maintained a high level of metmyoglobin-reducing ability and underwent a rapid conversion of pigment to deoxymyoglobin, with no visible or instrumental manifestation of metmyoglobin at any time. The conversion to the deoxy form went so completely that there was never enough brown pigment to be detected. With the 6 h exposure to air before vacuum packaging, a similar ability to change oxymyoglobin to deoxymyoglobin was noted, with a small amount of brown metmyoglobin being detected for approximately 4 h after the vacuum packaging. However, muscle samples held in air for 24 h before vacuum packaging took approximately 2.5 times as long (10 h) for the metmyoglobin to become reduced and 20% metmyoglobin was reported at 5 h after vacuum packaging. Moreover, muscles exposed to air for 48 h before vacuum packaging took 20 h for maximum conversion back to deoxymyoglobin, but only 80% of the total myoglobin reverted to the deoxy form. Analysis showed that up to 60% of the myoglobin was in the met form at 4 h after vacuum packaging. This study emphasizes the importance of vacuum packaging cuts rapidly after they have been fabricated. Ideally, plant organization and product flow should be organized to facilitate rapid packaging and cuts should never be held overnight or even for several hours before vacuum packaging. Stacking or throwing retail cuts into a combo or lug before vacuum packaging might result in partial blocking of the surface of some cuts so that part is exposed to oxygen and part is blocked from exposure. With some time in this condition, a single cut may demonstrate color variation on its surface, because different areas of the surface are in different stages of the change of myoglobin from one form to another. This could be a negative factor when product is displayed for sale.

Curing and Vacuum Packaging

In the curing process, nitric oxide formed from nitrite reacts with myoglobin to form red-colored nitric oxide myoglobin, which, on heating and in favorable conditions, is converted to nitrosohemo- or nitrosomyochrome, a pink-colored pigment. Although this cooked form is frequently described as relatively stable, it is susceptible to oxidation, leading to fading of the pink to a gray, tan, or sometimes colorless appearance. The oxidation is catalyzed by contact with oxygen, exposure to light, and higher temperatures. Such a product is especially vulnerable to ultraviolet (UV) light, especially shorter UV wavelengths. Packaging plays a critical role in stabilizing the cured meat pigment by keeping the within-package levels of oxygen low and by having a very small amount of headspace. A vacuum of 77–93.5% should be drawn for processed meat packages. The greater value is a much-preferred target to sustain product for a long shelf life and display life. Thus, very good evacuation of air or an effective gas flush, as well as having a package that is a good barrier to oxygen, is essential.

Choosing Vacuum or Gas Packaging

For a retail package, vacuum is useful for more solid or chunky cured products, such as ham pieces or even frankfurters and similar sausages, but gas packaging works better for sliced or fragile products. Fragile products may be broken by the pressure of vacuum packaging, whereas thin slices may be very difficult to separate if they are pressed together by the force of vacuum packaging. Gas packaging may also be favored over vacuum for product with uneven surface contour, as it may be difficult to draw the vacuum film down into a depression on the product surface. For example, products such as chicken are not well suited for vacuum packaging because of their very uneven contour and their relatively soft texture and also because of their small product mass in relation to surface area, which result in diffusion of larger amounts of oxygen into the meat surface proportion to scavenging of oxygen by the muscle.

Considerations whether vacuum can be used or whether gas packaging would be more appropriate depend on product characteristics. Size, shape, and regularity of product may determine whether an effective vacuum can be drawn; if not, these products should be considered for gas packaging.

Essential Film and Package Traits

Properties of Films and Oxygen Permeability

Important requirements for films used for vacuum packages include good barrier properties, for both gas and moisture, because of the necessity of minimizing oxygen permeability for chilled or frozen fresh meat and also when using film for cured meat packaging. Specifications for bags used for packaging beef subprimal cuts for domestic use in the United States frequently require permeability not exceeding 30 ml per 24 h per m^2. Some countries and some meat exporters favor or require an oxygen permeability not exceeding 3–4 ml per 24 h per m^2.

Some variation from the requirement for very low oxygen permeability might be in order for frozen meat packaging. When skintight film for frozen meat was used, a brighter red beef color was maintained with somewhat greater oxygen

permeability. Another approach to providing a bright red color, in this case for ground beef, was to expose the ground beef to hyperbaric oxygen immediately before packaging and freezing. Both approaches require acceptably cold freezer temperatures for storage, distribution, and display, because this product might be more vulnerable to oxidation; thus, resulting in an oxidative or rancid taste sooner than expected in product life.

Permeability of Films

To maximize storage life of fresh meats through marketing channels, permeability of films should be minimized. The literature suggests that greater than 15 weeks of storage life can be attained when permeability is 190 ml per 24 h per m^2 or less. Films that allow greater permeability will likely result in greater counts of *Pseudomonas* and putrid odors.

Films used for cured items also require very low oxygen permeability because of the vulnerability of the nitrosohemochrome pigment to oxidative fading. One study, reported in 1985, compared films ranging in oxygen permeability from 0.1 to 1600 ml per 24 h per m^2 and vacuum levels ranging from approximately 60.2% to 96.7% to determine the effects on color stability and rancidity in sliced bologna. Color was most stable with the highest-barrier film but remained acceptable for 35 days of storage if oxygen permeability was 7 ml per 24 h per m^2 or less. Vacuum at approximately 89.8% or higher resulted in the best cured color stability in all films, but if film permeability exceeded 60 ml per 24 h per m^2, color became unacceptable in only 10 days. At the lower vacuum levels in this study, only the best barrier film, one that had a foil layer and oxygen permeability of 0.1 ml per 24 h per m^2, retained acceptable color, whereas at 77–84.9% vacuum a film permeability of <60 ml per 24 h per m^2 was needed. Thiobarbituric acid residue measures of oxidation did not become significantly greater until a vacuum of 77% or less, or a film allowing more than 120 ml O_2 per 24 h per m^2, was used. A similar study with bacon showed no large effects, although a vacuum of 75% and a barrier film allowing 10.6 ml per 24 h per m^2 or less showed a somewhat better color. These authors suggested an 89.8% vacuum as a good target. The product life reported in these studies has been improved, and many commercial products today have a 'sell by' date based on a product life of 70 days or longer.

Physical Traits of Films

Materials must be flexible so as to conform closely to the shape of product being packaged and must have the ability to shrink and to be heat sealed. It must also be strong and puncture resistant. Special bone guard materials are sometimes used to cover sharp bone edges and some films are available that are very resistant to such punctures. Details of films and film uses are given in the article.

Research in Denmark showed that holding vacuum-packaged cured meat in the dark long enough for the meat to deplete oxygen stores by its metabolism before placing product under display lighting was very helpful in reducing cured color fade. Processed meat is frequently packaged in heat-shrinkable and heat-sealable bags with three layers, such as ethylene–vinyl acetate, polyvinylidene dichloride, ethylene–vinyl acetate or nylon, ethylene–vinyl alcohol, and ionomer coextrusions. Ionomers with zinc or sodium are very good in heat-sealing layers. These products are also packaged using thermoformed packages.

Vacuum Packaging Operations

Benefits of Vacuum Packaging

Vacuum packaging offers some special benefits because of the extended product life, reduced weight loss through the control of evaporation, protection against contamination, preservation of color, and improved eating characteristics because of the longer aging that is possible in vacuum bags. These benefits led to the 'boxed beef' program (vacuum packaging and placing beef subprimal cuts in conveniently sized boxes) developed originally by Iowa Beef Processors (later named IBP, Inc. and, today, Tyson Foods, Inc.). This system offered huge benefits because bones and fat trim were retained at the slaughter–fabrication plant and not shipped to retail stores. This allows huge savings in transport space and in cost and results in fresher fat trim and bones for rendering. Vacuum-packaged subprimal cuts in boxes holding approximately 9–27 kg were more easily handled at individual stores and their use facilitated quicker cutting and retail packaging of retail cuts. The system also lends itself to enhanced marketing flexibility, as individual retail market managers can order a product mix to suit their anticipated business.

Creating the Vacuum Package

Vacuum packaging can be accomplished by several techniques. The simplest method involves mechanically collapsing the film around the product, which does not result in a very satisfactory package or degree of vacuum. Steam evacuation or hot filling of the product can force atmospheric gases out of the package headspace, and a vacuum develops when the product cools and steam condenses. More commonly, vacuum packaging involves pumps to withdraw air from the package to create a negative pressure before sealing. For a very high degree of vacuum, multistage pumps may be needed. Mechanisms of air withdrawal include a snorkel device or single- or double-chamber systems. The snorkel has a tube or nozzle that is placed into the package to enable withdrawal of air, which is then withdrawn when package sealing is performed. For a single chamber, the package is placed in the chamber, a vacuum is drawn in the chamber so that the package atmosphere is drawn out, and sealing is performed inside the chamber. The double chamber is set up so that external pressure outside the bag produces better evacuation before sealing, resulting in a tighter drawdown. Although an earlier study indicated little difference in storage life of product from nozzle compared with chamber evacuation machines, and no major difference between nozzle/clip and chamber/heat seal for vacuum packages, the chamber-type approach seems to be highly favored.

An interesting and effective way to extend the vacuum clip technology is Pi-Vac, a 'vacuum-free vacuum pack,' which has recently been marketed in Europe. The film is fed into tubes and the film edge is stretched over 'fingers' in such a way that meat can be placed in the opened tube. The specially constructed barrier film is stretched while meat is introduced into the tube and the stretched film then contracts to its prestretch dimensions over the meat, forcing out air and bringing the film into close contact with the meat. The open end of the tube can be clipped or heat sealed to produce an air-free meat package.

Effective Sealing of Vacuum Packages

Technique is important in effective vacuum packaging. The heat seal bar should be as close as possible to the product in the vacuum bag to avoid collapse of the bag between the product and the seal bar resulting in trapping air in the bag. A clean bag at the seal location is highly desirable to produce a better closure, although some sealing systems are designed to function even with some interfering meat or fat in the seal location. Proper adjustment of seal temperature is important as some films can melt at high seal temperature, whereas others require a higher temperature to obtain a hermetic seal.

Shelf Life and Microbiology of Vacuum-Packaged Product

Shelf life of vacuum-packaged meat depends a great deal on the microbiological condition of the meat at the time of packing. Residual oxygen is metabolized by enzymes in the meat and carbon dioxide is produced. The resulting conditions severely limit the growth of aerobic microbes; thus, the growth of *Pseudomonas*, responsible for spoilage of meat under aerobic conditions, is lessened and lactic acid bacteria predominate. Holding vacuum-packaged product at very low temperatures favors growth of lactic acid bacteria more than that of *Brochothrix thermosphacta* and enterobacteriaceae, which can also grow under these anaerobic conditions. The more desired lactics are also favored by film with very low oxygen permeability, and these same conditions favor the formation of deoxymyoglobin. A lactic acid aroma on opening a vacuum bag of meat is a good sign of growth of lactic acid bacteria. This odor quickly dissipates on exposure to air. Beef of normal pH and of good condition could be stored for up to 12 weeks, but high-pH beef spoils much more rapidly due to growth of *Alteromonas putrefaciens* and of psychrotropes, such as *Serratia liquefaciens* and *Hafnia* and *Enterobacter* species. The latter two are detected by greening of the meat surface and off-odors, mainly from sulfur-containing compounds. The hydrogen sulfide produced results in green sulfmyoglobin and sometimes even further degradation of myoglobin to porphyrins and bile pigments. When the pH of beef is higher than 6.0, it should not be kept more than 2–3 weeks and actually should not be vacuum packaged at all. An important consideration in vacuum-packaged meat is the possible growth of psychrotropic pathogens, including *Yersinia enterocolitica*, *Listeria monocytogenes* and *Aeromonas hydrophila*, although *L. monocytogenes* grows very slowly below pH 6.0.

Vacuum Packaging of Larger Cuts

Many studies, especially with beef and pork, have been done on vacuum packaging, and its major commercial use has been for the distribution of larger cuts that are later cut into retail cuts and probably overwrapped for display. Major results include lower weight losses after 21 days of storage, less weight loss because less trimming is required, reduced discoloration, less off-odor and off-flavor, and improved palatability of the product. Some studies report an additional day of display time for cuts from a vacuum-packaged larger cut. Beef cuts produced under commercial conditions were reported to have a 70-days storage life, whereas another study reported an 11-weeks storage life when held at 0 °C, although undesired color was sometimes noted after 8 weeks. Black or brownish spots were noted on the fat at 6 weeks of storage, and authors attributed them to myoglobin breakdown products in the purge. On rare occasions, boxes of beef subprimal cuts were lost in the huge boxed beef warehouses, despite digital records of boxes and their locations. Sometimes product as old as 84 days had been 'found,' but was still able to be successfully used. Several reports have noted that a shorter storage life is appropriate for lamb with higher psychrotropic counts. Perhaps the smaller muscle mass in relation to surface area has an influence. One study indicates that the optimum time to vacuum package lamb cuts is at 4 days postmortem, and further delay before vacuum packaging reduces the intensity of later bloom and encourages metmyoglobin formation.

Some interest has been developed in the possibility of precutting wholesale or subprimal cuts into retail cuts and then placing chops or steaks back in their order and vacuum packaging them collectively. When these packages are opened on delivery at retail stores, they need no further cutting and are retail packaged. This approach is probably considered periodically by the industry, but a recent test of the procedure again resulted in considerable brown and green discoloration on most of the individual steaks within the vacuum packages. A likely contributing factor is rapid diffusion of oxygen into each retail cut so that, when these were vacuum packaged, some locations within the vacuum package have partial oxygen pressures that strongly favor oxidation.

Reducing Headspace with Shrink Packaging and Skintight Packaging

Alternative methods for reducing headspace in a package include shrink packaging and skintight packaging, which use oriented thermoplastic films that shrink onto the product surface or are drawn tightly around the product when they are in the heat-softened state. Skintight packaging is an appropriate method for packaging frozen meat, as the closeness of film to the meat does not allow buildup of ice between meat and film, so that product visibility is very good. The skintight film is also effective in minimizing desiccation of the meat surface, even under conditions where freezer temperature may have considerable variation. A concern with skintight packaging is having enough initial film thickness so that, when the film thins in the shrink process, it does not thin so much that it causes a blowout or reduces barrier properties to an extent that package barrier needs are compromised.

Frozen Meat Packaging

For frozen meat packaging, a more snug fit of film to meat should occur if packaging is done before freezing. However, packaged meat should be taken to the freezer immediately after packaging and subjected to rapid freezing, because muscle metabolism may cause a rapid darkening of color to dark red or even brown unless meat is quickly frozen to stop metabolic activity. A frozen meat retailing effort in Manhattan, Kansas was not successful because many cuts became too dark in color, and management failed to emphasize initiation of rapid freezing immediately after vacuum packaging. Cryogenic freezing via liquid nitrogen or carbon dioxide is highly recommended. Retailing of frozen, raw meat cuts has been relatively unsuccessful, even though with good management and handling, beef, pork, and lamb cuts can show an attractive color in display. A possible reason is that meat purchasers felt unable to evaluate quality in frozen meat and perhaps too many had experienced unfavorable impressions of discolored chilled meat that had been discounted in price and placed in a freezer case for quick sale.

Heat-sealable film bags are useful in packaging frozen meat. Heat-shrinkable polyolefin bags are appropriate for packaging frozen poultry products. Low-density polyethylene has a relatively low cost but needs improvement as an oxygen barrier. Coextruding or laminating these films or ethylene–vinyl acetate with barrier materials, such as polyvinylidene dichloride or ethylene–vinyl alcohol, creates a film that functions well to prevent oxidation and desiccation.

Choosing Proper Bag Size

An important consideration in vacuum packaging is to use the smallest possible size of bag into which the meat cut can be placed. The obvious justification is that larger bags are more costly, but another consideration is having the least possible bag surface area to reduce the area available for oxygen ingress. Although a larger bag might be shrunk onto the product, this larger area can still be a factor in allowing more oxygen into the package.

Commercial Difficulties in Marketing Vacuum-Packaged Fresh Meat

A major effort was made some years ago to market chilled vacuum-packaged cuts in retail stores by one of the largest US meat companies and offered in stores of a large retail chain in Midwest United States. Retail beef cuts were fabricated and packaged in a mid-western state and marketed in several states. As expected, the meat color was darker purplish red, which proved to be a large obstacle, even though a very strong effort was made by college students hired by a local retail store to educate consumers. The quality of the meat was very acceptable other than in terms of color, which did not meet expectations of many customers. Color was stable for a week, except for more rapid discoloration in stew beef and in steaks tenderized in a rotating-knife cubing machine. Both the large amount of surface related to meat mass for stew beef and the facilitation of oxygen diffusion into the tenderized beef created conditions favorable to early formation of metmyoglobin.

Recently developed technology has incorporated small quantities (5–90 mg m^{-2}) of sodium nitrite into the film. In a vacuum, oxymyoglobin is oxidized to metmyoglobin due to the low level of oxygen remaining in the package and ultimately converted to deoxymyoglobin via the natural reducing activity of the muscle. On reduction, nitrite forms nitric oxide in the meat that binds with deoxymyoglobin to form nitric oxide myoglobin, providing a red color visually similar to that of oxymyoglobin. The quantity of nitrite in the film does not cure the meat, nor does it provide antimicrobial properties.

In the authors' experience, vacuum-packaged pork retail cuts have an attractive meat color. Even though the muscle has darker color of deoxymyoglobin, the lower myoglobin content of this meat creates a situation in which the color is very acceptable. One negative aspect of vacuum-packaged retail pork cuts is that the purge may take on a greenish color.

In summary, vacuum packaging has added a new and very useful dimension to the distribution and marketing of red meat with benefits for food safety, appearance, and eating satisfaction.

See also: Additives: Functional. Chemical and Physical Characteristics of Meat: Color and Pigment. Curing: Physiology of Nitric Oxide. Packaging: Equipment; Modified and Controlled Atmosphere; Overwrapping; Technology and Films

Further Reading

Boegh-Sorensen, L., Zeuthen, P., Zeuthen, H., Bertelsen, G., 1986. The TTT-concept for chilled meats. In: Chilled Meat Conference Proceedings. Session 1986, pp. 209–214. Bristol, UK: International Institute of Refrigeration.

Breidenstein, B.B., 1982. Wholesale packaging for fresh meat. In: Proceedings of the International Symposium on Meat Science and Technology, pp. 343–351. Lincoln, NE: American Meat Science Association.

Faustman, C., Cassens, R.G., 1990. The biochemical basis for discoloration in fresh meat: A review. Journal of Muscle Foods 1, 217–243.

Gill, C.O., 1996. Extending the storage life of raw chilled meats. Meat Science 43, s99–s109.

Harte, B.R., 1987. Packaging of restructured meats. In: Pearson, A.M., Dutson, T.R. (Eds.), Advances in Meat Research, vol. 3. New York, NY: Van Nostrand Reinhold, pp. 433–468.

Hermansen, P., 1983. Comparison of modified atmosphere versus vacuum packaging to extend the shelf life of retail fresh meat cuts. In: Proceedings of the 36th Reciprocal Meat Conference, pp. 60–64. Savoy, IL: American Meat Science Association.

Hotchkiss, J.H., 1994. Packaging muscle foods. In: Kinsman, D.M., Kotula, A.W., Breidenstein, B.C. (Eds.), Muscle Foods: Meat, Poultry and Seafood Technology. New York: Chapman and Hall, pp. 475–496.

Jeremiah, L.E., 2001. Packaging alternatives to deliver fresh meats using short- or long-term distribution. Food Research International 34, 749–772.

Kropf, D.H., 1980. Effects of retail display conditions on meat color. In: Proceedings of the 33rd Reciprocal Meat Conference, pp. 15–32. Savoy, IL: American Meat Science Association.

Kropf, D.H., 1982. Retail packing of frozen meat. In: Proceedings of the International Symposium on Meat Science and Technology, pp. 367–386. Savoy, IL: American Meat Science Association.

Kropf, D.H., 1993. Meat Focus International. Colour stability. Wallingford, UK: CABI Publishers. pp. 269–275.

Newton, K.G., Rigg, W.J., 1979. The effect of film permeability on the storage life and microbiology of vacuum-packed meat. Journal of Applied Bacteriology 47, 433–441.

Renerre, M., Labadie, J., 1993. Fresh meat packaging and meat quality. In: Proceedings of the 39th International Congress of Meat Science and Technology, pp. 361–387. Ottawa, Canada: Clearbrook Communications.
Rizvi, S.S.H., 1984. Flexible packaging material properties that influence shelf life. In: Proceedings of the Meat Industry Research Conference, pp. 125–141. Savoy, IL: American Meat Science Association.
Sebranek, J.G., 1985. Stabilizing the properties of meat products with packaging systems. In: Proceedings of the Meat Industry Research Conference, pp. 150–167. Savoy, IL: American Meat Science Association.

Relevant Websites

http://www.cryovac.com/en/default.aspx
Cryovac Food Solutions and Food Packaging – Sealed Air.

http://www.curwood.com/
Curwood.

http://us.multivac.com/packaging-solutions/packaging-technologies-features/packaging-technologies/vacuum-packaging.html
Multivac.

http://meat.tamu.edu/color.html
Texas A&M Meat Science.

http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/the-color-of-meat-and-poultry/the-color-of-meat-and-poultry/ct_index
United States Department of Agriculture – Food Safety Inspection Service.

PARASITES PRESENT IN MEAT AND VISCERA OF LAND FARMED ANIMALS

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Introduction

The purpose of this article is to give an overview about the main zoonotic and nonzoonotic parasitic infections affecting meat and viscera (excluding gastrointestinal tract) of land farmed animals (cattle, small ruminants, swine, solipeds, poultry, and lagomorphs), providing insights on the main drivers for their transmission. As part of the Encyclopedia, this article is not intended to be exhaustive about all parasites associated with meat or viscera in all land farmed animal species, but up-to-date literature will be indicated for further information.

Ecological Patterns of Meatborne Parasite Transmission and Maintenance of Life Cycle in Farmed Animals

From the ecological viewpoint, parasites can shape host population structure through their effects on food webs, competition, and biodiversity. In the case of parasites encysted in muscle or in viscera, their development is necessarily based on carnivorism or scavenging behavior of at least one host in the life cycle. These parasites have generally indirect life cycles and are usually characterized by complex trophic interactions between different hosts and the environment. Infectious stages present in the environment (i.e., larvae, eggs, and oocysts) are ingested by the suitable intermediate hosts, where further development of the parasites and encystations in muscle or viscera occurs. The encystation usually occurs in the most oxygenated muscles (e.g., the diaphragm, tongue, masseter muscles, and heart) and in selected viscera that are usually the top choice for predators (e.g., the liver and the lungs). These evolutionary patterns are intended to maximize the survival of the parasitic larvae and their protection in privileged sites inside the host and to maximize the chance of transmission to the definitive carnivore or omnivore hosts. In the definitive hosts, the sexual maturation and reproduction of the parasites take place, and dissemination stages (i.e., eggs, larvae, oocysts, and sporocysts) are produced and shed to the environment, commonly with the feces. The number of stages disseminated by the definitive host is generally high, which guarantees the survival of sufficient number of parasite stages to be transmitted to the next intermediate hosts. In case of parasites with an indirect life cycle, the presence of a determined parasite in a host indicates that other required hosts are also present in that biotope. These facts should be taken into consideration when setting prevention programs, which should aim at interrupting the parasitic life cycle at the most convenient stage. Humans may serve as definitive, intermediate, or accidental hosts for several meat-associated parasites. The pathogenic impact of these parasites can be related to the different role of humans in the life cycle. Generally, the pathogenic impact is higher when humans act as intermediate or accidental hosts (i.e., larval encystation in meat or viscera) than when they are definitive hosts (harboring adult parasite stages in the gut) of these parasites.

A special case is represented by some parasite species that may also occur in the viscera of farmed animals (i.e., *Fasciola hepatica*, *Dicrocoelium* sp., Protostrongylids, *Dictyocaulus* sp., and *Eimeria stiedae*) but developed other evolutionary strategies other than carnivorism for their spread. These parasites affect mainly lungs or liver in their adult phase, and the dissemination stages (i.e., eggs, larvae, and oocysts) can reach the environment through the host feces and further develop into a direct or an indirect life cycle involving one or more intermediate hosts.

In Table 1, the main parasite species associated with meat or viscera of farmed animals are listed.

Farmed Animals as Intermediate Hosts

Because most farmed meat-producing animals are herbivores or omnivores, their role in the life cycle of parasites associated with meat is mostly as intermediate hosts (harboring parasite stages in muscles or organs), whereas definitive hosts are generally represented by domestic carnivores (i.e., dogs and cats), wild carnivores (i.e., foxes and wolves), or humans (i.e., for *Taenia saginata* and *Taenia solium*), which account for contamination of the farm environment with parasite stages, generally shed with the feces.

Farmed Ruminants

Farmed ruminants can harbor stages from several parasite species in edible tissues (meat and viscera). The most important zoonotic parasites affecting ruminants include *Taenia* and *Echinococcus* species and *Toxoplasma gondii*. Humans get infected orally by consumption of raw or undercooked tissues harboring immature stages of these parasites (i.e., cysticerci, *T. gondii* tissue cysts). Some trematodes, such as *F. hepatica*,

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Table 1 Main parasite species that can be present in meat or viscera of farmed animals

Parasite group	*Parasite species*	*Definitive hosts (DHs)*	*Localization and size of adult stages in DHs*	*Intermediate (IH), accidental (AH), and paratenic hosts (PH)*	*L = Larval stage (size); main localization in IH and AH*	*Zoonotic potential*[a]/ meatborne[c]	*Geographic distribution*
Cestodes	*Taenia solium* (pork tapeworm)	Humans	Small intestine; length: 3–4 m	Domestic and wild pigs (IH), humans, dogs, cats, and ruminants (AH)	L: Cysticercus cellulosae (0.5–1.5 cm, sometimes bigger in CNS); skeletal and cardiac muscle, seldom CNS, eye, subcutis, liver, kidney, and lung	Yes/yes	Latin America, Asia, Africa, and very rare in Eastern and Southern Europe
	Taenia saginata (beef tapeworm)	Humans	Small intestine; length: Up to 10 m (seldom longer)	Cattle, buffaloes, and other bovids (IH)	L: Cysticercus bovis (0.3–1 cm); skeletal and cardiac muscle, seldom esophagus, subcutis, and adipose tissue	Yes/yes	Worldwide
	Taenia asiatica	Humans	Small intestine; length : 4–8 m	Domestic and wild pigs, seldom cattle (IH), goats, monkeys (AH)	L: Cysticercus viscerotropicus (0.2 cm); liver, seldom other organs (lung and omentum)	Yes/yes	Asia
	Taenia hydatigena	Dogs, foxes, wolves, and other canids, and cats	Small intestine; length: 0.5–2.5 m	Domestic and wild ruminants, swine, and equines (IH)	Cysticercus tenuicollis (1–7 cm); liver, subserous tissues in abdominal cavity, and seldom thoracic cavity	No/no	Worldwide
	Taenia ovis	Dogs, foxes, and wolves	Small intestine; length: 0.6–1.5 m	Small ruminants (IH)	Cysticercus ovis (0.2–3.5 cm); skeletal and cardiac muscles	No/no	Worldwide
	Taenia cervi (for some authors syn. of *T. ovis)*	Dogs and foxes	Small intestine; length: 2–2.5 m	Cervids (IH)	Cysticercus cervi; striated muscles	No/no	Europe
	Taenia pisiformis	Dogs, foxes, wolves, and seldom cats	Small intestine; length:0.3–1.5 m	Rabbits, hares, rodents (IH), dogs, goats, and equines (AH)	Cysticercus pisiformis (0.5–0.7 cm); liver and subserous tissues in abdominal cavity	No/no	Worldwide
	Taenia multiceps	Dogs, foxes, wolves, and other canids	Small intestine; length: 0.2–1.2 m	Domestic and wild ruminants, swine, equines (IH), dog, and humans (AH)	Coenurus cerebralis (2–6 cm, sometimes bigger); brain, seldom spinal cord, in AH also subcutis and eye	Yes (rare)/no	Worldwide, but not in Central and Northern Europe, Northern America, and Australia
	Taenia serialis	Foxes, wolves, and seldom dogs and cats	Small intestine; length:0.2–0.7 m	Rabbits, hares, seldom rodents (IH), dogs, and humans (AH)	Coenurus serialis (1–6 cm, sometimes bigger); connective tissue and muscles (CNS in AH)	Yes (rare)/no	Worldwide
	Taenia brauni	Canids	Small intestine	Rodents (IH) and humans (AH)	Coenurus brauni; subcutis and eye	Yes (rare)/no	Africa
	Taenia polyacantha	Foxes, seldom dogs, and other canids	Small intestine; length:0.1–0.14 m	Rodents, rabbits, and hares (IH)	Cysticercus (up to 3 cm); body cavities	No/no	Northern hemisphere

(*Continued*)

Table 1 Continued

Parasite group	*Parasite species*	*Definitive hosts (DHs)*	*Localization and size of adult stages in DHs*	*Intermediate (IH), accidental (AH), and paratenic hosts (PH)*	*L = Larval stage (size); main localization in IH and AH*	*Zoonotic potential*[a]/ meatborne[c]	*Geographic distribution*
	Echinococcus granulosus (sheep strain G1; Tasmanian sheep strain G2; buffalo isolate G3)	Dogs, dingoes, and jackals	Small intestine; length: 2–7 mm	Small ruminants, cattle, buffaloes, yaks, and pigs (IH); humans and other mammals (AH)	Hydatid cyst (few mm–> 20 cm); liver, lung, and other organs	Yes/no	Worldwide
	Echinococcus equinus (horse strain G4)	Dogs	Small intestine; length: 2–7 mm	Equines (IH)	Hydatid cyst; liver	Unknown/no	Europe, Africa, and the Middle East
	Echinococcus ortleppi (cattle strain G5)	Dogs	Small intestine; length: 2–7 mm	Cattle, small ruminants, buffaloes (IH), and humans (AH)	Hydatid cyst; lung and liver	Yes (rare)/no	Europe, Asia, Africa, and Central and South America
	Echinococcus intermedius (camel strain G6; pig strain G7; strain G9)	Dogs	Small intestine; length: 2–7 mm	Pigs, goats, camels, cattle (IH), and humans (AH)	Hydatid cyst; liver and lung	Yes/no	Europe, Asia, and South America
	Echinococcus canadensis (cervid strain G8, 10)	Wolves and dogs	Small intestine; length: 2–7 mm	Cervids (IH) and humans (AH)	Hydatid cyst; lung and liver	Yes/no	Northern Eurasia and North America
	Echinococcus multilocularis	Red foxes, arctic foxes, wolves, raccoon dog, dog, and cat	Small intestine; length: 1.2–4.5 mm	Rodents (IH), pigs, horses, humans, and other mammals (AH)	Alveolar hydatid cyst; liver and other organs	Yes/no	Northern hemisphere
	Spirometra spp.	Dogs, cats, and other carnivores	Small intestine; length: 0.1–1 m	Copepods (1st IH) ; reptiles, amphibians, birds, and mammals (i.e., rodents, pigs, humans, dogs, and cats) (2nd IH)	Procercoid in 1st IH, plerocercoid (syn. sparganum) in 2nd IH (1–40 cm); body cavities, viscera, subcutis, intermuscular connective tissue, CNS, and eye	Yes/yes	Europe, Asia, and America
Trematodes	*Alaria alata*	Foxes, wolves, raccoon dog (dog and cat)	Small intestine; length: 2.5–6 mm	Planorbid snails (1st IH) ; frogs (2nd IH) ; amphibians, reptiles, birds, and mammals (i.e., wild pigs and humans) (PH)	Cercaria (1st IH), mesocercaria (2nd IH and PH) (0.4–0.7×0.2 mm); striated muscles and associated connective tissue	Yes (rare)/yes	Europa and Asia
	Fasciola hepatica (common liver fluke)	Domestic and wild ruminants, horses, pigs, rabbits, hares, humans, and other mammals	Liver (juveniles in parenchyma and adults in bile ducts); length: 2–5 cm	Snails (IH) (*Lymnaea* spp.)	Sporocist, redia, and cercaria	Yes/no–yes[b]	Worldwide

	Parafasciolopsis fasciolaemorpha (moose fluke)	Moose, bison, cervids, and sheep	Small intestine and liver (bile ducts and gall bladder); length: 3–7 mm	Snails (IH) (*Planorbarius corneus*)	Sporocist, redia, and cercaria	No/no	Eastern and Southeastern Europe
	Fascioloides magna (large American liver fluke)	Cervids; moose, bison, and domestic ruminants: Parasites do not reach maturity	Liver (juveniles in parenchyma and adults in cysts communicated with bile tract); Length: 7–10 cm	Snails (IH) (*Lymnaea* spp.)	Sporocist, redia, and cercaria	No/no	North America and Europe
	Fasciola gigantica (large liver fluke)	Cattle, buffaloes, sheep, goats, horse, camel, humans, and other mammals	Liver (juveniles in parenchyma and adults in bile ducts); Length: 2.5–7.5 cm	Snails (IH) (*Radix auricularia*-complex)	Sporocist, redia, and cercaria	Yes/no–yes[b]	Subtropical and tropical regions in Africa and Asia
	Dicrocoelium dendriticum (lancet fluke)	Domestic and wild ruminants, camelids, rabbits, hares, horses, humans, and other mammals	Liver (bile ducts); length: 0.5–1 cm	Snails and slugs (1st IH); ants (2nd IH)	Miracidium, sporocyst, and cercaria in 1st IH; cercaria and metacercaria in 2nd IH	Yes (rare)/no	Regional in North America, Europe, North Africa, Asia, and some South American countries
Nematodes	*Trichinella* spp.	Domestic and wild pigs, horses, dogs, cats, foxes, raccoon dogs, rat, other wild animals, and humans (AH)	Small intestine; length; 1–3. 7 mm	The whole cycle occurs in the same host	Encapsulated larva I (nonencapsulated for some *Trichinella* spp.) (capsule: 300–700×200–300 μm; larva I up to 1 mm); skeletal muscle	Yes/yes	Worldwide (some *Trichinella* species have regional distribution)
	Protostrongylus sp., *Muellerius* sp., *Cystocaulus* sp., and *Neostrongylus* sp. (small lung worm)	Small ruminants, cervids, rabbits, and hares	Lungs (parenchyma, small bronchi, and bronchioles) length: 0.5–3 cm	Snails and slugs (IH)	Larvae I–III	No/no	Regionally worldwide
	Dictyocaulus filaria (large lung worm)	Small ruminants and cervids	Lungs (trachea and bronchi); length: 5–10 cm	No	No	No/no	Worldwide
	Dictyocaulus viviparus (large lung worm)	Cattle, other bovids, and cervids	Lungs (trachea and bronchi); length: 3.5–7 cm	No	No	No/no	Worldwide
	Metastrongylus spp. (swine lung worm)	Wild and domestic pigs	Lungs (trachea and bronchi); length: 2.5–5 cm	Earthworms (IH)	LI–III	No/no	Worldwide
Pentastomids	*Linguatula serrata*	Dogs, foxes, wolves, and humans (AH)	Nasal cavity and paranasal sinuses; length: 2–13 cm	Domestic and wild ruminants, pigs, horses, and rodents (IH)	Encysted larva (up to 5 mm); abdominal and thoracic organs	Yes (rare)/yes	Regionally worldwide

(*Continued*)

Table 1 Continued

Parasite group	*Parasite species*	*Definitive hosts (DHs)*	*Localization and size of adult stages in DHs*	*Intermediate (IH), accidental (AH), and paratenic hosts (PH)*	*L = Larval stage (size); main localization in IH and AH*	*Zoonotic potential*[a]/ meatborne[c]	*Geographic distribution*
Protozoans	*Sarcocystis hominis*	Humans; other primates	Small intestine; (sporocysts: Approximately 13–16 μm)	Cattle (IH)	Merozoite (6×3 μm); endothelial cells of many organs only during acute infection phase, cyst (sarcocyst) (7 mm); Striated muscle	Yes/yes	Worldwide
	Sarcocystis suihominis	Humans; other primates	Small intestine; (sporocyst: Aprroximately 13–16 μm)	Pigs (IH)	Merozoite (6×3 μm); endothelial cells of many organs only during acute infection phase, cyst (Sarcocyst) (1.5 cm); striated muscle	Yes/yes	Worldwide
	Sarcocystis bertrami (syn. *S. fayeri*)	Dogs	Small intestine; (sporocysts 12–14 μm)	Equids (IH)	Cysts (up to 9 mm); skeletal muscle	No/no	Worldwide
	Sarcocystis equicanis	Dogs	Small intestine; (sporocysts 15–16 μm)	Equids (IH)	Cysts (up to 0.6 mm); skeletal muscle	No/no	Worldwide
	Toxoplasma gondii	Cats and wild felids	Small intestine; (oocyst: 10×12 μm)	Virtually all warm-blooded animals (i.e., pigs, small ruminants, etc.) (IH); humans (AH)	Tachyzoite (4–7×2–4 μm); virtually all types of tissues generally during acute infection phase; cyst (up to 100 μm); striated muscle, SNC, and other organs	Yes/yes	Worldwide
	Neospora caninum	Dogs, coyotes, and wolves	Small intestine; (oocyst: 10×12 μm)	Cattle, small and wild ruminants, dogs (IH); other mammals (birds)	Tachyzoite (4–7×2–4 μm); mainly liver, lung, brain, and other tissues during acute infection phase.; cyst (up to 100 μm); SNC, skeletal muscle (stages morphologically similar to *T. gondii*)	No/no	Worldwide
	Eimeria stiedae	Rabbits	Liver (bile ducts); (oocysts 30–40×15–25 μm)	No	No	No/no	Worldwide

[a]Exceptional or unconfirmed human cases were not considered in this classification.
[b]Potential in case of consumption of liver infected with immature stages.
[c]Only the transmission to humans (and not to all DH) through edible tissues was considered for this classification.
Source: Reproduced from Deplazes, P., Eckert, J., von Samson-Himmelstjerna, G., Zahner, H., 2013. Lehrbuch der Parasitologie für die Tiermedizin. Stuttgart: Enke Verlag; OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, third ed. Paris: OIE (World Organisation for Animal Health); and Schnieder, T., 2006. Veterinärmedizinische Parasitologie. Stuttgart: Parey.

Fasciola gigantica, and *Dicrocoelium* sp. that are present in the liver of ruminants can also be zoonotic, but they are usually not transmitted directly to humans by consumption of infected tissues. These parasites undergo an indirect cycle with involvement of one (*Fasciola*) or more (*Dicrocoelium*) invertebrate intermediate hosts (i.e., snails and ants). Definitive hosts (humans and ruminants) get infected by accidental ingestion of larval stages (metacercariae) present mainly on vegetables (*Fasciola*) or in ants (*Dicrocoelium*).

Ruminants are intermediate hosts of many *Taenia* species and harbor the larval stages or metacestodes (cysticercus or coenurus depending on the *Taenia* species) in their tissues. These vesicular stages have a macroscopic size and can be detected during meat inspection at slaughterhouse. Humans are the definitive hosts of *T. saginata*, whose larval stages, Cysticercus bovis, are found in skeletal and cardiac muscle of cattle, buffaloes, and other bovid species worldwide. Cysticercus from other *Taenia* species (i.e., *Taenia hydatigena* and *Taenia ovis*) or coenurus present in ruminants' tissues are infectious for their definitive canid hosts but normally not infectious for humans. Humans can be accidental hosts of some *Taenia* species from canids (i.e., *Taenia multiceps* and *Taenia crassiceps*) and some cases of larval development in human tissues after ingestion of eggs from canids' feces have been observed. Ruminants are also intermediate hosts for *Echinococcus* species (i.e., *Echinococcus granulosus*, *Echinococcus ortleppi*, and *Echinococcus canadensis*), with development of hydatid cysts mainly in the liver and the lungs. Dogs and other canids get infected through ingestion of these larval stages with development of adult worms in the small intestine and shedding of eggs with the feces, accounting for environmental contamination. Humans, who serve as accidental intermediate hosts, can develop hydatidosis after ingestion of *Echinococcus* eggs, with development of hydatid cysts in the liver, lung, central nervous system (CNS), or other tissues.

Toxoplasma gondii is a worldwide-distributed, cyst-forming protozoan parasite that affects warm-blooded animals and humans. Ruminants get infected during grazing through ingestion of sporulated oocysts shed by the definitive hosts (cats and wild felids). Sheep along with goats possess the highest incidence of cysts in meat, playing an important role as a source of infection for humans. In these animals, the parasite can persist asymptomatically in the form of microscopic bradyzoite-containing tissue cysts, mainly in the brain and muscles. *Toxoplasma gondii* is morphologically similar to *Neospora caninum*, a nonzoonotic parasite that can occur in ruminants' tissues (mainly CNS and muscle) and represents a major cause of abortion in cattle worldwide and can also affect small and wild ruminants and other animal species. Cattle can harbor cysts of zoonotic (*Sarcocystis hominis*) and nonzoonotic *Sarcocystis* species (*Sarcocystis cruzi* and *Sarcocystis hirsuta*), mainly in striated muscle tissue. Humans get infected by ingestion of undercooked meat containing cysts of *S. hominis* and can develop gastrointestinal signs. A differentiation of these species is important from a public health and economic point of view because only *S. hominis* is zoonotic. *Sarcocystis cruzi* and *S. hirsuta* are highly prevalent worldwide; they have canids and cats as definitive hosts, respectively, and have no clinical consequence for the definitive hosts. Therefore, condemnation of meat infected by nonzoonotic *Sarcocystis* species not affecting meat quality (i.e., microscopic cysts and no visible macroscopic changes) would not be required. Tissue cysts of *Sarcocystis* species can be differentiated by microscopic and electron microscopic examination and by molecular methods. Small ruminants can be affected by many *Sarcocystis* species, sometimes with macroscopic cysts (i.e., *Sarcocystis gigantea*), but they are not zoonotic.

Farmed Swine

All major meatborne parasites of public health importance (*Trichinella* spp., *T. solium*, *T. gondii*, and *Sarcocystis suihominis*) are associated with pork from domestic pigs and wild boars. Although the risk of porkborne parasites, particularly for *T. solium*, may be currently considered limited in countries with high sanitation standards and where pigs are mostly raised under controlled housing conditions, these parasites are still endemic and highly prevalent in humans in some geographical regions, representing important public health concerns.

Owing to its public health importance and ubiquitous pattern, *Trichinella* is one of the most-studied parasite worldwide affecting domestic and wild swine. Its low host specificity and wide distribution increase opportunities for interspecific transmission, whose patterns have been extensively studied. At the present time, it is recognized that the epidemiology of trichinellosis is characterized by a considerable complexity with 12 *Trichinella* taxa described so far.

Trichinella spp. is one of the few meatborne parasites with all developing stages occurring within one host. It has a very broad range of host species (mammals, birds, and reptiles), mainly those with cannibalistic and scavenger behavior; however, only humans become clinically affected. Because *Trichinella* transmission involves carnivorism at each stage, the only farmed animals involved in the transmission are swine apart from accidental ingestion of contaminated feed by herbivores (horses). Following ingestion of infected raw or undercooked meat containing larvae, these are released in the stomach, they mature in the small intestine developing to adult female and male worms, and reproduce and give progeny to new larvae (LI) that enter the blood circulatory system and invade skeletal muscle. Here most of the *Trichinella* species/genotypes become encapsulated (excluding *Trichinella pseudospiralis*, *Trichinella papuae*, and *Trichinella zimbabwensis*) until being ingested by the next host. The tongue, diaphragm, and masseter muscles are considered sites of choice for *Trichinella* encystation, but this may vary according to the host species.

Among the cestode species associated with pork, *T. solium* is one of the most investigated due to its peculiar public health implication where humans act both as definitive and intermediate hosts. In fact, the larval stage (Cysticercus cellulosae) can develop in swines and humans after accidental ingestion of eggs shed by humans harboring adult parasites in the small intestine; moreover, autoinfections are also possible. *Taenia solium* as adult and larval infection (leading to taeniosis and cysticercosis, respectively) is estimated to infect millions of people worldwide, particularly in countries with inadequate sanitary infrastructure and insufficient health education.

Cysticercus cellulosae in humans develop mainly in the muscles, eyes, and the CNS, causing neurocysticercosis, which is one of the most serious parasitic diseases characterized by a wide range of neurological disorders.

The relatively new species *Taenia asiatica* has also been found to infect humans in addition to *T. solium* and *T. saginata*, whose distribution is restricted to Asian countries. Although according to the World Health Organization (WHO)/Food and Agriculture Organization (FAO)/ World Organization for Animal Health, *T. asiatica* does not cause human cysticercosis, due to its similarity with *T. solium*, humans could be potential candidates as *T. asiatica*'s intermediate hosts.

Pork is considered one of the major sources for *T. gondii* infections in humans. The infection in swine is generally asymptomatic, but in some occasions clinical disease (anorexia, apathy, fever, cyanosis, dyspnea, hind limb weakness, and even death) and abortion or neonatal mortality were reported. The prevalence of infection in swine varies enormously according to the categories and age of pigs tested, for example, piglets versus market pigs or sows, and the management system, for example, free range versus biosecure indoor systems. *Toxoplasma gondii* infections have also been described in wild boars.

Among the species of *Sarcocystis* found in pork, *S. suihominis*, with humans as definitive hosts, is of public health importance and can cause gastrointestinal symptoms, whereas both *S. suihominis* and *Sarcocystis miescheriana* (syn. *Sarcocystis suicanis*) with dogs and other canids as definitive hosts can be pathogenic for pigs, causing myositis, myocarditis, lameness, dyspnea, thrombocytopenia, disseminated intravascular coagulation, and even death.

Equine Species

Horses and donkeys are considered meat animals in many European, South American, and Asian countries; however, in some parts of the Western world, particularly in the US, the UK, and Ireland, they are forbidden food because of the role of equids as companion animal. In Europe, France, and Italy account for 71% of the total horsemeat consumed in the European Union (EU).

The parasites associated with horsemeat of public health importance are *Trichinella* spp. and *T. gondii*. Concerning *Trichinella*, as equine species are herbivores, the possible ways of infection are grazing in pastures contaminated with infected carcasses or ingesting infected flesh from pigs and wild carnivores, even as practice of using carcasses of carnivores bred in captivity, or hunted for their fur, for a mash to fatten horses before slaughter. Human cases of *Trichinella spiralis* in Western Europe have occurred in Italy and France in the 1990s due to consumption of meat from horses imported from Eastern Europe.

Toxoplasma gondii is uncommon in horses, although viable cysts have been isolated from horses slaughtered for export. Three cases of human toxoplasmosis caused by atypical strains probably acquired by ingestion of raw horsemeat imported from Brazil and Canada were detected in France.

Sarcocystis bertrami, *Sarcocystis equicanis* (with canids as definitive hosts), and *Sarcocystis neurona* (with opossums as definitive hosts) can infect equids, although the latter does not encyst in muscle but affect nervous tissue.

Poultry

Parasites are rarely found in poultry meat with the exception of some *Sarcocystis* species and *T. gondii*. Free-range chickens can be considered indicators for environmental contamination with *T. gondii*, but the infection rarely causes clinical disease. Ingestion of infected (raw or undercooked) chicken meat can be a potential source of infection for *T. gondii*.

Besides, poultry organs (mainly the liver and the lungs) and muscles can harbor *Toxocara canis* and *Toxocara cati* larvae and are potential sources of human toxocariasis if these tissues are ingested raw or undercooked.

Histomonas meleagridis is a frequent protozoan parasite of turkeys. It is not zoonotic but induces macroscopic changes in the liver, characterized by round (3–10 mm) necrosis foci that can be seen during inspection at slaughter. Histomoniasis may have high morbidity and mortality, mostly in young birds, and can cause important economic losses.

Farmed Lagomorphs

Protozoa, such as *T. gondii* and *E. stiedae*; larval stages of cestodes, i.e., cysticerci of *Taenia pisiformis, Taenia serialis*, and *Taenia polyacantha*; liver flukes, such as *Dicrocoelium dendriticum* and *F. hepatica*; and lung nematodes, such as *Protostrongylus pulmonalis* are among the most common and studied parasites present in meat and viscera in rabbits and hares.

Detection at Slaughter: New Approaches

Parasitic meatborne infections that can be detected macroscopically, such as cysticercosis in cattle and pigs, are traditionally controlled at the end of the meat production chain by visual inspection of single carcasses. Nevertheless, such an approach may be obsolete in countries with high biosecurity farming systems (e.g., swine production) where prevalence of meatborne parasites in farmed animals and the risk of human infection may be very low. This consideration is also valid for the monitoring of individual swine carcasses to control and prevent trichinellosis in humans, as currently prescribed in Europe as described by the EU legislation 2075/2005, where the current examination method for the detection of *Trichinella* spp. larvae is based on isolation of the larvae by artificial digestion and microscopic identification. Although derogations from the testing program for herds or regions where the risk of *Trichinella* infection in domestic swine is officially recognized as being negligible can be foreseen, the *Trichinella* monitoring program imposes huge economic costs. Modern food production requires a risk-based approach in which several critical control points are monitored and where quality assurance systems are designed considering cost effectiveness. These are some of the factors leading the European regulatory body to revise the current meat inspection practice. As a consequence, the European Food Safety Authority (EFSA) is performing a systematic risk assessment for the modernization of meat

inspection in the EU in order to introduce a risk-based approach to meat inspection at all relevant stages of the meat production chain.

Drivers for Transmission of Meatborne Parasites among Farmed Animals and to Humans

The maintenance of the life cycle of meatborne parasites among farmed animals and their transmission to humans is influenced by, for example, farming system and eating habits, both factors facilitating transmission to intermediate and definitive host, respectively.

Farming System

Farming and agriculture systems represent a major artificial ecosystem in which parasite development and transmission among farmed animals can be influenced by many factors. Because meatborne parasites are transmitted to farmed animals through carnivorism, scavenging on infected preys, or through feeding on pasture or feed contaminated with infective eggs or oocysts, parasite-free farming implies strict indoor housing of animals, including pest control, proper feed preparation and storage, proper slurry management, and general good hygiene. Nevertheless, where biosecurity regimes are not sufficient, meatborne parasite transmission may be facilitated. This may be even truer where the intensification of agriculture is increasing rapidly, especially for poultry and pigs. Moreover, with increasing ecological awareness and request for higher animal welfare standards, food production is evolving toward more sustainable agriculture and organic farming. This increased environmentally sound or animal-friendly livestock production often implies outdoor rearing, which brings livestock in close contact with both the environment and wildlife and may increase the liklihood parasite transmission.

Eating Habits

Among the raw and undercooked foods that may pose a risk for parasitic infection to humans, meat preparation plays a major role. Meat consumption from different animal species is strongly influenced by religious and cultural factors. Pork, which may be a source of infection with *Trichinella* spp., *T. gondii*, and *T. solium*, is avoided by Jews, Orthodox Christians in Ethiopia, and Muslims due to religious beliefs. Beef, which may contain cysticerci of *T. saginata*, is not consumed by Hindus as cows are sacred in their culture. This has a major impact on distribution of infections in various societies throughout the world.

Public Health Impact of Zoonotic Meatborne Parasites from Domestic Animals

The major contributors to the global burden of parasitic zoonoses are cysticercosis, echinococcosis, toxoplasmosis, foodborne trematode infections, leishmaniosis, and zoonotic schistosomosis. In the long list of approximately 100 foodborne parasite species of zoonotic importance, meatborne parasitic species represent a minority and could be restricted to *Trichinella* spp., *T. gondii*; *S. hominis* and *S. suihominis*; and *T. saginata* and *T. solium*. Nevertheless, in a recent evaluation of the global public health impact of 24 preselected foodborne parasites by the FAO/WHO experts, three of the above-mentioned meatborne species are ranked in the top 10 foodborne parasitic species, namely *T. solium* (1st place), *T. gondii* (4th place), and *T. spiralis* (7th place). Public health importance was the primary driver of ranking with almost equal importance given to illness and severity of disease. The disease burden of the above-mentioned species has been thoroughly studied worldwide.

Disclaimer

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See also: Manure/Waste Management: Manure Management. Meat, Animal, Poultry and Fish Production and Management: Meat Production in Organic Farming. Meat-Borne Hazards, Concepts and Methods for Mitigating Risks Related to. Microbiological Safety of Meat: Emerging Pathogens

Further Reading

Alban, L., Pozio, E., Boes, J., *et al*., 2011. Towards a standardised surveillance for *Trichinella* in the European Union. Preventive Veterinary Medicine 99, 148–160.
Broglia, A., Kapel, C., 2011. Changing dietary habits in a changing world: Emerging drivers for the transmission of foodborne parasitic zoonoses. Veterinary Parasitology 182, 2–13.
Deplazes, P., Eckert, J., von Samson-Himmelstjerna, G., Zahner, H., 2013. Lehrbuch der Parasitologie für die Tiermedizin. Stuttgart: Enke Verlag.
Dorny, P., Murrell, K., 2005. WHO/FAO/OIE Guidelines for the Surveillance, Prevention and Control of Taeniosis/Cysticercosis. Paris: OIE (World Organisation for Animal Health).
Dorny, P., Praet, N., Deckers, N., Gabriel, S., 2009. Emerging food-borne parasites. Veterinary Parasitology 163, 196–206.
Dubey, J.P., 2010. Toxoplasmosis of Animals and Humans. Boca Raton, FL: CRC Press.
Dubey, J.P., Speer, C.A., Fayer, R., 1989. Sarcocystosis of Animals and Humans. Boca Raton, FL: CRC Press.
European Food Safety Authority, 2011. Scientific opinion on the public health hazards to be covered by inspection of meat (swine). EFSA Journal 9, 2351.
FAO/WHO, 2012. Multicriteria-based ranking for risk management of foodborne parasites (preliminary report). Report of a Joint FAO/WHO Expert Meeting. Rome: Food and Agriculture Organisation.
Fayer, R., 2004. *Sarcocystis* spp. in human infections. Clinical Microbiology Review 17, 894–902.
Gottstein, B., Pozio, E., Noeckler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clinical Microbiology Review 22, 127–145.
Murrell, K.D., Pozio, E., 2011. Worldwide occurrence and impact of human trichinellosis, 1986–2009. Emerging Infectious Disease 17, 2194–2202.
OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, third ed. Paris: OIE (World Organisation for Animal Health).
Schnieder, T., 2006. Veterinärmedizinische Parasitologie. Stuttgart: Parey.
Tenter, A.M., 2009. *Toxoplasma gondii* in animals used for human consumption. Memórias do Instituto Oswaldo Cruz 104, 364–369.
Torgerson, P.R., Macpherson, C.N.L., 2011. The socioeconomic burden of parasitic zoonoses: Global trends. Veterinary Parasitology 182, 79–95.

PATENTING PRODUCTS, PROCESSES, AND APPARATUSES

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Introduction

Inventors have always, exclusively, wanted the right to own their inventions since time immemorial, but the evolution of a justice system, with statutory rules for the protection of rights, is more recent.

The modern democratic political principles of the nineteenth century brought new legislative initiatives, which also embraced patent law. Today, patent law comes under the law governing intellectual property, which in turn comes under property law.

Intellectual property is so called because it is intangible. Intellectual property law grants the exclusive right to that which one person or group of persons have personally created through intellectual effort, for example, an invention.

However, it was not until the end of the nineteenth century, when industrialism was well established, that patent law succeeded as an important, general legal discipline, marked by international agreements and conventions. As a result, the countries of Europe (cf. the European Patent Convention (EPC)) have experienced an overall harmonizing of their patent rules.

Legal Requirements for Patentability of Inventions

Patents are an integral part of the world of technology. According to patent law, an invention is defined as a technological product capable of being reproduced. For an invention to be deemed patentable in Europe, three criteria must be adhered to:

- The invention must be new, i.e., it must never have been publicly disclosed anywhere in the world.
- The invention must comprise an inventive step, i.e., it must differ essentially from the state of the art before the date of filing of the patent application.
- The invention must be capable of industrial application.

Patents are granted for products, methods/processes, apparatuses/devices, and use of products. Each country has certain exceptions to the rule as to what can be regarded as inventions, for example:

- plant or animal species;
- essentially biological processes for the production of plants or animals; and
- inventions, the commercial utilization of which is contrary to the morals and public order of society.

Patent – An Exclusive Right

A person who obtains patent protection for an invention gains an all-embracing exclusive right, as others are forbidden from producing, marketing, importing, or selling the invention for commercial purposes. In return, the invention becomes publicly available by way of the patent application, allowing anyone the opportunity, on the basis of the invention, to further develop the technology. The application is published 18 months after the filing date. This is the price the inventor pays to society for his protection.

Patent protection is thereby a contract between the inventor/company and the rest of society. Inventors and companies are given the incentive to develop their products, as the return on development costs is achieved by preventing competitors from copying. In return, the publication of the invention allows society to be fully informed about the invention, thus promoting technological development.

The patent is a legal document valid for 20 years commencing from the date the application is filed, on the condition that the annual fees are paid to maintain the patent. Should it be decided that maintaining the patent during its term of validity is no longer advantageous, the patent can be allowed to expire by ceasing payment of the annual fees.

Nowadays, the vast majority of inventions are built on other patented inventions. This could necessitate being involved with other patent holders, i.e., agreements will have to be made with the parties concerned before being able to utilize one's own patent rights. In fact, this rule applies whether one owns a patented product or not. Therefore, it is always wise to be sure there is no risk of infringing the rights of others, as this can cost dearly if investing in production equipment and marketing only leads to the discovery that one does not have the right to produce or sell the product.

In addition, a patent does not provide its owner with the right to utilize the invention if it opposes other laws or rules of society.

Patents are important to economics. First, companies increase their expenses for research and development when innovations can be protected. This gives rise to new inventions and higher levels of productivity. Second, patents increase the spread of knowledge by the publication of the patent. The disadvantage of the patent system is the lack of competition owing to the patentee's (time-limited) exclusive right. However, most economic literature points to the economic advantages of patenting clearly outweighing the disadvantages;

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i.e., a stronger patent protection system provides the means for greater economic growth.

The Patenting Process

The patenting authority examines the patent application, and determines whether a patent can be granted for an invention. The patent authority determines whether the invention is new (novelty search) in relation to that which already exists, and then decides whether the invention differs essentially from prior art (inventive step) in such a way as to achieve a surprising technical effect. The invention must not be an obvious adaptation of prior art. Finally, it is determined whether the invention is capable of industrial application. An element of the examination looks to recognize the invention as progressive improvement on prior art. Depending on the conclusions of the examination, the application is either approved or refused. Before the decision is finally made, a series of correspondence may be made between the parties. A refusal can be appealed against to the appeal board.

Regardless of whether a patent application is approved or refused, it is published 18 months after the filing date, unless the applicant has withdrawn the application before this time.

The invention is protected from the day the patent application is filed. It is very important that the invention has not been made public in any way whatsoever before filing the application, as this could affect the novelty of the invention and lead to refusal of the application. Once the application has been filed, as far as novelty is concerned, the application can be made public, but many choose not to do so, as this could damage the possibility of patenting further development of the invention.

Patent Strategy

A patent is only valid in those countries where the patent has been granted.

Therefore, careful consideration must be given to determining in which countries a product is to be produced and sold. In addition, the cost of patenting depends on the number of countries designated for patent protection.

There are three ways of applying for a patent:

- national patent application;
- European patent application (EPC); and
- international patent application (PCT).

A national patent application is filed separately in each of the countries where patent protection is required.

EPC stands for the European patent convention. A patent application filed under this convention, via the European Patent Office (EPO) will, if the patent is granted, be effective in all of the member countries of the EU, or in those countries specifically designated. For each country designated, a translation of the application is required, plus the payment of an initial fee and subsequent annual fees.

PCT stands for the Patent Cooperation Treaty. The PCT is a collaboration between 120 member countries. The aim of the PCT is to centralize the examination of patent applications. The PCT carries out a novelty search and on request, serving as a guide, a patentability evaluation. After this, the applicant forwards the application to each of the countries designated in the application. The patent authority of each country, according to its own national law, will decide whether or not to grant the patent.

These international patenting systems offer different options for combining the application filing process. Which process is selected depends on the company's individual needs and economic means.

Figure 1 shows an example of a common patent strategy.

The process begins with the filing of a national patent application in the country in which one resides. The patent authority will, within the first 12 months, perform a novelty search and a patentability evaluation. Based on the information obtained from this examination, a decision can be made as to whether one wishes to file the application internationally (e.g., PCT or EPO) or nationally to those countries

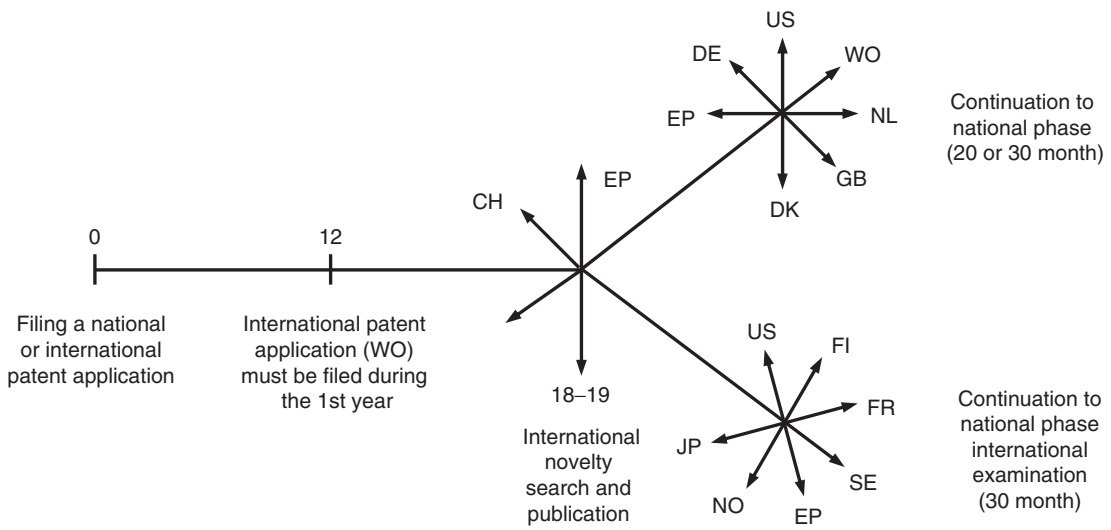

Figure 1 An example of a common patent strategy.

of market interest. The further-extended application, if filed within a 12-month period from the initial date of filing (parent application), will be given the priority date of the first filing date, i.e., the application is deemed valid from the date the application was first filed. Extending the patent application to a PCT application allows a 30-month period in which to decide in which countries the application is to be continued.

Patents as a Source of Information

Nowadays, patent applications that have been published are registered in databases, where they are systematically filed so that they are easy to locate. Apart from using the databases when examining new patent applications for the novelty and inventive step, it is also possible to use them for extracting useful information on, for example:

- innovative activity within a specific field;
- which companies are key players;
- technical areas of current interest; and
- markets of interest to competitors.

The many million items of information in the patent system provide countless opportunities for every company individually to gather invaluable knowledge about the market and the competition.

In the patent applications, the inventions are thoroughly described so as to allow clear insight into new technology, thereby stimulating the inspiration to formulate new ideas that can then be developed.

Patenting in the Meat Technology Field

The field of meat technology has seen a steady rise in patenting worldwide, depicted in Figure 2.

Patent activity varies greatly from country to country. Overall, the US, Japan, and Germany are substantially more active in the meat area than other industrialized countries. This activity can be seen in Figure 3.

Typically, patents are products, processes/methods, and apparatuses/devices and parts/components thereof. The patents span a broad spectrum of technologies from the anaesthetizing of animals before slaughter to processed meat products.

1. Patented slaughter processes: equipment and methods directly associated with the slaughtering process, for example:
   - slaughtering pens and fettering;
   - stunning;
   - fixing;
   - antimicrobial treatment of the carcass;
   - cutting and slaughter equipment and methods with simple tools such as knives, fetters and hooks, and more complex slaughterhouse machinery, for example, for splitting or eviscerating;
   - identification systems; and
   - meat quality measuring equipment.
2. Patenting for meat processing: equipment, methods, and products, for example:
   - cutting meat or bones, bone cleaning, cutting off rind;
   - hanging up meat or sausages;
   - pounding, forming, pressing, tenderizing, or mixing meat;
   - sausage-making;
   - sausage casings;
   - cleaning or working up intestines;
   - marking of meat;
   - extrusion of meat;
   - processing poultry; and
   - working up protein from meat.
3. Patents for meat preservation processes, for example:
   - preserving by heating;
   - irradiation;
   - drying;
   - smoking;
   - freezing/thawing;
   - preserving by inorganic salts, acids, or other chemicals; and
   - preserving with microorganisms or enzymes.

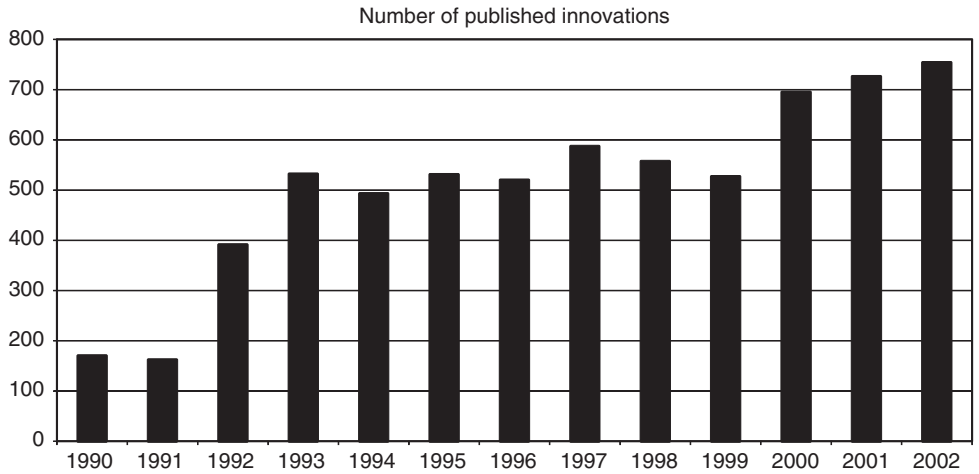

Figure 2 Inventions in the meat technology field registered in the *World Patents Index* from 1990 to 2002.

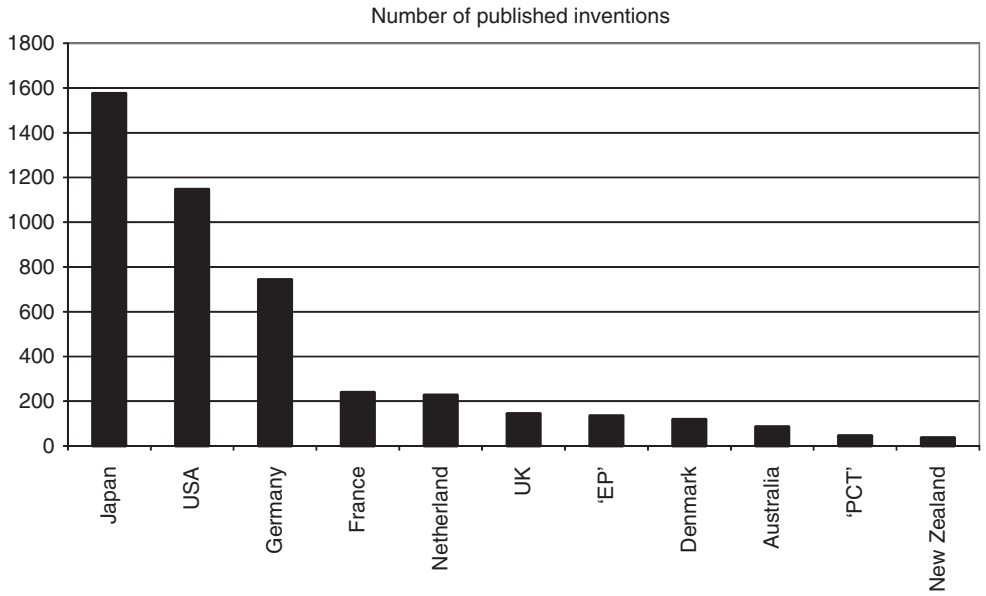

Figure 3 Country of priority for the 6645 meat technology inventions from 1990 to 2002.

4. Patents for meat products, for example:
   - meat meal, convenience food;
   - meat extract;
   - tenderized or flavored meat pieces; and
   - comminuted or emulsified meat products including sausages.

Also patented are methods and equipment for analysis, packaging and packaging methods, food additives, etc.

Activity in the different areas of meat technology is shown in Figure 4, where it can be seen that inventions are far more abundant in the areas of preservation and meat products. As in many other fields of technology, the inventions are typically for optimizing processes and apparatus, for example, to achieve greater cutting precision, simplified processes, improved quality sorting of meat, and easier cleaning, as well as products with improved shelf-life, taste, and appearance.

Examples of Patents

As mentioned earlier, to achieve patent status, an invention must be new and differ essentially from that which is already known (prior art). This means that the invention must comprise a novel technical feature (improvement on a product's properties) in relation to the known product, process, or apparatus.

The following gives a number of examples to illustrate the types of technical features necessary for obtaining a patent for an invention. The examples are divided into products, processes/methods, and apparatuses/devices. Note that the patents/applications in each of these categories may also contain other categories. Therefore, an application for a product can also incorporate the production method and the production apparatus/device. Patents for product use can also be included in the same application.

Products

Patented products are characterized by their composition, for example, providing improved taste, shelf-life, appearance, or texture, or easier and quicker handling of products, such as convenience products.

Low-fat meat product

There is an ever-increasing interest among consumers in food products containing less fat than products having a traditional, full-fat content. Unfortunately, merely eliminating the fat from minced meat food products results in a disagreeable chewy texture as well as a bland taste. There are many inventions in this area aiming to solve the problem, for example, an American patent describes low-fat minced meat products. The products comprise particles of minced meat and fat-like particles made of hydrolyzed milk protein gel particles. The low-fat products have much of the flavor, texture, and appearance of comminuted meat products that have a traditional full-fat content. The hydrolyzed milk protein gels can be combined with meat and processed into meat products using conventional techniques.

Low-fat meat product

An American patent describes a fat-free ham product. This product is made from whole muscle meat cuts and finely textured reduced fat meat combined with brine to give a meat product block. The product is not comminuted or minced, thus giving a more acceptable score at taste testing. Appearance and texture are more acceptable than in comminuted products.

Pizza topping

An American patent describes an uncooked meat product for topping pizzas. It comprises nuggets with a sealing layer containing denatured protein on its external surface and an uncooked central portion. The nuggets retain their shape, do not stick together, and have an improved texture.

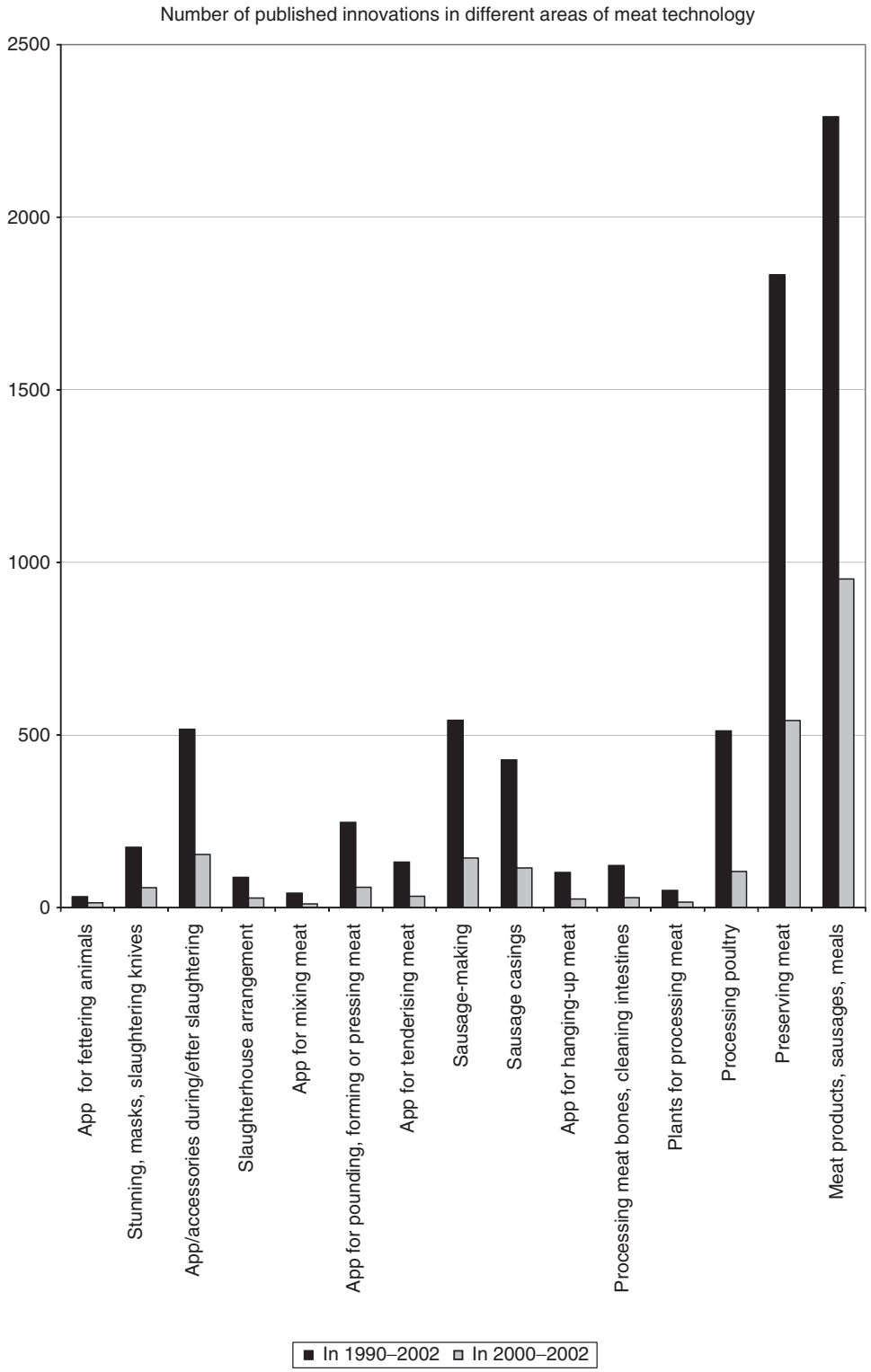

Figure 4 Distribution by area of meat technology of the inventions for the period 1990–2002 (**Figure 2**) and for the subperiod 2000–2002.

Composition for meat

A European patent describes a composition comprising an inorganic propionate salt and a bacteriocin from *Pediococcus acidilactici* and a method comprising inoculation of unspoiled processed meat at pH 6.0 to pH 6.5 using the composition. The composition inhibits the growth of spoilage bacteria in meats, allowing storage at temperatures above freezing. The propionate salts and bacteriocin do not impart any taste to the meat and the additives may be incorporated in a marinade or sauce that dresses a packaged meat.

The method/process and the apparatus for producing the product can also be patented, and can also be included in the same application as for the product.

Processes/Methods

Processes/methods are characterized as those steps that are necessary for achieving the desired technical effect, for example, in the form of a better product or quicker, simpler, or cheaper product production.

Preserving meat by microorganisms

During the past few years there has been increased attention to the occurrence of pathogenic germ cells in food. An international patent application describes a method and an apparatus for the production of sliced food, for example, meat products. During slicing, sufficient amounts of a preparation inhibiting the development of pathogenic germ cells are automatically added and spread onto the surface of the cut-off slices, which results in a product that is safe to use after having been stored for a considerable amount of time at refrigeration temperature. The preparation-inhibiting pathogens may comprise bacteria that are directly or indirectly, via a manufactured bacteriocin, effective against pathogenic germ cells, for example, listeria organisms.

Preserving meat by salts

The treatment and packaging of fresh meat has been a subject of intense research and development for many years. An American patent describes a method for packaging fresh meat. The fresh meat is treated with a predetermined amount of aqueous solution consisting of water, alkali metal lactate, and alkali metal diacetate microbial growth inhibitors in order to increase the weight of meat to approximately 125 wt% of its original weight. The treated meat is then packaged in a closed film package. The shelf-life, taste, tenderness, and freshness of the packaged meat are improved.

Preserving meat with high pressure

A German patent describes a process for production of stable meat and sausage products prepared in the presence of microorganisms, subjected to maturing and drying, wherein on reaching the desired pHs, the semifinished product is exposed to a high-pressure treatment at room temperature and then further processed in the usual way. The method is useful for fixing the pH. The high-pressure treatment kills microorganisms, insects, and parasites present in the product and inactivates enzymes without the formation of toxic components or off-odors.

Prefermentation of meat

A European patent describes the production of a fermented, protein-based meat product – comprising a prefermentation step to reduce the length of the subsequent fermentation. The starter culture comprises a mixture of *Pediococcus pentosaceus* and *Staphylococcus carnosus*. The fermentation step used is shorter without compromising the quality or microbiological safety of the product.

Sausage production

A European patent describes a method and an apparatus for coagulating sausage skins uniformly by spraying brine over extruded sausage as it passes down a serpentine conveyor. The sausage strand is not stretched during transport, and precise weight control is therefore possible. The time during which the sausage is exposed to the brine is precisely controlled, unlike the situation with known methods.

Ham production

A European patent describes a method and an apparatus for curing and cooking of hams without churning and tenderizing as the meat is vacuum-packaged and placed in stackable molds and vibrated. The churning and tenderizing stages of curing meat are replaced by vibrating, which greatly reduces the handling requirements, especially as the meat can be cooked and chilled while still in the molds.

Process for classification of meat

A German patent describes a visual imaging process for the evaluation of slaughtered pig carcass. In an imaging process to assess the distribution of meat, especially pork, within a slaughtered animal carcass, the image is fully assessed and evaluated solely using an electronic data processing system. The process provides reliable classification of the meat for retail purposes.

The product that is being produced by the new process and the apparatus can also be included in the same application as for the process.

Apparatus

Apparatus/device is characterized as the constructive means necessary for the apparatus/device to function as intended, i.e., a description of the precise structural form. This could, for example, be a new apparatus for a new product, or a simplified or in some way changed apparatus or parts thereof that facilitate, speed up, or enable continual production, facilitate cleaning, improve safety, fine-tune the process, improve hygiene, improve the fixing/holding of the product, etc.

Apparatus for evisceration

A European patent describes an apparatus for evisceration of carcasses, especially pigs. The apparatus cuts open the abdomen and breast, allowing the intestines to hang from the abdominal cavity before being separated from the carcass. The need for manpower and heavy work is reduced, and better hygiene is achieved than in traditional organ removal, whereby the connection between the pluck set and the intestines is cut through inside the carcass.

Cutter for splitting animals

A Dutch patent describes a cutter for dividing an animal carcass. The apparatus has a bracket positioning the carcass and a knife driven in a continuous movement to cut longitudinally through the bone. The device makes the carcass division proceed steadily and reliably, avoiding bone splintering, smearing, and cutter-jamming. Sideways displacement

of the cutter can be prevented and replacement of the cutter is easy.

Guide wheels

A European patent describes a guided cutter for dividing animal carcasses into two equal parts. The apparatus has guide wheels that pass either side of the outer surface of the animal's spine, keeping the cutter central with the spine. The guide wheels ensure that the carcass is cut into two symmetrical parts, each with an equal ratio of flesh to bone. The cutting operation can be carried out at high speed.

Sausage-cutting machine

A German patent describes a sausage-cutting machine for cutting strands, especially with natural gut skins, into individual sausages or sausage links. The cutting machine has cutting elements preceded by mold elements holding the sausages apart during cutting. The machine provides reliable cutting of sausages, independently of the link joint properties and without damaging the individual sausages.

Apparatus for antimicrobial treatment

A European patent describes an apparatus for antimicrobial treatment of animal carcasses with directed sheets of heated water. The apparatus comprises housing and two sets of distributing elements for dispensing water sheets downwardly and upwardly on top/under the carcass and systems for supplying water. The apparatus accepts carcasses of variable geometry without significant modification.

Markings for identification

A German patent describes identifying carcasses in slaughterhouses by applying machine-readable markings to abattoir hooks. The hooks can be machine-identified in a rational and inexpensive manner without the need for detachable reusable labels.

Product Use

Product use is defined as the product's specific intended purpose. However, patents for product use are rare in the meat technology field.

How to Prepare a Patent Application

A patent application must, apart from the formalities of name of applicant and inventor, contain the following:

- a title;
- a description of the invention;
- drawings or photographs where necessary to understand the inventions;
- a set of claims; and
- an abstract.

The application must only concern one invention.
The title must be short and specific.

The description must state the technical area with which the invention is concerned and the technique on which the invention builds. The invention must be explained in such a way that the technical problem and its solution can be comprehended, and it must be stated what, specifically, can be achieved by the invention based on prior art, and the means necessary for achieving this. The invention must be supported with examples, and should not be obvious from the nature of the invention, it must be stated how the invention can be industrially applied.

The claims of the patent must state concisely the technical features necessary to achieve the intended effect. There are two types of claims: independent and dependent. A dependent claim states the features that are additional to the features comprised in the independent claim. An application may contain several independent claims as long as there is technical unity between them. The independent claims can be of the categories: a product, a method of production, a production apparatus and a product use for a special purpose.

The abstract must be constructed in a clear and precise manner to cover the technical problem for which the invention has been designed, the method for solving the problem, and the scope of the invention.

The Patent Subsequent to Publication

Opposition

Once the patent authority has completed the examination of the application and the patent has been granted, the patent will be published. It will now be possible to file opposition against the patent within a period of no more than 9 months from the date of publication. Anyone, even the applicant, can oppose the patent, subject to payment of a fee. However, oppositions are usually filed by competitors.

Oppositions are filed in 10% of European patents and many of these lead to the patent being revoked, either in part or entirely.

See also: Automation in the Meat Industry: Cutting and Boning; Slaughter Line Operation. Classification of Carcasses: Beef Carcass Classification and Grading; Pig Carcass Classification. On-Line Measurement of Meat Composition. Packaging: Technology and Films. Preslaughter Handling: Design of Stockyards, Lairages, Corrals, Races, Chutes, and Loading Ramps. Tenderizing Mechanisms: Mechanical

Further Reading

Banss, K.G., Maschf, D.E., 1993. German Patent No. 4132830 C. Identifying Carcasses in an Abattoir by Applying Machine-Readable markings, C & F Packing Company.

C & F Packing Company Inc. (US), 2000. US Patent No. 6,086,941A. Meat Product Having Denatured Protein Surface.

CBS Software Entwicklung (DE), 2002. German Patent No. 19847232 C2. Visual Imaging Process for the Evaluation of Slaughtered Pig Cadavers.

Chabot, B.P., Gill, C.O., Canada Agriculture (CA) and McGinnis DS (CA) (1998) European Patent No. 0738109 B1. Pasteurization of Carcasses with Directed Sheets of Heated Water.

Chartered Institute of Patent Agents, 1998. European Patents Handbook, second ed. London: CIPA
Chr. Hansen, A.S., Lammert, L., Jacobsen, T., Granly, K.A., Slagteriernes Forskningsinstitut, 2002. International Patent Application No. 02056694 A1. A Method and Apparatus for Inhibiting of Pathogens when Producing Sliced Food.
Databases: EPOQUE (EPODOC, WPI), QUESTELL.
Durand International (FR), 2002. European Patent No. 0801900 B1. Method and Means for Dorsal Guiding and Carcass Splitting.
Gall, G., 1989. European & International Patent Applications, Questions and Answers. London: Longmans
Handtmann, A.M. (DE), 1999. German Patent No. 19802101 C1. Device and Method of Separating Sausages.
Hormel Food Corporation (US), 1999. US Patent No. 5989610 A. Chemical Treatment and Packaging Process to Improve the Appearance and Shelf Life of Fresh Meat.
Jehan, R., 1998. European Patent Decisions, 1992–1997. Chorleywood: Goan Publishing.
Kaufler, S.A. (FR), 2002. European Patent No. 0952774 B1. Methods for Treating Pickled Meat Slices, such as Ham Slices.
Kortschack, F. (DE), 1997. German Patent No. 19653677 C1. Method of Producing Stable Products and Sausages, Produced in the Presence of Microorganisms.
Kraft Foods Inc. (US), 1997. US Patent No. 5674550 A. Fat-Free Meat Product.
Ouest International Flavors An (US), 1993. European Patent No. 0463284 B1. Composition for Extending the Shelf Life of Processed Meats.
Patentdirektoratet, 1994. Putting Pep into Patents. Taastrup, Denmark: Danish Patent and Trademark Office
Paterson, G., 1992, 1994, 1997. The European Patent System, 1992 and Supplements 1994 and 1997. London: Sweet and Maxwell.
Slagteriernes Forskningsinstitut (DK), 2001. European Patent No. 0879558 B. Methods and Device for Evisceration of Carcasses.
Stork, R.M.S. BV (NL), 1996. Dutch Patent No. NL 1000260 C. Apparatus for Dividing a Piece of Meat Containing a Bone Structure.
Swift Escrich Inc. (US), 1998. US Patent No. 5,773,057 A. Low-Fat Ground Meat Products.
Townsend Engineering Company (US), 2001. European Patent No. 0932340 B1. Methods and Apparatus for Coagulating Surface of Sausage Strand from a Sausage Extruding Machine.
Unilever, P.L.C., Unilever, N.V., 2002. European Patent No. 0776611 B1. Fermented and Pre-Fermented Protein Based Products.

Relevant Websites

http://www.epo.org/service-support/useful-links/databases.html
European Patent Office.
http://www.oecd.org/sti/innovationinsciencetechnologyandindustry/oecdpatentdatabases.htm
Organisation for Economic Co-operation Development.
http://patft.uspto.gov/
United States Patent and Trademark Office.
http://www.uspto.gov/patents/process/search/
United States Patent and Trademark Office.

PHYSICAL MEASUREMENTS

Contents

Other Physical Measurements

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This article is a revision of the previous edition article by QT Pham, volume 3, pp 1004–1012, © 2004, Elsevier Ltd.

Glossary

Absolute humidity Ratio of the mass of water vapour in the air to that of the other gases, expressed as kilogram moisture per kilogram dry air.
Dew point Temperature to which the air has to be cooled before moisture starts to condense out.
Frost point Temperature to which the air has to be cooled before moisture starts to freeze out as frost.
Humidity ratio See absolute humidity.
Relative humidity (RH) Ratio of water vapour pressure in the air to the saturation water vapour pressure at the same temperature, usually expressed as a percentage.
Water activity RH of air in equilibrium with a product with a given moisture content, usually expressed on a scale from 0 to 1.
Wet bulb depression Difference between temperature of the air and that of a wet surface.

Measurement of Humidity and Water Activity

What are Humidity and Water Activity?

Air humidity influences microbial growth, drying rate, evaporative weight loss, condensation, the heat load on cooling coils, the performance of dryers, and the comfort of workers. Air humidity can be expressed in several ways. Absolute humidity or humidity ratio is the ratio of the mass of water vapor in the air to that of the other gases, expressed as kilogram moisture per kilogram dry air (sometimes also as gram moisture per kilogram dry air or as ppm). Relative humidity (RH) is the ratio of water vapor pressure in the air to the maximum possible (saturation) water vapor pressure at the same temperature, usually expressed as a percentage. Humidity can also be expressed as dew- or frost-point, the temperature to which the air has to be cooled before moisture starts to condense out or freeze out, as the case may be. The relationships between these concepts are illustrated in Figure 1, where air at 25 °C and 60% RH is shown to have an absolute humidity of 0.012 kg kg^{-1} and a dew point of 16.7 °C.

The water activity (a_w) of a product is defined as the RH of air in equilibrium with that product. Conventionally, water activity is measured on a scale from 0 to 1 rather than 0–100%. Water activity measures the degree to which water molecules in the food are free and available to biological processes, rather than bound to other molecules. As such, water activity is a critical factor in determining the shelf life of

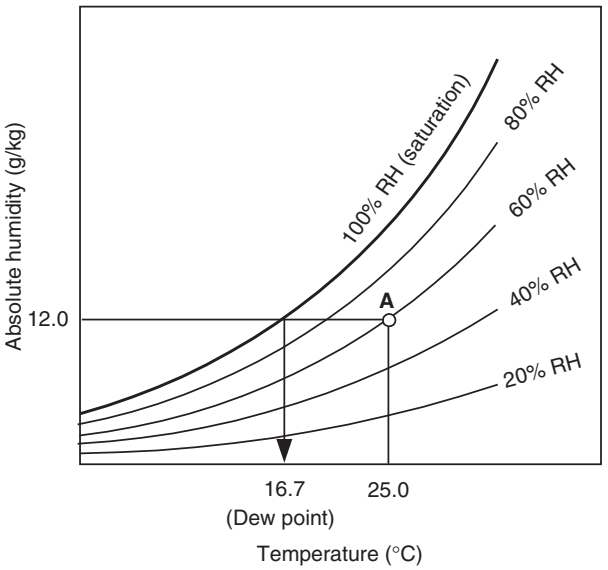

Figure 1 Relationships between dew point, absolute humidity, and RH.

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foods. Most bacteria do not grow at water activities below 0.91, and most molds and enzymatic reactions cease at water activities below 0.80. The range of water activity of most interest to meat technologists is from 0.6 to 1. Fresh meat has a water activity close to 1, whereas below 0.6 most biological reactions cease.

Water activity also determines the transfer of moisture (by evaporation, absorption, and desorption) between different food components and between food and the air. In an isothermal environment, moisture always moves in the direction of decreasing water activity or RH, irrespective of the absolute moisture content. This is important to consider if meat is mixed with other ingredients.

Water activity measurement usually involves equilibrating the product in a small air space and then measuring the humidity of that air space. Water activity measurement will therefore be treated together with air humidity measurement.

A review in 1881 by Symons listed no fewer than 138 devices and methods for humidity measurement, and more have been invented since then. This article will review only the most common methods.

Types of Humidity Instruments

Psychrometers (wet and dry bulb thermometers)

A psychrometer consists of two thermometers, one of which is covered with a wet wick. The wet sensor will read a lower temperature owing to the cooling effect of the evaporating water. This 'wet bulb depression' is a function of the air temperature and humidity, and can be converted to a humidity reading by using a psychrometric chart.

The psychrometer is inexpensive and very popular. It is rather inaccurate at low temperatures, where wet bulb depression tends to be small. Using thermopiles to multiply the temperature difference signal can overcome this drawback, even at subzero temperatures. In general, for reliable results, the following precautions must be taken:

- The wick and water must be clean and free of solute. To ensure this, the wick can be boiled in water for several minutes.
- The wick must be completely wet but not dripping.
- There must be sufficient air velocity (3 m s^{-1} or more) over the wet bulb. In still air, a fan is used or the thermometer is whirled manually.
- The sensors must be shielded from thermal radiation.
- The air must be clean and free of volatiles that may condense on the sensors.

Psychrometers are obviously not suitable for measuring food water activity owing to the requirement for a high airflow over the sensor.

Salt dew cells

These are also called thermoelectrolytic sensors or Dunmore sensors. A cloth impregnated with a hygroscopic (strongly moisture-absorbing) salt is heated by passing an electric current through it. When the salt's dew point temperature (the temperature to which it has to be heated before it stops absorbing moisture from the air) is exceeded, the cloth starts to dry and its electrical resistance increases. This causes the electrical power to fall (according to $P=V^2/R$) and the cloth starts to cool, causing moisture to be absorbed and electrical resistance to fall. Eventually the system comes to equilibrium and its temperature is then equal to the salt's dew point, which is related to absolute humidity (Figure 1).

Because these instruments have a simple control circuit and need no cooling, they are robust and relatively inexpensive. An accuracy of approximately 0.5–1 °C is usually obtainable. Salt dew cells are usable over the dew point range −40 °C to +70 °C, but their response is slow at low temperature (up to 30 min). It is important to ensure that the cloth is clean and free of solutes other than the salt being used. At high air velocity, a windshield has to be used to avoid excessive cooling and temperature gradients in the probe. If current to the sensor is cut off for half an hour or more, water may condense on the cloth and drip off, leading to loss of salt and corrosion problems. Salt dew point instruments are not used in water activity instruments owing to their heating effect.

Capacitance and resistance hygrometers

Capacitance and resistance hygrometers measure the variation in the capacitance or resistance of a material (such as metal oxides or polymers) with the amount of absorbed moisture, itself a function of RH of the air. These hygrometers, therefore, give a reading of RH. They are relatively inexpensive and are widely used in industry. Their accuracy varies from approximately 1–3% RH depending on model and RH range. Exposure to high RH (more than 95%) may affect their calibration but in any case they should be regularly calibrated. Capacitance and resistance sensors can be used in water activity instruments.

Chilled mirror dew point meters

These instruments have a mirror that is cooled until dew or frost forms on it. This is detected by a change in the intensity of a reflected light beam. An electronic controller maintains the mirror at the dew or frost point. Chilled mirrors use a very fundamental and stable measurement principle and the best models are very accurate, though rather expensive. Their accuracy can be as good as 0.1 K dew point. When used for water activity measurement, this would lead to errors of approximately 0.005 in a_w for most meat products. Less well-built models have a greater temperature measurement error for the dew point and may also have systematic errors owing to temperature gradient in the mirror.

Larger errors might be introduced by soluble impurities on the mirror (e.g., salt solution may have been splashed on it during calibration) and its cleanliness should be checked. In the range of approximately −20 to 0 °C, there may be uncertainties as to whether the mirror is covered with frost or (supercooled) dew; for example, if air at 0 °C is being monitored with a chilled mirror instrument and a reading of −10.0 °C is obtained, this could indicate an RH of 42.5% or 46.9%, depending on whether frost or dew has formed on the mirror.

Direct dielectric measurement

If the air being measured is hot and moist (as in most dryers), the moisture in it can be measured directly by a capacitance

instrument without first absorbing it into a solid sensor (as in most capacitance hygrometers). The Dewcon humidity meter uses this principle. The gas is filtered through a self-cleaning membrane and passed between two capacitor plates. The instrument is claimed to be linear, drift free, and resistant to dust.

Selection of Hygrometers

The price, performance, and application range of hygrometers, including water activity instruments, vary widely, and it is important to select one that is suited to the application and the budget. The user should ask the following questions:

1. What is the purpose of measuring humidity/water activity?
2. What are the temperature and humidity ranges likely to be encountered?
3. What is the most important variable: dew point, absolute temperature, or RH?
4. What accuracy is required?
5. How maintenance-free should the instrument be? How frequently can calibration be done?
6. Are there special environmental problems, such as dirt, solvent-rich atmosphere, high or low pressures, or widely fluctuating temperatures?
7. Is continuous monitoring required?

At this time, capacitance and resistance sensors are the most widely used industrial hygrometers. Chilled mirrors occupy the top-of-the-range position, but their limitation in the dew point range immediately below 0 °C owing to dew/frost point uncertainty must be kept in mind. In any case, all hygrometers should be regularly calibrated.

Using Hygrometers to Measure Air Humidity

Sensors that measure RH directly (e.g., capacitance and resistance sensors) should be put directly in the air to be measured. Those that measure dew point (salt dew cells, chilled mirrors) or wet and dry bulb temperatures can be used on air sampled via a tube, because changes in air temperature will not affect their reading. If air is sampled in this way, the following must be kept in mind:

- The tube should be made of nonmoisture-absorbent material. Stainless steel, glass, and nickel alloys are best, but polyethylene or Teflon is acceptable at higher dew points. Most other plastics or rubber should be avoided.
- Air tightness is essential when the system is at neutral or negative pressure.
- To avoid condensation, the air sample must not be cooled below its dew point at any point.
- Large pressure drops in the sampling line should be taken into account.
- The air velocity requirements of wet and dry bulb psychrometers and salt dew cells must be satisfied.

Using Hygrometers to Measure Water Activity

Because a_w sensors actually measure the humidity of the air surrounding both sample and sensor, the main problem is in ensuring that temperature and humidity of sample, air, and sensor are the same, i.e., equilibration is as complete as possible. At 25 °C and 0.9 a_w, a temperature difference of 0.1 K between sensor and sample will cause an error of 0.005 in a_w. As far as possible, the sensor must be surrounded by sample and there must be no leakage from the chamber containing both. For faster response, the volume of the measuring enclosure must be as small as possible and the product should occupy a significant fraction of the enclosure's volume. Some a_w meters have a small fan that circulates air in the chamber (Figure 2), but even this may not guarantee equilibration in the air. Equilibration of the product is another limitation, because the diffusivity of water in meat is very slow, of the order 10^{-10} m^2 s^{-1}. It may take up to an hour or more for a_w in meat as thin as 1 mm to equilibrate. Thus, if a reading is taken within a few seconds, it will be the a_w at or near the surface that will be obtained. This effect may be used to advantage if it is the surface value of a_w that is wanted (e.g., when surface microbial growth at the surface is the main concern, as it is for carcass meat): a sensor contained in a cup that is pressed against the meat surface will give a reading of the surface a_w within a few seconds.

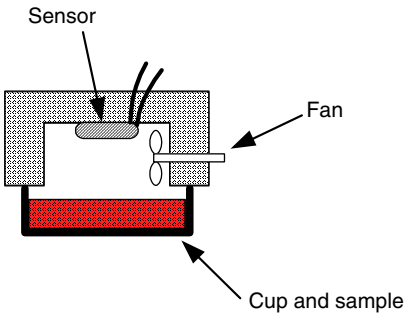

Figure 2 Water activity (a_w) measuring chamber.

Calibration of Hygrometers

For best results, the calibration of hygrometers should be checked immediately after the instrument is purchased and then at least annually. This is particularly important for instruments that rely on changes in the resistance, capacitance, or other physical properties of the sensor.

Salt solution method

The most common way to calibrate hygrometers is to put the sensor in a flask over a saturated solution of a salt for which the moisture sorption isotherm is known. Equilibration can be slow, and complete airtightness must be ensured. Several salts can be used to cover the whole range of RH. To ensure that the solution is saturated, some solid particles must be observable. Nonsaturated salt solutions can also be used if changes in concentration can be avoided. Users can make their own calibrating flasks, but to obtain good results and quick equilibration, the flasks must be as small as possible, leakage must be minimized, and the sensor must be as close to the solution as possible. Extreme care must be taken to ensure that

the solution does not splash on the sensor. Most hygrometer manufacturers supply a range of calibrating cells.

Two-pressure RH generator

In this method, air at high pressure is saturated by bubbling it through water, and then expanded to a lower pressure. If the air temperature is constant, the RH obtained is the ratio between the two pressures.

Dew- or frost-point generator

Air is saturated by bubbling it through water or columns of crushed ice, and then heated and passed over the sensor.

Chilled mirror dew point meter

Because it uses a fundamental measurement principle, a good-quality chilled mirror dew point meter can be used as a secondary standard for calibrating other sensors.

Measurement of Product Moisture Content

Product moisture content can be measured in kilogram water per kilogram wet product, kilogram water per kilogram dry product, or any multiple of these. Although there are very accurate analytical methods for moisture measurement, this article will consider only common industrial measuring instruments.

Gravimetric Method

Modern computerized moisture balances will weigh a sample, dry it under an infrared lamp, and reweigh and display the moisture content. They are more expensive than hygrometric instruments but are not subject to calibration changes owing to variations in composition or temperature.

Water Activity Meters

All a_w meters can be used for measuring product moisture content because the a_w of a given material depends on moisture content and temperature. This relationship is termed the 'sorption isotherm' of the material. An example is shown in Figure 3. A calibration of reading versus moisture content, the latter being measured with the gravimetric method, must be carried out. The sorption isotherm can also be obtained by a dedicated method such as a dynamic vapor sorption instrument. For best results, certain conditions must be fulfilled:

- The product composition must be reproducible.
- The temperature must not vary too much (normally, isotherms are not greatly affected by a few degrees' variation)
- The moisture content must be in a range where a_w is highly sensitive to moisture content. For fresh meat, a_w changes very little with moisture content in the range 0.9–1.0 a_w, and therefore moisture content cannot be measured by this method for that range (Figure 3). However, for dried meat or meat meal, a_w is highly sensitive to moisture content.

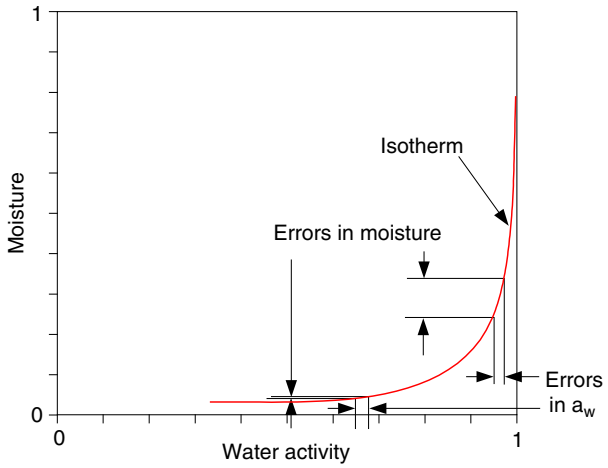

Figure 3 Typical isotherm for fresh meat and errors in using a_w to measure moisture content.

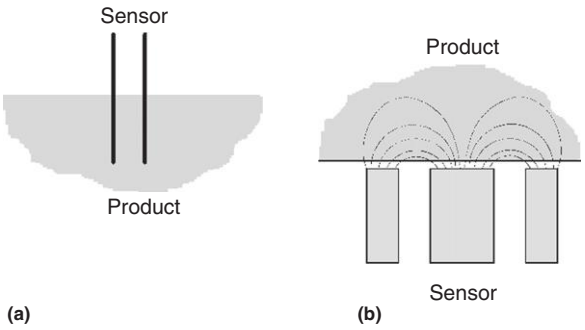

Figure 4 Dielectric moisture sensors: (a) immersed; (b) external.

Dielectric Moisture Sensor

Water molecules are highly polar and tend to reorient themselves in an alternating electromagnetic field. This principle can be used to directly measure the moisture content of foods. The basic sensor consists of an electrode transmitting a radio frequency electric field and a sensing electrode. Both electrodes may be inserted into the product or be outside the product for noncontact measurement (Figure 4). The electric field is wholly or partly immersed in the product and is affected by its dielectric properties. The moisture content being measured is averaged over the domain of electric field, not necessarily the bulk value, and hence the reading is reliable only if the moisture content is uniform.

Microwave absorption instruments are a subclass of dielectric sensors. A microwave transmitter is positioned on one side of the product and a sensor on the other, and the attenuation of the microwave beam by water is measured. Microwave attenuation measures the average or bulk moisture content of the product. Measures should be taken to minimize the effect of scattering and reflection of microwave by the product and surrounding objects, as well as variations in product temperature, composition, thickness, and shape. Microwave absorption works best when the thickness of the product is constant and much smaller than other dimensions, such as with sheet materials or layers of particles.

Near-Infrared Reflectance

A near-infrared beam is shone on the product and the reflected light is analyzed for absorption in certain wavebands owing to water. These instruments measure only surface moisture. For best results, the material should be in powder or flaky form. In a high-humidity environment, accuracy might be affected by infrared absorption by water vapor in the air. Dust or condensation must be avoided.

Determining Moisture Content from Product Temperature

When food is heated by hot air, its temperature will vary between the wet bulb and dry bulb temperature of the air, whereas if it is heated by contact with a hot surface, its temperature will be governed by the boiling point elevation relationship. The higher the moisture content, the lower the product temperature (owing to evaporative cooling); hence, other things being equal, temperature will give an indication of moisture content. This method will work only if

- operating conditions are similar to those under calibration conditions,
- the product is communited and well mixed, so that temperature is uniform, and
- material composition remains constant.

Measurement of Air Velocity

Types of Anemometers

Air velocity sensors in common use include Pitot tubes, mechanical anemometers, thermal anemometers, ultrasonic anenometers, and Laser Doppler anemometers (LDA).

Pitot tubes

Pitot tubes (Figure 5) measure the pressure difference owing to the kinetic energy of the moving gas. The Pitot tube is pointed directly against the airflow so that air impinges on a hole at the tip of the tube. Immediately in front of the hole, the air is stationary and its pressure increases as a result of the conversion of kinetic energy to pressure. Other holes on the side of the tube measure the static pressure. Velocity is related to the pressure difference by eqn [1].

$$v = C\sqrt{2\Delta P/\rho} \qquad [1]$$

where ρ is the gas density in kilogram per cubic meter, ΔP is measured in Pascal, and C is a constant ranging from 0.98 to 1.0. The pressure difference is very small at low air velocities. At $v = 1\ \mathrm{m\ s^{-1}}$ it is only 0.6 Pa or approximately 0.06 mm of water. The resolution of a Pitot tube therefore depends on what is used to measure the pressure difference. An inclined U-tube can at best be read to a few tenths of a mm (1 Pa), whereas micromanometers have resolutions down to 0.1 Pa or less. Pitot tubes are therefore not useful at low air velocities.

Pitot tubes are inexpensive and do not need calibration. They are highly directional instruments (they should be aligned to within 5°) and can be used only when the airflow direction is well defined and accurately known, as in pipes and ducts.

Mechanical Anemometers

Mechanical anemometers measure the rotational speed of vanes or cups driven by the wind. Because of static friction, they only operate at air velocities above approximately 0.2–0.3 $\mathrm{m\ s^{-1}}$. Vane anemometers (Figure 6) are highly directional. As with all mechanical devices, they should not be subject to shock, dust, etc. that may affect bearing friction. Cup anemometers (Figure 7) consist of three or more cups mounted symmetrically about a vertical axis. They are insensitive to airflow direction as long as it is horizontal, and are therefore widely used in meteorological measurements.

Thermal Anemometers

Thermal anemometers measure the cooling effect of airflow over an electrically heated sensor. There is a vast difference in price and performance between laboratory thermal anemometers and industrial models, this article being only concerned with the latter. Thermal sensors come in several shapes: hot wire, hot film, or hot bead (Figure 8). A hot wire sensor consists of a very thin wire made of a noble metal such as platinum or tungsten, tensioned between two metal prongs. A hot film sensor consists of a thin film of a noble metal usually

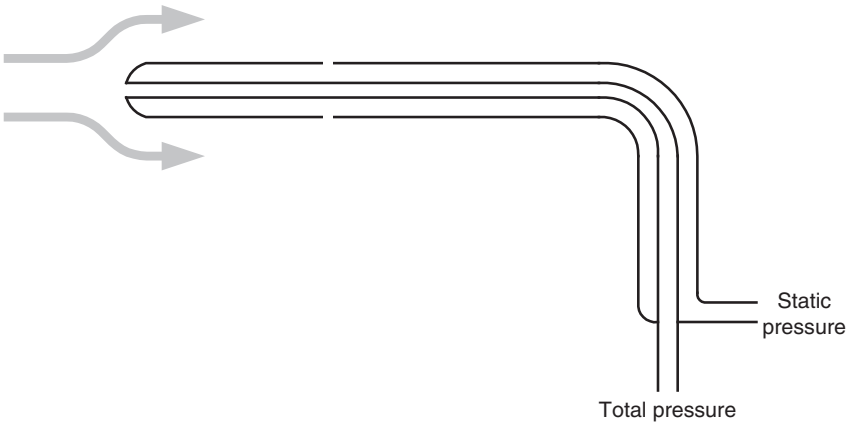

Figure 5 Pitot tube.

Figure 6 Vane anemometer (TSI Inc.).

Figure 7 Cup anemometer.

deposited near the tip of a thin ceramic probe. A hot bead sensor usually consists of a thermistor because of the requirement for high resistance in spherical shape.

Thermal anemometers are better at low velocities than vane anemometers. Good instruments will provide temperature compensation to ensure that the calibration is independent of air temperature. Hot wire and hot film sensor readings are dependent on airflow direction, whereas hot beads are almost omnidirectional, except when a probe component is in the way. All thermal anemometers are affected by turbulence in the air, which increases the cooling rate.

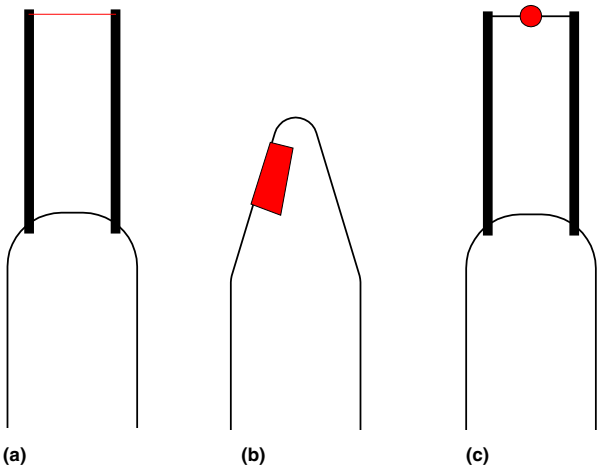

Figure 8 Thermal anemometers: (a) hot wire; (b) hot film; (c) hot bead.

Ultrasonic anemometers

Ultrasonic anemometers measure the effect of wind on the speed of sound traveling between a pair of transducers. By using three orthogonal pairs of transducers, they can measure all components of velocity. Some models can measure air turbulence as well as mean velocity. These anemometers are robust, do not suffer calibration shift, have no moving parts, and can measure quite low velocities (0.01 m s^{-1}). They are still fairly expensive but are gaining popularity, especially in meteorological applications.

Laser Doppler anemometers

LDA use two laser beams that intersect to create a fringe pattern. As a micron-sized particle passes through these fringes, the scattered light from it fluctuates in intensity. The frequency of this fluctuation is measured with a photodetector and converted to particle velocity. Laser Doppler systems are very accurate and can measure very low velocities, down to a few millimeter per second, but they are very expensive and require expertise as laser beams are dangerous to handle.

Practical Guidelines in Using Anemometers

To measure airflow from a fan, coil, or in a duct, several readings must be taken to take into account the nonuniform airflow profile. The flow cross-section area should be divided into several equal subareas. How this is done depends on the geometry of the flow. In a pipe, the variation is mainly in the radial direction and the cross-section should be divided into annuli of equal areas. A rectangular duct or a coil face can be divided by a rectangular grid. Air capture hoods and airflow horns are devices that, in combination with an anemometer, will give the total flows from diffusers, fans, etc.

When the airflow direction is not well known, a nondirectional instrument such as a bead-type thermal anemometer should be used.

In fluctuating flow, modern instruments often have an integrating or averaging capability to give a steady reading. Most thermal anemometers, however, will give high readings in strongly turbulent flow.

All mechanical and thermal anemometers will be adversely affected by dirty or corrosive environments. In such cases, a noninvasive method such as ultrasonic measurement is recommended.

Low air velocities are difficult to measure. Many manufacturers specify a range from 0 m s^{-1} upward for their thermal and mechanical anemometers, but readings below 0.2 or 0.3 m s^{-1} (depending on the model) are unreliable. If it is absolutely necessary to measure at these low ranges, an ultrasonic anemometer or Laser Doppler system may be necessary.

Calibration of Anemometers

Pitot tubes use a fundamental physical principle and do not need to be calibrated. Mechanical and thermal anemometers are usually calibrated in wind tunnels that are carefully designed to give very low turbulence and a flat velocity profile. The air enters via a bellmouth and several turbulence-reducing devices with mesh screens and honeycombs. After the measuring section, the flow is channeled into a smaller tube where the velocity is increased and measured with an accurate flowmeter. Such equipment is not widely available, although some models are now sold commercially and, therefore, anemometers must usually be calibrated professionally in scientific laboratories. For best results, calibration should be checked immediately after the instrument is purchased and then annually thereafter, or more frequently if in heavy use.

See also: Physical Measurements: Temperature Measurement

Further Reading

ASHRAE, 2009. Psychrometrics ASHRAE Fundamentals Handbook. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers Chapter 1.

Benedict, R.P., 1984. Fundamentals of Temperature, Pressure, and Flow Measurements, third ed. New York: Wiley-Interscience.

Considine, D.M., 1993. Process/Industrial Instruments and Controls Handbook, fourth ed. New York: McGraw-Hill.

Greenspan, L., 1977. Humidity fixed points of binary satured aqueous solution. Journal of Research of the National Bureau of Standards – A. Physics and Chemistry 81A (1), 89–96.

Lovatt, S.J., Hill, H.K., 1998. Surface water activity during meat cooling. Proceedings Meeting Commissions B2 & C2 with D1 & D2/3, Sofia, Bulgaria. Paris: International Institute of Refrigeration.

Pomeranz, Y., Meloan, C.E., 1994. Food Analysis: Theory and Practice, third ed. New York: Chapman & Hall.

Relevant Websites

http://www.ima.co.uk/theory.htm
IMA.

http://www4.uwsp.edu/papersci/biasca/currentpages/
University of Wisconsin.

Temperature Measurement

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Glossary

Electromotive force (Emf) Voltage generated by a circuit.
Ice point Freezing temperature of water.
PRT Platinum resistance thermometer.
Stem error An error due to conduction along the stem or lead wire of a temperature sensor.
Stray voltage Unexpected or undesired voltages caused by imperfections in the electrical circuit or exposure to electrical fields.
Thermal center The location in an object where temperature change is slowest during heating or cooling.

Types of Thermometers

Liquid-in-Glass (or Liquid-Filled) Thermometers

Liquid-in-glass thermometers consist of a glass bulb attached to a glass stem containing a narrow bore channel. As temperature rises, the liquid expands and rises along the channel. It must be borne in mind that, when temperature rises, both the liquid and the glass containing it expand to different extents. Good quality thermometers are constructed of high-quality glass that has been properly annealed to avoid changes in volume after manufacture. Precision liquid-in-glass thermometers may be designed for total immersion or (more commonly) partial immersion, and it is important that the correct immersion mode is used, because they have been calibrated in that mode.

Liquid-in-glass thermometers can lose their calibration when exposed to very high temperatures due to changes in the volume of the bulb, which may not relax back to its original dimensions. Liquid-filled thermometers also tend to have large response times, corresponding to the time it takes for the liquid in the bulb to equilibrate. Another limitation is the size and shape of the bulb, which means that measurement of surface temperature, for example, is not very accurate. Also, liquid-in-glass thermometers must be read by eye and do not produce an electric signal. Safety is a concern because they break easily.

Owing to these disadvantages, liquid-in-glass thermometers are not widely used in industry, except in a laboratory environment.

Thermocouples

When two dissimilar metal wires are joined, the difference in the energy levels of the electrons in each metal causes electrons to migrate from one metal to the other and a voltage (EMF) develops. This voltage is dependent on temperature. When a circuit is formed by joining the wires at both ends (Figure 1), providing the junctions are at the same temperature, equal but opposite EMFs appear at the two junctions and cancel each other. However, if one junction is hotter than the other, the EMFs will be of different values and a net EMF will develop in the circuit. If the temperature at one junction is known, the other junction's temperature can be found from the circuit voltage by using a conversion equation, reference table, or chart specific to the two metals used.

Several types of thermocouple material pair are in common use, each identified by a single letter: T (copper/copper–nickel), J (iron/copper–nickel), K (nickel–chromium/nickel–aluminum), and so on.

In practice, thermocouple circuits are seldom as simple as shown in Figure 1. To be able to use thermocouples correctly in a variety of situations, a number of 'laws' have been proposed but the most important one to remember is: "A homogeneous piece of wire of any material inserted into the circuit will not change the EMF, if the two ends of this wire are at the same temperature."

An application of the above law is in the use of compensating or extension wires. Ideally, there should be only two metal wires in a thermocouple circuit. However, often the measured temperature is far away from the voltmeter, and it would be prohibitively expensive to use high-quality thermocouple wires all through. In such case, wires of lower quality and cost may be used (Figure 2). These wires, A' and B', are usually made of the same materials as the instrument wires A and B, but to less stringent standards. As long as the temperatures at the terminal blocks 1 and 2 are not very different, the error introduced will be negligible.

A thermocouple only measures the temperature difference between two points. To determine the actual temperatures, that of at least one point (known as the reference junction) must be known. In Figure 3, the reference junction is kept at a known temperature, such as a mixture of ice and pure water (0 °C). This is the most accurate method for measuring temperature with a thermocouple. To measure several temperatures, the circuit of Figure 4 is used. All the wires made of

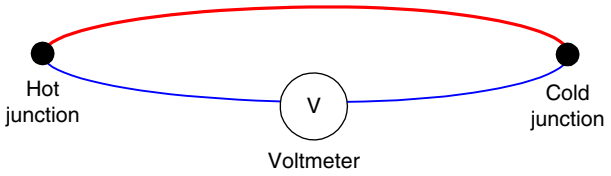

Figure 1 Basic thermocouple circuit.

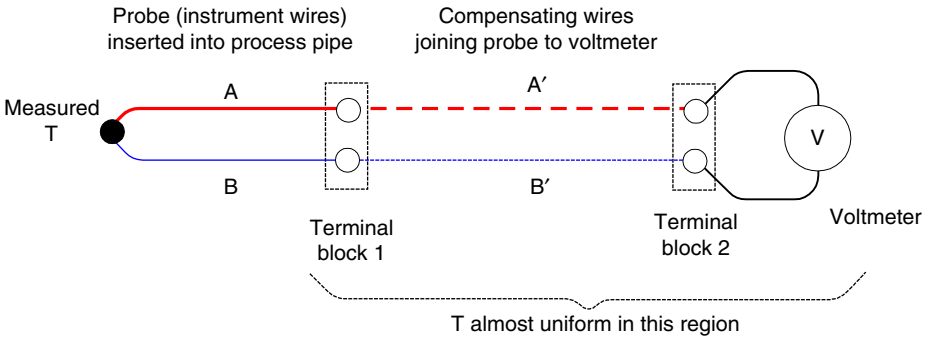

Figure 2 Using compensating wires.

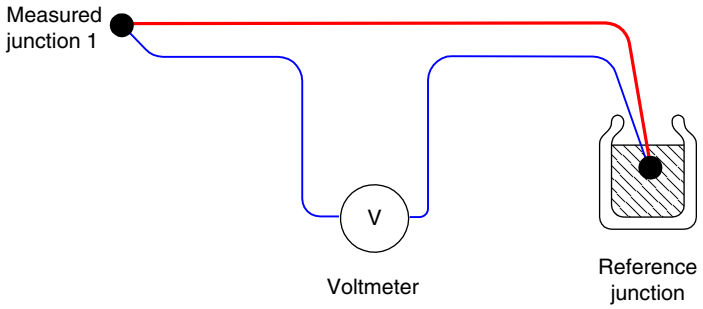

Figure 3 Measuring temperature with thermocouples and external reference.

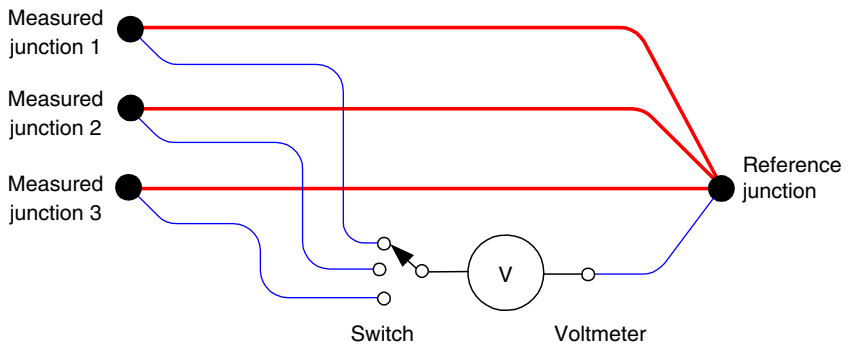

Figure 4 Measuring multiple temperatures with external reference.

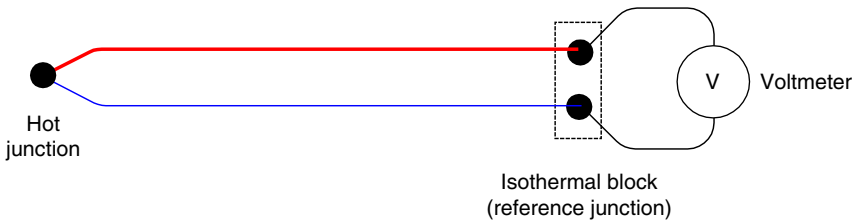

Figure 5 Measuring temperature with thermocouples and internal reference.

material A are joined together at the reference junction, and a single wire of material B connects this junction to a voltmeter. The voltmeter is connected to any of the measured junctions via a selector switch, which may be manual or automatic (automatic switching is standard in commercial dataloggers).

Modern instruments often have internal temperature compensation, which dispenses with the need for an external reference junction (Figure 5). The two ends of the dissimilar thermocouple wires are connected to an isothermal connector block in the instrument, which serves as the reference junction.

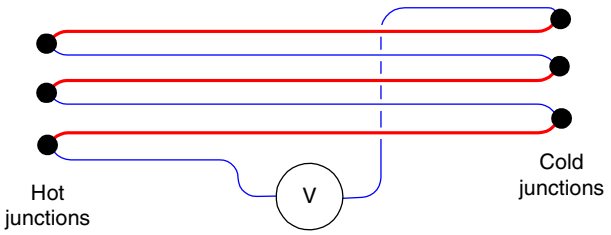

Figure 6 Thermopile.

The temperature of this connector block is measured with, for example, a thermistor, and the instrument computes the temperature of the hot junction from the voltage difference. However, this method is less accurate than using an external reference junction, due to uncertainty about the temperature of the connector block.

In some applications, such as when measuring wet bulb depression or heat flux, one may be more interested in measuring 'temperature differences' rather than temperature. Thermocouples are ideally suited for this. For measuring very small temperature differences, a 'thermopile' made of several pairs of junctions connected in series can be used. The voltage will be multiplied by the number of thermocouple junction pairs present; thus the voltage obtained from the six-junction circuit of Figure 6 is three times that of a single pair. It is vital that the junctions are electrically insulated from each other.

Thermocouples are widely used because of their low cost, robustness, wide range, and flexibility. They are sufficiently accurate for most industrial purposes but can also be used for high-precision measurements. An inherent error in thermocouples is due to the variation in the composition of the wires and this is given in the manufacturer's specification. However, there are many other possible sources of errors that should be taken care of:

1. Reference junction error: In single-junction instruments, the temperature of the terminal block is used as reference temperature. The accuracy then depends on how isothermal the terminal block is and how its temperature is measured. Typically, a 0.5–1 K error is involved. Sometimes the reference temperature sensor can be several centimetres away from the terminal block and the error can be higher (this happens in dataloggers with long arrays of terminals). External references using an ice point can be much more accurate, but this depends on the experience of the user (see Calibration and Use of Thermometers).
2. Short circuiting between different parts of the thermocouple circuit, or between the thermocouples and the electrical ground of the measuring instrument. If there is any possibility of this happening, the junctions must be coated with an electrically insulating coating.
3. Electromagnetic interference causing stray voltage.
4. Changes in sensitivity caused by aging of the thermocouple wires.
5. Stresses in the wires (kinking, work hardening), which cause local changes in composition.
6. Using low-grade (compensating) wire in a highly non-isothermal environment. Compensating wires should be used only if no significant temperature variation is expected along these wires.

Resistance Thermometers

There are two types of resistance thermometers: pure metal resistance thermometers and thermistors.

The platinum resistance thermometer (PRT) is the most well known and accurate of the first type. The resistance of a pure platinum wire increases almost linearly with temperature (the temperature coefficient specified in international standards is 0.385% per Kelvin; however, it should always be checked with the manufacturer). In the past, a Wheatstone or Mueller bridge was used to measure the resistance. Modern instruments measure resistances by sending an accurately known electric current through the sensor, and measuring the voltage across it. For maximum accuracy, a four-wire connection must be used (Figure 7). Two of the wires carry the current, the other two connect the ends of the sensor to a high impedance (low current) voltmeter. If the sensor is close to the voltmeter, or if high accuracy is not needed, a two-wire connection can be used (Figure 7). The resistance measured will then be that of the sensor plus those of the lead wires, which will decrease accuracy. Another source of errors in using a PRT is ohmic heating by the measuring current. If the PRT is in good thermal contact with the product being measured, this may not matter but, if thermal contact is poor (e.g., if the sensor is in still air), the error may be more significant – although still small in most cases. PRTs are highly accurate and stable but costly.

Thermistors come in various sizes and shapes and are usually made of some semiconducting material. Their advantages over PRTs include lower cost, smaller size, and much higher sensitivity (several percent per Kelvin). The accuracy and stability of thermistors vary, but the best models can be comparable to those of PRTs. They have high resistances (thousands of ohms, vs. 100 Ω for standard PRTs) and large temperature coefficients, so that a simple two-wire measuring circuit can be used (Figure 7b). They are very nonlinear and require linearizing hardware or software; however, that is not a big problem these days. They should not be exposed to high temperatures. Thermistors are widely used in hand-held industrial instruments and miniature loggers. Thermistors are not as highly standardized as PRTs, so it is important to use the correct hardware, calibration curve, or equation, as supplied by the manufacturer. As resistive devices, they are subject to the same self-heating problem as PRTs.

Infrared Thermometers

Infrared thermometers measure temperature by sensing the infrared radiation emitted by a surface. For a given surface, the amount of radiation, I, increases with temperature according to Plank's equation, $I = \sigma\varepsilon T^4$, where σ is Plank's constant, ε the surface's emissivity (which can have a value between 0 and 1), and T the absolute temperature. Surfaces differ in their emissivity and emission spectrum; therefore, the infrared sensor must be calibrated for each product surface. The surface can be coated with a paint or tape of known emissivity to ensure reproducible readings. Unwrapped meat and other unwrapped

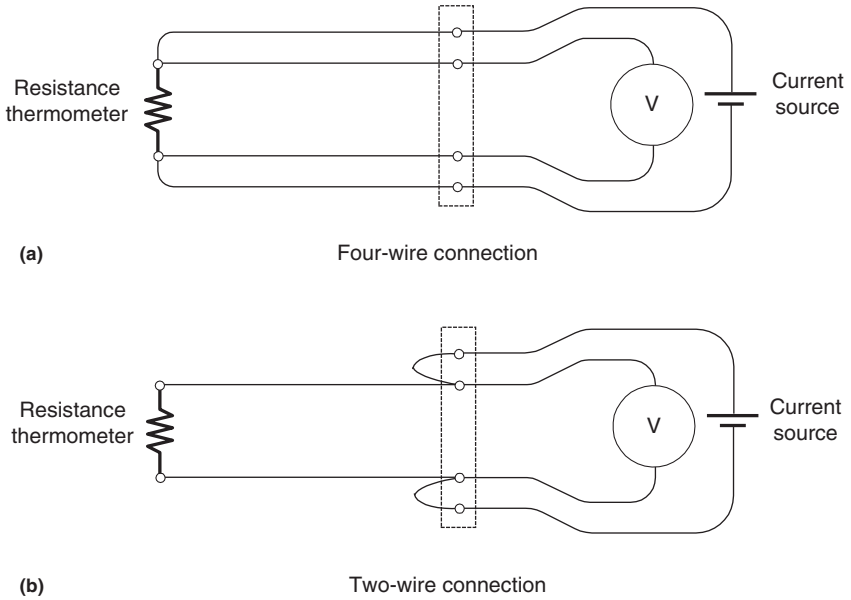

Figure 7 Connecting resistance thermometers.

foods usually have high emissivities (above 0.9), which make them suitable for infrared measurement.

The sensitivity and resolution of infrared thermometers fall off at low temperatures. Most models have a lower limit of −20 or −30 °C, although some will work down to −50 °C, whereas upper limits of up to 400 °C are common. At a low temperature, the error may be ±2 K or more. In view of the difficulties in measuring true surface temperature by other means (location errors and stem errors), this is not as bad as it sounds.

Errors arise when changes in emissivity are not taken into account, for example, when a surface fogs up as it cools below dew point. Smoke, dust, and vapors in the atmosphere can also affect the reading. For wrapped product, it is the wrapping's temperature that is read, even if it is optically transparent. With low-emissivity surfaces such as polished metal, reflected radiation may cause large errors.

There is a wide range of infrared sensors in the market, differing in size, capabilities, and cost. Technical factors that should be considered in purchasing a unit include accuracy, range, response time, emissivity adjustment, distance to target size ratio, availability of a targeting aid such as a laser spot, and portability. Instruments with wider temperature ranges and which can focus on smaller targets at larger distances will be more expensive.

For accurate measurement, the infrared thermometer should be aimed perpendicular to the surface. The field of view must be adjusted to avoid viewing unwanted areas. The thermometer itself should not be exposed to subfreezing temperature.

The advantages of infrared sensors are as follows:

- They are noninvasive – no hole drilled, no damage to wrapping, no contamination.
- There is no contact with food, hence no danger of contamination of the food or risk of burns in the case of very hot surfaces (e.g., charcoal, cooked meat, hot soup).
- They can be used to scan the surface quickly and locate hot or cold spots. Thus, they can be used for scanning product in a cold room or a retail display, or hot spots on the wall of a cold store, which indicate heat leaks.
- They can measure the temperature of objects that are moving or vibrating.
- They can measure very high temperature (e.g., burning charcoal).
- Their field of view can be adjusted (like a camera lens) for a small or large target area.
- They can read the true surface temperature of meat and other surfaces, unaffected by location errors or stem errors as with other instruments.

Bimetallic Thermometers

Bimetallic thermometers consist of two strips of metals with different thermal expansion coefficients, joined side by side. When the temperature changes, differential expansion causes the strips to bend. For increased sensitivity, the bimetallic strip may be formed into a coil or spiral. Bimetallic thermometers are not particularly accurate (typically ±1 K to several Kelvin), but they involve no electrical parts, hence they are robust and popular as cooking indicators, oven indicators and thermostats, and freezer thermometers.

Thermochromic Materials

Thermochromic materials, whose color changes with temperature, are used as temperature indicators in the food industry. There are two basic types, liquid crystals and leuco dyes, the former being more sensitive and accurate. Thermochromic indicators are available as dyes, inks, paints, paper, tapes, sticky labels, and so on. The color change may or may not be reversible. Some materials change color at a sharp temperature and can only be used as single temperature

indicators, whereas others (known as thermochromic materials), such as some liquid crystals, go through a range of colors as temperature changes and can be used for quantitative measurement.

Color change sensors can be used as disposable temperature indicators with respect to some critical temperature. Sensors that change colors irreversibly are used to detect and record temperature abuse in packaged foods during storage and transport, or to indicate that a cooking or sterilization process has reached a specified temperature. Color change materials usually measure surface temperature but have also been used in cooking probes to indicate when meat has been properly cooked.

Temperature Logging

Data loggers are widely used in industry to collect data on the cold chain. The data are stored in nonvolatile memory for downloading later to a computer. Some data loggers may also be accessed remotely via telephone lines, mobile phone, or the Internet for continuous or intermittent downloading. Data loggers range from miniature (as small as 16 mm diameter × 5 mm thickness) low-cost portable units with one or a small number of inputs, often used in the transportation industry, to larger modular units that can be expanded to many channels. Miniature loggers are often dedicated to one type of input (such as temperatures), have lower accuracy and can store less data. Larger units can usually handle multiple types (temperature, humidity, voltage, current, and so on). Some data loggers have a digital display allowing instant reading, as with an ordinary digital thermometer. Because digitization normally converts a temperature or other signal into an electrical signal using a high-precision digital voltmeter, some data loggers – especially those of the generic input type – may record temperature or other variables to several decimal places more than would be justified by the inherent accuracy of the sensor. Caution must be exerted in interpreting these outputs.

Calibration and Use of Thermometers

Thermometer Calibration

In any physical measurement, the signal from the sensor must be processed into an analog (dial) or digital reading. This processing may introduce errors of three different kinds (Figure 8): 'zero error,' 'span error,' and 'nonlinearity error.' A zero error remains constant throughout the measuring range. It can be corrected for by doing a single temperature calibration. A span error is an error in the sensitivity of the instrument. Calibration consists of taking the reading at two known temperatures (e.g., the ice point and the boiling point of water). To correct for nonlinearity, more reference temperatures have to be measured.

An ice point reference is obtained by filling a vacuum flask with a crushed ice-distilled water mixture. To ensure equilibrium, it is important that both phases are present. If liquid is not present, the ice may be below freezing point, which can be detected by a frosty appearance, whereas a two-phase

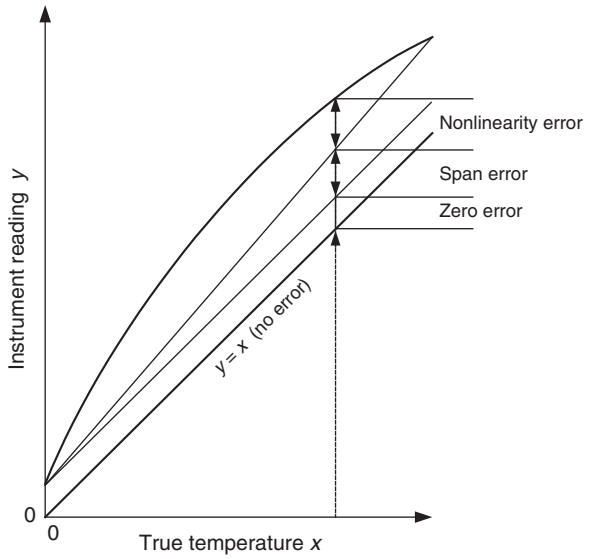

Figure 8 Types of calibration errors.

mixture will be transparent. If there is too much liquid, the ice will float on top and the liquid below may be above freezing point due to conduction from the wall. The sensor must be immersed into the ice slush as much as possible and kept away from the wall of the container.

The ice point is fairly insensitive to atmospheric pressure. On the contrary, the boiling point is highly sensitive to ambient pressure, a +1% change in pressure causing a +0.28 K change in boiling point. Thus, when calibrating a thermometer with boiling water, it is important to measure the ambient pressure and correct for its effect.

Commercial thermometer calibration units are now offered by many manufacturers. Calibration can also be made against a reference thermometer, usually liquid-in-glass or platinum resistance. To ensure that the two thermometers are at the same temperature, they should be inserted into a well-stirred bath or an insulated block of aluminum with holes drilled for the sensors. Good thermal contact between the aluminum block and the sensor must be ensured.

Reading Digital Displays

Except for liquid-in-glass thermometers, bimetallic thermometers, and thermochromic indicators, practically all commercial thermometers use a digital display. The number of displayed decimal digits may be greater than the accuracy of the sensor, especially when the latter varies over the range. The user should always refer to the manual to get an indication for accuracy and, if necessary, perform a calibration or commission an accredited laboratory to do it.

Error Sources in Temperature Measurement

Many factors will influence the reading of temperature, including sensor location, type, and construction of the probe used, and method of probe insertion. To get reproducible results, it is important when developing a protocol for

measuring product or environment temperature to pay attention to all these factors.

The temperature at the center (more accurately the 'thermal center') of the product is of interest because it is often used to define the end of a cooling or heating process. However, it is not always easy to locate the thermal center. This problem may arise because of the irregular shape of meat components (leg, shoulder, or loin), irregular packing, or asymmetrical ambient conditions (e.g., when a carton is cooled mainly from the bottom due to an air gap at the top) (Figure 9). In these cases, the thermal center must often be found by trial and error. Fortunately, a small error in locating it will usually not lead to very large errors in temperature, because the temperature profile tends to be flattest around the thermal center (Figure 10). Often, the best one can do is to define a protocol that will measure temperature at a reproducible location. With beef, pork, and lamb, the temperature next to the bone in the thickest part of the hind leg is usually taken as the 'deep meat' temperature.

A frequent source of error is 'stem errors.' Stem error is a combination of two effects. First, sensors do not 'sense' the temperature at one point; rather, the sensor's temperature, which we read is an average of both the product surrounding it (what one wants to read) and the stem of the thermometer or thermocouple/thermistor wires to which it is attached (which one does not want). Second, the stem is a heat leakage path, which will modify the temperature of the product surrounding the sensor (Figure 10). An error of several degrees may result when measuring food cooling. Stem error is more severe when the stem is larger and a better heat conductor, immersion is shallower, and the length exposed to ambient is longer. Stem error is also worse in sheathed sensors, because thermal contact between the sensor and the product immediately around it is not as good as with unsheathed sensors.

To ensure the most accurate possible measurement, the following precautions must be taken:

1. If necessary, the thermal center must be located by trial and error.
2. The thermometer stem or leading wires must be parallel to isotherms within the meat (i.e., perpendicular to the direction of heat flow) (Figure 10). In an elongated product, the wires should lie in the major direction.
3. The leads should be as fine as possible.

The steeper the temperature gradient (small products, high cooling rates), the more difficult it is to minimize measurement errors. For large products, such as a beef leg, errors due to stem conduction are usually negligible, but some experience is still needed to locate the center accurately.

Surface temperature is difficult to measure with a probe. Temperature will vary along the surface depending on shape, airflow pattern, and so on. In air blast cooling, for example, the surface will cool fastest on the upstream side. For irregular products, thinner parts cool more quickly than thicker parts. Temperature gradients tend to be largest near the surface and in the boundary layer (Figure 10), so errors due to sensor location and stem conduction are more severe than when measuring center temperature. The sensor should be as small as possible and inserted at a shallow angle, to be located as close to the surface as possible (Figure 10). Gluing or taping the sensor to the surface with adhesive tape may alter the surface temperature, especially if evaporative cooling is taking place. The same problem arises when using a thermochromatic material. An infrared thermometer is perhaps the best choice but, for good accuracy, the emissivity should be determined. For a spot manual measurement, it may be possible to sandwich the probe between two pieces or packs. The probe should be as thin as possible (a thermocouple is best) because a thick probe may not fit snugly and will change the surface temperature. A reading must be taken quickly before the surface temperature changes, due to the change in surface heat flux.

The mean product temperature cannot be measured with an ordinary thermometer. However, by leaving the product to equilibrate in an insulated box, it will eventually reach the average temperature (allowing for some heat loss or gain). A microwave attenuation detector developed by AgResearch-MIRINZ (New Zealand) can give an approximate indication of the mean temperature of a frozen carton by measuring its microwave transmittance, which depends strongly on the ice content.

When measuring air temperatures in chillers, cold stores, transport vehicles, and retail displays, temperature variation is a big problem. With cold rooms, temperatures tend to be highest above doors, near ceiling and walls, in dead spots shielded from the airflow by stacked products, near lights, and near warm product. For control purposes, the temperature

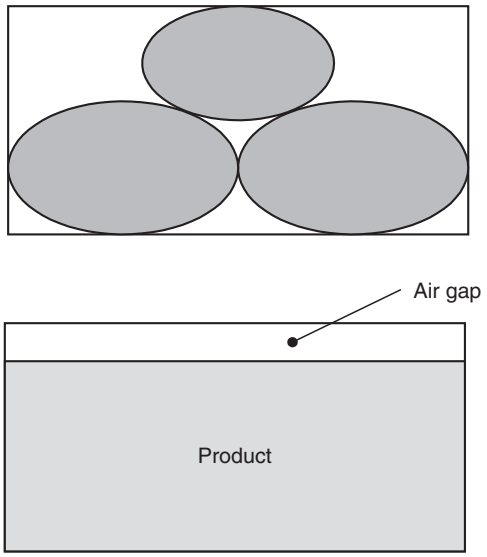

Figure 9 Causes of error in locating thermal center.

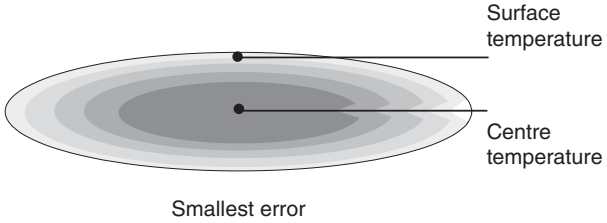

Figure 10 Measuring surface and center temperatures.

sensor should be on the return air to the cooling coils, but there should be other sensors at critical spots if product is stacked nearby. In transport vehicles and containers, the highest temperatures are found at the door end, especially toward the top, due to air infiltration through the door seals and the distance from the cooling unit. Retail display units are particularly susceptible to large temperature variations, which depend on cabinet design and the stacking of product. One cost-effective method may be to use color change temperature indicators at various positions to locate hot spot areas. With chilled meat, the coldest temperature is also of interest, because freezing must be avoided and therefore product should not be allowed to go below −1 °C.

Advances in sensor technology and computer miniaturization have enabled the development of inexpensive miniature loggers that can accompany the product during storage and transport, which is important in the long distance transport of temperature-sensitive product such as chilled meat.

See also: Physical Measurements: Other Physical Measurements

Further Reading

ASTM, 1993. Manual on the Use of Thermocouples in Temperature Measurement. revised ed. Philadelphia, PA: American Society for Testing and Materials.

Bentley, R.E., 1998. Handbook of Temperature Measurement. Heidelberg, Germany: Springer Verlag.

Kinzie, P.A., 1973. Thermocouple Temperature Measurement. New York: Wiley.

Nicholas, J.V., White, D.R., 1982. Traceable Temperatures: An Introductory Guide to Temperature Measurement and Calibration. Wellington, New Zealand: DSIR Science Information Division.

Schooley, J.F., 1986. Thermometry. Boca Raton, FL: CRC Press.

Valentas, K.J., Rotstein, E., Singh, R.P., 1997. Handbook of Food Engineering Practice. Boca Raton, FL: CRC Press.

POTENTIAL CHEMICAL HAZARDS ASSOCIATED WITH MEAT

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Glossary

Antibiotics Chemical substances used to inactivate bacteria.
Antihelmintics Chemical substances used to inactivate parasitic helminths (worms).
Curing A method of food preservation that, by the addition of curing agents (salt, nitrites, nitrates, and sugar) occasionally in conjunction with smoking, results in reduction of water activity as well as in desirable organoleptic changes in the food product.
Hazard Analysis Critical Control Point A systematic approach in food safety that aims for the reduction of biological, physical, and chemical risks in food products to negligible levels.
Maximum Residues Level The upper legal concentration of residues of veterinary medicinal products in food of animal origin for human consumption.
Nitrates and nitrites Salts used as curing agents in food products. They not only have antimicrobial activity, especially against *Clostridium botulinum*, but also give food organoleptic characteristics that improve acceptability of cured products (color and flavor).
Quaternary ammonium compounds (QACs) The most commonly used chemicals in industrial environments worldwide. These amphoteric compounds have a broad-spectrum bactericidal effect, which is related to disruption of cell membranes, resulting in cytolysis. However, if QACs are used on an unclean surface, the contact time required for them to be effective could be as long as 10 min. If QACs have been used for disinfection of food contact surfaces, these should be rinsed before use in order to reduce the risk of toxicity.
Smoking A method of preservation of food products. The two most common smoking methods are cold and hot smoking. The process not only acts as a flavor enhancer but also has antimicrobial and antioxidant effects.
Standard Operation Procedures (SOPs) In the food industry, SOPs are written procedures that aim to produce safe food products. The SOP written document must include the purpose and frequency of a task, who is responsible to execute the task, a description of the procedure to be performed (which must include all the steps), and corrective actions to follow if problems are identified in the running of the task.
Standard Sanitation Operation Procedures (SSOPs) In the food industry, SSOPs are written procedures for sanitation (cleaning and disinfection) of food processing facilities.

Introduction

Paracelsus in the fifteenth century stated, "The dose makes the poison;" hence, depending on the level of exposure to a specific substance, any substance could have a detrimental effect on health. When talking about food products in general, and specifically meat and meat products, it is important to assess not only the potential chemical risks but also the likelihood of these risks to be harmful for human health (the hazard level).

Chemical risks in food products are not easy to evaluate. Chemical contaminants do not always produce clinical signs/conditions that can be identified during antemortem inspection in abattoirs, neither organoleptic alterations of the food product that can be detected during postmortem inspection or during consumption. Unfortunately, most of the current protocols for surveillance in chemical residues in food products require costly equipment and highly trained personnel; prerequisites can be found mostly in developed countries but not necessarily in the developing ones, where the population could be, unknowingly, exposed to unsafe levels of chemical contaminants.

For some chemicals, there is a lack of evidence on the risk level for human health; for other chemicals the risks might be related to the accumulation of that substance over a long period of time, rather than being the result of a single exposure. Hence, Maximum Residues Limits (MRLs) are not always available, and some countries have decided to use a precautionary principle banning the use of some chemical compounds in food-producing animals. However, these precautionary measures can be considered and can be used as a tool to protect local markets, instead of human health. Strong scientific evidence is required in order to carry out proper risk assessments, evaluate the impact on human health, and choose the most appropriate tools to control and reduce these chemical risks.

Veterinary Drug Residues

The kind and amount of veterinary drugs used in meat-producing animals vary, amongst other factors, with the species of animals used for production, the production system

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in which these animals are bred, and also the country where these animals are produced. The veterinary drugs covered in this section include the following: antibiotics, antihelmintics, and growth promoters.

There are multiple reports highlighting the potential detrimental effects on human health when consuming products of animal origin that contain veterinary drug residues at unacceptable levels. Some of the potential effects and examples are as follows:

Anaphylactic reactions: Allergic reactions after consumption of food products of animal origin have been reported primarily for dairy products. Nevertheless, meat products could potentially trigger an anaphylactic shock in susceptible consumers.

Antibiotic resistance: Presence of antibiotic resistance in intestinal microflora has been reported in animals after ingestion of subclinical doses of antibiotics. However, the impact of antibiotic resistance in animal intestinal microflora on human health is not yet fully understood.

Chronic and acute toxicity: Human health problems have been reported after consumption of meat products from animals that have been administered growth promoters, such as clenbuterol, a drug currently banned for use in food-producing animals in the United States, European Union (EU), and other countries. Toxicity problems associated with clenbuterol include neurological signs (gross tremors of the extremities), tachycardia, nausea, headaches, and dizziness.

Imbalance in human intestinal microflora: Reports show that human intestinal microflora might change not only after ingestion of food products that contain antibiotic residues but also when working in an environment where antibiotics are used in large amounts as prophylactic treatment on food-producing animals. The presence of antibiotic residues might affect the balance of not only intestinal microflora but also oropharyngeal, skin, and vaginal microflora. However, more research is needed to elucidate whether these changes might affect animal and human health.

The EU periodically reviews new information available on veterinary drugs in order to revise the list of veterinary drugs included in the list of forbidden substances, whereas an MRL is established for other substances. A conscientious checking of drug residues by national surveillance systems is not easy, as it may result not only in a large number of samples to analyze but also in a wide variety of residues to check for, long waiting times for results, and a high economic cost. Additionally, the accuracy on the detection of noncompliances can be affected by the mixing of drugs at smaller doses by unscrupulous people, in order to get a synergistic effect of some drugs as well as avoiding detection of residues by the current analytical methods. Hence, the existence of fast screening techniques is critical for the implementation of effective residues control.

Antibiotics

Antibiotics are widely used in meat-producing animals to control bacterial diseases, and in some countries they are also used as growth promoters. Regardless of the positive effect on animal health and welfare, food production, and human health, indiscriminate use of antibiotics might result in undesirable effects, such as bacterial antibiotic resistance, technological problems in food production (Figure 1), or anaphylactic reactions in consumers. To reduce the risk of human health problems associated with the presence of antibiotics in meat products, antibiotics should be used responsibly in food-producing animals, and withdrawal periods should be respected.

Figure 1 Damage in ovine carcase in an injection point due to bad practice.

There is a great concern about antibiotic resistance in human pathogens due to use of these drugs in animal production. For example, resistance to beta-lactamic antibiotics due to the presence of extended-spectrum beta-lactamases (ESBLs) enzymes in food-producing animals could potentially be extremely detrimental for human health as these proteins provide resistance to bacteria against antibiotics used in the treatment of humans affected by bacterial infections unresponsive to first-generation antibiotics. There is, however, no clear evidence that bacteria carrying ESBLs in animal and human infections are genetically related.

Efforts have been made to reduce the use of antibiotics used in veterinary medicine. For example, in the EU it is not possible to advertise prescription only veterinary drugs to the general public; that restriction includes animal keepers. The ban aims to reduce pressure on veterinary professionals by animal keepers to prescribe antibiotics that might have a short withdrawal period, instead of an election drug. Also, at EU level there is a legal requirement to monitor resistance levels in *Salmonella* obtained from food-producing animals. Moreover, from 1 January 2014, that requirement will include the monitoring of antibiotic resistance levels in *Campylobacter* spp. and in commensal *Escherichia coli* isolated from food-producing animals.

Additionally, the World Health Organization (WHO) and the World Organisation for Animal Health (OIE) have produced guidance documents of international relevance on the responsible use of antibiotics considered critically important antibiotics (CIA) for human and veterinary medicine. This high-priority antibiotic group includes fluoroquinolones, third and fourth generation cephalosporins, macrolides, and glycopeptides. CIAs should be used only in veterinary medicine in clinical cases unresponsive to other antibiotic groups. Furthermore, antibiotic sensitivity tests must be carried out before using a CIA. Fortunately, in EU, level CIAs are not normally used, where tetracycline and penicillins are the most widely used antibiotics in food-producing animals.

Although in developed countries there is awareness, monitoring, and enforcement on antibiotic residues in products of animal origin, in many developing countries that might not be the case. This situation does not necessarily affect only human health but also the potential presence of antibiotics in food products of animal origin for human consumption. In addition, factors such as lack of strength of veterinary services, animal health status, and traceability of food chain could reduce the chances of some developing countries to commercialize food products in international markets. Although the antibiotic residues situation in developing countries is widely unknown, some studies in cattle abattoirs report levels as high as 44% of positive samples for antibiotic residues, with penicillin (14%), tetracycline (8%), and streptomycin (4%) the most common antibiotics found in the analyzed samples, and with 18% of multidrug resistance. This example clearly highlights that more international support is needed to encourage responsible use of antibiotics.

Antihelmintics

Antihelmintic drugs are widely used in food-producing animals to increase animal production by reducing animal disease burden (Figure 2). Some of the most widely used antihelmintics are benzimidazoles, such as triclabendazole (effective against *Fasciola hepatica*), and drugs from the avermectin group, such as ivermectin (effective against nematodes and ectoparasites).

Although there are reports of toxicity in animals and humans due to treatment with antihelmintics, the risks for human health due to the presence of antihelmintic drugs in meat products are either low or unknown. In fact, there are many studies in the pharmacokinetics of these drugs, but not many studies have been carried out in the potential human side effects after ingesting meat with residues. More importantly perhaps, from the point of view of human health, is the fact that missed doses might result in the selection of resistance for these parasites with drugs that might be an elected treatment for human health.

Figure 2 Applying antihelmintics in beef cattle.

Growth Promoters

Growth promoters are drugs used in meat-producing animals in order to get higher meat yields and better feed conversion in a shorter period of time. Antibiotics, hormones, and beta agonists, amongst other drugs, have been used and are used in animal production with that aim. The legal frame that allows or bans the use of these compounds in food-producing animals varies. These variations are related not only to scientific evidence but also in the precautionary principle and pressures from consumers.

Owing to the increased risk of antibiotic resistance, and the associated risk for animal and human health, using a precautionary principle, from 1 January 2006, the EU has implemented a wide ban on the marketing and use of antibiotics as growth promoters, allowing the use of antibiotics in feed for only veterinary purposes. However, countries such as the United States still widely use antibiotics as growth promoters is some species. Nevertheless, the United States has also started its own initiatives, where the Food and Drugs Administration has proposed a voluntary program on labeling and use of antibiotics in food-producing animals. More evidence is needed on the use of antibiotics as growth promoters for food-producing animals and on the risk to generate antibiotic resistance in potentially dangerous human pathogens.

Hormones are widely used in some countries as growth enhancers for food-producing animals. The five hormone types most commonly used in meat production, either as injection or as ear implants (slow release), include three natural hormones, oestradiol 17-β, testosterone, and progesterone, and two synthetic substances, trenbolone and zeranol.

In the United States and other countries, natural and synthetic hormones are normally used as growth promoters in cattle, whereas in the EU for decades there have been concerns about the potential human toxicity risk associated with the likely presence of extremely minute quantities of exogenous hormone residues in meat products of animal origin for human consumption. However, there have not been any human health issues associated with consumption of beef from cattle properly implanted with hormone implants. Furthermore, as a result of these concerns in food-producing animals in the EU, these chemicals can be used for only therapeutic purposes and there is a wide ban on all hormone products to be used as growth promoters. Meat produced in non-EU countries where hormones are used as growth promoters cannot be exported to the EU.

It has been suggested that hormone growth promoters would pose endocrine, developmental, immunological, neurobiological, immunotoxic, genotoxic, and carcinogenic effects, particularly for prepuberal children. However, because there is

no conclusive scientific evidence on the human health impact, market restrictions imposed by the EU have resulted in a long international debate.

There are a number of beta agonists currently in use as growth promoters in the farm industry in some countries. Some of the most commonly used are ractopamine hydrochloride and zilpaterol hydrochloride. These drugs enhance feed conversion to muscle, with a faster growth, better yields, and leaner meat. Although improper use of these two drugs might negatively affect meat quality, they do not have a reported detrimental effect on human health as other beta agonists, such as clenbuterol. Nevertheless, some countries do not allow their use and will not allow imports of meat from animals fed with ractopamine hydrochloride and zilpaterol hydrochloride.

Disinfectants

To obtain the desired effect and to ensure food safety and health, cleaning and disinfection procedures in the meat industry should follow specific written protocols (Hazard Analysis Critical Control Point, standard operation procedures, and standard sanitation operation procedures), and these protocols should be carried out only by trained personnel. Additionally, the election of a specific chemical product must consider the material of which the surface is made, the nature of the contamination to be removed, the effectiveness at reducing microbial contamination under specific conditions, risk assessment, feasibility to use, cost of the product, and whether or not there is a need for rinsing before reusing the food contact surface.

Chemical risks associated with the presence of disinfectants are mostly related to human exposure during cleaning and disinfection processes rather than intoxications due to the presence of residues on food products. After exposure to some of these chemicals, people could suffer from skin, eye, and respiratory irritation; also, some disinfectants can have a corrosive effect on the material being cleaned, or can have a carcinogenic effect in humans.

Some disinfectants, like quaternary ammonium compounds (QACs), one of the most widely used sanitizers in the food industry, when used in the appropriate concentrations, have a low toxicity risk, and additionally they are biodegradable (Figure 3). However, there is a number of other cleaning/disinfection products, such as sodium hydroxide, which might represent a higher risk for toxicity for humans, even when used at the recommended concentrations.

Figure 3 Use of QACs foam products for disinfection of food processing premises.

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) comprise a group of chemical compounds widely distributed in the environment. PAHs are mostly produced as a result of incomplete combustion; in food production, there is a latent risk that these chemicals could be present in a larger than recommended amount in smoked products.

For decades, concerns have been raised on the genotoxic and carcinogenic risk for humans exposed to PAHs. However, the tolerable daily intake for PAHs has not been set yet; as a result, the current recommendation is that human exposure to PAHs through consumption of food products should be as low as reasonably achievable. However, exposure to PAHs has been related to a higher risk to develop lung, skin, and bladder cancer. When a case of toxicity occurs, it is not easy to relate human health problems to PAHs present in a specific food product, as the clinical effects are a result of an accumulation of these residues during a period of time (chronic cases rather than an acute intoxication).

Previous EU legislation targeted only the presence of benzo(a)pyrene in food products, which was used as an indicator of PAHs; hence, maximum levels in food were regulated only for that compound. However, after a scientific opinion of European Food Safety Authority suggesting that benzo(a)pyrene on its own was not a good indicator of PHAs, current EU legislation requests to monitor PAHs concentration in food products by measuring the levels of a combination of four substances (PAH4) (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene) while maintaining a separate maximum level for benzo(a)pyrene.

Other Chemical Risks

Lead

Lead is a heavy metal that can affect animal and human health. The highest frequency of intoxication with lead in animals is associated with the use on farm buildings of paints that contain a lead base as well as the rare instance of animals grazing in areas where car batteries might have been disposed. Although there are more important routes of human exposure to lead, consumption of contaminated meat products can pose a risk for human health. However, human consumption of game meat might represent a higher risk for human health than consumption of meat produced from farmed animals; that higher risk is related to the use of bullets that contain lead for hunting.

Signs of acute lead poisoning include not only gastrointestinal signs, such as abdominal pain, nausea, vomiting,

diarrhea, and constipation, but also neurological signs, which are associated with encephalitis. Signs of chronic lead intoxication might have a similar presentation as acute intoxication; however, neurological problems (short memory loss, depression, lack of concentration, and stupor) and anemia can also be seen.

Dioxins

The presence of these chemical compounds in food products is generally associated with industrial processes. In fact, dioxins can be produced as a by-product of waste management through incineration. Animals can be easily exposed to dioxins present in the environment, and as these molecules are lipophilic, they will accumulate in fatty tissues.

If humans consume food products contaminated with a higher than acceptable amount of dioxins, they might be affected by an acute intoxication, where skin lesions as well as alteration of liver function could occur. As dioxins are ubiquitous in the environment, human health can also be affected by long-term exposure not related to meat from contaminated animals. A chronic intoxication might result in an impaired immune system, endocrine and reproductive system, and in younger people could result in alteration in the development of the nervous system.

A number of episodes of dioxin contamination in meat products have occurred in the past years; hence, official contingency plans in order to identify, detain, and dispose of contaminated feed and food products should be in place in each country in order to reduce human and animal health risk. However, the current laboratory methods required for dioxin detection are expensive and not necessarily available worldwide.

Mycotoxins

Mycotoxins are metabolites produced naturally by fungi and molds. They have a special relevance for animal and human health as many of these mycotoxins have a carcinogenic effect. The highest risk for animals is by consumption of feed that has neither been prepared nor stored properly, allowing the growth of microorganisms, such as *Aspergillus* spp. Although human health can be at risk by the consumption of food products of animal origin that contain mycotoxins, the highest risk of exposure is not through meat consumption but through the consumption of contaminated cereals.

Nitrites/nitrates

Nitrates and nitrites are food additives used during curing of meat products in order to extend their shelf life. They are used for improving organoleptic characteristics of products (redness) and palatability as well as for the control of bacterial pathogens (*Clostridium botulinum*). Human consumption of food products that contain a high concentration of these chemicals might result in nausea and vomiting, dizziness, headaches, confusion, cyanosis, and methemoglobinemia. Additionally, nitrites and nitrates can be metabolized in the organism and produce nitrosamines, a potential carcinogenic compound.

See also: Chemical Analysis for Specific Components: Curing Agents; Veterinary Drug Residue Analysis. Environmental Contaminants. Equipment Cleaning. Hazard Analysis Critical Control Point and Self-Regulation. Microbial Contamination: Decontamination of Fresh Meat; Decontamination of Processed Meat. Processing Equipment: Smoking and Cooking Equipment. Residues in Meat and Meat Products: Feed and Drug Residues. Risk Analysis and Quantitative Risk Management

Further Reading

Barbosa, J., Cruz, C., Martins, J., *et al.*, 2005. Food poisoning by clenbuterol in Portugal. Food Additives and Contaminants 22, 563–566.

Commission Regulation (EC), 2003. No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (Text with EEA relevance). European Commission, OJ L268-29.

Commission Regulation (EC), 2006. No. 1881/2006 of 19 December setting maximum levels for certain contaminants in foodstuffs. European Commission, OJ L364/5.

Commission Regulation (EU), 2011. No. 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. European Comission, OJ L215/4.

Dayan, A.D., 1993. Allergy to antimicrobial residues in food: Assessment of the risk to man. Veterinary Microbiology 35 (3–4), 213–226.

Dewdney, J.M., Maes, L., Raynaud, J.P., *et al.*, 1991. Risk assessment of antibiotic residues of beta-lactams and macrolides in food products with regard to their immuno-allergic potential. Food and Chemical Toxicology 29 (7), 477–483.

EFSA, 2008. Polycyclic aromatic hydrocarbons in food scientific opinion of the panel on contaminants in the food chain. EFSA Journal 724, 1–114.

Ibrahim, A., Junaidu, A., Garba, M., 2009. Multiple antibiotic residues in meat from slaughtered cattle in Nigeria. Internet Journal of Veterinary Medicine 8, 1.

Jacob, C.J., Lok, C., Morley, K., Powell, D.A., 2011. Government management of two media-facilitated crises involving dioxin contamination of food. Public Understanding of Science. 20, 261–269.

Levy, S.B., FitzGerald, G.B., Macone, A.B., 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. New England Journal of Medicine 295, 583–588.

OIE, 2013. OIE list of antimicrobial agents of veterinary importance. Available at: http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/OIE_list_antimicrobials.pdf (accessed 10.09.13).

Pain, D.J., Cromie, R.L., Newth, J., *et al.*, 2010. Potential hazard to human health from exposure to fragments of lead bullets and shot in the tissues of game animals. PLoS One 5 (4), e10315. doi:10.1371/journal.pone.0010315.

Perrin-Guyomard, A., Cottin, S., Corpet, D.E., Boisseau, J., Poul, J.M., 2001. Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (hfa) mice model. Regulatory Toxicology and Pharmacology 34 (2), 125–136.

Reig, M., Toldrá, F., 2008. Veterinary drug residues in meat: Concerns and rapid methods for detection. Meat Science 78 (1–2), 60–67.

Sullivan, A., Edlund, C., Nord, C.E., 2001. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infectious Diseases 1 (2), 101–114.

Relevant Websites

http://www.apvma.gov.au/about/index.php
Australian Pesticides and Veterinary Medicines Authorities.

http://fsrio.nal.usda.gov/pathogens-and-contaminants/chemical-and-physical-contaminants
Chemical and Physical Contaminants. United States Department of Agriculture (USDA).

http://ec.europa.eu/food/food/chemicalsafety/index_en.htm
Chemical Safety of Food. European Union (EU).

http://www.daff.qld.gov.au/plants/agvet-chemicals-and-residues/chemical-residues-and-contaminants/monitoring-and-regulating-chemical-residues-and-contaminants
Department of Agriculture, Fisheries and Forestry. Queensland Government.
http://www.ema.europa.eu/ema/index.jspcurl=pages/audience/alp_audiencetype_000003.jsp&mid
European Medicines Agency.
http://www.fda.gov
Food and Drug Administration, United States of America.
http://www.noah.co.uk/
National Office for Animal Health.
http://www.vmd.defra.gov.uk/
Veterinary Medicines Directorate (VMD).

PREDICTION OF MEAT ATTRIBUTES FROM INTACT MUSCLE USING NEAR-INFRARED SPECTROSCOPY

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Glossary

Chemometrics A science discipline dedicated to develop and apply multivariate data analysis, numerical analysis, and statistics to interpret data from chemical systems.
Isosbestic A point where extinction coefficients are the same.
Metmyoglobin Ferric state (Fe^{3+}), the oxidized form of myoglobin responsible for brown–red oxidized surface color.
Myoglobin The primary oxygen-binding pigment protein found in the muscle tissue of animals.
Near-infrared spectroscopy A spectroscopic technique that uses the near-infrared region of the electromagnetic spectrum (from 700 to 2500 nm).
nm and cm^{-1} The units of wavelength (λ) and wavenumber ($\tilde{\nu}$), respectively, used to identify the frequency of the electromagnetic radiation in spectroscopy ($\tilde{\nu} = 1/\lambda$).
Oxymyoglobin Ferrous state (Fe^{2+}), the reduced form of myoglobin responsible for bright-red bloomed surface color.
Principal component analysis (PCA) Multivariate data analysis technique based on data reduction, which is able to compress and organize most of the information using a few variables, thus facilitating the interpretation of the data.
Water-holding capacity The ability for fresh meat to retain its own water.

Introduction

After slaughter, the muscle undergoes a series of biochemical and structural changes until it reaches the state of rigor. Following rigor a new series of changes take place, including proteolysis. The combination of these pre- and postrigor changes define the meat quality attributes in muscle from that carcass. Although there are processes to control and direct these changes toward consistent meat quality, not all factors affecting the final meat quality can be controlled in the slaughter plant as it is also influenced by preslaughter factors such as breed, sex, weight, and environment, which can lead to inconsistencies of the final meat quality. A strategy to deal with these inconsistencies is to identify abnormal carcasses early on in the process and manage them to achieve a desirable set of attributes. To be successful, this strategy requires the carcass to be evaluated within a time frame that allows it to be managed toward a desired use.

Near-infrared spectroscopy (NIR) is a technique based on the interaction of a radiation with the sample through an absorption and scattering process. As a result, the NIR spectrum is a rich source of information about the chemical composition of the sample as well as its microstructure. NIR is a rapid, noninvasive, and nondestructive spectroscopic method suitable for online measurement and has become widely used across the food and fiber industries including dairy, grains, oil, meat, and wool industries for proximate analysis. For the meat industry, however, there is no current commercial online NIR measurement system to accurately predict key meat quality indicators (pH, glycogen, and temperature) and/or meat quality attributes (tenderness, color, or water-holding capacity).

A large number of studies have investigated NIR technology for this purpose showing variable results. Hence, this article focuses on application of NIR spectral measurements taken from intact muscle and placed in a controlled environment to evaluate changes in meat during its conversion from muscle to meat as it relates to a meat quality indicator and the attributes relating to the conversion process The underlying principles enabling NIR to be used as an assessment criterion for the evaluation of meat quality attributes are presented to understand and develop the opportunities for the use of NIR by the meat industry.

Near Infrared

NIR spectroscopy is based on changes in vibrational status of molecules resulting from interaction with incident radiation. These changes are detected in the form of bands associated with vibrational modes of the molecules (Figure 1). Within the NIR spectral range, overtones and combinations of fundamental vibrations are observed, which lead to high degree of overlap between bands. The water molecule, for example, has the fundamental vibrations shown in Figure 1. In bulk water, the band observed at approximately 840 nm

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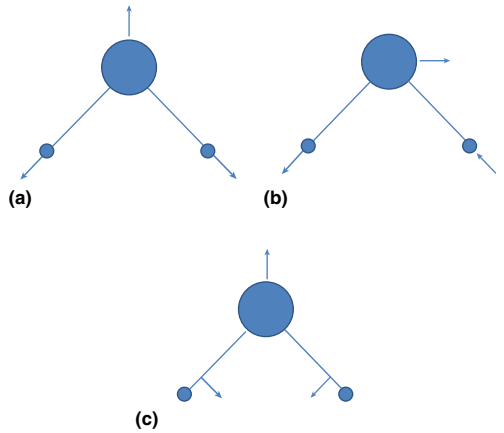


Figure 1 Fundamental vibrational modes of water (a) symmetrical and (b) asymmetrical stretch and (c) scissoring.

results from the combination of the asymmetrical stretch (Figure 1(b)) at 3500 cm^{-1} (2860 nm) and the scissoring (Figure 1(c)) band 1645 cm^{-1} (6079 nm), i.e., 3500 +1645 = 5145 cm^{-1} (1943 nm). Although molecules containing C–H, N–H, S–H, and O–H are highly active in the NIR region, the interpretation of NIR spectra is rather complex due to overlap among bands resulting from overtones and combinations.

NIR allows high penetration of the incident radiation into the sample, allowing the noninvasive detection of the backscattered radiation or transmitted radiation through the sample. For complex solid samples such as meat, the radiation–sample interaction involves mainly two principles: scattering and absorption; the former related to the microstructure of the sample and the latter resulting from the chemical composition of the sample. This allows NIR to be applied for the study of meat attributes, which depend not only on the chemical composition of meat but also on structural components.

In the NIR instrument, a beam of light from a powered radiation source (a tungsten coil or halogen lamp) is directed at the meat sample and the transmitted or backscattered radiation from the sample is returned to the spectrometer (Figure 2). The ratio between the intensity of returning radiation and the intensity of incident radiation is obtained for a range of light wavelengths to produce the NIR spectrum.

A wide range of detection systems is available to NIR spectroscopy and in particular, those based on sensor arrays without moving parts are portable so that the spectrum is collected in milliseconds. In this type of instrument, the returning radiation, following interaction with the sample, is diffracted and directed to a sensor(s) with a series of independent elements, which thus allows the scanning of an entire spectrum to be captured in a few milliseconds (Figure 3). A range of measurement modes are available, including: transmittance, where the radiation is transmitted through the sample, and reflectance, where the radiation penetrates the sample and the backscatter radiation is detected by the spectrometer (Figure 2). Reflectance mode is the type more commonly used for measurement of meat, as it allows a noninvasive evaluation of the sample.

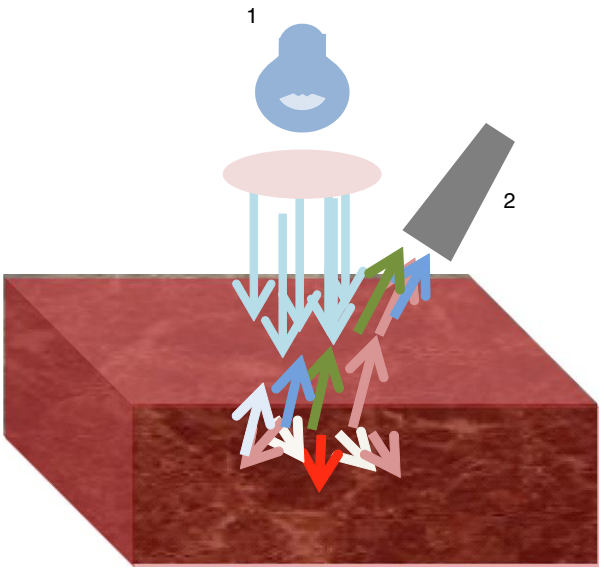


Figure 2 Schematic illustration of the reflectance measurement. (1) Incident polychromatic light beam from the probe. (2) Fiber optics to capture backscattered light.

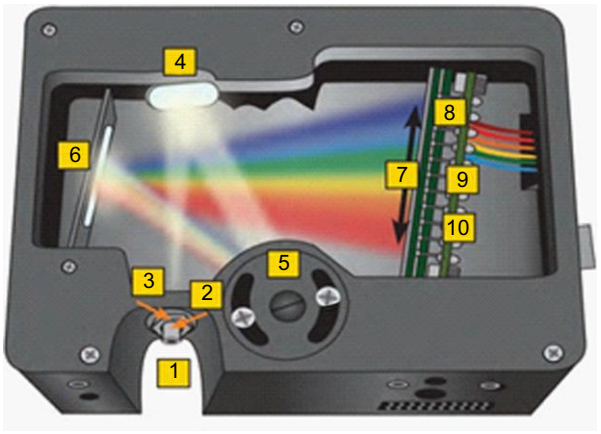


Figure 3 Overview of spectrometer. (1–3) Connection system to receive light from fiber (see Figure 2(2)). (4) Collimating mirror to reflect the entering light toward the grating. (5) Grating and selector for wavelength range. (6) Focusing mirror to focus spectra on the detector. (7) Detector collection lens. (8–10) Detection system. Drawing courtesy of Ocean Optics, Dunedin, FL, USA.

Although these instrumental characteristics allow NIR to be used as a noninvasive fast technique for evaluation of meat attributes, its spectrum is rather complex to easily interpret. This has restricted the interest in NIR for decades until the development of chemometrics that expanded the use and interpretation of NIR spectra in wide range of applications. Chemometrics utilizes multivariate data analysis to extract information from NIR spectra and to develop mathematical models for the prediction of attributes. In the approach based on the use of exploratory data analysis to extract information, a series of spectra are analyzed, and features, or responses, from the spectra associated to sample traits are identified and interpreted. The most popular methodology is based on

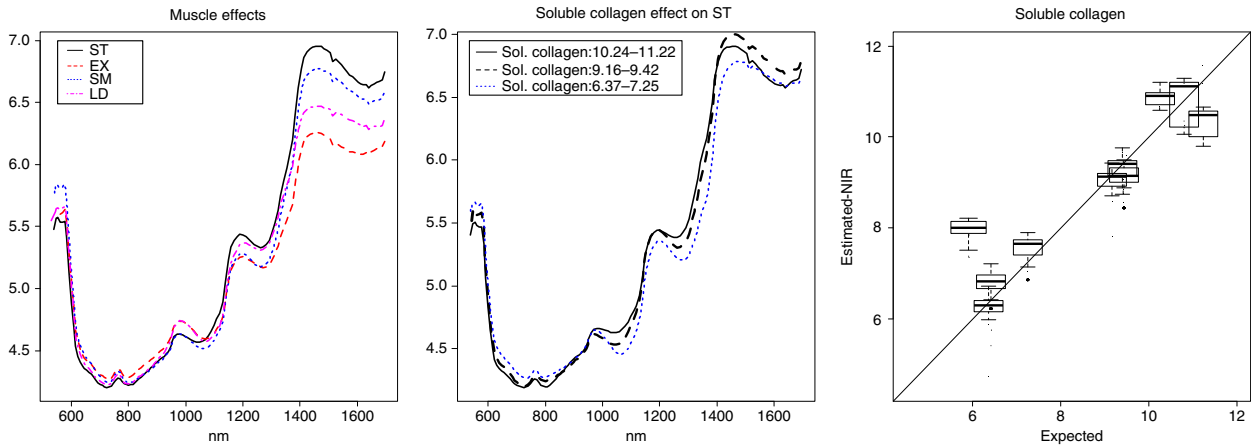


Figure 4 Effect of muscle type and concentration of soluble collagen on NIR spectra. On the left-hand side, the effect of muscle type on NIR is shown. ST, Semitendinosus; EX, Extensor capri; SM, Semimembranosus; LD, Longissimus dorsi. In the middle, a set of spectra is averaged according to range on soluble collagen content (solid line 10.24–11.22, dashed line 9.16–9.42, and dotted lines 6.37–7.25). On the right-hand side is shown the predictions of soluble collagen in ST, showing that NIR is able to predict the presence of soluble collagen, for details of the experiment see Reis *et al.* (2012).

principal component analysis (PCA). The second approach enabling the rapid prediction of sample attributes from NIR spectra involves the development of a chemometric model used to translate the NIR spectrum into the property of interest (e.g., tenderness).

The NIR spectrum is affected by different characteristics of the sample, such as chemical composition, microstructure, temperature, homogeneity, to mention a few. All of these effects may not be required for a given application. Thus, the NIR spectrum is corrected before the multivariate analysis. This correction is also known as preprocessing, with the most common being multiplicative light scattering used to reduce effect of unwanted light scattering.

The development of prediction models from NIR spectra requires a representative dataset that includes similar sources of variation to those found in new samples. Once a representative dataset is obtained, the development of the model is performed in two steps: calibration and validation. To perform these steps, one part of the dataset, the validation dataset, is initially put aside. The other part called calibration dataset is used to fit the model, which can involve different mathematical techniques such as partial least squares, neural network, and others. Once the best calibration model is selected and the model is applied to the validation dataset, its performance is assessed. There are several processes to perform the model selection. Among them, cross-validation is widely used. In this case, the calibration dataset used to develop the model is split in subsets of samples. One subset is used to fit a calibration model, which is then applied on the cross-validation subset of samples left out. This process is repeated until all samples are left out once. The predictions of these samples are then used to evaluate the performance of the model. In the meat science literature, it is common to use cross-validation to assess the ability of NIR to predict a given attribute. This is not an ideal process as cross-validated samples are not completely independent and care must be taken when interpreting these results.

Meat Microstructure and Near Infrared

As the NIR portion of the incident radiation penetrates the meat, it undergoes light scattering and absorption. In particular, light scattering is dependent on the microstructure of the meat and absorption results from the chemical composition. In Figure 4, for example, the effect of four different muscle types on the NIR spectra and the effect of three different concentration ranges of soluble collagen on the NIR spectra are shown. Although muscle type is associated to an additive effect on spectral range over 1200 nm, the effect of soluble collagen does not show such additive effects. It has been found that distinction between muscle types is related to the light scattering (Figure 5) and that the amount of scattered light is associated with shear force (Figures 6 and 7). This ability to capture two types of information makes NIR a useful tool for meat quality evaluation where most of quality attributes evolve from changes in the chemical composition as well as in the meat microstructure.

Pre- and Postrigor Changes

PCA of NIR spectra collected on beef M. longissimus lumborum from slaughter up to 90 h postmortem is shown in Figure 7. The scores of first principle component (PC1) are separated between pre- and postrigor, where prerigor samples present negative scores and postrigor samples have positive scores. The loadings from PC1 show high positive values for bands associated with the OH (water) functional group and negative values for bands in the visible region and CH functional group (Figure 8). This suggests that postrigor samples (positive scores) are associated with more water when compared with prerigor samples. It also indicates that prerigor samples (negative scores) are richer in the CH functional group compared with postrigor (positive scores), i.e., postrigor must present a higher degree of breakdown of its

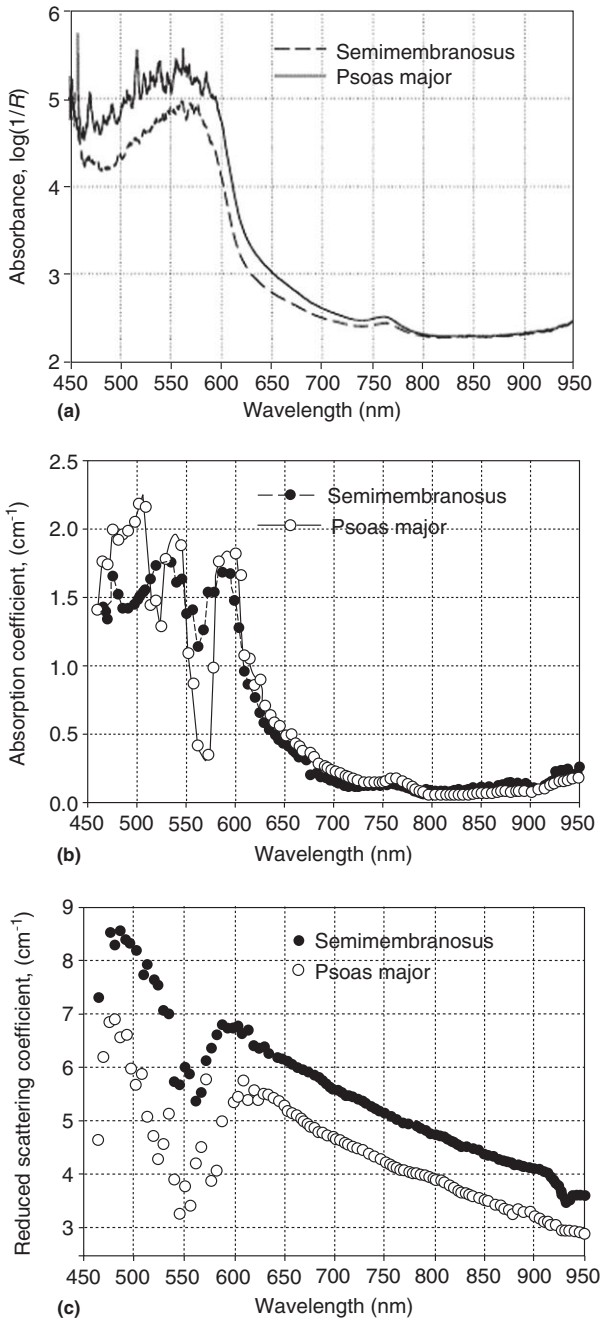


Figure 5 (a) NIR spectra of samples of two different muscle types, (b) absorption coefficient, and (c) scattering coefficient. The scattering coefficient corresponds to an additive effect over spectral range as the two scattering spectra are similar in shape but shifted by an additive constant. Reproduced from Xia, J.J., Berg, E.P., Lee, J.W., Yao, G., 2007. Characterizing beef muscles with optical scattering and absorption coefficients in VIS–NIR region. Meat Science 75, 78–83.

microstructure that reduces the level of the CH functional group. The bands in the visible spectral range (below 600 nm) are associated with oxidation status of myoglobin where 572 and 600 nm are the isosbestic points for deoxymyoglobin/oxymyoglobin and metmyoglobin/oxymyoglobin, respectively. The reflectance at the isosbestic point is the same for the two or

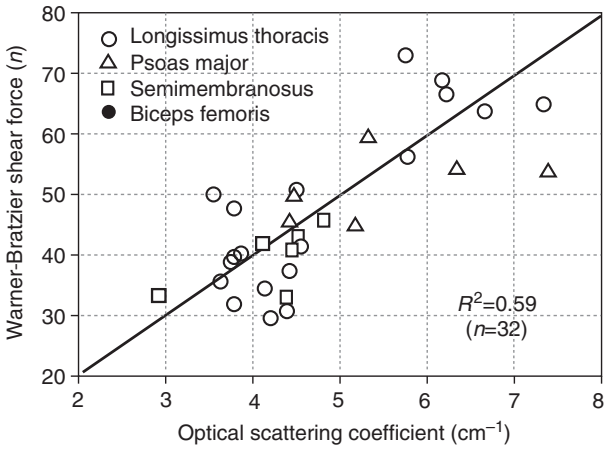


Figure 6 Relationship between scattering coefficient and shear force. The values for M. longissimus are spread over the whole range of shear force, showing the relationship due to confounding between muscle type and shear force. Reproduced from Xia, J.J., Berg, E.P., Lee, J.W., Yao, G., 2007. Characterizing beef muscles with optical scattering and absorption coefficients in VIS–NIR region. Meat Science 75, 78–83.

three forms of myoglobin, which suggest that the negative loadings at 572 and 600 nm are an indication of higher degree of oxidation of myoglobin for the postrigor samples that would reduce the overall reflectance in this spectral region (Figure 7).

Prerigor Changes

Figure 7 also shows that NIR is associated with pH as there is a trend in prerigor samples where the decrease in pH is related to an increase the scores of PC1. To investigate this relationship, a quantitative approach was applied to the data shown in Figure 7. This experiment included carcasses electrically stimulated and nonstimulated with samples kept at three different temperatures, resulting in six rates of pH decline. The pH predictions by NIR, from 1 to 90 h postslaughter, demonstrate the ability of NIR to capture the temporal variation as well as the differences among the applied treatments (Figure 9).

It has been shown that NIR is able to predict glycogen in prerigor meat (R^2 validation 0.72, RMSEP = 2.68 mg g^{-1}), which combined with the ability to predict pH might underlie key principles that allow NIR to be used prerigor to predict ultimate pH.

Postrigor Changes

The development of full rigor occurs as each muscle fiber sequentially enters rigor over the preceding period. At this point, new changes take place in the meat, especially in its microstructure. During postrigor aging, meat loses increasing amounts of water and an increase in drip with aging is most likely due to the release of water tightly bound up in the cytoskeletal proteins as they degrade and progressively unravel, releasing their bound water. In a study on lamb, NIR was

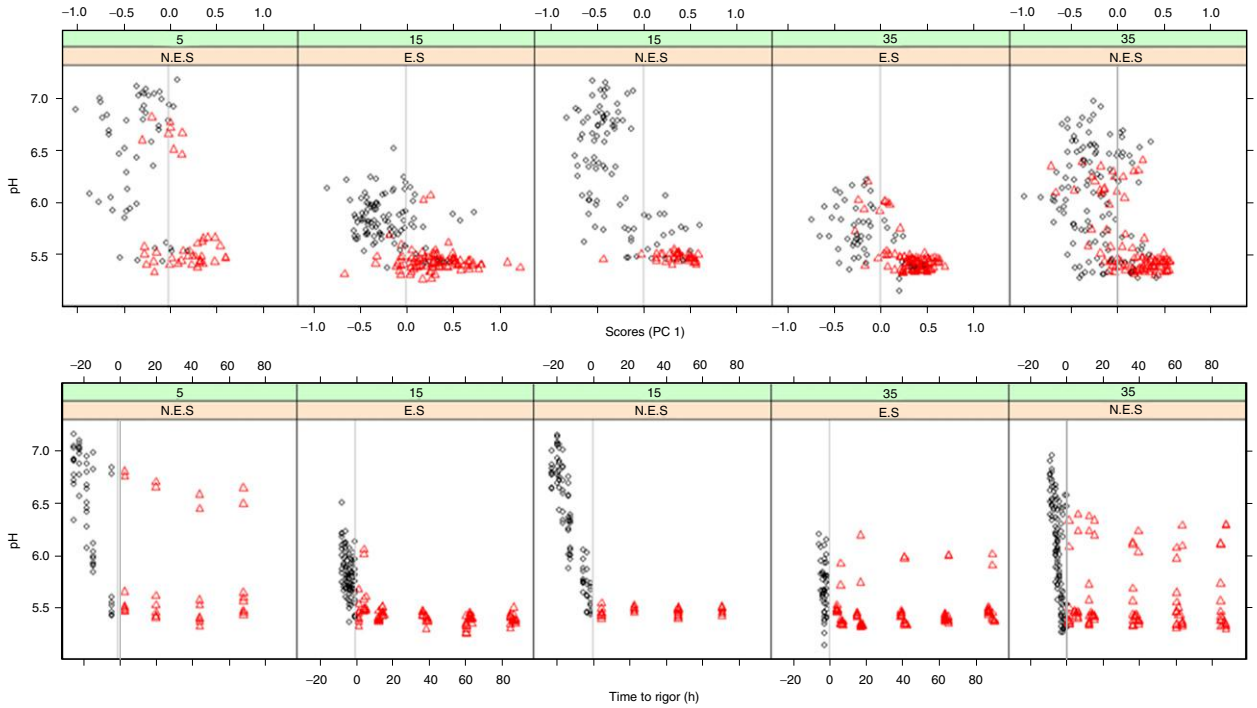


Figure 7 Relationship between scores of the first PC1 with pH and time to/from rigor. Open circles represent spectra collected prerigor and triangles correspond to samples postrigor. The temperature of the samples is shown on the top of each panel, whereas E.S and N.E.S refer to electrical and nonelectrical stimulated carcasses, respectively, for details of the experiment see Rosenvold *et al.* (2009).

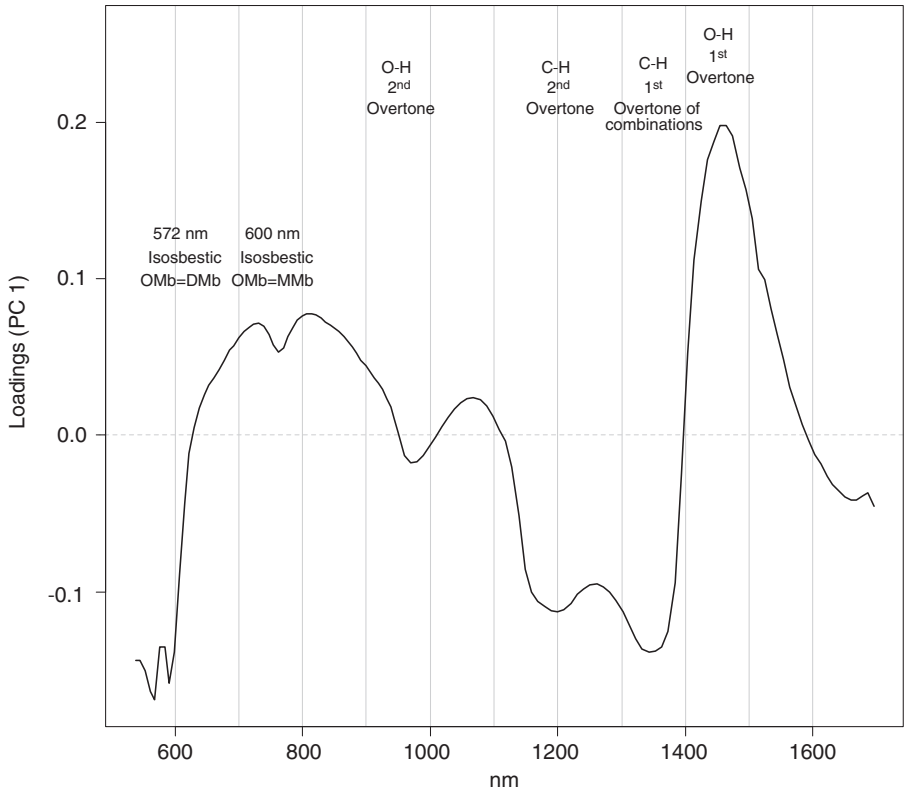
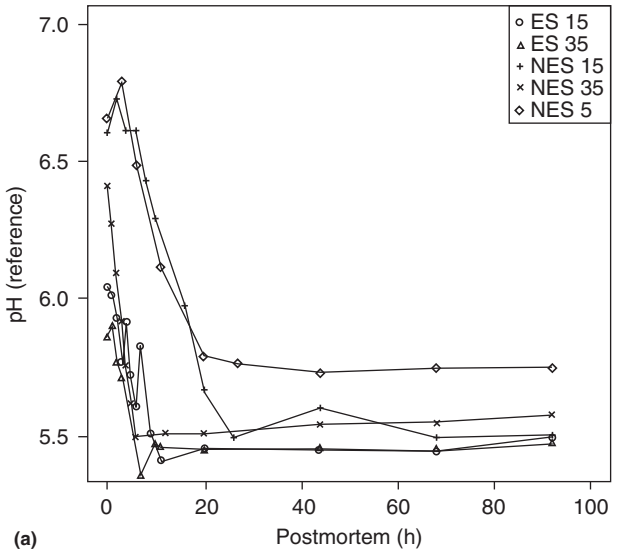


Figure 8 Loadings for PC1 shown in **Figure 7**. The isosbestic points for deoxymyoglobin, oxymyoglobin (OMb), and metmyoglobin (MMb) occur at 572 and 600 nm. 'O–H' and 'C–H' refer to spectral region where there are overtone and combinations of fundamental molecular vibrations for the functional groups 'O–H' and 'C–H'.



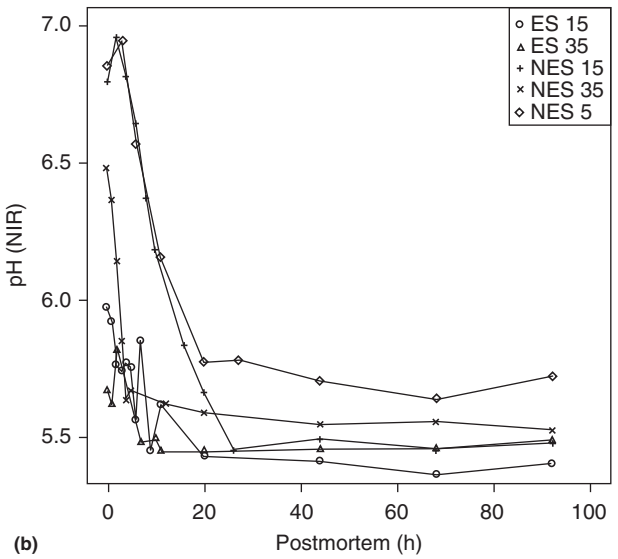
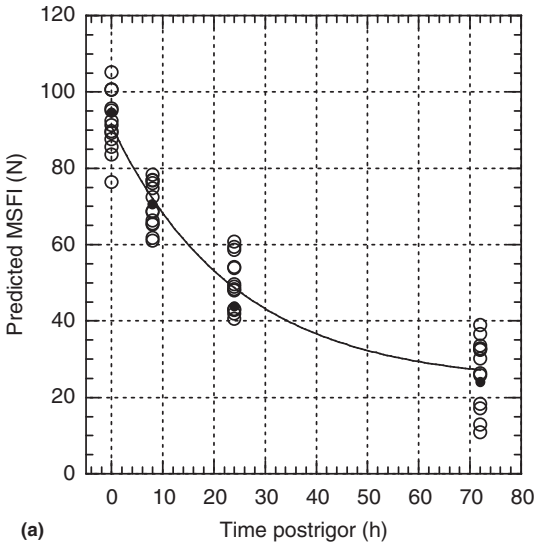
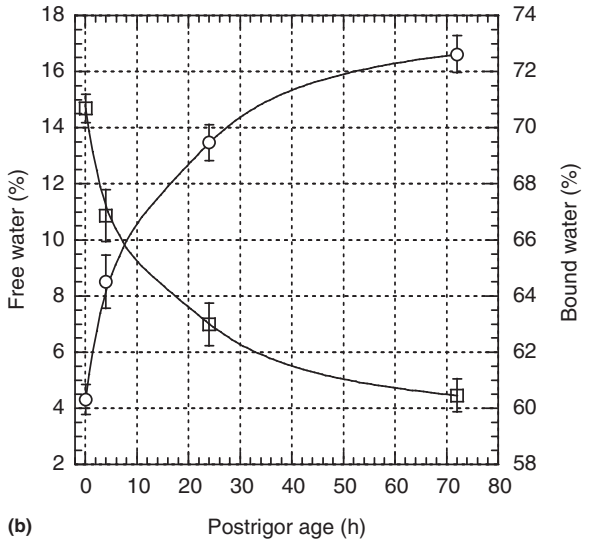


Figure 9 (a) Reference measurement and (b) NIR measurement of pH. ES15: electrically stimulated and held at 15 °C until rigor. ES35: electrically stimulated and held at 35 °C until rigor. NES15: nonstimulated and held at 15 °C until rigor. NES35: nonstimulated and held at 35 °C until rigor. NES5: nonstimulated and held at 5 °C until rigor. Reproduced from Rosenvold, K., Micklander, E., Hansen, P.W., *et al.*, 2009. Temporal, biochemical and structural factors that influence beef quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.





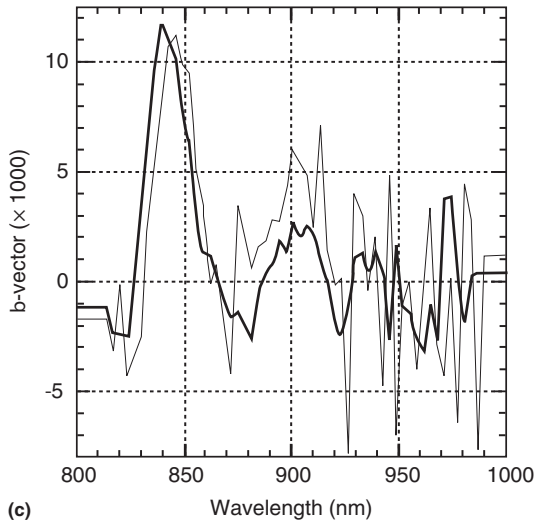


Figure 10 (a) Scatterplots of predicted Mean Shear Force index (MSFI) versus postrigor age for a validation subset. The curve was generated using an exponential model. (b) Mean percentages of free (○) and bound (□) water plotted against postrigor age for the same data as in (a). (c) Regression coefficients for the models to predict shear force from NIR from two independent datasets. Error bars represent ±1 standard error of the means. Reproduced from McGlone, V.A., Devine, C.E., Wells, R.W., 2005. Detection of tenderness, postrigor age and water status changes in sheep meat using near infrared spectroscopy. Journal of Near Infrared Spectroscopy 13, 277–285, with permission from IM Publications.

able to predict the drop in shear force during the aging process (Figure 10(a)). In this model, the dominant feature was the OH band at 840 nm, suggesting that the underlying chemistry is related to the postrigor changes in the water status of the meat. These samples also showed an increase in the amount of free water and a decrease in the amount of bound water (Figure 10(b)). It suggested that drip was related to a degradation of the tertiary structure of the cytoskeletal proteins during aging. This observation is in agreement with Figure 8, where NIR was able to identify reductions of CH, more likely to happen due to degradation, and there was an increase in the amount of water in the postrigor samples. Figure 8 shows that key difference between pre- and postrigor samples in NIR is associated with higher amount of water and reduced amount of CH in the samples. To further investigate this observation, an experiment was set up with three different treatments applied to the carcass/sample: electrical stimulation, wrapping, and prerigor temperatures. The results from six aging regimes were obtained, where shear force was monitored with a conventional tenderometer and predicted by NIR. In this experiment, each sample was monitored to identify starting point of rigor. NIR was able to predict the time from rigor (Figure 11) and the decline of shear force similarly to measured values (Figure 12), i.e., NIR was able to predict the stage and monitor the aging process.

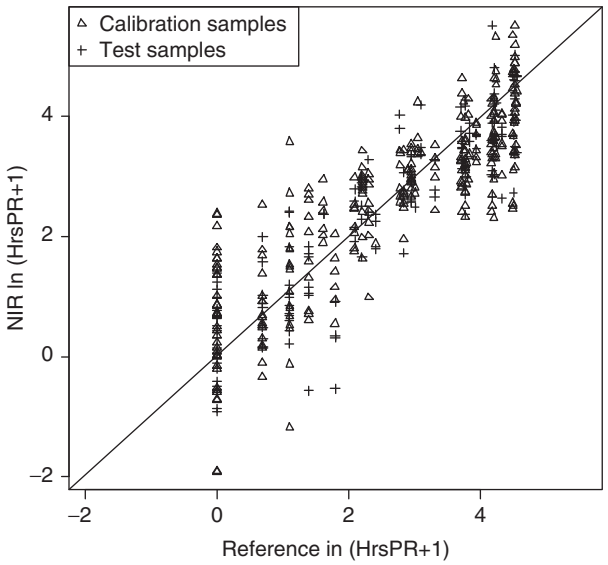


Figure 11 Prediction of time from rigor measured as ln (HrsPostRigor+1) in beef M. longissimus lumborum samples from rigor mortis to 90 h postrigor from the same samples as in Figure 12 Reproduced from Rosenvold, K., Micklander, E., Hansen, P.W., *et al.*, 2009. Temporal, biochemical and structural factors that influence beef quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.

Use of Near Infrared to Predict Meat Quality in Commercial Situations

The experiments described in this article show the ability of NIR to capture changes in the meat throughout the conversion of muscle to meat that are related to final quality attributes. This ability to predict pre- and postrigor changes enables NIR to be considered as:

1. a tool to monitor rigor and aging processes to manage carcasses in order to set quality standards,

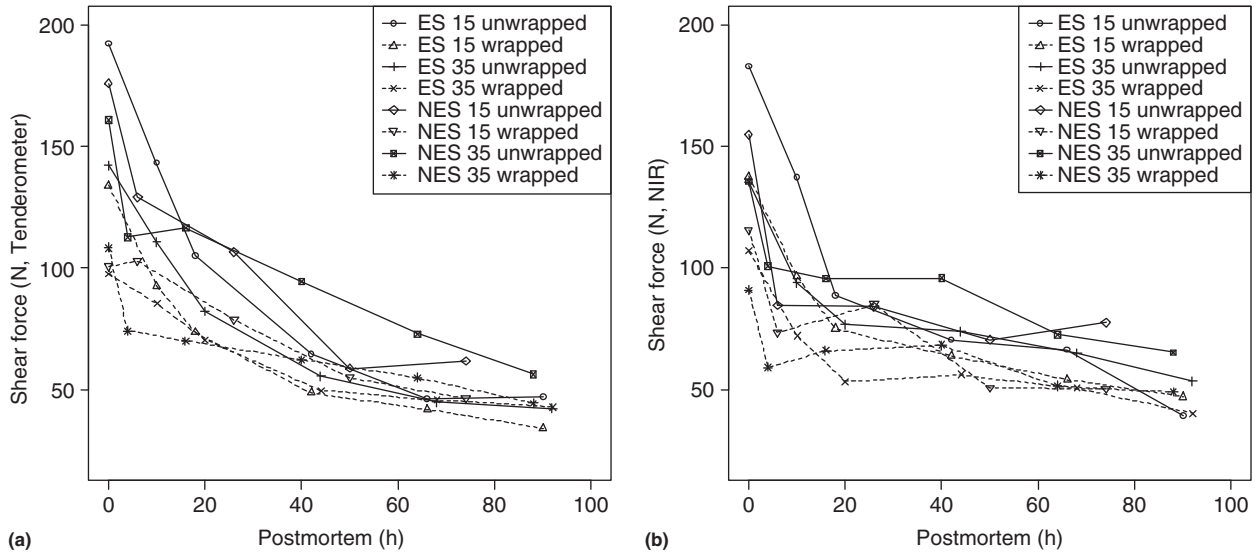


Figure 12 Shear force measured from rigor mortis to 90 h postmortem in steer M. longissimus lumborum samples to which electrical stimulation, wrapping, and prerigor temperatures were applied to create a variation in meat quality. (a) Reference measurement and (b) NIR measurement. ES15: electrically stimulated and held at 15 °C until rigor. ES35: electrically stimulated and held at 35 °C until rigor. NES15: nonstimulated and held at 15 °C until rigor. NES35: nonstimulated and held at 35 °C until rigor. NES5: nonstimulated and held at 5 °C. Unwrapped/wrapped refers whether the muscles were wrapped or not. The calibration for shear force (N) measured in beef M. longissimus lumborum used samples from rigor mortis to approximately 90 h postrigor. Reproduced from Rosenvold, K., Micklander, E., Hansen, P.W., *et al.*, 2009. Temporal, biochemical and structural factors that influence beef quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.

2. a grading tool to identify quality carcasses for sale into higher value markets (e.g., higher quality bulls with normal pH),
3. a filtering tool to pick out a small percentage of inferior quality carcasses to ensure that they do not go to discerning customers (e.g., dark cuts identified from NIR prediction of ultimate pH), and
4. a selection tool for producers and processors, leading to improved quality over time through better genetic selection, improved finishing regimes, or process control.

NIR has been investigated for prediction of meat quality attributes for more than two decades, where a variety of independent studies have been carried out. Results are not always in agreement across studies, and it is very rare to identify studies using similar experimental design and processing conditions, which makes it difficult to evaluate and compare outcomes. Indeed, the development of meat quality attributes involves a complex biological process affected by several factors (e.g., seasonality, processing factors, and animal-to-animal variation). Thus, the success of any of NIR applications to predict meat quality attributes will rely on the design of experiments which must necessarily accurately represent the environment in a meat-processing plant. Good experimental design will lead to representative datasets which account for most of the factors affecting the conversion of muscle into meat, such as environmental (e.g., seasonality) and processing factors (e.g., chain speed and temperature) must be taken into consideration and included in the final analysis. Thus, the variation of results observed in the literature should not deter the development science underpinning NIR spectroscopy for application in meat industries and shows that there is need of some harmonization of the measurement of meat quality attributes.

See also: Chemical and Physical Characteristics of Meat: Chemical Composition; Color and Pigment; Water-Holding Capacity. Classification of Carcasses: Beef Carcass Classification and Grading. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Electrical Stimulation. On-Line Measurement of Meat Composition. Tenderness Measurement

Further Reading

Barton, F.E., 2002. Theory and principles of near infrared spectroscopy. Spectroscopy Europe 14, 12–18.
Hildrum, K.I., Nilsen, B.N., Mielnik, M., Naes, T., 1994. Prediction of sensory characteristics of beef by near-infrared spectroscopy. Meat Science 38, 67–80.
McGlone, V.A., Devine, C.E., Wells, R.W., 2005. Detection of tenderness, postrigor age and water status changes in sheep meat using near infrared spectroscopy. Journal of Near Infrared Spectroscopy 13, 277–285.
Osborne, B.G., Fearn, T., Hindle, P.T., 1993. Practical NIR spectroscopy with applications in food and beverage analysis, second ed. Harlow, UK: Addison-Wesley Longman Ltd.
Pasquini, C., 2003. Near infrared spectroscopy: Fundamentals, practical aspects and analytical applications. Journal of Brazilian Chemical Society 14, 198–219.
Prevolnik, M., Potokar, M.Č., Škorjanc, D., 2004. Ability of NIR spectroscopy to predict meat chemical composition and meat quality. Czech Journal of Animal Science 49, 500–510.
Prieto, N., Roehe, R., Lavín, P., Batten, G., Andrés, S., 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science 83, 175–186.
Ranasinghesagara, J., Nath, T.M., Well, S.J., *et al.*, 2010. Imaging optical diffuse reflectance in beef muscles for tenderness prediction. Meat Science 84, 413–421.
Reis, M.M., Mercer, G.J.K., Wiklund, E., Rosenvold, K., North, M.F., 2012. The ability of NIR to detect simultaneous changes in meat collagen and shear force. 15th Australian NIR Spectroscopy Conference (ANISG). Queenstown, New Zealand: The Australian Near Infrared Spectroscopy Group.
Rosenvold, K., Micklander, E., Hansen, P.W., *et al.*, 2009. Temporal, biochemical and structural factors that influence beef quality measurement using near infrared spectroscopy. Meat Science 82, 379–388.
Weeranantanaphan, J., Downey, G., Allen, P., Sun, D.W., 2011. A review of near infrared spectroscopy in muscle food analysis: 2005–2010. Journal of Near Infrared Spectroscopy 19, 61–104.
Williams, P., Norris, K., 1987. Near Infrared Technology in the Agricultural and Food Industries. St. Paul: American Association of Cereal Chemists Inc.
Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: A basic tool of chemometrics. Chemometrics and Intelligent Laboratory Systems 58, 109–130.
Xia, J.J., Berg, E.P., Lee, J.W., Yao, G., 2007. Characterizing beef muscles with optical scattering and absorption coefficients in VIS-NIR region. Meat Science 75, 78–83.

Relevant Websites

http://cran.r-project.org/web/views/ChemPhys.html
R Foundation for Statistical Computing, Vienna, Austria.
http://en.wikipedia.org/wiki/Near-infrared_spectroscopy
Wikipedia: The Free Encyclopedia (Wikimedia Foundation Inc.).
http://en.wikipedia.org/wiki/Vibrational_spectroscopy
Wikipedia: The Free Encyclopedia (Wikimedia Foundation Inc.).
http://en.wikipedia.org/wiki/Chemometrics
Wikipedia: The Free Encyclopedia (Wikimedia Foundation Inc.).
http://en.wikipedia.org/wiki/Principal_components_analysis
Wikipedia: The Free Encyclopedia (Wikimedia Foundation Inc.).
http://en.wikipedia.org/wiki/Multivariate_statistics
Wikipedia: The Free Encyclopedia (Wikimedia Foundation Inc.).

PRESERVATION METHODS OF ANIMAL PRODUCTS

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Glossary

Binders Reduce cost and some have water binding and emulsifying properties.
High-pressure food preservation Extremely high pressure can preserve fresh appearance and reduce microbial content.
Hurdle technology Using several bacterial limiting factors in one product to reduce bacterial growth.
Irradiation Used to reduce the microorganisms content for feed, spices, and meat.
Modified atmosphere packaging Reduction of oxygen and replacing it with carbon dioxide or nitrogen within a package.
Reducing compounds Ascorbic acid and erythorbic acid and their salts have antioxidant effects and resist color fading.
Smoking Placing in an environment with smoke to improve flavor, color, and to obtain a slight bacteriostatic effect.

Introduction

Animal products preservation is the system of treating and handling these products to stop or retard spoilage, loss of quality, oxidation while maintaining the nutritional value and desirable edibility, and extending shelf life.

Preservation usually involves prevention or reduction of growth of microorganisms (although in some cases benign bacteria are taken advantage of to achieve desired results). Preservation can also include inhibiting or retarding color deterioration, which may be caused by pigment oxidation, heating, the nonenzymatic Millard reaction (combination of an amino acid and a reducing sugar), or enzymatic action (however, enzymes can be helpful in tenderizing meat). Maintaining or improving nutritional value, texture, and flavor is also important in preservation. Some preservation techniques drastically alter product characteristics, and these changes are often desirable. Many other preservation techniques are often combined (hurdles) to accomplish the desired results. For example, a few of the hurdles might include cooking, reduction in water content, additives such as salt or nitrite, and modified atmosphere or vacuum packaging.

Individual Techniques Utilized

Drying

Drying is one of the oldest preservation techniques and reduces water activity to a level in the product that will prevent bacterial growth (Table 1). American-Indians hung meat in their campfire smoke not only to smoke it but also to dry it (reduce water activity, a_w), so that it would keep for later use. Today, jerky is an example of this dehydration. Modern dehydration is normally carried out by blowing filtered warm air over the product until the desired water activity level is achieved (Table 1). Even though bacterial growth will not occur if the water activity is low enough, recent discoveries reveal that some of the bacteria may still survive. Therefore, combined treatments are now required, and a moist high temperature treatment must also be

Table 1 Influence of water activity (a_w) on microorganism's growth

$a_w = \frac{\text{55.5 mol of water per kilogram}}{\text{Number of moles of solute} + \text{55.5 mol of water}}$	a_w *or minimum* a_w *for growth*
Fresh meat (optimum for most bacterial growth)	0.99
Most bacteria	0.90
Molds	0.85–0.60
Yeast	0.01–0.88
Rapid spoilage can occur	1.0–0.92
Spoilage can occur, but few pathogens will grow	0.85–0.92
Some spoilage can occur, but generally the product is stable without refrigeration	0.85–0.80
Spoilage is greatly delayed	0.7–0.65

Source: Reproduced from Ockerman, H.W., 1996. Chemistry of Meat Tissue, eleventh ed. Columbus, OH: The Ohio State University.

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included in the drying procedure, for example, for jerky. As a_w is lowered below the optimum, it will increase the lag phase, decrease the growth, and decrease the amount of cell substance synthesized. Each organism has its own a_w range of growth. Combinations of environmental factors (surface drying and migration of salts to the surface, curing salts, pH, and temperature) influence a_w effect.

Water activity is usually obtained by measuring the relative humidity of the atmosphere in a sealed container in equilibrium with the food in the container.

Refrigeration

Microbial growth and the action of enzymes are primarily controlled by time and temperature. In some areas of the world, refrigeration (both commercial and domestic) is utilized, giving food a longer shelf life and improving diets by allowing fruit, salads, dairy products, and meat to be safely stored for longer periods, particularly when the weather is warm. Meat freezes at −2.0 °C and the closer the refrigerated food can keep to this temperature, the longer the food will be protected. The food danger zone is between 4.4 and 60 °C (40 and 140 °F), so food should not be kept in this temperature range for any extended period of time. In other areas of the world, time is controlled and little refrigeration is utilized, lowering the carbon footprint. This practice requires early morning slaughter, meat sales by noon, cooking for lunch or early afternoon, and consumption that day. This is particularly important if the weather is warm. Lack of refrigeration also requires daily shopping, which reduces human efficiency.

Freezing

Freezing is a continuation of refrigeration except at a lower temperature, with an even greater extension of the food's storage life. In evaluating storage temperature, usually colder is better; however, the colder temperature requires a greater expender of energy, so a compromise must be achieved and usually −23.3 °C (−10 °F) is recommended. The rate of freezing is also important; the faster the better (less time for water migration and production of large ice crystals, which causes dehydration of other areas). In most cases, microorganisms will not grow at recommended freezer temperatures and therefore the food is usually safe to eat. However, oxidation, even though retarded in frozen products, will continue and off-flavors will develop. Oxidation rate is not only reduced at lower temperatures but is also controlled by a substrate that is susceptible to oxidation, such as unsaturated fat, so higher fat content will result in shorter acceptable storage life. Also, sodium chloride (salt) added to food will speed up oxidation. Therefore, different products have different recommended frozen storage times. Food packaging is also important, and a material with reduced moisture transmission is desirable. If this is not used, dehydration will occur and in extreme cases freezer burn will produce a white area that will allow oxygen penetration and increased oxidation. The product is usually still safe to eat but flavor and texture is reduced.

Vacuum and Modified Atmosphere Packaging

Vacuum packaging in an oxygen impermeable package will exclude most of the oxygen, thus reducing oxidation and off-flavors and retarding the growth of microorganisms that require oxygen for growth (aerobe). This will significantly extend the shelf life of refrigerated, frozen, and dried foods. Vacuum packaging changes the color of muscle tissue. Tissue that has been exposed to the oxygen changes from myoglobin (purplish red) to oxymyglobin (bright red), and consumers associate this bright red color with a fresh product. When meat is vacuum packaged, this reaction is reversed and a purplish-red color is obtained. Modified atmosphere packaging changes the environment around the food in the package. In this system the oxygen (O_2) concentration is often reduced and the carbon dioxide (CO_2) concentration is increased. Increase of CO_2 may be obtained by depositing a block of dry ice in the package before sealing or more frequently the food container can be purged by gaseous carbon dioxide from a cylinder. The reduction of oxygen reduces oxidation. Nitrogen (N_2) gas is also sometimes used instead of CO_2. Various combinations of gases are currently being researched to find the ideal mixture for each type of product and storage condition.

Salt

Salt is one of the oldest additives to extend the shelf life of a food item. It also alters flavor, which is desired by most humans, decreases the water activity value, which gives better bacterial protection, extracts protein, and assists in emulsion formation. If salt is placed on the outside of food, it attracts food moisture by osmosis and dehydration results. If mixed with the food, salt will retard bacterial growth. When salt is added to muscle tissue, the tissue will have a higher salt concentration than the bacterial cell; most bacterial cell walls are semipermeable, which will allow water but not salt to pass through. Water will pass from the less salt dense concentration (bacterial cell) to the more salt dense concentration in the food, and the bacterial cell will become dehydrated, shrivel, and die. Other effects of salt addition include toxicity of the chlorine ion, reduced oxygen solubility in food, and reduced effectiveness of bacterial proteolytic enzymes. Table 2 indicates the approximate salt level for bacterial inhibition.

Because this high salt level is objectionable for most products, it is usually used in combination with other bacterial

Table 2 Upper limits of salt for bacterial growth

Microorganism	*Salt (NaCl)*
Clostridium botulinum	10%
Staphylococcus aureus	15%
Salmonellae	8%
Most bacteria	8%
Fermentative yeast	10%
Oxidative yeast	Up to 25%
Molds	18%, some up to 22%

Source: Reproduced from Ockerman, H.W., 1996. Chemistry of Meat Tissue, eleventh ed. Columbus, OH: The Ohio State University.

inhibiting factors. Disadvantages of salt are that it promotes oxidation and fades desirable meat color to a stale one. Owing to the fact that many people consume more salt than they require, most processors are trying to reduce the salt level in their products to promote better health.

Sweeteners

Sugars (sucrose, glucose, corn sirup solids, corn sirup, glucose, sirup, and malt sirup) and artificial sweeteners are used in food to contribute to flavor. Sugars result in food energy, altered color on cooking, and reducing conditions, which aid in color development in meat. The level of sugar needed to retard bacterial growth is from 20% to 80%, which is used in jams and jellies, but this level is too high for most animal products. The only exception to this rule in the meat area is some mincemeat. In fact, the 1–10% sugar level usually used in meats will encourage bacterial growth.

Smoking

Smoking is used primarily with meat (including sausage), fish, and cheese and is usually, but not always, combined with heating. Its main purpose is to add flavor and produce a desirable external color. The smoke usually affects only the external surfaces and has little penetrating power. Smoke has a slight bacteriostatic effect, adds a protective coating that acts as a physical barrier for the product and retards penetration of undesirable microorganisms, and deposits some phenol compounds, which retard oxidation. However, smoking alone does not have much preservative power unless it is combined with heating or drying. Sausages that are moist cooked, showered, or sliced after smoking lose some of the advantages of smoking. Liquid smoke, which is obtained by burning hardwood and capturing a fraction of the smoke volatiles in a suitable solvent, can be mixed with the food, resulting in more penetration into the product. Liquid smoke is usually acidic and the lowering of pH can result in some preservative effect. As smoking is a potential carcinogenic risk, it is continuously evaluated for its safety.

Additives

Many food additives influence preservation, of which two (salt and sweeteners) have already been mentioned. Many additional food additives make present day food formulations possible, and without additives many products would be difficult to manufacture and most animal food products' shelf lives would be drastically affected. A few of the more important and often used food additives are listed here.

Nitrite (nitrate that can be converted into nitrite in the meat and its typical microbial environment) and salt (sodium chloride) convert fresh meat into cured meat. Sodium (Na) or potassium (K) nitrite is primarily used. Na and K nitrates are primarily used as a reservoir for nitrite in long-cured meat (e.g., dry-cured meats that will keep without refrigeration). These cured meat products are not as easily found today as in the past. In meat curing, the nitrite is partly converted into nitrous acid, which is then converted into nitric oxide, which combines with the muscle pigment (myoglobin) to form the desirable pink color of cured meat. In addition to the color change, nitrites are highly bacteriostatic (retard bacterial growth). Five percent salt plus 200 ppm of nitrite is equivalent in preserving effect to approximately 15% salt in meat products. A number of genera of bacteria are retarded by nitrite in the pH 5.7–6.0 range. Flavor is also altered by nitrite to produce the desirable, typical, cured meat flavor. Nitrite is also an excellent antioxidant, which reduces 'warmed-over flavor' and other oxidation problems, giving cured products a much longer shelf life than their uncured counterparts. Nitrite usage in the US is limited to 156 ppm (in some countries this value is lower) in most products and 120 ppm in bacon. After heat processing and storage, this level is usually reduced to much less than 50 ppm. In fermented products, the nitrite is used at a lower level to keep it from deactivating the starter culture bacteria. Nitrite in large dosage is toxic, but there are several safeguards built into its usage in meat processing. Nitrite is kept in a locked 'bonded room,' and if nitrite is used in excess in a product, 'nitrite burn' will result in an undesirable color, which would warn the processor. Nitrite is usually mixed with salt (Europe, 0.6% and USA, 6.25% nitrite), and if too much is added, the product would be too salty to eat. This mixture is also colored pink so it would be difficult to confuse it with 100% salt or sugar. Nitrite oxide (HNO_2) could also combine with secondary amines (R_2NH) to produce nitrosamines ($R_2N–NO$), which are carcinogenic. Till date, these have only been found in extremely low levels in sausage products. In a few cases, fried bacon has been found to have slightly higher levels, so to combat this, you can only add 120 or less ppm of nitrite and must also contain 500 ppm of erythorbate (a reducing compound to retard this nitrite/secondary amine combination). Also, new processing techniques are now required to reduce this possibility. In the US, products are sampled periodically by the inspection service to make sure that they do not contain excessive levels of nitrosamines and are, therefore, safe.

Reducing Compounds

Ascorbic acid, erythorbic acid (isoascorbic acid), and their salts (sodium ascorbate and sodium erythorbate (sodium isoascorbate)) are used in cured meats (not allowed in the US in fresh meat products) and have water-soluble antioxidant effects, which result in faster color development and resist color fading during storage. These compounds can be used at 500 ppm (some countries allow higher levels); this quantity is required in bacon. Citric and fumaric acids (acidulates) lower the tissue pH, influence the flavor, reduce bacterial growth, and speed up color development. Glucono delta lactone (GDL) has a neutral pH when added to meat but is slowly converted to gluconic acid, which causes a delayed influence in meat due to a delayed pH decline that speeds up color development similar to the other acid compounds. GDL can be added in the US at the rate of 8 oz per 100 lbs of meat. In several countries, the permissible rate is much higher. Sodium (or potassium) lactate (salt of lactic acid) lowers water activity,

which has an antimicrobial effect, without changing the pH or color of the meat tissue.

Phosphates (Na or K) fall into two categories: those that raise pH, increase water-holding capacity, increase juiciness, reduce cooking shrink, reduce cooking time in mature poultry and white tuna, have antioxidant properties, and reduce refrigerator and thaw drip. The greater the pH increase, the greater the effect. The other category is acid phosphate, which lowers pH, speeds up color development, reduces bacterial growth, increases muscle texture and emulsion stability, and improves sliceability but does not increase water-holding capacity. Phosphates in the US may be added at the rate of 0.05% but are usually used at a 0.03% level. In the EU, the maximum level is 5 g kg^{-1} (0.5%), but the amount used is usually 2.5 g kg^{-1} as phosphor pentoxide.

Binders

Binders that can be added to sausage emulsions include corn, wheat, oats, rye, rice, sunflower meal, vital wheat gluten, corn gluten, potato, barley, rapeseed, rusk, soya, and possibly many more. Many of these have subdivisions depending on how they are processed. In addition to the plant kingdom, many animal additives are also used, such as nonfat dry milk, sodium caseinate, calcium-reduced dried skim milk, dried whey, gelatin, and blood. Binders and additives are added to reduce the cost per unit weight. A few have emulsifying power and several of the dried products can absorb moisture and/or fat, reduce shrinkage, increase juiciness, and alter color and flavor.

Spices

Spices (whole or extract) incorporated into sausage often determine its characteristic (flavor, visual effect, flavor impact, and label requirements), and the addition of spices supplies a tremendous variety of products.

Color

Color additives are usually placed on the surface and can be extracts of plant material or coal tar dyes, titanium dioxide, or caramelized material. Other color additions such as hydrogen peroxide (used to bleach tripe), federal inspection ink, and branding ink are utilized and approved as safe.

Other Additives

Other additives include starter cultures (that cause fermentation) and/or added acid to produce a tangy flavor.

Antioxidants are sometimes used to reduce rancidity and are usually selected for their 'carry through' effect, which will be determined by the environment in which the product is stored.

Meat tenderization can be improved by weak acids or by mechanically breaking the fibers or enzymes (both naturally in the meat or added). A few preservatives are legal in the US primarily to reduce mold growth on the surface of dried products.

Casings to contain sausage fall into three major categories: natural casing obtained from animal intestines or internal organs, collagen made from cotton linters (often removed before sale), or cellulose (extracted from animal tissue). Each casing category has its market place. Stockinets from knitted cotton are often used to maintain the meat's shape during cooking and smoking.

Pickling

Pickling is used more in the vegetable area than in animal products, but a few examples can be found, such as corned beef, muscle tissue from many species, herring, and eggs. Pickling can be divided into two main categories: chemical pickling and fermentation pickling. In chemical pickling, the food is placed in an edible liquid that kills microorganisms and includes brine (high in salt), vinegar alcohol, or oil. Pickling often involves heating or boiling so that the food becomes saturated with the pickling agent. Fermentation pickling involves microorganisms (starter culture or natural culture) that on growth lower the pH to such a point that bacteria will not grow. This type of pickling is used extensity for fermented sausages.

Canning

Canning in the meat area is most successful with cured products, such as canned or chopped ham (e.g., spam) or fish or poultry. With uncured products, the time/temperature requirements for sterilization are so high that the flavor is not very acceptable. A few products have been successful by masking this flavor with spices.

The classic example for bottling is dairy products that are pasteurized and, along with refrigeration, allow the product to have several days of shelf life. Conditions often used are 71.7 °C (161 °F) for 15–20 s or for ultra-high temperature processing, the milk is exposed to a temperature of 135 °C (275 °F) for a minimum of 1 s. Some countries use sterilized milk, but in most cases due to heat-induced flavor, the milk is used for cooking and usually not for drinking. Condensed milk is cow's milk from which some of the moisture has been removed. It is most often found in the form of sweetened condensed milk, in which sugar has also been added.

Irradiation

A very wide range of dry herbs and spices can be processed using irradiation or treated with ethylene oxide gas to reduce microbial contamination. Irradiation is frequently used in spices utilized in the food industry and usually not used in spices sold in the supermarket.

Irradiation effectively controls or eliminates microorganisms in animal feeds and is approved by the US Food and Drug Administration (FDA) to treat feeds for any animal. The irradiation of poultry feed to control *Salmonella* is approved by

the FDA (21 CFR § 579.40). The FDA is amending the food additive regulations to allow safe use of irradiation on unrefrigerated (as well as refrigerated) uncooked meat, meat byproducts, and certain meat food products in order to reduce levels of foodborne pathogens and to extend shelf life. The FDA also increased the maximum dose of ionizing radiation permitted in the treatment of poultry products and clarified packaging requirements.

Pulsed Electric Fields Processing

Use of pulsed electric fields (PEFs) for inactivation of microorganisms is a nonthermal processing method. Microorganisms are exposed to high-voltage PEFs that react with the electromechanical instability of the bacterial cell membrane. Electric field strength and treatment time are the two most important factors involved. This system works with liquid and semiliquid products and encouraging results are reported at the laboratory level, but scaling up to the industrial level escalates the cost. A successful continuous PEF processing system for industrial applications is currently in the design stages. The high initial cost is the major obstacle.

High-Pressure Food Preservation

High-pressure food preservation (high hydrostatic pressure processing; high-pressure pasteurization) refers to placing food inside a vessel and exposing it to 70 000 lb per square inch or more. Food processed by this technique retains its fresh appearance, flavor, texture, and nutrients while disabling harmful microorganisms and delaying spoilage. A few of the foods preserved by this method include fruits, juices, vegetables, sea food, sauces, and ready-to-eat meats. Average high-pressure pasteurization systems employ pressures up to 700 MPa (100 000 psi) to destroy many foodborne pathogens, such as *Listeria*, *Escherichia coli*, and *Salmonella*, with little or no change in the organoleptic properties or nutritional value. Products may be packaged in consumer packaging before processing, thus eliminating recontamination problems. A good fact sheet on this process can be located at Ramaswamy *et al.* (2012).

Biopreservation

Biopreservation is the use of natural or controlled macrobiotics or antimicrobials as a way of preserving food and extending product shelf life. Desirable bacteria or the fermentation products produced by these bacteria are used in biopreservation to control spoilage and to inactivate pathogens in food. This is a benign ecological approach that is gaining increasing attention. Lactic acid bacteria (LAB), which have antagonistic properties that make them useful as biopreservatives, are the organisms mostly used. When LAB compete for nutrients, their metabolites often include active antimicrobials, such as lactic and acetic acids, hydrogen peroxide, and peptide bacteriocins. Some LAB produce the antimicrobial nisin, which is an effective preservative. Fermented (usually dried) sausage is a classic example of this type of preservation. Fish preservation is achieved by adding antimicrobials or by increasing the acidity (lowering the pH) of the fish muscle; most bacteria stop multiplying when the pH is less than 4.5. Traditionally, acidity has been increased by fermentation, marination, or by direct addition of acetic, citric, or lactic acid to food products, but most consumers do not like the flavor of the acid-added products as well as naturally fermented products. Other preservatives include benzoates, essential oils, nitrites, sorbates, and sulfites, to name but a few. This type of processing is fundamentally in the dairy industry and, currently, is being extended to other fermented foods, such as meat, spirits, vegetable products, and juices.

Hurdle Technology

Hurdle technology means combining various bacteria-inhibiting or bacteria-killing factors (some of the 'hurdles,' e.g., are salt, reduced pH, reduced water activity, heat treatment, and appropriative packaging) to achieve a safe product with acceptable shelf life and an acceptable taste and consistency. This means that the level of an individual hurdle can be reduced from the height (intensity or contrition) it would require for the same safety if used alone. An appropriate combination of hurdles can work in synergy and provides a good antibacterial effect, even though the 'height' (level) of each individual hurdle may be smaller than would be appropriate by itself.

See also: Biofilm Formation. Canning. Cooking of Meat: Cooking of Meat. Drying. Electrical Stimulation. Fermentation. Foodborne Zoonoses. Foreign Bodies. Hazard Analysis Critical Control Point and Self-Regulation. Irradiation. Microbiological Safety of Meat: Hurdle Technology. Refrigeration and Freezing Technology: Thawing. Risk Analysis and Quantitative Risk Management. Sausage Casings. Sensory Assessment of Meat. Spoilage, Factors Affecting: Microbiological

Further Reading

ANAB, 2012. Sterigenics International. Available at: http://www.sterigenics.com/food1.htm (accessed 14.11.13).

Huff, E.A., 2012. FDA quietly increases allowed radiation doses of meats for human consumption. Available at: http://www.naturalnews.com/038255_radiation_meats_fda.html#ixzz2kixLNzrK (accessed 29.11.13).

Jeyamkondan, S., Jayas, D.S., Holley, R.A., 1999. Pulsed electric field processing of foods: A review. Journal of Food Protection 62 (9), 1088–1096.

Leistner, I., 2000. Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology 55, 181–186.

Membré, J-M., Bassett, J., Gorris, L.G.M., 2007. Applying the food safety objective and related standards to thermal inactivation of *Salmonella* in poultry meat. Journal of Food Protection 70 (9), 2036–2044.

Ockerman, H.W., 1996. Chemistry of Meat Tissue, eleventh ed. Columbus, OH: The Ohio State University.

Ramaswamy, R., 2004. High pressure processing. Fact Sheet for Food Processors. The Ohio State University. Available at: ohioline.osu.edu/fse-fact/pdf/0001.pdf (accessed 29.11.13).

Relevant Websites

http://www.gpo.gov/fdsys/pkg/FR-2012-11-30/html/2012-28967.htm
Federal Digital Systems.
http://www.naturalnews.com/038255_radiation_meats_fda.html
Natural News.
http://www.naturalnews.com/038255_radiation_meats_fda.html#ixzz2kixLNzrK
Natural News.
http://www.ncbi.nlm.nih.gov/pubmed/10492486
US National Library of Medicine.

PRESLAUGHTER HANDLING

Contents

Behavior of Cattle, Pigs, Sheep, Bison, and Deer during Handling and Transport

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Glossary

Crowding pen A small pen for directing animals into a single-file race. It may also be referred to as a forcing pen, push pen, or funnel pen. In systems where animals are stunned in groups for controlled gas atmosphere stunning, the crowd pen is defined as the last small pen before the animals enter the gondola or other apparatus that conveys them into the gas.

Driving aids These are the tools for moving livestock. Some examples of acceptable driving aids are flags, plastic paddles, solid panels carried by the handlers, plastic bags, or trained leader animals.

Electric prod (goad) A stick that delivers a shock for moving animals. Other driving aids for moving animals are preferable because electric prods are stressful.

Flight zone An animal's safety zone or personal space. Animals that are not completely tame will move away when a handler enters their flight zone.

Lairage These are the pens for holding animals after they have been unloaded from the trucks. They are sometimes called stockyards, holding pens, antemortem pens, or yards.

Point of balance All types of farm animals will move forward when a person is located behind the point of balance at the shoulder and they often back up when a person stands in front of the shoulder.

Race An alley where animals move to the stunner while walking in single file. It may also be called a chute or a lead-up alley. Single-file races are also used in some countries as the loading ramp for loading animals onto trucks.

Staging alley An alley for holding animals that is located between the lairage pens and the crowd pen.

Stunner A device or method for rendering animals insensible to pain and unconscious before slaughter.

Stunning box A small stall or restrainer where an animal is held for application of the stunning method.

Introduction

This article is concerned with behavior of cattle, pigs, sheep, bison, and deer during handling and transport. However, this is only part of the way animals need to be handled and this article needs to be read in association with design of stockyards, lairages, corrals, races, chutes, and loading ramps to facilitate the movement of animals for both optimum welfare and productivity.

Behavior Is Important

People who understand animal behavior will be able to move all species of livestock more easily. A skilled person uses behavioral principles instead of forcing the animals to move. The use of behavioral methods will also help to improve animal welfare. Animals that become stressed shortly before slaughter will have higher lactate levels and be more likely to have tougher meat. A calm animal that has not become agitated and fearful will also move more easily and be safer for people to handle. Handlers should be aware of the behavioral signs of fear and distress in the different species of animals.

Behavioral Indicators of Stress

Behavioral indicators of stress can be used to detect welfare problems during preslaughter handling. One of the most obvious and useful indicators of stress in cattle and pigs is

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vocalization during handling and restraint. In both cattle and pigs, high levels of distress are associated with vocalization (moo, bellow, and squeal) during handling and restraint. Research studies have shown that vocalization during handling, restraint, or surgical procedures is associated with physiological indicators of stress, such as increased levels of cortisol, lactate, or glucose in the blood. A basic principle is that measuring glucose or lactate within 5 min after a stressor is applied will result in higher levels. When lactate and glucose are measured several hours after a stressor is applied, such as truck loading at the farm, they will be lower in stressed animals. Cortisol is a time-dependent measure and requires 15–20 min to peak after a stressor is applied.

In cattle and pigs, vocalization during movement by a person through the race and vocalization in the stunning box or restrainer are associated with aversive events. Some examples of aversive events are use of electric prod, excessive pressure from a head holder or body restraint, slamming gates on animals, or being held too long in a restraint device. There are species differences in vocalization behavior. Sheep are the most defenseless prey species animal and they do not vocalize when painful or distressing stimuli are applied to them. Goats will often vocalize loudly when they are distressed by either handling or restraint. When vocalization is being used as an indicator of poor welfare and distress during handling, it should only be measured when people are actually moving animals or when the animal is being held in a stun box or restraint device. It should not be measured in the lairage where animals are quietly resting. Animals will sometimes vocalize to each other during lairage. This often happens in bulls and it is not associated with the stress of preslaughter handling.

There are other less obvious signs of stress in animals. When the whites of an animal's eye show, it is starting to become fearful. In cattle, eye white will often be shown before the aversiveness of the situation becomes high enough to elicit vocalization. Scientists were able to verify that eye white is associated with fear because antianxiety drugs will block the eye white response. The best scientific documentation for eye white is in cattle. The author has also observed eye white during handling and restraint in horses and bison. Other obvious indicators of distress are struggling during restraint and increased heart rate and respiration. Tail switching in cattle and raised tails in bison are also signs of stress. Heart rate can be easily monitored with electronic monitors designed for athletes. Handlers should be observant for struggling and behavioral agitation associated with a slippery floor. A common problem is an animal that refuses to stand still in the stunning box. This is often due to a slick floor and one or more of the animal's feet doing a series of rapid small slips. Providing a nonslip floor in the stunning box will prevent this and the animal will remain still and be calmer.

Stress during Slaughter

A common question that people ask is, "Do the animals know they will die?" The author has observed that the behavior of animals moving to the stunning area at a slaughter plant is the same as animals moving into a veterinary restrainer on the farm. Data collected during many studies, both on the farm and in slaughter plants, indicate that the level of cortisol is similar after stunning and after restraint on the farm. However, epinephrine (adrenaline/adrenalin) will be elevated after stunning compared with on-farm restraint, and it increases almost instantly after the brain is disrupted by stunning. This does not cause an animal welfare issue, because correct application of either electrical stunning or a captive bolt shot will render the animal instantly unconscious. Cortisol measures taken immediately after slaughter will not be affected because cortisol takes 10–20 min to reach peak values.

Researchers have found that the novelty of the new environment at the slaughter plant may be associated with stress at slaughter. Cattle that had the biggest reaction to a sudden novel stimulus on the farm also had the highest physiological indicators at slaughter in the plant.

Animal Perception

Vision Affects Livestock Movement

Cattle, pigs, bison, deer, and sheep all have wide-angle vision. Panoramic vision enables them to see all around themselves. Farm animals are also dichromatic and they do not see the color red. Dichromatic vision improves both night vision and seeing contrasts of light and dark. All the species discussed in this article are sensitive to visual distractions that can slow down animal movement through handling facilities. In many existing slaughter plants, locating and removing one or more visual distractions greatly facilitated the movement of cattle and pigs. In one pork plant, moving ceiling lights to eliminate a sparkling reflection on a wet floor improved movement through the single-file race. Modification of lighting can often greatly improve animal movement. All animal species will often refuse to enter a dark race or stun box. Indirect illumination of the entrance of the race or stun box encourages the animals to enter. The basic principle is that livestock are attracted toward more brightly lighted areas, but they will not walk toward a blinding light. People should experiment with portable lamps to find the best position for additional illumination. Sometimes turning off a ceiling light will improve movement. Animals are often attracted to a 'light at the end of the tunnel' effect. It is also essential to prevent animals from seeing moving equipment or people up ahead. The installation of solid panels or curtains made from discarded conveyor belting can be used to block these visual distractions. Large pieces of cardboard can be used to experiment with shields for blocking the view of moving people or equipment. At one cattle plant, four different distractions had to be located and fixed before the animals would move easily.

The four modifications were:

1. block sunbeams from a hole in the lairage roof;
2. block the cattle's view of a person checking cattle ID's with cardboard;
3. install a light on the restrainer entrance;
4. install a curtain to prevent seeing people walking by.

People need to be observant. A calm animal will stare directly at a distraction such as a reflection, a hose on the floor,

a coat on a fence, or dangling chains. Frightened agitated animals are too stressed to stare at the distraction. Instead, they will immediately balk and turn back.

Auditory Effects

Yelling at livestock is stressful and raises the animal's heart rate more than the sounds of gates slamming. One study showed that yelling in the ear of a cow was as stressful as an electric prod. Handlers need to be quiet with no yelling, whistling, or banging on the side of the race.

Effect of Odor

Novel odors will often make animals balk and refuse to move. The author has observed that both odors from the slaughter process itself and odors such as fresh paint that are not related to the slaughter process will both increase balking and retard animal's movement. Two studies have shown that cattle or pigs that have been stressed for 10–15 min with aversive stimuli will secrete substances that will be avoided by other animals. During numerous new equipment startups, the author has observed that if an animal got jammed in a malfunctioning piece of equipment for 10–15 min, other animals would refuse to enter the equipment. The next day after the equipment was completely washed, cattle would enter easily. A single poke with an electric prod is usually not sufficient to cause this effect. At one plant, the author observed that after one steer had flipped over on its back in the race and was stuck for 10–15 min, the other cattle refused to walk over the spot in the race where this severely stressed steer had urinated and salivated. After the race was washed, the cattle started moving easily again.

Behavioral Methods for Moving Livestock

People who work with animals at a slaughter plant and during loading of animals onto trucks need to use and understand some basic principles of animal behavior. Two of the most important principles that apply to all species are flight zone and point of balance. These two principles are used in all livestock species. The only time they do not work is in very tame animals that are trained to lead. Tame animals have no flight zone and they can be led into the slaughter plant or onto a truck instead of being driven. The flight zone and point of balance principle should be used in all animals that are not completely tame.

The Flight Zone

The flight zone is like a bubble around the animal. When a person enters the flight zone, the animal will move away and when a person retreats from the flight zone, the animal will stop. A major cause of animals rearing up in stun boxes and races is due to a person being deep in the flight zone. The animal will often settle back down and stop rearing if the handler backs away. The handler should back away instead of attempting to push the animal back down. The size of the flight zone is determined by both the animal's genetics and its previous experience with people on the farm of origin. Animals that live in close contact with people will have smaller flight zones than extensively raised animals that seldom see people. Species such as deer and bison often have larger flight zones than cattle and pigs. Covering the side of the race with a solid panel will also reduce the size of the flight zone. Even a partial solid side where the animal can still see over the top will usually reduce the flight zone compared with a completely open-sided race.

Animals tend to have a larger flight zone when approaching novel new things compared with familiar things. For example, cattle or pigs that are accustomed to people walking through them will have smaller flight zones than animals that are not accustomed to. The reactions of animals are highly specific. Cattle that have become accustomed to a person on a horse may have a small flight zone of 2 m, but when they are suddenly confronted with the novel experience of a man on foot, the flight zone may expand to more than 10 m. On pig farms, it is important for people to actually walk through the fattening pens; walking only in the aisles is not sufficient. On farms where the caretakers have only walked in the aisles, the pigs may become agitated and pile up the first time a person enters their pen. This is especially a problem with certain genetic lines of lean more excitable pigs. To reduce stress during truck loading, for several months before shipment, the producer should walk quietly through the pens and get the pigs accustomed to quietly moving away when a person walks through them.

Point of Balance

One of the most common mistakes that handlers make when moving an animal through a single-file race (chute) is to stand at the animal's head and attempt to make it go forward by poking it in the rear. To induce all species to move forward, the handler should be positioned behind the animal's point of balance at the shoulder. When a person is standing close to an animal in a single-file race, the point of balance will usually be at the shoulder. If the handler is in the lairage and further away from the animal, the point of balance may be just behind the eye. The point of balance will never be in front of the eyes. All handlers should be trained on how to use the point of balance. One of the most effective methods for inducing all species to move forward in a race is to quickly walk back past the shoulder in the opposite direction of desired movement. This seems counter intuitive, but it really works. To make it work, the handler must walk quickly. If the handler walks too slowly, the animals will back up. The forward movement will start when the point of balance is crossed. If the handler stops in front of the point of balance, the animal will back up.

Species Differences in Behavior between Sheep and Other Livestock

Most basic principles of moving animals are the same for all species, but there are some important differences. Sheep are intense followers and they can be moved in large groups. They will keep following and can be moved through a handling

system in a continuous flow. They have an intensive behavioral motivation to follow other sheep. On progressive farms and slaughter plants, specially trained lead sheep are used to lead sheep out of pens, unload trucks, and move sheep through the race to the stunner. There are two basic methods for using lead sheep. On some large farms, the trained lead sheep is led like a dog with a lead rope and a halter (head collar). Another method is a trained leader that knows the routine when it is released by a handler. In one well-run large sheep plant, they had three types of specialist leader sheep.

They were:

1. truck unloaders,
2. leaders to lead sheep out of the lairage pens, and
3. a leader that led sheep up the race to the stunner.

To avoid being slaughtered, the trained leader escaped through a side door. Each leader is trained for its specialist job. The most progressive sheep slaughter plants have stopped using dogs.

Move Small Bunches of Other Species

Cattle, pigs, goats, bison, and deer should all be moved into the crowd pen in small separate bunches. This applies to both handling systems that have a single-file race (chute) and systems where the animals are stunned in groups. One of the most serious handling problems is moving groups that are too large. Good handling is going to require more walking to bring animals up to the staging alley to the crowd pen. Each plant will need to determine the correct number of animals that should be moved through each stage in the handling system. The most common mistake is to overfill the crowd pen that leads to the single-file race. Animals need to have room to turn. If animals balk and refuse to move out of the crowd pen into the race, there is probably a distraction that needs to be removed. One of the most common distractions is an antibackup gate. Try tying it open. If animals constantly back up, there is a distraction in the race that needs to be removed.

Use Following-Behavior

Animals will enter the single-file race (chute) more easily if there is space in the single file before the crowd pen is filled. This will enable the animals that are entering the crowd pen to move into the race without stopping. If the crowd pen is filled when the race is full, the animals may turn around. They turn around because it is natural behavior to return to where they came from. This same principle applies to truck loading. Before the animals are brought to the crowd pen, the truck should be backed up and ready to receive them. The animals can then be moved through the crowd pen and onto the truck without stopping.

Isolated Lone Animal Problems

All species get frightened and agitated when they are isolated from their herdmates. Many accidents have occurred that have injured both people and animals when a single isolated animal charges fences or attempts to jump out of a facility. Handlers should never leave a single animal alone in a race or a stun box during breaks. If a lone animal becomes agitated, some other animals should be put in with it.

Use of Electric Prods (Goads)

This is a controversial area from an animal welfare standpoint. The OIE World Animal Health Organization bans their use on horses, sheep, small calves, and piglets. Only battery operated electric prods should be used. They should be applied no more than three times on the hindquarters. An electric prod should never be a person's primary driving tool. In the most progressive slaughter plants, electric prods are banned in the lairage and in the truck unloading area. A single electric prod is kept at the stunner entrance. It is only picked up to move a stubborn animal and then put away. If animals refuse to enter the stun box, it has either too many distractions or design problems that must be corrected.

Recommended Driving Aids

Some of the recommended driving aids for cattle, sheep, or bison are plastic paddles or flags. A plastic paddle looks like a small boat oar. Flags of various types are also excellent for moving animals. A simple, easy-to-use driving aid is a plastic bag tied to a light stick. These driving aids should be used to quietly guide animals. When animals are calm, they can be turned by moving the flag or paddle next to the eye. The handler should not wildly wave the driving aid. Blocking the animal's vision on one side will make the animal turn. If the animals are agitated and fearful, they will stick together and be hard to turn. Good driving aids for pigs are solid panels and large flags. The following aids for driving animals are not acceptable – iron rods, bats, sticks with nails in them, heavy whips, or electric prods wired into the main current.

Abusive Acts that are Detrimental to Welfare

The following abusive acts are banned according to many international and private standards. They are dragging downed nonambulatory animals, throwing animals, dropping animals off the back of a large truck, beating animals, poking sensitive areas such as the rectum, nose, ears, or eyes, deliberately driving animals over the top of downed animals, and deliberately slamming gates on animals.

The Need and Impact of Understanding Animal Behavior

Animals have been part of human culture for thousands of years and to a large extent pastoralists have understood much of what was required for domestication. With intensive farming systems and a large focus on production and processing in modern abattoirs, the need has arisen for a better understanding behavior for welfare and sensible movements of animals through various facilities. This article and its companion article have pulled together many aspects of behavior

Table 1 The impact of Temple Grandin's work

Design of animal handling facilities	Dr. Grandin is one of the world's leaders in the design of livestock handling facilities. She has designed livestock facilities throughout the USA and in Canada, Europe, Mexico, Australia, New Zealand, and other countries. In North America, almost half of all cattle-processing facilities include a center track restrainer system that she designed for meat plants. Her curved chute systems are used worldwide and her writings on the flight zone and other principles of grazing animal behavior have helped many producers to reduce stress during handling. Temple has also designed an objective scoring system for assessing handling of cattle and pigs at meat plants. This system is being used by many large corporations to improve animal care.
Industry consulting	Dr. Grandin has consulted with many different industry organizations each year for the past 10 years. These efforts represent the majority of her time as she has a part-time appointment at Colorado State University but a thriving business as a consultant. The majority of her work is involved with large feedlots and commercial meat packers. She has worked with Cargill, Tyson, JBS Swift, Smithfield, Seaboard, Cactus Feeders, and many other large companies. Her company also does design work for many ranches. She was also involved with several major packing companies. Her consulting has led to work with companies such as Wendy's International, Burger King, Whole Foods, Chipotle, and McDonald's Corporation, where she has trained auditors regarding animal care at processing plants. She also has consulted with organic and natural livestock producers on animal care standards The animal handling guidelines that she wrote for the American Meat Institute are being used by many large meat-buying customers to objectively audit animal handling and stunning.
Research	Dr. Grandin maintains a limited number of graduate students and conducts research that assists in developing systems for animal handling and, in particular, with the reduction of stress and losses at the packing plant. She has published her research in the areas of cattle temperament, environmental enrichment of pigs, livestock behavior during handling, reducing dark cutters and bruises, bull fertility, housing dairy cattle, and effective stunning methods for cattle and hogs.
Media exposure	Dr. Grandin has provided worldwide media exposure for the livestock industry and, in particular, with issues relating to animal care. She has appeared on television shows such as 20/20, 48 h, CNN Larry King Live, 60 min, and has been featured in People Magazine, the New York Times, Forbes, U.S. News and World Report, and Time magazine. Interviews with Dr. Grandin have been broadcast on National Public Radio (NPR) and she has been taped for similar shows in Europe. She was named one of Time Magazine's 100 Most Influential people. HBO has made a movie about her life starring Claire Danes.
Outreach	Dr. Grandin maintains an appointment with Cooperative Extension at Colorado State, where she has been active in making presentations to Colorado ranchers and farmers as well as those interested in the packing industry. She is sought after to discuss issues of quality assurance. Privately, she has developed her own website (www.grandin.com), which has been expanded to include information on livestock handling in addition to information relative to the design of handling systems. A section on bison handling and one in Spanish have been popular. More than 2000 people visit the website every month and approximately 1000 download significant amounts of information. As many as 1431 files were downloaded daily and more than 42 000 have been downloaded in a single month. The website has been accessed by people from more than 50 countries worldwide. She also did a TED talk in 2010 entitled, 'The World Needs All Kinds of Minds.'
International activities	It is clear from the wide variety of information accessed via the website, presentations made in international settings, and interest in livestock handling systems developed by Dr. Grandin that her work has reached an international audience. She typically travels to make presentations internationally three to five times annually.

of animals and design of facilities for optimum handling of animals. The author, Dr. Temp"le Grandin, has had a major impact on the meat and livestock industries worldwide and the editors have compiled six specific examples that document this influence from student involvement to international presentations – these provide a template on Table 1 for others to follow and improve.

Conclusions

Improvements in animal handling of all species of livestock will have benefits for the welfare of the animals, quality of the meat, prevention of bruises, and safety for plant employees.

See also: Growth of Meat Animals: Metabolic Modifiers; Physiology. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists. Nutrition of Meat Animals: Pigs; Ruminants. Preslaughter Handling: Design of Stockyards, Lairages, Corrals, Races, Chutes, and Loading Ramps; Preslaughter Handling; Welfare of Animals. Species of Meat Animals: Cattle; Sheep and Goats. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning

Further Reading

Grandin, T., 1997. Assessment of stress during handling and transport. Journal of Animal Science 212, 36–39.
Grandin, T., 2007. Livestock Handling and Transport. Wallingford, Oxfordshire, UK: CABI Publishing.
Grandin, T., 2010a. Improving Animal Welfare: A Practical Approach. Wallingford, Oxfordshire, UK: CABI Publishing.
Grandin, T., 2010b. Auditing animal welfare at slaughter plants. Meat Science 86, 56–65.
Grandin, T., 2012. Auditing animal welfare and making practical improvements in beef, pork, and sheep slaughter plants. Animal Welfare 21 (Suppl. 2), 29–34.

Grandin, T., Deesing, M., 2008. Humane Livestock Handling. North Adams, MA, USA: Storey Publishing.
Gregory, N.G., 1998. Animal Welfare and Meat Science. Wallingford: CABI Publishing.

Relevant Websites

www.grandin.com
Temple Grandin.

www.animalhandling.org
The American Meat Institute.

Design of Stockyards, Lairages, Corrals, Races, Chutes, and Loading Ramps

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Introduction

The behavioral principles of animal handling have been discussed in the article 'Behavior of Cattle, Pigs, Sheep, Bison, and Deer during Handling and Transport'. In addition, the cross-references in 'see also' and the websites cover the design and layout of chutes and races and these references are an important supplement to this article. Visual distractions discussed in this article can ruin the efficiency of a well-designed facility because animals constantly balk, back up, or turn around. Low stress humane handling will be impossible until the distractions are removed. The distractions that inhibit animal movement are the same for all species. Some of the most common distractions that must be removed are:

1. Air blowing into the faces of approaching animals – This must be stopped. Airflow at the entrance of a stun box or restrainer must either be still or move with the flow of the animals.
2. The 'dark movie theater' Effect – When animals are moving from bright sunlight into a dark building, they will often balk because they cannot see where they are going. This can be fixed by building a shade over the entrance to reduce the brightness of the sunlight.
3. Seeing Moving People or Equipment – Look through the chutes and races and if moving people or equipment is visible, solid shields should be installed. Experiment with cardboard.
4. Indoor Dark Race or Chute Entrance – In indoor facilities where the animal's eyes have adjusted to lower light levels, lamps can be installed to attract animals into a dark stun box or race. Experiment with portable lights.
5. Reflections on shiny metal or wet surfaces – Reflections can often be eliminated by either moving existing ceiling lamps or turning off selected lamps. Experiment with lighting while a person looks through the facility at an animal's eye level. Some shiny surfaces will need to have a dull nonreflective finish applied.

Further information and photos can be found in the websites.

A Case Study of A Beef Plant

It is often required to find and fix two or more distractions before animals move easily through a plant. Below is the list of distractions that had to be eliminated at one plant that processed cattle.

1. Installed 80% light-blocking shade cloth over the single file chute that led up to the stun box. This blocked bright sunlight and eliminated the 'dark movie theater' problem. The shade cloth was stretched very tightly to prevent flapping. Flapping materials will cause balking.
2. Installed an easy-to-use latch so the one-way backstop gate between the crowd pen and the single file race could be held open. This facilitated cattle entry into the single file because it prevented approaching cattle from seeing the moving gate.
3. Installed a solid shield to prevent cattle entering the stun box from seeing moving people and equipment up ahead.
4. Installed nonslip flooring in the entire facility.

Stun Box and Restrainer Design for Cattle, Pigs, and Sheep

Drawings of restrainers, head-holders, and other equipment for holding animals for stunning or religious slaughter can be found in websites, and other articles.

1. Nonslip flooring is essential – Animals become agitated when they slip. Agitation in stun boxes is often caused by a series of small rapid slips. Pictures of good nonslip flooring are on the websites and references. Steel rods welded to the floor of the stun box make a good nonslip floor. Embossed steel or broom finish concrete is too slick. When steel rods are used, do not crisscross them. This will damage hooves. The rods must flush against the floor.
2. The stun box must not be too wide – Average sized cattle will fit in a 76 cm (30 in.) wide box (Figure 1). Larger bulls and some European Continental cows will require more width. A good design is to have an adjustable side.

Figure 1 Well-designed stun box that is the correct width to prevent animals from turning around. A common mistake is to make stun boxes too wide. Stun boxes must have nonslip flooring. Animals remain quieter on nonslip flooring.

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3. Head-holders and other mechanical restraint devices must not cause stress – If cattle or pigs vocalize (bellow or squeal), or struggle when a restraint device is applied, it has a design problem such as excessive pressure or a sharp edge. Animals may also vocalize or struggle if they are held tightly for more than 15–30 s in any mechanized restraint device. This is why stunning or religious slaughter should occur immediately after animals are fully restrained. There is a problem with the design or operation of the device if animals struggle or vocalize in direct response to application of the restraint of which there are several designs (see 'Relevant Websites'). Hydraulic or pneumatic systems that control rump pusher gates, head-holders, and other devices for restraining animals should move with steady, smooth motion. Sudden jerky motion frightens animals.
4. Use features of design which will encourage animal entry into a restrainer – Prevent the incoming animal from seeing the visual cliff effect under a conveyor restrainer. The installation of a false floor will facilitate entry because it prevents the animal from seeing that the conveyor restrainer is high up above the plant floor and drawings are on websites. Incoming animals must not be able to see out onto the slaughter floor. A shield should be installed to prevent incoming animals from seeing activity on the slaughter floor.
5. Engineer equipment to reduce noise – On pneumatic equipment, either pipe air exhausts outside or install silencers. Silencers (mufflers) need to be replaced periodically because over time they lose their ability to reduce noise. Use rubber stops to reduce metal to metal clanging and banging. Noise from moving parts of equipment can be reduced by using plastic for door tracks. Plant buildings constructed from foam core cooler boards are often quieter than prestressed concrete. Hydraulic pumps should be located away from the animals. Use larger diameter plumbing to reduce high pitched noise that occurs when hydraulic fluid moves rapidly through smaller diameter pipes.

Layout Principles for Races, Chutes, and Crowd Pens

Chutes or races leading up to the stun box or restrainer must be laid out correctly. Layout mistakes can wreck the efficiency of the system. Below is a list of layout principles.

1. Never, never dead-end the single file chute or race – The design of the junction between a single file race and the crowd pen is very critical. When an animal is standing at the entrance of either a single file or double file race, it must be able to see three body lengths up the race. If the single file or double file race is bent too sharply where it joins the crowd pen, animals will back up or turn back because it looks like a dead-end. Diagrams of correct and wrong layout of this critical junction can be found in references.
2. Correct crowd pen/forcing pen angle at the single file race/chute entrance – For all species, a transition that is angled too gradually will cause jamming. For cattle and sheep, the best angle for the transition between the single file race and the crowd pen is one side straight and the other side on a 30° angle. For pigs, the transition should be abrupt because pigs jam in a funnel-shaped transition. Diagrams can be found in references.
3. Proper layout of curved races – Curved single file or double file races work well for cattle and sheep because they take advantage of the animal's natural tendency to go back to where they came from. They also prevent the animals from seeing activity up ahead (Figure 2). To be effective, they must be laid out correctly. Diagrams can be found in references.
4. Never build a crowd pen or forcing pen on a ramp – Cattle, pigs, sheep, and other animals will stand quietly if they are held in single file on a ramp. Groups of animals that are held standing on a wide, 3° or greater sloped alley will tend to pile up on the back gate. Crowd pens and the wide staging alleys before the single file part of the system should be level. If there is a ramp in the system, it should be in the single file race part of the system. For pigs, complete elimination of ramps is recommended. Pigs handle best in level systems. A slight drainage slope will be required for washing in all facilities.
5. Design of ramps to the stunner – Stair steps are recommended (designs are covered in references). The advantage of stair steps is that when they wear out, the animals can still walk on them without slipping. Ramps with grooved concrete without stair steps wear out and get slick. Dimensions for stair steps for cattle are 3.5 in. (10 cm) rise and 45 cm long or longer tread length. A 30 cm (12 in.) tread length will work if space is restricted. For pigs and sheep, use a 2.5 cm rise and 8 in. (25 cm) or longer tread width. Maximum ramp angles to the stunner are: cattle 20°, pigs 15°, and sheep 25°. More gradual ramps are strongly recommended.
6. Crowd pen and forcing pen layout – If a circular round crowd pen (tub) is used, it should be laid out in a 180° full half circle to take advantage of the natural behavior of animals to go back to where they came from. The recommended radius for the crowd gate is 12 ft (3.5 m) for cattle, and 8 ft (2.5 m) for pigs and sheep. Longer and shorter lengths can be less efficient.
7. Use solid fences – There are many activities and distractions in a meat plant, so the use of solid fences on the

Figure 2 Well-designed, curved cattle race/chute with high solid sides. A curved race prevents the cattle from seeing activity up ahead.

single file races and crowd pen is strongly recommended. The most important fences to make solid is the outer perimeter to prevent animals from seeing people, moving vehicles, or equipment. The crowd gate in the forcing pen or crowd pen should be solid. This will help prevent animals from turning back towards the yards.

8. Correct single-file race length – The chute or race for holding animals in single file should be long enough so that natural following behavior can be used. Cattle and pigs will enter a single file race more easily if they keep moving through the crowd pen without stopping. When a group of animals can enter a partially empty, single file race, natural following behavior will facilitate movement. If animals are allowed to stand in a crowd pen, they tend to turn around. The recommended capacities for single file races for cattle and pigs are shown below. For sheep, shorter races can be used because sheep can be handled in continuous flow. Cattle and pigs are moved in small, separate bunches.

   Under 25 per hour – should hold 3 or 4 animals
   26–100 per hour – should hold 6–10 animals
   Over 100 per hour – should hold 10–15 animals

   For large plants over 200 per hour, the single file race should hold 15 cattle.

   For higher speeds, do not make it longer. This will require handler to walk too far. These capacities are recommended in systems where the stunned animals are indexed onto a power chain at fixed intervals. In pork systems, with loose shackles, batches of pigs can be handled with a waiting period between batches. In these systems, a shorter race might work because the processing floor is kept supplied by the stunned pigs that are batched on the rail. These capacities will make it easier to use following behavior because the races can be allowed to become partially empty before more animals are brought up. To determine the correct length, one market weight pig that weighs 275 lbs. will fit in a 4 ft (1.2 m) section of single file race. One adult bovine will fit in an 8 ft (2.5 m) section of single file race. One lamb will fit in a 3 ft (1 m) section.
9. Batch floor stunning systems – In these systems, the single file race is eliminated. This system is used mainly for pigs or lambs in smaller plants. In these systems, a group of 5–10 animals is held in a small pen. Electric stunning tongs are applied to the head. To prevent return to sensibility, a second stunner application should be applied to the chest to stop the heart. To allow enough time to do an initial head stun and then a second cardiac arrest stun, 10 s per animal will be required. Group electric stunning should be used in systems processing less than 120 pigs or lambs per hour. Larger plants should install a V-conveyor or center track conveyor restrainer system.
10. Do not exceed these maximum capacities – On a single line, the following maximum capacities should not be exceeded:
    - Pigs and sheep – 800 per hour
    - Cattle – 390 per hour

Exceeding a speed of 800 pigs per hour on a single V-conveyor restrainer will cause serious handling problems. Faster speeds more than 800 per hour exceed the normal slow walk of the pig. In plants that use CO_2, when the line speed going into the cooler is 1000 per hour, two large group CO_2 machines with separate handling systems will be required. For plants using electrical stunning, two V-conveyor restrainers and two complete handling systems will be required.

Design of Stockyards and Lairages

Diagrams are available in references (Figure 3). A well-designed beef stockyard with a herringbone layout can be seen on YouTube Beef Plant video Tour with Temple Grandin. Different countries have different requirements for the capacity of stockyards. In the US, the minimum stockyard or lairage capacity is 4 h of production, which is equal to half a production shift. In countries where longer times in the lairage are required, stockyards with greater capacity will be needed. More space will also be required if deliveries of animals all occur during the same time of the day. For all species, there should be sufficient space in the stockyard so that all the animals can lie down. This is especially important for animals that are held overnight or on weekends. Below are recommended space requirements.

- Adult cows and fed cattle 20 ft^2 (1.85 m^2). Cattle that weigh over 1200 lbs (545 kg) will require more space.
- Market weight pigs 6 ft^2 (0.55 m^2). Very large pigs over 275 lbs will require more space.
- Sheep – 5 ft^2 (0.46 m^2).

The US Humane Slaughter Act requires that every holding pen has a waterer. Hanging nipple waters will work well for pigs. Cattle and sheep should have water troughs. It is best to lay out the pens so that each pen holds either one truckload or two truckloads of animals. This will prevent the problem of jamming too many animals in a pen that holds a fractional proportion of a truckload. In plants where many small groups are processed, some smaller pens will be required. Boars or bulls that are mounting other animals or fighting will need to be put in a separate small pen.

Flooring Surface in the Stockyards

For cattle and other large animals, grooved concrete should be used. The recommended grooving pattern is an 8 in. (20 cm)

Figure 3 Herringbone stockyard/lairage with long narrow pens. Cattle enter through one end and exit through the other end. The correct angle for the pens is 60–80°.

diamond or square pattern. V-shaped grooves that are 1 in. (2.5 cm) to 1.5 in. (3.5 cm) deep are recommended. Instructions and pictures for making a grooving tool are in the references. For sheep and pigs, a good concrete finish is to print the pattern of expanded metal mesh into the wet concrete. For the small animals, epoxy grit finishes can also be used. Epoxy grit is not recommended for cattle because it will not provide sufficient traction. Do not use a broom finish. It will wear out quickly and become slick. In the United States, mats made from woven tire treads are available. They provide excellent nonslip flooring on unloading ramps. Some plants have installed a custom-made woven tire mat on the stun box floor. Unfortunately, these mats are more difficult to clean than grooved concrete.

Layout of Stockyards

The best stockyards have one-way traffic through the yards. Animals enter through one alley and leave to go to the stunner through an alley that is at the other end of the pen. Pens can be laid out either straight or on an angle (herringbone). Angled pens work well but they must be laid out correctly. The correct angle for all species is pens on a 60–80° angle (Figure 3). Never use a 45° angle. Animals might get stuck in the corners. To further eliminate corners, gates can be built that are longer than the width of the alley so that they open on an angle. The recommended alley and gate lengths are:

- Cattle – 10 ft (3 m) alley with 12 ft (3.5 m) gates
- Pigs and sheep – 8 ft (2.5 m) alley with 10 ft (3 m) gates

Some plants use narrower alleys for pigs. When narrow alleys are used, pigs should be moved in much smaller groups of 10 or less.

The Use of Powered Gates

Gates that are operated by either pneumatics or hydraulics are used in some facilities. In group CO_2 stunning systems, powered gates are used to move pigs up to the chamber and into the chamber. There is a tendency to use too much automation, which is expensive and completely unnecessary. In the group CO_2 pig stunning system, only five powered gates are required. They are:

1. A powered push gate to bring pigs up the main alley
2. Entrance gates to the holding pen by the stunner entrance
3. Indexing gate
4. Push gate to move the pigs into the chamber
5. A vertical sliding gate on the chamber entrance. Additional powered gates are a needless expense.

A video of a well-designed CO_2 with powered gates is on YouTube (use keywords Temple Grandin, pigs). If the pigs are difficult to handle, automated push gates might need to be converted from fully automatic to manual. A hand switch that is controlled by a person is used to start and stop gate movement. Even if fully automated gates have controls to prevent excessive pressure from being applied to animals, they still tend to overcrowd the animals. Powered gates must not knock animals over or push a fallen animal along the floor. This is abusive and must be corrected. This applies to all species.

There are often many problems with powered vertical gates (guillotine gates) on the entrance of beef stun boxes. When poorly designed and operated, they can cause many bruises on cattle. Shoving an animal down to the floor with a vertical slide gate is not acceptable. Controls should be designed so that the operator can immediately stop downward movement of the gate. The bottom of the gate should be padded. For all species, bruises can also be reduced by making the bottom portion of a vertical slide gate a flexible curtain. Animals will perceive the curtain as a solid barrier and not go through it. For pigs, make the bottom 18 in. (45 cm) from lightweight conveyor belting. For cattle, the bottom 24 in. (60 cm) can be a conveyor belt curtain.

Design of Unloading Ramps

For new construction, it is strongly recommended to eliminate all ramps in the system. In many existing facilities, ramps will be required to unload trucks and for moving animals to the stunner. Diagrams and designs of unloading ramps are available. For all species, concrete ramps for unloading should not exceed 20°. Stairstep ramps described previously should be used. Ramps with less than 20° slope are better. An unloading ramp should have a level dock at the top so that animals step out onto a level surface when they leave the truck. The minimum length of the level dock is 10 ft (3 m), and 20 ft (6 m) is recommended to prevent exiting cattle from jumping on the ramp. Man gates should be installed so that people can easily get away from charging animals.

A well-designed unloading area for all species has unloading pens that hold an entire truckload before they go to the main lairage stockyard pens. The unloading pens must not be on a ramp. Holding groups of cattle on a ramp may cause animals to pile up on the back gates.

Design of Drains and Wash Down

Animals tend to balk and refuse to step over any changes in the floor surface or texture. A drain located in the middle of a drive alley might cause animals to stop moving. Drains should be located outside the areas where animals walk. Many well-designed stockyards are laid out with a concrete ditch drain that is located outside the alley fence. To facilitate washing of the pens, there should be a 12 in. (30 cm) high concrete curb along the bottom of each pen fence. This curb contains the wash water within the pen that is being washed. Pens are much more difficult to clean, if wash water from one pen runs over into the next pens.

See also: Growth of Meat Animals: Metabolic Modifiers; Physiology. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists. Nutrition of Meat Animals: Pigs; Ruminants. Preslaughter Handling: Behavior of Cattle, Pigs, Sheep, Bison, and Deer during Handling and Transport; Preslaughter Handling; Welfare Including Housing Conditions;

Welfare of Animals. **Religious Slaughter. Slaughter, Ethics, and the Law. Slaughter-Line Operation:** Cattle; Pigs; Sheep and Goats. **Species of Meat Animals:** Cattle; Sheep and Goats. **Stunning:** CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning; Slaughter: Immobilization

Further Reading

Bourquet, C., Deiss, V., Tannugi, C.C., Terlouw, E.M., 2012. Behavioral and physiological reactions of cattle in a commercial abattoir relationship between organizational aspects of the abattoir and animal characteristics. Meat Science 68, 158–168.

Grandin, T., 1982. Pig behavior studies applied to slaughter plant design. Applied Animal Ethology 9, 141–151.

Grandin, T., 1990. Design of loading facilities and holding pens. Applied Animal Behavior Science 28, 187–201.

Grandin, T., 1992. Observations of cattle restraint devices for stunning and slaughter. Animal Welfare 1, 85–91. (Contains design information on restrainers for religious slaughter).

Grandin, T., 1993, 2000, 2007, and 2013. Livestock Handling and Transport Wallingford, Oxfordshire: CABI Publishing. (Different editions of this book contain additional diagrams and photos).

Grandin, T., 1998. The feasibility of using vocalization as indicator of poor welfare during slaughter. Applied Animal Behavior Science 56, 121–138.

Grandin, T., 2001. Cattle vocalizations are associated with handling and equipment programs in beef slaughter plans. Applied Animal Behavior Science 71, 191–201.

Grandin, T., 2003. Transferring results of behavioral research to industry to improve animal welfare on the farm, ranch, and slaughter plants. Applied Animal Welfare Science 81, 215–228. (Contains center track restrainer diagrams and design information).

Grandin, T., 2008. Engineering and design of holding pens, loading ramps and handling facilities for land and sea transport of livestock. Veterinaria Italia 44, 233–245. (Contains unloading ramp designs).

Grandin, T., 2010. Improving Animal Welfare: A Practical Approach. Wallingford, Oxfordshire: CABI Publishing.

Grandin, T., Deesing, M., 2008. Humane Livestock Handling. North Adams, MA: Storey Publishing. (Contains many diagrams of chutes, races, crowd pens, unloading ramps, and stockyards).

Grandin, T., Regenstein, J.M., 1994. Slaughter: A Discussion for Meat Scientists Meat Forum International, pp. 115–123. (Contains diagrams of restrainers for religious slaughter).

Vogel, K.D., *et al*., 2011. Head only followed by cardiac arrest electrical stunning is an effective alternative to head only electrical stunning in pigs. Meat Science 89, 1412–1418.

Relevant Websites

www.grandin.com
: Design Information is in the Behavior, Design, Stunning, and Ritual Slaughter Sections.

www.animalhandling.org
: Recommended Animal Handling Guidelines and Audit Guide, American Meat Institute.

Preslaughter Handling

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Glossary

Animal welfare The state of an individual as regards its attempts to cope with its environment.
Journey The entire transport operation from the place of departure to the place of destination, including any unloading, accommodation, and loading occurring at intermediate points in the journey.
Lairaging Keeping animals in stalls, pens, covered areas, or fields associated with or part of processing plants.
Stress Biological response elicited when an individual perceives a threat to its homeostasis.
Transport Movement of animals affected by one or more means of transport and related operations, including loading, unloading, transfer, and rest, until the unloading of the animals at the place of destination is completed.

Introduction

Preslaughter handling refers to the interaction between humans and animals during the phases of preparation for transport, loading, transportation, unloading, lairage, and moving to the place of stunning and slaughter. Stunning and sticking are discussed in other articles. Potential stressors include fasting and water deprivation, mixing of unacquainted individuals, exposure to a different environment, noise, forced physical exercise, and extremes of temperature and humidity. No animal shall be transported unless it is fit for the transportation. Poor conditions during transport might cause high levels of fear and pain, inducing psychological and physical stress or even death, particularly in pigs. These problems can be exacerbated during longer journeys. Lairage at abattoirs permits animals to recover from the stress and physical activity resulting from deprivation of food and water, handling on the farm, loading, transport, and unloading, which can be beneficial to meat quality and welfare. However, the benefit of providing animals with a resting time can be lost if the animals are subjected to poor handling and stressful environmental conditions in lairage. To safeguard animal welfare during transport and at the abattoir, preslaughter handling should guarantee the four basic requirements for animal welfare defined by Welfare Quality®:

1. Good feeding (absence of prolonged hunger and thirst).
2. Good housing (comfort around resting, thermal comfort, and ease of movement).
3. Good health (absence of injury, disease, and pain induced by management procedures).
4. Appropriate behavior (expression of social and other behaviors, good human–animal relationships, and absence of general fear).

Methods of Measuring Preslaughter Stress

The most common definition of animal welfare states that the welfare of an individual is its state as its attempts to cope with the environment. The environmental stimuli that lead to an imbalance of homeostasis are defined as 'stressors' and the corresponding physiological and behavioral defense reaction as 'stress response.' The stress response produces an activation of the adrenal gland, which results in an increase in glucocorticoid and catecholamine secretion. Depending on the nature, duration, and intensity of the stressor, the stress response can vary. Parameters such as heart rate, respiration rate, or cortisol concentration in blood or saliva can be used to assess the magnitude of the stress as they are valid indicators of the levels of activation of different pathways associated with stress responses. Other factors used are the concentrations of some acute phase proteins, such as haptoglobin, pigMAP, or serum amyloid A. In addition, biochemical parameters, such as total proteins in blood, albumin, urea, lactate dehydrogenase, vasopressin, creatine kinase, lactate, free fatty acids, β-hydroxybutyrate, osmolality, packed-cell volume, or glucose also give information about the stress response. Species-specific behavioral responses must be also taken into account, such as vocalizations induced by poor management, fleeing reactions, reluctance to move due to poor design and maintenance of handling facilities, or muscle tremors due to fear. Other factors to be considered are based on the general state of the animals, such as exhaustion, sweating, panting, shivering, lameness, or unable to walk. The presence of deep injuries, bruising, scratches, lacerations, or broken bones are also indicators of poor welfare, as well as high or low body or skin temperature. After slaughter, carcass and meat quality can also be used to assess preslaughter stress. Elevated body temperatures and fast glycolysis (low pH in muscles) just before or after slaughter produce pale, soft, and exudative (PSE) meat in pigs. However, the incidence of dark, firm, and dry (DFD) meat with a high ultimate pH is linked to the depletion of glycogen stores of the muscle before slaughter and can be due to severe and long exercise periods, long fasting periods, or any combination of stressors that lead to a high demand for energy for prolonged time.

Preparation for Transport

Inspection and selection of fit and healthy animals are the most important factors to maintain an adequate level of

Figure 1 Pigs loading on farm. Reproduced from IRTA.

welfare during transport. Animals should be checked at the place of departure and before the loading for fitness for transport. Sick, injured, weak, disabled, or fatigued animals; females in advanced state of pregnancy; and newborn animals with unhealed umbilical cords are unfit animals to be transported as the stress response will be impaired.

Water should always be available at the farm until loading on transport vehicles and in lairage until animals are stunned and bled. However, animals might be fasted to prevent the release and spread of bacterial contamination through feces within the group during transport and lairage as well as through the spillage of gut contents during carcass evisceration. Fasting before transport, within reasonable limits, is also beneficial to the welfare of pigs as it prevents them from chocking due to vomiting in transit or developing hyperthermia. Some studies recommend fasting pigs from 4 to 12 h before transport and a maximal feed withdrawal of 16–24 h before stunning and slaughter. After this time, animals mobilize fat and proteins as the main source of energy, increasing the tendency to form DFD meat and reducing carcass weight.

Pig groups are usually mixed on farm before loading in order to obtain groups of uniform weight and to adjust the group size to that of the truck compartments. Beef cattle are sometimes collected in the same lorry from several farms and mixed together. Mixing unacquainted animals leads to aggression to establish a new social rank. However, it impairs animal welfare. Firstly, aggression may result in injuries, pain and, in extreme cases, the death of animals. Secondly, aggression leads to physiological stress and immunosuppression within the whole group. To avoid fighting, animals should be kept in stable social groups. If mixing is unavoidable, the recommendation is to mix animals at loading rather than later in the abattoir and to maintain in lairage the same groups from the transport. To reduce aggression at arrival in the abattoir, cattle should be grouped again by farm of origin.

Loading and Unloading

Loading and unloading are the most stressful phases of transport (Figure 1). As animals meet new situations, they may be mixed with unknown animals, and finally there is the physical exertion involved with the physical exercise. Animals jumping, running, slipping, or falling might increase the risk of injuries, but also an animal balking, turning back, or going backward are indicators of fear and these behavioral indicators must be observed during loading and unloading, and appropriate actions should be taken immediately to rectify defects.

Cattle, sheep, and pigs go up better than they go down, but they have considerable difficulty negotiating steep ramps. Between 0° and 20°, slope has little effect on the time taken for pigs and cattle to ascend, and the time taken to ascend increases linearly when the slope is above 20°. Furthermore, pigs' heart rates increase as the angle of a loading ramp increases. In pigs, cleats (2.5×2.5 cm) on ramps must be spaced 20 cm on the centers to fit the normal walking stride of animals, and missing cleats can cause leg injuries in the animals due to slipping and falling. In cattle, inter-cleat distances should be at 20–30 cm. Steps between the loading ramp and floor should not be higher than 15 cm. Unloading ramps should have a level dock before the ramps go down so that animals have space to walk on when they exit the truck. Another possibility is the use of a deck lift, in which the whole deck moves upward or downward.

The stockperson and others associated with animal handling should have adequate knowledge and understanding of

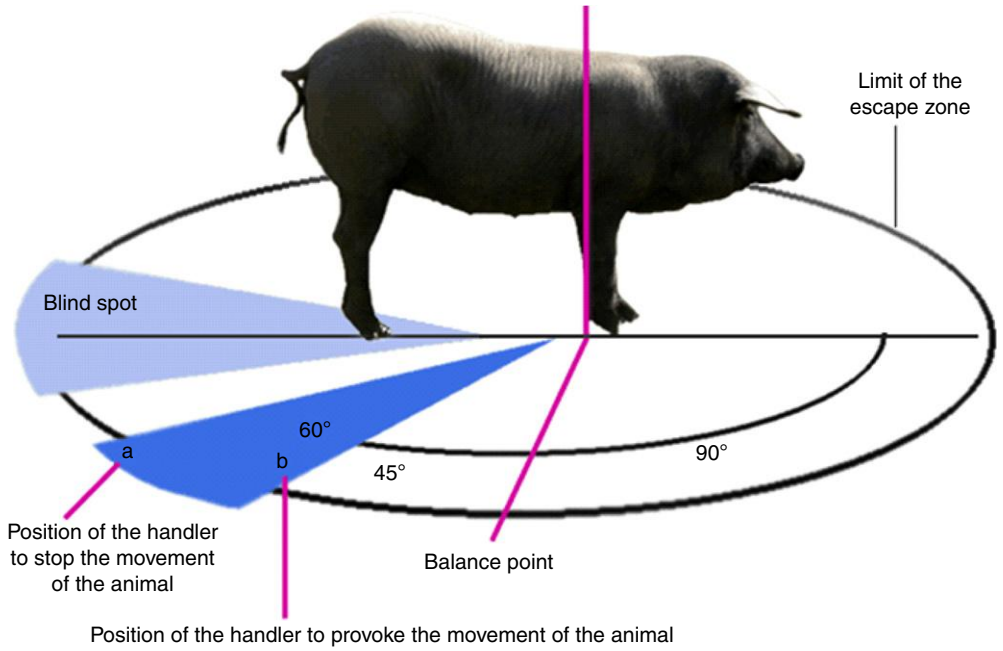

Figure 2 Balance point of the pig. If the intention is to move the pig in a forward direction, the animal handler should be situated at point b. Reproduced from IRTA.

the species-specific behavioral patterns. Red meat animals have wide-angle vision but have only limited forward binocular vision and poor depth perception (Figure 3). This means that they can detect objects and movements in front and beside them but can only judge distances directly ahead. Therefore, animal movements can be affected by shadows, discontinuities on the floor, and lighting. Animals have a tendency to move from a darker area toward a brighter area, but they will not approach blinding light. However, these animals can hear over a greater range of frequencies than humans and are more sensitive to higher frequencies.

Proximity to humans remains to be one of the most potentially alarming experiences for many farm animals that are reared under industrial farming conditions. When a person approaches closer than a certain distance, domestic animals try to escape. This critical distance, which defines the flight zone (Figure 2), varies among species and individuals of the same species and depends upon previous experience with humans. Animals reared in proximity to humans may have a short flight zone, whereas those kept in free range or extensive systems may have more a distant flight zone. The point of balance at the animals' shoulders could be used to move animals by adopting a position behind the point of balance to move an animal forward and in front of the point of balance to move it backward. The sudden penetration of the flight zone may cause a panic reaction. During a threatening situation, animals exhibit adaptive behaviors such as escape, balk, back off, shake, or other fear-induced behaviors including lying down. Social animals such as pigs collaborate with conspecifics in defense against predators and vocalize a lot when caught or hurt. Species that are unable to defend themselves, such as sheep, vocalize far less or none at all (Figure 3).

Transportation

During transport, effective temperature (which is the end result of the interaction between air temperature, relative humidity, ventilation, and flooring), space allowance, vehicle design, driving quality, journey duration, and road conditions are important factors influencing the welfare of animals and resultant meat quality.

The effective temperature inside the transport vehicle generally increases when vehicles are stationary for prolonged periods with lack of forced vents because of the lack of sweat glands in animals. In this case, lying behavior is the most important tool within behavioral thermoregulation. When it is too warm, pigs lie down quickly, maintain relatively wide separation between individuals, and increase their respiration rate. Therefore, high temperature increases the space each animal needs to rest.

In addition, the physical activity and stress response may contribute to the increases in body temperature. The physical activity will depend on the management of the animals during loading, difficulties in maintaining balance, the presence of unknown animals in the compartment, and the general fearfulness and excitability of the animals. Vehicle motion and vibration produces motion sickness in pigs, which contributes to increase in body temperature and the risk of vomiting, which can cause death due to asphyxiation (acute) or aspiration pneumonia (chronic). Practical strategies to reduce the risk of heat stress in pigs include transporting early in the morning or at night, when temperature and humidity are low; substitute deep bedding materials or straw by wet sand or small amounts of wet shavings; use of additional ventilation and showers; reducing stops; and unloading the animals as soon as possible at arrival. Cattle and small ruminants are less

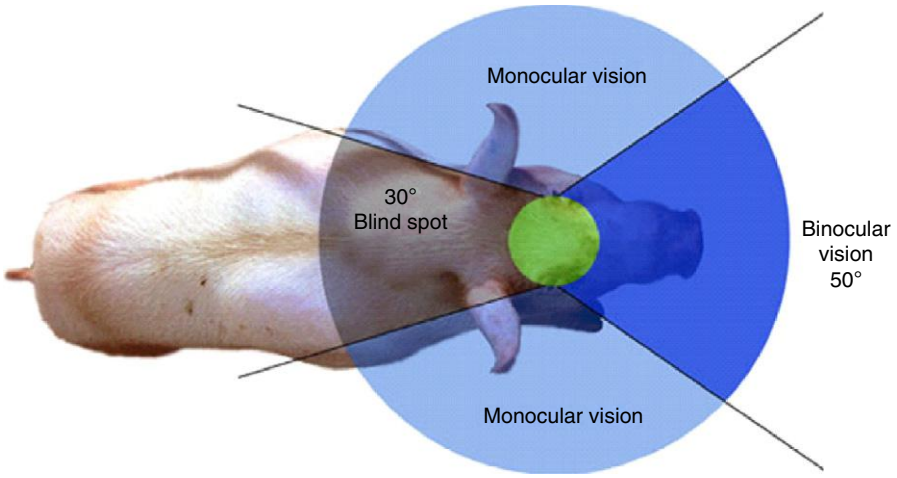

Figure 3 Monocular and binocular vision zones of the pig. Reproduced from IRTA.

vulnerable to high environmental temperatures, but in case of high temperatures it is also recommended to use additional ventilation, reduce stops, unload promptly, and drive during the cooler parts of the day. Low temperatures and wind chills can provoke cold stress, especially in young animals and pigs. Keeping livestock dry is important to protect them from wind chill. Animals can also have frostbite in severely cold weather.

Transported animals require a certain floor space allowance in order to stand comfortably, lie down, and keep their balance during vehicle movement. Once journeys start, pigs explore the compartment in which they are placed and try to find a suitable place to sit or lie down. The space allowance for each animal that needs to lie during transport should allow it to adopt a comfortable lying position without a significant risk of being walked on or smothered by other animals. Cattle usually remain standing during transport of up to 20 h duration, even if the vehicle is driven carefully. The space allowance provided for each animal that prefers to stand during transport should be such that it can adopt a position maintaining balance without any contact with other animals or with the vehicle or partition walls and without a high risk of falling. Avoidance of contact with other animals is also important if overheating is a potential problem in hot climates. Space allowances are calculated according to the size, bodyweight, and type of animal. However, the relationship between bodyweight and required floor area is not linear as the animal's weight is proportional to its volume. For cattle with horns, space allowance should be 7% higher than dehorned/hornless cattle. Attempts to reduce transport costs by overloading in the trucks produce a reduction in carcass weight, downgrading of carcasses due to bruising, and increased risk of injuries or death in worst cases.

Vehicle design and driving quality are other important factors to consider. When animals are transported in vehicles where the internal height is too low, they are prevented from standing in their natural position and may be in constant contact with the ceiling (Figure 4). This may result in wounds and bruises to the animals and may also hinder adequate ventilation above the animals. Furthermore, where there is not enough space between animals and the ceiling, the possibility to access the animals and allow them to be inspected may be reduced.

Figure 4 Transport of sheep with low internal height. Reproduced from IRTA.

The flooring surface should be designed to maintain balance and thermoregulation of animals and minimize the leakage of urine or feces. In motion, truck vibration can be reduced by installing a pneumatic suspension. Overinflated tires must be avoided as it increases severity of vibration, and driving must be with care to prevent bruises and injuries by avoiding sudden stops and acceleration, unpaved roads, and potholes.

Journey time per se is unlikely to be a risk factor but it becomes a risk when other aspects related to transport, such as animal fitness, fasting, vehicle design, driving style, stocking density, weather condition, ventilation, etc., are neglected. According to the EU Regulation 1/2005, journey times shall not exceed 8 h, except when road vehicles meet special requirements. In the EU, cattle and sheep can be transported for 28 h (with a rest of at least 1 h after 14 h, after which they must be unloaded and given food, water, and at least 24 h rest). If the higher vehicle standards are attained, pigs can be transported for 24 h, after which they must be unloaded and given food, water, and at least 24 h rest before continuing the journey.

Lairaging at the Abattoir

It is possible that fit animals at the start of the journey might fall sick, become ill, or be injured during transport. Animals should be inspected while they are still in the vehicle upon arrival at the abattoir for any welfare and health problems (Figure 5). Animals that have an injury or a disease associated with severe pain or suffering, and when there is no practical possibility to alleviate it, should be killed humanely whilst on the vehicle within the shortest possible time.

If animals arrive exhausted, for example, due to prolonged period of deprivation of food and water, long transport distance/time, and adverse weather conditions, their ability to cope with the lairage conditions would be impaired. To avoid the negative welfare effects of lairage, mixed groups of animals should be unloaded as quickly as possible after arrival and subsequently slaughtered without undue delay. In unmixed groups of pigs, however, a short period of lairage permits animals to recover from the stress and activity resulting from transporting and unloading, which can be beneficial to meat quality. A recommendation of a minimum 2–3 h has been made on the basis of physiological measurements in pigs.

The lairage environment should meet the welfare needs of animals in terms of resting and thermal comfort (neither too hot nor too cold) as well as by providing enough space for animals to be able to move around freely. To satisfy its welfare needs, each animal shall have enough space to stand up, lie down, and turn around. High stocking densities also increase aggression because the easy escape of attacked individuals is thwarted. When pigs are kept in small pens without the space and opportunity for attacked animals to escape, it leads to distress and physical damages in them, predominantly caused by dominant animals.

Where holding-pen space is limited, it is important to schedule truck arrivals precisely. Otherwise, animals would have to wait in the truck on arrival at the processing plant, which increases the chances of compromising animal welfare. Longer times in lairaging can demand a great use of energy in all the species due to the fact that animals are in a new situation and usually without food. Under this situation, prolonged periods of preslaughter stress can increase the incidence of DFD meat and, therefore, feeding animals and giving a resting period in lairage would be economically beneficial to the meat industry. EU legislation states that animals must be fed if the lairage time exceeds 12 h.

During transport, animals are usually deprived of water. Therefore, sufficient quantity of drinking water should always be available in the lairage/holding pen. The water supply system should be designed and constructed to allow all sizes of animals easy access (i.e., in terms of comfortable drinking height and number of water troughs or drinkers) to clean water at all times, without being injured or limited in their movements, and so that the risk of the water becoming contaminated with feces or urine is minimized. Suckling animals are particularly susceptible to dehydration because they might not have learned how to drink from a trough and so they fail to drink the water provided at the abattoir.

During hot weather, the practice of spraying pigs with cold water (10–12 °C) at lairage limits the risk of hyperthermia and consequently reduces the mortality rate in lairage pens (Figure 6). Showering also reduces aggressive behavior and facilitates greater ease of handling into the stunning chute. The shower regimen should be intermittent (i.e., once at arrival and once just before moving to stunning) and not longer than 30 min in total in order to get the greatest cooling effect and reduce activity and aggression. For maximum cooling effect, the sprinklers should have a spray coarse enough to penetrate the hair and wet the skin. Sprinklers that create a fine mist can

Figure 5 Inspection of animal at the slaughterhouse. Reproduced from IRTA.

Figure 6 Showering of pigs at arrival to the lairage pens. Reproduced from IRTA.

increase humidity in the lairage pen without penetrating the hair and should not be used. Showering in lairage is not recommended when the ambient temperature is below 5 °C as it causes thermogenesis by shivering to maintain body temperature in pigs.

Lighting/illumination in the lairage area/holding pens should be sufficient to observe all the animals and that they are able to move without fear and distress. All the animals kept in lairages or holding pens should be protected from inclement weather.

Movement from Lairage to Stunning Pen

Pens, passage ways, and races should be designed and constructed to allow the animals to move freely in the required direction using their behavioral characteristics and without distraction. Pigs are calm during handling when they can walk side by side for as long as possible. The flow of animals proved to be better when the corridors are well lit, wide, straight, and with few bends. Solid-walled fences eliminate contact between pigs walking through the alleys and those held in pens and prevent stops due to distraction.

Occasionally, animals are moved too fast through the raceway in order to meet high throughput. The combination of high throughput and poorly designed and maintained handling systems (sharp protrusions on the walls, gaps, and potholes on the floor) lead to rough handling and excessive and inappropriate (e.g., when there is no empty space to move forward) use of electric goads. Application of electric goads, even with low voltages, causes pain and distress as evidenced by significant raises in heart rate, open mouth breathing, squealing (in pigs), and many other physiological measures. The routine and excessive use of electric goads is an indicator of poor attitude of the stockperson and/or serious lack of species-specific infrastructures. Training personnel of the abattoir on the behavioral principles of handling can greatly reduce the use of electric goads. Similarly, abattoirs should be designed and constructed to meet the species-specific demands of handling and throughput rates.

The number of pigs and cattle moved per group can affect stress and ease of movement as any disruption in the movement and consequent distress can spread through the whole group. When animals are handled in large groups, those close to the transporter might be excited whereas there is a lack of control over the animals that are far away. Moving pigs in groups of five or six at a time seems to be optimum. Cattle are calmer and show less turning around when driven in groups with more than three cattle per group and when they have uniform side protections of equal height (minimum 1.5 m). Owing to the flocking and following behavior of sheep, it is better to manage them in big groups and give them a clear and unobstructed view toward the exit.

Body trembling and vocalizations are highly correlated with physiological stress indicators, and the level of squealing in pigs is correlated with meat quality problems. Pig squeals can be measured by determining the percentage of time pigs are quiet in the stunning pen, restrainer, and race and crowd pens, or with a sound meter. Vocalization can also be used in cattle but is not a good stress indicator in sheep. Reduction of noise in the driving area facilitates the movement of animals calmly. In pigs, high sound levels in lairage (>100 dB) increase the levels of lactate and creatine phosphokinase in blood. Noise produced by machinery, pressure hoses, and humans represent a source of stress. Provision of rubber pads on gates help to reduce noise levels. Prevention of noises higher than 80 dB and high-pitched sounds from hydraulic systems must be avoided in the live animal handling area. Lairages with high concrete ceilings and precast concrete walls have more echoes and noise than those built from foam-core insulation board. Races, stunning boxes, and restrainers must be illuminated with indirect, shadow-free light. Any disturbance or distraction, including at the entrance to the stunning box or restrainers, will affect the normal movement of animals and, in turn, will increase the pressure on operatives leading to poor animal welfare.

Improvements in the practical handling of pigs have been achieved with the introduction of the group handling system and lairage design, which incorporate smaller pens holding only 15 pigs, and automatic push gates to move the animals from lairage pens up to the point of loading into the gas stunning system. The lower stress imposed on the pigs and reduced interaction with the handlers have been shown to improve welfare and meat quality.

Effects of Preslaughter Handling on Meat Quality

Stressful conditions during transport and at slaughter adversely affect meat quality. Both PSE and DFD meat are related to poor animal welfare conditions in pigs. At slaughter, the supply of oxygen to the muscle ceases, and any subsequent metabolic process is anaerobic. Therefore, energy (adenosine triphosphate) is generated through glycolysis, which results in the accumulation of lactic acid in the muscle. Because this is not removed by the blood system the muscle gradually acidifies. The drop in the muscle pH causes the denaturalization of the muscle protein and conversion of the muscle into meat. The final pH is inversely proportional to the concentration of lactic acid.

Antemortem short-term or long-term stressors impair normal muscle metabolism and affect fresh meat color, water-holding capacity, shelf life, and yields. Acute stress around the time of slaughter causes PSE meat, determined using a pH at 45 min postmortem of lower than 6. PSE usually occurs in pigs that are genetically sensitive to stress when subjected to acute preslaughter stressors immediately before stunning, though it can occur in normal pigs subjected to stressful conditions before slaughter. The risk of PSE meat decreases with decreased durations of transportation, though its effect depends on the stocking density. For transits longer than 3 h, the risk of PSE increases with stocking density during transport whereas the opposite occurs for shorter transits.

Animals that are subjected to chronic stress before slaughter suffer severe depletion of muscle glycogen, leading to less lactic acid formation during postmortem. DFD meat is often defined as having an ultimate pH, measured after 24 h, higher than 6. The extent of glycogen depletion antemortem depends on physical exhaustion and psychological preslaughter stress in cattle. Various stress factors have been reported as

responsible for glycogen depletion: time and handling during transportation from farm to abattoirs, lairage time, climatic factors, social disruption, and the novelty of the preslaughter environment. In cattle, mounting behavior, stimulated by social regrouping, and fighting are associated with dark-cutting beef.

See also: Animal Health Risk Analysis. Chemical and Physical Characteristics of Meat: pH Measurement. Conversion of Muscle to Meat: Slaughter-Line Operation and Pig Meat Quality. Exsanguination. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Modeling in Meat Science: Meat Quality. On-Line Measurement of Meat Quality. Preslaughter Handling: Welfare Including Housing Conditions; Welfare of Animals. Quality Management: Abattoirs and Processing Plants. Slaughter, Ethics, and the Law. Species of Meat Animals: Cattle; Pigs; Sheep and Goats. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning

Further Reading

Dalmau, A., Temple, D., Rodríguez, P., Llonch, P., Velarde, A., 2009. Application of the Welfare Quality® protocol at pig slaughterhouses. Animal Welfare 18, 497–505.

Fàbrega, E., Corna, J., Tibau, J., Manteca, X., Velarde, A., 2007. Evaluation of parameters for monitoring welfare during transport and lairage at the abattoir in pigs. Animal Welfare 16, 201–204.

Fàbrega, E., Manteca, X., Font, J., *et al*., 2002. Effects of halothane gene and preslaughter treatment on meat quality and Welfare from two pig crosses. Meat Science 62, 463–472.

Faucitano, L., Schaefer, A., 2008. Welfare of Pigs from Birth to Slaughter. The Netherlands: Wageningen Academic Publishers

Fraser, A.F., Broom, D.B., 1990. Welfare measurement. In: Fraser, A.F., Broom, D.M. (Eds.), Farm Animal Behaviour and Welfare, third ed. London: Bailliere Tindall, pp. 266–279.

EFSA, 2011. Scientific opinion concerning the welfare of animals during transport. EFSA Journal 9 (1), 1996. Available at: http://www.efsa.europa.eu/en/efsajournal/pub/1966.htm (accessed 16.10.13).

Grandin, T., 1997. Assessment of stress during handling and transport. Journal of Animal Science 75, 249–257.

Grandin, T., 2000. Livestock Handling and Transport. Wallingford: CABI Publishing.

Gregory, N.G., 1998. Animal Welfare and Meat Science. Wallingford: CABI Publishing.

Guàrdia, M.D., Estany, J., Balasch, S., *et al*., 2004. Risk assessment of PSE condition due to pre-slaughter conditions and RYR1 gene in pigs. Meat Science 67, 471–478.

Guàrdia, M.D., Estany, J., Balasch, S., *et al*., 2005. Risk assessment of DFD meat due to pre-slaughter conditions in pigs. Meat Science 70, 709–716.

Mach, N., Bach Velarde, A., Devant, M., 2008. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Science 78, 232–238.

Manteca, X., Velarde, A., Jones, B., 2009. Animal welfare components. In: Smulders, F., Algers, B. (Eds.), Welfare of Production Animals: Assessment and Management of Risks. The Netherlands: Wageningen Academic Publishers, pp. 61–77.

Marahrens, M., Kleinschmidt, N., DiNardo, A., *et al*., 2011. Risk assessment in animal welfare – Especially referring to animal transport. Preventive Veterinary Medicine 102, 157–163.

Velarde, A., Geers, R., 2007. On Farm Monitoring of Pig Welfare. The Netherlands: Wageningen Academic Publishers

Warriss, P.D., Brown, S.J., Adams, M., 1994. Relationship between subjective and objective assessment of stress at slaughter and meat quality in pigs. Meat Science 38, 329–340.

Relevant Websites

http://qpc.adm.slu.se/Driving_to_stunning/
Anne Algers. Swedish University of Agricultural Sciences.

http://ec.europa.eu/food/animal/welfare/slaughter/index_en.htm
European Commission.

http://ec.europa.eu/food/animal/welfare/transport/index_en.htm
European Commission.

http://www.grandin.com/references/handle.stress.html
Temple Gradin. Department of Animal Sciences, Colorado State University.

Welfare Including Housing Conditions

AM de Passillé and J Rushen, University of British Columbia, Agassiz, BC, Canada

Glossary

Catabolism The process of breaking down complex molecules (e.g., starches, proteins, or fats) into simpler ones (e.g., glucose and amino acids) with the release of energy.

Farrowing crate A metal crate or cage in which pregnant pigs are housed for a short period before giving birth and in which they can remain for several weeks after. The crate is narrow so that the sow cannot turn around. Farrowing crates are used with the intent of reducing crushing of the piglets when the sow lies down.

Feedlot A dirt-floored, outdoor pen in which livestock (most often cattle) are fattened by being fed a high grain diet before being sent to slaughter.

Hypothalamic–pituitary–adrenal (HPA) axis It consists of the hypothalamus, the anterior pituitary gland and the adrenal cortex, and controls one of the main sets of physiological responses to stress. HPA activity is most often measured through blood concentrations of adrenocorticotropic hormone (ACTH) and the corticosteroids (e.g., cortisol).

Innate behavior The behavior patterns that are characteristic of a particular species and which are strongly influenced by genetics.

Periparturient The period of time just before and just after birth.

Stereotypic behavior Behavioral patterns that are repeated in a relatively fixed and repetitive manner, and which appear to have little purpose. When seen among captive animals, the occurrence of stereotypic behavior is thought to indicate reduced welfare.

Stress-induced immunosuppression A reduction in the capacity of the immune system to fight against disease as a result of the animal being exposed to stress.

Introduction

Animal welfare is important to meat production because poor animal welfare is associated with poor animal production or health, and because consumers' concerns may influence market access. Throughout the world, animal welfare is the topic of legislation, retailer standards, and codes of practice. An animal has good welfare if it is in good health and feeling good, and the psychological component must not be ignored. Challenges to animal welfare differ between species and production systems. Concern about animal welfare is highest for intensive production but extensively housed animals also have welfare problems. Poor welfare is apparent in the animal's health, behavior, production, and physiology. Different welfare indicators detect specific challenges to animal welfare, rather than measuring the overall welfare.

What Is Animal Welfare?

Healthy animals that are not experiencing unpleasant emotions should be in a good state of welfare. Disease is a major cause of poor welfare but animal welfare is more than just poor health. Animal welfare is at risk whenever animals are suffering as a result of pain, fear, or when they cannot behave in a way that they are motivated to do. Suffering is an emotional state of animals and animals' emotions are difficult to assess. Alternative definitions of animal welfare that ignore animal emotions have been proposed, but these do not address the main concerns of the public.

Various definitions exist for animal welfare. A good inclusive definition is the 'five freedoms' viz: freedom from discomfort, freedom from fear and pain, freedom from thirst and hunger, freedom to express most normal patterns of behavior, and freedom from injury or sickness. Although complete 'freedom' may be impossible to attain, this definition indicates how animal welfare can be improved. The World Organization for Animal Health (OIE) defines an animal experiencing good welfare if it is healthy, comfortable, well nourished, safe, able to express innate behavior, and is not suffering from unpleasant states, such as pain, fear, and distress. The European Welfare Quality project involves 12 animal welfare criteria, namely:

- absence of prolonged hunger,
- absence of prolonged thirst,
- comfort around resting,
- thermal comfort,
- ease of movement,
- absence of injuries,
- absence of disease,
- absence of pain induced by management procedures,
- expression of social behavior,
- expression of other behaviors,
- good human–animal relationship, and
- absence of general fear.

These criteria are grouped into four welfare principles, namely, good feeding, good housing, good health practices, and an environment for natural behavior. The overlap between these definitions shows that there is considerable agreement as to what constitutes good animal welfare.

The Importance of Animal Welfare to Consumers

The existence of animal-friendly niche markets suggests that some consumers are sufficiently concerned to alter their

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buying habits if not convinced that animals were raised in a way that leads to good animal welfare. However, there is little evidence that animal welfare issues affect the normal buying habits of consumers in general, and most consumers are not willing to pay substantially more for animal-friendly products. Younger generations tend to be more sensitive to animal welfare issues, suggesting that consumer concerns about animal welfare will grow in the future, and 'scandals' or stories of animal abuse reported in the press have potential to affect buying habits of all consumers. Consumer concerns currently impact the meat industry most through legislation or through retailer standards.

Legislation, Standards, and Codes of Practice

The European Union countries have comprehensive animal welfare legislation, focusing on contentious issues, for example, mandatory group housing of calves after 8 weeks of age and prohibiting tethering of pregnant sows. Historically, North American governments were hesitant to pass farm animal welfare legislation, but recent referenda in a number of US states, most notably California, have resulted in welfare legislation being introduced at a state level. Legislation is a direct way of preventing particular unacceptable practices, but has limited ability to improve overall animal welfare. Often, legislation deals with issues that concern the public most and overlooks issues of which the public is less aware. Therefore, legislation might only serve to establish minimum acceptable levels of welfare. In addition, legislation is time consuming to pass, requires public resources to police, and can be inflexible.

Some food retailers have animal welfare standards that their suppliers must meet. Auditable standards are now an accepted way of dealing with animal welfare in North America. A number of food retailers and chain restaurants are promoting 'best practices' for each animal species, with adherence verified through independent audits. Auditable standards are more comprehensive than legislation and often aim at a higher level of animal welfare. Standards can be more flexible and easier to revise, but unless the auditing process is transparent, consumers might have less confidence in them.

Codes of practice are usually comprehensive and cover most aspects of housing and management. These vary from purely voluntary to where compliance is mandated by legislation, as in the UK. Codes often serve as an educational tool helping producers to improve practices and can form the basis of self-audits whereby producers can identify potential animal welfare issues on their own farms. However, the codes are often complex, which makes compliance difficult to assess and it is doubtful whether purely voluntary codes will do much to reassure skeptical members of the public or improve animal welfare when producers are reluctant to implement voluntary codes.

With increased global trade, there is pressure to harmonize animal welfare standards in different countries, and the OIE has included animal welfare in its mandate and has begun to develop international animal welfare standards. The requirement to meet international regulations and standards that deal with animal welfare will be of increasing importance to the meat industries in regions that export meat or animal products.

Animal Welfare and Food Safety

Concern with the on-farm contribution to food safety might increase attention to animal welfare because there are close links between animal welfare and animal health. Endocrine changes that occur when animals are chronically stressed can inhibit immune responses to infection, rendering animals more susceptible to disease. Overcrowding might increase disease transmission within groups of animals but confinement housing might minimize transmission of diseases to other farms. Reduced reliance on antimicrobials will require housing and management techniques that improve the welfare of meat animals. However, some changes in housing or management practices to improve animal welfare might increase on-farm risks to food safety; for example, increased use of group housing might increase disease transmission unless the groups are well-managed. Animal welfare standards and their application must be consistent with on-farm food safety standards.

Threats to Animal Welfare

Challenges to animal welfare vary between species and production systems. The list in Table 1 is not exhaustive but focuses on animal welfare issues that have gained most attention.

The use of surgical procedures that cause pain to animals are of particular concern to the public. These include tail docking, castration, and tooth clipping of pigs; castration, branding, and dehorning of cattle, as well as aversive handling, such as use of electric prods. Dehorning of cattle is done partly to safeguard animal welfare, but pain should be reduced preferably by combinations of local anesthetic, antiinflammatory agents, and sedatives. Dehorning should be done as early as possible, because young animals recuperate better than older ones. Polled cattle breeds are available that perform as well as nonpolled breeds, thus removing the need for dehorning. In other cases, for example, hot-iron branding, the procedures are not done to protect animal welfare and alternative methods of identifying animals should be sought.

Good stockmanship is essential for good welfare, but is often overlooked. Poor handling often arises from mistaken beliefs about the difficulties of handling animals and can be reduced by training. In addition, good stock people recognize the importance of routine tasks, such as cleaning of facilities to protect animal health and welfare. Table 2 shows that poor handling and painful practices can make animals frightened of people and contribute to poor welfare and reduced production, possibly due to the increases in stress hormone levels, like those of cortisol.

Concern about animal welfare is highest when animals are kept intensively. This is particularly true when large numbers are kept in small areas and prevented from exercising their natural behavior. However, intensive housing systems can have advantages for animal welfare if designed and managed properly. Animals under extensive conditions can suffer from inclement weather, natural disasters, poor feeding, parasitism, and predation. These threats might not be the direct result of human action, but because such animals are kept to benefit

Table 1 Some of the more widely recognized animal challenges to animal welfare in meat production

Beef production
Early weaning
Respiratory disease at feed lots
Lameness at feedlots
High grain diets and acidosis
Heat stress (and lack of shelter)
Buller syndrome
Lack of bedding
Concrete and slatted floors
Stocking density
Branding
Dehorning
Castration
Rough handling
Nonambulatory animals
Veal production
Individual housing
Tethering
Stall dimensions
Stall flooring
Ventilation
Group size
Respiratory and GI tract disorders
Low roughage diets
Anemia
Pork production
Breeding animals
Individual housing for gilts and sows
Tethering
Stall dimensions
Lameness in sows and boars
Intestinal torsion in sows
Breeder sow mortality
Tusk removal
Low roughage diets
Lack of bedding
Meat animals
Stocking density
Preweaning mortality
Tail docking
Tooth clipping
Castration
Aggression in groups
Lack of bedding
Concrete and slatted floors
Respiratory disease in weaner/growers
Ventilation
Rough handling
Poultry meat production
Skeletal disorders
Dermatitis and skin disorders
Cardiovascular problems (ascites)
High mortality
Stocking density
Ventilation
Food restriction of breeding animals

their owners, welfare remains the responsibility of the owners. Alternative housing systems might not improve overall welfare but change the nature of the threats to animal welfare. Group housing of pigs and calves provides opportunities for social contact, but increases the risk of aggression, competition, and disease transmission. The increased use of such housing systems requires that effective ways of controlling aggression and disease transmission are implemented. This can be achieved by appropriate group sizes. For example, disease incidences in calves in groups <10 are similar to those of individually housed animals. In addition, appropriate stocking rates and feed space can minimize competition. Among broilers, incidence of mortality and pathologies starts to rise when stocking densities are above 30 kg m^{-2}. Growth rates of 250–500 kg beef cattle are reduced at space allowances less than 3–4 m^2 per animal.

Table 2 Some of the effects of rough handling on swine production and welfare

Variable	*Positive handling*	*Rough handling*
Growing pigs		
Growth 7–13 weeks (g d^{-1})	455	404
Growth 11–22 weeks (g d^{-1})	709	669
Cortisol concentration (ng ml^{-1})	2.1	3.1
Adrenal weight (g)	3.82	4.81
Time to approach human (s)	10	160
Gilts		
Pregnancy rate (%)	88	33
Cortisol concentration (ng ml^{-1})	1.7	2.4
Time to approach human (s^{-1})	48	120

Source: Based on results from experiments described in Hemsworth, P.H., Coleman, G. J., 2011. Human-Livestock Interactions. Wallingford: CABI International.

Providing an appropriate physical environment is essential: animals must be protected against temperature extremes, provided with good quality air, and given stalls and pens that are sufficiently large and designed to avoid injury and ensure comfort. Heat stress is a particular problem for feedlot cattle: providing shade can be necessary to reduce mortality in areas where heat waves are common. A lack of proper, clean bedding and use of concrete floors increase foot and leg injuries in cattle and pigs. Slatted floors in beef production increase mortality and tail tip necrosis. However, excessive mud in extensive systems can result in poor animal welfare.

Farm animals continue to suffer from a variety of endemic diseases that pose a major challenge to their welfare (Table 1). Reducing their incidence will improve animal welfare and profitability. Continued genetic selection for high growth rates can increase the risk of various 'production diseases,' such as leg weakness and osteochondrosis in pigs and leg and cardiovascular problems (ascites) in poultry and may be a risk factor for poor welfare.

Behavioral deprivation is a problem in some housing systems. That an animal on a farm behaves differently from one in the wild does not mean its welfare is threatened: behavior is an adaptation to the local environment and varies between different environments. However, farm animals have retained much of their ancestral behavior and research has identified some behaviors that animals are highly motivated to perform but are unable to perform due to housing environment. Periparturient domestic sows, in the appropriate environment, show the nest building behavior typical of wild boar. In farrowing crates, this behavior is sometimes expressed as rooting and pawing at the floor or chewing at metal bars. Providing

suitable farrowing accommodations or nesting materials does not prevent these behaviors. The sows appear to need to perform the behaviors themselves. However, farrowing crates may help prevent injury or death to baby pigs, which are also major welfare issues. Milk-fed veal calves fed from a bucket will suck at each other or on pen fixtures. The motivation to perform this nonnutritive sucking is reduced by allowing the calves to suck for an adequate period of time, either while drinking milk or by sucking a dry teat after a meal.

Feeding methods and nutrition can be responsible for some welfare problems. Dairy heifers often remain hungry because of restricted milk feeding. Milk-fed veal calves are at the risk of being anemic if hemoglobin levels are not adequately monitored. Beef cattle fed large amounts of grain can develop acidosis and liver abscesses. Broilers grow too heavy without feed restrictions. Breeding sows fed low volume concentrates can remain hungry after the meal and develop stereotypic behavior possibly due to a delay in the physiological signals that register satiety. This behavior can be reduced by increasing the energy content or the bulk of the meal.

Assessing Animal Welfare

Recently, the European Food Safety Authority has begun to develop a risk-assessment approach to identify the most serious threats to animal welfare in various species of farm animals. Many protocols are now available for conducting on-farm animal welfare assessments or audits looking at threats that are apparent in animals' health, behavior, production, and physiology. Animal welfare indicators detect specific challenges to animal welfare, rather than measuring overall welfare and different welfare indicators are required to detect different problems. To provide more positive outcomes, there is an increasing interest in having measures of good welfare rather than measures of poor welfare only. By using certain measurable indicators of animal welfare, different systems or operations can be compared. These indicators or criteria do not measure absolute levels of welfare, nor can they determine whether the level of welfare is acceptable because this is more of an ethical question.

Input- Versus Outcome-Based Criteria

Animal welfare assessment requires a combination of input-based criteria, which describe how animals are kept (e.g., spatial allowances and size of stalls), and outcome-based criteria (or animal-based criteria), which assess the animals themselves. Input-based criteria are easier to audit and can describe the level of risk that exists for poor welfare, but they provide no direct evidence about the actual state of the animals, and it is difficult to compare different production systems. Outcome-based measures are closely linked to the actual state of the welfare of the animals, irrespective of how they are housed or managed, but are harder to audit. Both input- and outcome-based criteria are required for an overall animal welfare assessment but they must be validated, that is, shown to actually measure animal welfare. At present, there is considerable research aimed at developing reliable and outcome-based animal welfare indicators for use in on-farm animal welfare assessments.

Health Indicators

Poor health, measured by mortality or disease incidence, is the least controversial indicator of poor welfare. However, it is unlikely that all threats to animal welfare (such as behavioral deprivation or social isolation) will reduce animal health. Thus, a high incidence of illness is a clear sign of welfare problems, but the absence of illness is not sufficient to determine that welfare is good. Obtaining reliable data on health problems on farms is difficult. Record keeping can be time consuming, but would do much to help farmers control welfare problems and improve production and profitability. Indirect measures of health can be obtained from records of veterinary treatments but underestimate the incidence of illness, and are influenced by the farmer's judgment of the need for treatment.

Behavioral Indicators

Injurious behaviors

The least controversial behavioral indicator is behavior that results in injury (Table 3). Problems arise from the difficulties of recording these behaviors on farms. Usually, injurious behaviors occur sporadically and animals must be observed for long periods to accurately estimate how much aggression is occurring. It is easier to observe the consequences of the behavior, for example, by counting the types of wounds or injuries that are likely to result from aggression.

Abnormal behavior

Farm animals perform a variety of behaviors that appear abnormal, for example, milk-fed veal calves that suck at each other or at metal bars and gestating sows root the floor of the pen or mouth the bars of the cage (known as 'stereotypic behavior'). When these behaviors occur excessively, it can indicate a welfare problem but to use such abnormal behaviors to assess welfare, their causes and consequences need to be properly understood.

Table 3 Some of the more common behavioral indicators of poor welfare

Injurious behaviors
Aggression/bullying
Tail biting (pigs)
Vulva biting (sows)
Bulling (cattle)
Feather pecking (poultry)
Vent pecking (poultry)
Cross sucking (veal calves)
"Abnormal" behavior
Belly nosing (pigs)
Stereotypic feeding behavior (sows)
Sucking pen fixtures (veal calves)

Changes in normal behavior

Changes in normal behavior can be used to assess animal welfare, for example, if animals feed or sleep less. Some behavioral changes are related to illness or disease. Animals respond to illness by feeding less, resting more, and showing less social behavior. These behavioral changes are controlled by cytokines from the immune system and can be considered as part of the immune response. They occur simultaneously with physiological components of the acute phase response to illness and are adaptive responses that help the animals recover from the illness. Therefore, a better understanding of illness behavior can help in the early detection of illness. Some behavioral changes can be monitored automatically so that morbidity can be detected several days earlier than with conventional methods. For example, feedlot cattle suffering from bovine respiratory disease show an increased number of drinking bouts and a reduced number of visits to the feed bunk.

Anatomical problems or injuries could be detected by a change in normal movement of an animal; for example, changes in gait can be used to detect leg problems.

Physiological Indicators

Animals respond to various challenges by physiological responses, which have been used to assess animal welfare. The main components of the physiological response are:

- perception by the animal of the stressor,
- biological defense reactions, and
- long-term consequences of the stress response.

The physiological changes might show that the animal is suffering from some aversive emotional experience, or the physiological changes might themselves result in suffering, for example, stress-induced immunosuppression leading to disease. The main physiological defense responses involve the sympathetic nervous system (SNS) (assessed by heart rate, blood pressure, or plasma concentrations of catecholamines) and the hypothalamic–pituitary–adrenal (HPA) axis (assessed by plasma concentrations of corticosteroids or adrenocorticotropic hormone (ACTH)). Not all changes in SNS or HPA activity are specifically stress responses but occur in many normal physiological events; for example, after feeding or sexual activity. Physiological responses vary between different types of stressors and some stressors require specific measures, for example, heat stress in cattle is detected by changes in tympanic temperature or respiration rates.

An animal's perception of a threat triggers both the emotional and the physiological stress response. Animals respond to pain or fear with increased SNS and HPA activity, which can be used to assess the animals' response to painful treatments or acute stressors (Table 4). However, physiological measures are less useful in assessing longer lasting stress. Firstly, secretion of cortisol is pulsatile and follows circadian rhythms, requiring multiple blood samples over the day. Secondly, HPA activity can adapt when stress is prolonged. For example, when bulls are first tethered, plasma cortisol increases, but this is absent 1 month later due to a reduced sensitivity of the adrenal gland to ACTH.

Chronic physiological changes can lead the animal to enter a 'prepathological' state that can involve increased catabolism leading to weight loss, or immunosuppression. However, stress has complex effects on immune responsiveness, with moderate stress often improving immune competence, and it is still difficult to predict that any change in the immune system will indicate increased susceptibility to diseases. Without better understanding of the relationship between stress, immune competence, and disease susceptibility, caution is necessary when using physiological measures to assess animal welfare.

Table 4 Increases in plasma cortisol concentrations in cattle following various painful or stressful procedures[a]

Treatment	*Increase in plasma cortisol (ng ml^{-1})*
Hot iron branding	26
Surgical castration	31
Castration + LA	21
Castration + AI	3
Dehorning	28
Dehorning + LA + AI	5
Handling (3–4-week-old calves)	6

[a]Comparison of the values gives some indication of the relative degree of pain or stress and shows the relative advantages of providing local anesthetics (LA) and/or anti-inflammatory agents (AI) to reduce the pain.

Source: Data from Early, B., Crowe, M.A., 2002. Journal of Animal Science 80, 1044–1052; Fisher, *et al.*, 1996. Journal of Animal Science 74, 2336–2343; McMeekan, C. M., *et al.*, 1998. Research in Veterinary Science 64, 147–150; Schwartzkopf-Genswein, K.S., *et al.*, 1997. Canadian Journal of Animal Science 77, 369–374; and Wohlt, J.E., *et al.*, 1994. Journal of Dairy Science 77, 3725–3729.

Measures of Production

Animal productivity is a controversial welfare indicator. Reduced growth rate in pigs and cattle or even weight loss are common response mechanisms to chronic stress. However, measures of the production of individual animals must be used and not measures of profitability. For example, increasing the stocking density of broilers can reduce growth as a result of stress, whereas profitability continues to increase. Figure 1 shows mean body weight of broilers at 7 weeks of age housed at different stocking densities as well as an estimate of profit potential. Although the body weight of individual birds began to decline as space allowance dropped below 0.09 m^2 per bird, the profit potential continued to increase: having more birds in a given area compensates for the reduced gain of individuals. Furthermore, one must understand the cause of the change in productivity, and relate this to the animal's welfare. The reduction in growth that occurs when a calf has diarrhea indicates a welfare problem, but increased growth due to growth promotants would not necessarily indicate improved welfare. Beef cattle respond to high temperatures by reducing feed intake and growth is reduced. This would not necessarily indicate a welfare problem if the reduced feed intake helps the animal adapt to high temperatures by reducing metabolic heat production. Finally, the rapid growth of meat animals can result in welfare problems. Among fast growing broilers, lameness, sudden death syndrome, and cardiovascular disease are serious welfare problems. High productivity can, therefore, be a risk factor for poor welfare.

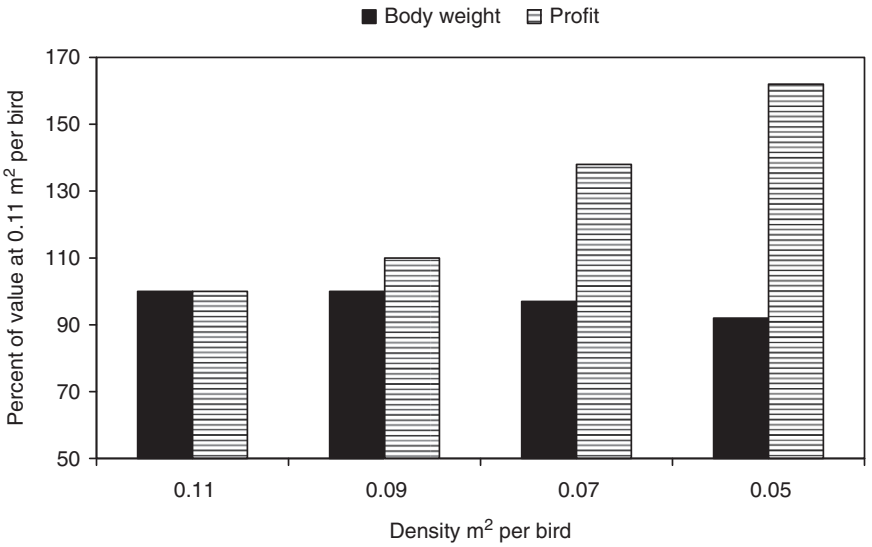

Figure 1 Mean body weight of broilers at 7 weeks of age housed at different stocking densities as well as an estimate of profit potential. The values are expressed as a percent of the value at 0.11 m^2 per bird. Drawn from data presented in Cravener, T.L., Roush, W.B., Mashaly, M.M., 1992. Broiler production under varying population densities. Poultry Science 71, 427–433.

See also: Growth of Meat Animals: Growth Patterns. Meat, Animal, Poultry and Fish Production and Management: Poultry; Red Meat Animals. Species of Meat Animals: Cattle; Pigs; Poultry

Further reading

Appleby, M.C., Mench, J.A., Olsson, I.A.S., Hughes, B.O. (Eds.), 2011. Animal Welfare, second ed. Wallingford: CABI.
Duncan, I.J.H., Hawkins, P. (Eds.), 2010. The Welfare of Domestic Fowl and Other Captive Birds. Dordrecht: Springer.
Fraser, D., 2008. Understanding Animal Welfare: The Science in its Cultural Context. Oxford: Wiley-Blackwell.
Hemsworth, P.H., Coleman, G.J., 2011., Human-Livestock Interactions, second ed. Wallingford: CABI International.
Marchant-Ford, J. (Ed.), 2009. The Welfare of Pigs. Dordrecht: Springer.
Moberg, G.P., Mench, J.A., 2000. The Biology of Animal Stress. Wallingford: CABI Publishing.
Rushen, J., de Passillé, A.M., von Keyserlingk, M.A.G., Weary, D.M., 2008. The Welfare of Cattle. Dordrecht: Springer.
Smulders, F.J.M., Algers, B. (Eds.), 2009. Welfare of Production Animals: Assessment and Management of Risks. Wageningen: Wageningen Academic Publishers.

Relevant Websites

www.efsa.europa.eu
European Food Safety Authority.
www.oie.int
World Organization for Animal Health.

Welfare of Animals

P Lawlis, Ontario Ministry of Agriculture and Food, Woodstock, ON, USA
A Allen, Canadian Food Inspection Agency, Saskatoon, SK, Canada

Glossary

Animal-based measures Scales developed and validated to measure an animal's welfare by observing the animal (e.g., lameness scoring and body condition scoring).

Immunosuppression Something that reduces the effectiveness of the immune system to fight disease or infection.

Management-based measures A method of assessing the level of animal welfare being provided through the verification of select protocols that have been demonstrated to be important (e.g., treatment protocols).

Resource-based measures A method of assessing the level of animal welfare by examining and investigating what has been provided for the animal. The necessary resources for good welfare are established through research.

Sentience The ability to feel or experience.

Utilitarian A term used in the study of ethics to describe an approach whereby a person selects the option which provides most happiness and reduces suffering. Also defined as the practical and material value of animals to serve some human purpose and that the practical demands outweigh emotional considerations, but there is not necessarily lack of affection toward animals.

Introduction

The human–animal relationship is complex. Humans filter information through narratives that are used to make sense of the world, people learn how to think about animals from their parents and elders at an early age. There have been significant changes in agriculture in the last 30 years and there is no longer a definitive universal understanding of how society should see the use of animals for human benefits. There is no single pervasive cohesive world view about how we should think, feel, and behave toward them. Hal Herzog explores many of the paradoxical and inconsistent beliefs we hold in his 2010 book: *Some We Love, Some We Hate, Some We Eat*. Similarly, interpretation of the concepts of animal welfare related to livestock production varies among interest groups and stakeholders who have differing experiences, knowledge, values, and vested interests.

The Evolution of Philosophical Thinking about the Human–Animal Bond

Interest in the human–animal relationship and concern about the welfare of animals is not new – the human–animal relationship has been of interest to concerned members of civil society for generations. Attitudes toward animals and how they differ from humans have been explored by many philosophical thinkers.

The Pythagorean School (Greek) viewed man and animals as having souls (animism). They believed that souls were finite in number and as an animal died, the soul took up residence in either another animal or another human. Followers of Aristotle held the belief that there was a scale or ladder of nature, with humans at the top and lower life forms at the bottom. This theory, known as vitalism, shared many similarities with the (much later) theory of evolution. Teleological anthropocentrism, described by Xenophon, decreed that everything on the earth was put there for the pleasure and exploitation of humans. Later Christian thinkers allowed that animals 'lower' than man could experience pain without fear and anticipation of its future continuation – but this condition was not equivalent to suffering.

Rene Descartes, a prominent French philosopher in the early seventeenth century compared animals to complex organic machines, devoid of mind and consciousness and the ability to feel pain and whose behaviors were dictated by the laws of physics. Descartes believed in a great divide between man and animals and that mankind had the authority to use animals as a means to an end. Like Descartes, Emmanuel Kant believed that animals were not capable of rational thinking and that man was set apart from animals because he possessed a 'categorical self,' which directs him to do the right thing. Although Kant did not consider animals to be moral agents, he did believe that humans ought not to cause suffering in animals because this increased their potential to do so to other humans.

John Locke, an English philosopher in the late 1600s with interests in social contract theory and the definition of consciousness, made some influential observations in his 1693 treatise 'Some Thoughts Concerning Education.' He based his theories on direct observation that animals, unlike machines, are sentient beings: that is that they can feel pain. John Locke was one of the first to highlight that disregarding animal pain had severe consequences for humanity. Locke's sentiments contributed to the development of the societies for prevention of cruelty to animals and to anticruelty legislation in Britain and Europe. Anticruelty legislation mirrors societies' concerns that animals deserve at least this minimal protection.

Jeremy Bentham and Modern Utilitarianism

Bentham was a British philosopher, social reformer, and early advocate of animal rights who argued that the ability to suffer

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and not the ability to reason should be the benchmark for attitudes toward animals. Bentham introduced the notion of utility as a guide to making moral decisions in 1789. Bentham's principle of utility was based on the desire to produce the greatest happiness for the greatest number. In his view, the calculation of the total amount of pleasure and pain should include all creatures capable of feeling pain, including animals. Bentham raised the question of how the pain of animals should be entered into the calculation of utility (but did not answer it). The difficulty with adopting this utilitarian, cost–benefit calculation approach is deciding what objective method should be used to calculate the experiences of pain and pleasure.

Brumbaugh argues that moral thinking about animals is divided between Descartes approach, a utilitarian, anthropocentric commodification of animals for food production and research, and the philosophical approach more aligned with John Locke's, which is a more animal-centric approach applied to animals that are kept as pets.

Recent philosophers, scientists, and activists have challenged the existing treatment of animals and rekindled the discussion concerning the morality of animal use and why we should care about the animals that we use.

The Animal Rights Movement

Peter Singer advocated for equality for all animals. Singer argued that man's treatment of animals (including intensive agriculture and animal experimentation) arose from our prejudices about them. Tom Regan, an American philosopher, promoted the granting of rights to all animals based on the issue of equality. For Regan, the granting of rights should be based on the similarities between humans and animals, not the differences. The animal rights movement, of which Regan is a prime supporter, is not focusing on larger cages, but cage-free egg production. Proponents argue that "what is wrong is not the pain" but the entire system of animal use.

Bonnie Steinbock argued that the granting of rights to animals would not immediately or directly improve their welfare. Steinbock makes the case that a cow would not benefit from a university education. Steinbock argued that there are valid reasons for treating animals with respect that are independent of inherent 'rights.' Steinbock gives the example that if we punch our neighbor it will hurt him (her), and that this is enough of a reason not to do it. This, Steinbock stated, is the reason that we should not hurt animals.

The Definition of Animal Welfare

A succinct and exhaustive definition of animal welfare is difficult to arrive at, because animal welfare, indeed human welfare, is subjective, i.e., 'in the eye of the beholder.' There are many criteria that influence opinions about animal welfare. Animal welfare is difficult to define because it not only involves information about animals, it also involves values about what is better or worse for them. Swanson asks whether the appropriate term is animal 'well-being' rather than 'welfare.' Swanson points out that definition of animal welfare will depend on such factors as cultural, scientific, religious, and political backgrounds. Broom believes that the welfare of an individual depends primarily on its ability to cope with its environment. Furthermore, intensity, duration, and incidence of any welfare relevant condition need to be considered.

There have been many attempts by governments and policy makers to create a working definition for animal welfare that has the consensus of stakeholders. Article 7.1.1 of the OIE (World Organisation for Animal Health) Terrestrial Animal Health Code provides a good working definition:

> *Animal welfare* means how an *animal* is coping with the conditions in which it lives. An *animal* is in a good state of *welfare* if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress.

The Five Freedoms

The Five Freedoms were first introduced in the Brambell Report that was commissioned by the UK government in 1965 to investigate concerns raised over factory farming. The concept of the five freedoms spawned countless debates and was the underpinnings for new animal welfare legislation in the UK and Europe, and the definition adopted by the OIE. The five freedoms include both physical and mental factors and stress the importance of animals being able to express 'natural behaviors' or 'telos' (see Section Relevant Websites):

1. Freedom from hunger and thirst;
2. Freedom from discomfort;
3. Freedom from pain, injury, or disease;
4. Freedom to express normal behaviour;
5. Freedom from fear and distress.

Animal Welfare Science

The 1990s saw the rise of a new field of Animal Welfare Science, an area of study that David Fraser refers to as "mandated science" because it was deliberately brought into existence to address policy issues and make recommendations to agribusiness and government about if and how changes will be made. Duncan and Fraser stated that animal welfare "is not a term that arose in science to express a scientific concept. Rather it arose in society to express ethical concerns regarding the treatment of animals." Animal welfare science has produced a growing body of objectively verifiable data ascertained using the scientific method, which helped to increase our understanding of how animals perceive the world. The use of quantitative, objective studies and results that can be expressed with a degree of mathematical certainty can provide information about resources required to ensure good welfare (such as stocking density); however, measurements relating to welfare outcomes (such as fear) are subjective and therefore can be influenced by observer bias.

Understanding Animal Welfare

Fraser proposed three different approaches to understanding animal welfare:

1. The 'nature' of animals.
2. Biological functioning.
3. The subjective experience of animals.

Appleby expressed these categories as mind, body, and nature (Figure 1).

The 'Nature' of Animals

This approach begins with the assumption that good animal welfare will flow from allowing animals to act naturally. Acting 'naturally' includes all natural biological functions, such as eating, drinking, and reproduction, so that the 'nature' approach mimics the biological scheme to some degree.

Fraser pointed out that this approach is disappointing, because many natural behaviors, such as sweating, shivering, and fighting, are negative and are not necessarily in the best interest of the animal. McBride stated that the natural approach requires the ability of an animal to adapt to its environment.

Welfare Is about Biological Functioning

Biological functioning is compatible with the 'nature' approach to animal welfare. Many guidebooks and codes on animal care stress the importance of good health and biological functioning. A view of animal welfare based solely on biological functioning is no longer satisfactory. Scientists, ethicists, and others have begun to provide insight into the mental state of animals and how the presence of a mental state must also be included in the evaluation of the welfare of an animal.

The Subjective Experience of Animals

In addition to the physical criteria that affect welfare (e.g., freedom from disease, injury, and hunger) science has demonstrated that animals are sentient beings, that is, they can feel pain and are motivated by the need to avoid suffering. The subjective experience of animals, that they have feelings and emotions, has been ignored, most likely due to the difficulties of defining and measuring these things objectively. Perhaps it has been because its existence or nonexistence cannot be proven in a scientifically recognized way. Fraser pointed out that some advocates of the biological functioning approach claim that the subjective experience of animals is so closely linked with their (animals') biological functioning that the two things are interchangeable. Despite this, many scientists and philosophers have considered the dimensions of a possible mental state of animals and are actively attempting to discover what exactly is going on in the brains of animals.

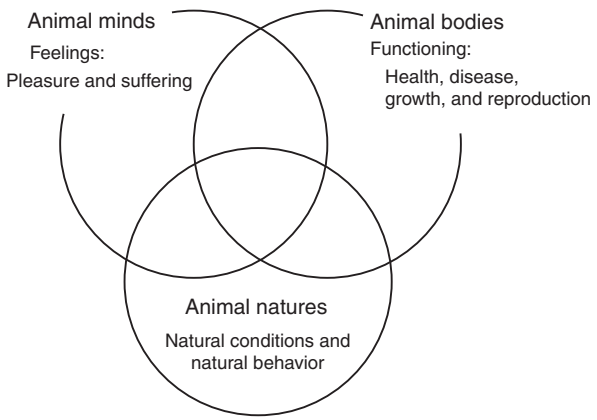

Figure 1 The three approaches to understanding animal welfare. Reproduced from Appleby, M., 1999. What Should We Do about Animal Welfare? Oxford: Blackwell Science, pp. 36–37.

The Welfare Continuum

Seamer argues that welfare means well-being, so that there can be no such thing as 'bad' welfare. However, Broom stated that welfare exists on a continuum from good to bad. Broom used the two extremes, good and bad, as basis points for the assessment and examination of animal welfare. Broom stated that the majority of work done had primarily focused on those conditions (pain, abnormal behavior, etc.) that lead to poor welfare. Broom believed that poor welfare is the result of overtaxing of an individual's ability to cope. Some responses to poor welfare are easy to recognize and assess – like sickness and death. Other responses, such as pain, immunosuppression, and abnormal behaviors, are difficult to measure and assess. Being able to recognize how animals react to certain elements of bad welfare is the key to an appropriate assessment of their welfare. However, it is important to keep in mind that even though an animal may look 'normal' it may be suffering.

Pain and Suffering

Most would agree that pain and suffering would be equivalent to 'bad welfare.' Bath claims that all those working with production animals are ethically bound to make their best assessment of animal pain and act to reduce suffering. Direct measurement of pain is difficult due to its subjective nature and the variability of the pain experience among individuals. How animals experience pain and how they react to it has been studied and this research has led to recommendations and legislation. The behavior of an animal will contribute to how it reacts to pain – prey animals (e.g., sheep) will hide their pain to avoid being picked out as being weak and vulnerable.

Molony and Kent maintain that there is still the thought that some pain is necessary; any pain experienced would be part of a learning experience. Beckoff and Bath suggest that although our (humankind's) ability to assess the pain and suffering might be inadequate, we should always err on the side of the animals and never question the ability of animals to experience pain.

Abnormal Behaviors as Indicators of Poor Welfare

Abnormal behaviors have been used as indicators of poor welfare. There are several abnormal behaviors that can signal to the observer that an animal is experiencing suffering. For example cribbing, weaving, and box walking are abnormal 'stereotypical' behaviors in horses that are associated with the confinement in individual stalls. Stereotypies are fixed sequences of behavior performed repetitively with no obvious function. Stereotypical behaviors are seen in most species and include such things as bar biting in sows and excessive grooming in dogs and cats. Stereotypies indicate that animals are highly motivated to perform a particular behavior, but the opportunity to perform this behavior has been denied to them.

Vacuum activities are another example of natural behaviors that are performed when animals are stressed (e.g., dust-bathing in hens and nest-building in sows that are performed in environments without the appropriate stimuli – dust for hens and straw for sows).

What Is Good Welfare

Duncan introduced the notion that animal welfare (be it good or bad) is what an animal is feeling (mental state). This concept is important because it focuses the debate from the external, physical contributors to welfare including how animals are bred, agricultural practices and management practices that affect disease, injury, and incapacity to the internal, the mental state of sentience; but how can one determine what an animal is feeling?

Using Animal Behavior to Explore Animal Welfare

Through various techniques, researchers have been able to draw conclusions about the relationship between what an animal is experiencing and its environment. Rushen notes that mental states of animals can be better understood by using aversion techniques to measure how an animal is feeling in a particular situation. Rushen looked at various handling and restraint methods of sheep and was able to show that sheep that were treated roughly when passed through a chute were very reluctant (averse) to returning. The difficulty with using aversion techniques to study the mental state of animals is that the results will be affected by the learning abilities of the animals.

Operant methods allow researchers to study how the consequences of a behavior affect future behavior. During operant testing, a particular behavior of an animal is selected – for example, the key-peck of a pigeon or a bar-press by a laboratory rat. Operant technology has been used to obtain information related to preference for prepared feeds, flavors, and on animals' abilities to smell and hear.

Other behavior research involves observation, with no manipulation of animals. Weary and Fraser explored the use of various signals – any feature of an animal or its behavior shaped by natural selection to influence the behavior of other animals – to calculate how animals were reacting to their environment. Weary and Fraser have focused on acoustic signals, as does Grandin in her research on the relationship between vocalization and animal handling during slaughter.

Dawkins asked animals how much they would 'spend' in terms of energy, time, etc., to perform certain behaviors. The cost that an animal is willing to pay for the opportunity to perform different behaviors can tell researchers which behaviors are more important to the animal. Dawkins found that the manipulation of time-budgets (the daily routines of wild animals and the amount of time they devote to different activities) provided the best measure of animal's motivation.

More recent research is combining the measurements from mind, body, and nature to draw conclusions. Bokkers used observation and slaughter data to uncover the suitability of three different environments for veal calves. Zulkifli measured the effects of regular visual contact with human beings on fear, stress, antibody, and growth responses on broiler chickens.

Animal Welfare Legislation and Standards

Laws are created in response to changing attitudes in society. A change in societal attitude comes first, the law follows. Good laws are intuitive, objective, and easy to communicate and can be used to modify human behavior to adapt to societal norms. Until the 1960s anticruelty legislation, transport, and slaughter were the only areas where the welfare of animals was protected.

There have been many changes in international standards and laws related to animal welfare in the last 20 years. The OIE has outlined standards for animal welfare and many countries have reviewed and updated their legislation recently. In some countries, the laws related to animal welfare are prescriptive (e.g., some countries define the required minimum current and voltage to stun animals), whereas other countries define the outcome that needs to be achieved (i.e., that the animals are effectively and immediately rendered unconscious during stunning and do not regain sensibility before death).

Germany's lower house of parliament recently voted in 2002 'to give animals constitutional rights.' The amendment will add the words 'and animals' to a clause in the German Basic Law or constitution that obliges the state to respect and protect the dignity of 'life.'

The Swiss Animal Welfare Act was passed in 1978. The essence of the five freedoms is noticeable in the principles of that Act, although the Act takes the protection of animal welfare much farther:

1. "Animals shall be treated in the manner which best accords with their needs."
2. "Anyone who is concerned with animals shall, in so far as circumstances permit, safeguard their welfare."
3. "No one shall unjustifiably expose animals to pain, suffering, physical injury or fear."

In 1981, legislation was adopted in Switzerland that prescribed that all housing system manufacturers had to submit their designs for preapproval before systems could be marketed. They chose to test proposed animal housing systems

using veterinary, Physiological, and behavioral measurements. Article 5 of the Swiss Animal Welfare Act states that:

> mass-produced housing systems and installations for the keeping of animals for purposes of profit may not be advertised and sold without prior authorisation from a service designated by the Federal Council. Authorisation shall only be granted if such systems and installations provide proper living conditions for animals.

More recently, consumer concerns about animal welfare has contributed to the development of legislation and voluntary industry standards designed to improve food animal welfare. For example, space allowances for production and prescriptive handling and transporting conditions have been legislated in the European Union (EU). Conventional battery cages and gestation stalls for sows are no longer permitted in the EU countries. The Royal Society for Prevention of Cruelty to Animals' 'Freedom Food Program' is an example of animal welfare schemes. The Freedom Foods seal is used by major supermarket chains in the UK to assure consumers that the meat, eggs, and dairy products are from animals reared under specified welfare conditions.

Practical Assessment of Animal Welfare

All segments of the livestock industry, from producers through to retailers, are becoming increasingly interested in animal welfare and some meaningful assessment of an animal's welfare status. Producers position themselves as knowledgeable and rational actors while they dismiss the concerns of the lay person as emotional and uniformed. The public, however, associates industry's interests in animal welfare as economic and profit oriented. The public believes their questioning the methods used in livestock production is ethically based. Spedding advises the industry to address these public concerns if they wish to ensure the sustainability of their industry.

If animal welfare science is to be of any practical use to animals, it must be applicable in the real world. The increased interest in animal welfare in livestock production in Western Society has been driven in part by the fact that the food supply has exceeded demand, which allows the development of demand-driven economies where consumers' needs and preferences have an impact. Animal welfare and acceptable production methods emerge as issues. Economic realities of animal agriculture have created a need for on-farm assurance programs, such as biosecurity and food safety. The knowledge built by animal welfare science can be used in the development of welfare-based quality control procedures. There is a need for cost effective, effective protocols to assess the conditions of good husbandry and for welfare assurance programs that consumers can trust and can use to make decisions about welfare.

There are three methods that can be used to assess animal welfare on-farm: resource-based, management-based, and animal-based measures. The first two measures have several common advantages, the most important being that these types of measures are objective and highly repeatable. Animal-based measures require more training before assessors can reliably use them. Grandin developed a basic 'yes' or 'no' scoring system to eliminate the confounding human factor. Grandin based her scoring system of handling in slaughter plants on one parameter: vocalization. She tabulated the vocalizations of cattle in the forcing pen and stunning box at six commercial slaughter plants ($n=1125$) and determined that a large percentage of vocalizations were the result of the use of excessive force (electric prods) by plant employees. When the use of prods was prohibited, vocalizations dropped dramatically. Therefore, by auditing the vocalization levels in slaughter plants, animal welfare can be assessed. Grandin has reported that the audit system has improved handling practices at some major US plants. Improvements are due to the simplicity of the system (i.e., a yes or no score) and because 'you manage what you measure.'

The type of measure used to assess animal welfare often, though not always, is dependent on the goals of the assessment. Mollenhurst demonstrated that resource-based measures are valid and sensitive enough to show differences in hen welfare between housing systems but not differences in hen welfare within systems.

In 2006, the European Union announced an Action Plan on the Protection and Welfare of Animals. One of the key features of this community Action Plan was the development of an EU-wide animal welfare label. In October 2009, this labeling program, Welfare Quality®, was unveiled. The program assesses animal welfare from farm to fork using animal-based measures – animal-based measures are valued by consumers because they actually assess the animals. The four welfare principles and the 12 welfare criteria of the Welfare Quality® project are listed below:

Welfare principles	Welfare criteria
Good feeding	1 Absence of prolonged hunger
	2 Absence of prolonged thirst
Good housing	3 Comfort around resting
	4 Thermal comfort
	5 Ease of movement
Good health	6 Absence of injuries
	7 Absence of disease
	8 Absence of pain induced by management procedures
Appropriate behavior	9 Expression of social behaviors
	10 Expression of other behaviors
	11 Good human–animal relationship
	12 Positive emotional state

See also: Environmental Impact of Meat Production: Primary Production/Meat and the Environment. Meat, Animal, Poultry and Fish Production and Management: Exotic and other Species; Poultry. Preslaughter Handling: Behavior of Cattle, Pigs, Sheep, Bison, and Deer during Handling and Transport; Design of Stockyards, Lairages, Corrals, Races, Chutes, and Loading Ramps; Preslaughter Handling; Welfare Including Housing Conditions. Quality Management: Farm Level: Pork Quality; Farm Level: Safety and Quality of Beef. Slaughter, Ethics, and the Law.

Species of Meat Animals: Cattle; Game and Exotic Animals; Pigs; Poultry; Sheep and Goats. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning; Slaughter: Immobilization

Further Reading

Anonymous, 2001. Scientists' assessment of the impact of housing and management on animal welfare. Journal of Applied Animal Welfare Science 4, 3–52.

Appleby, M., 1999. What Should We Do about Animal Welfare? Oxford: Blackwell Science, pp. 36–37.

Appleby, M.C., 1997. Life in a variable world: Behaviour, welfare and environmental design. Journal of Applied Animal Welfare Science 54, 1–19.

Bath, G.F., 1998. Management of pain in production animals. Applied Animal Behaviour Science 59, 147–156.

Bekoff, M., Gruen, L., Townsend, S.E., Rollin, B., 1992. Animals in science: Some areas revisted. Animal Behaviour 44, 473–484.

Bokkers, E.A.M., Koene, P., 2001. Activity, oral behaviour and slaughter data as welfare indicators in veal calves: A comparison of three housing systems. Applied Animal Behaviour Science 75, 1–15.

Broom, D.M., 2001. Coping, stress and welfare. In: Broom, D.M. (Ed.), Coping with Challenge: Welfare in Animals Including Humans. Berlin, Germany: Dahlem University Press, pp. 1–9.

Brumbaugh, R.S., 1978. Of man, animals and morals: A brief history. In: Morris, R. K., Fox, M.W. (Eds.), On the Fifth Day. Washington, DC: Acropolis Books.

Dawkins, M.S., 1985. The scientific basis for assessing suffering in animals. Defence of Animals. Oxford, UK: Basil Blackwell Publisher Ltd.

Dawkins, M.S., 1988. Behavioural deprivation: A central problem in animal welfare. Applied Animal Behaviour Science 20, 209–225.

Dawkins, M.S., 1993. Through our eyes only? In: Livestock, Ethics and Quality of Life. Oxford, UK; Cambridge, UK: W.H. Freeman and Company Limited; University Press.

Duncan, I.J.H., 1993. Welfare is to do with what animals feel. Journal of Agricultural and Environmental Ethics 6, 8–14.

Duncan, J.H., Fraser, D., 1997. Understanding animal welfare. In: Appleby, M.C., Hughes, B.O. (Eds.), Animal Welfare. New York, NY: CAB International.

Fraser, A.F., 1988. Animal suffering: The appraisal and control of depression and distress in livestock. Applied Animal Behaviour Science 20, 127–133.

Fraser, D., 2008. Understanding Animal Welfare: The Science in its Cultural Context. Oxford: Blackwell Publications (now UK: Wiley-Blackwell).

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N., 1997. A scientific conception of animal welfare that reflects ethical concerns. Animal Welfare 6, 187–205.

Grandin, T., 2000. Effect of animal welfare audits of slaughter plants by a major fast food company on cattle handling and stunning practices. Journal of the American Veterinary 216, 848–851.

Kendall, H., Laboa, L., Sharp, J., 2006. Public concern with animal wellbeing: Place, social structural location and individual experience. Rural Sociology 71 (3), 399–428.

Main, D.C.J., Kent, J.P., Wemelsfelder, F., Ofner, E., Tuyttens, F.A.M., 2003. Applications for methods of on-farm welfare assessment. Animal Welfare 12 (4), 523–528.

McBride, G., 1984. Feral animal studies in animal science: The uses and limitations of feral animal studies on contemporary animal science. Journal of Animal Science 58, 474–481.

Mellor, D., Patterson-Kane, E., Stafford, K., 2009. The Sciences of Animal Welfare. UFAW Animal Welfare Series. John Wiley & Sons: UK.

Molony, V., Kent, J.E., 1997. Assessment of acute pain in farm animals using behavioural and physiological measurements. Journal of Animal Science 75, 266–272. Written Paper of Presentation Given by V. Molony at 87th Annual meeting of the American Society of Animal Science, Florida, USA, July 1995 (Abstract).

Regan, T., 1983. The Case for Animal Rights. Berkely, LA: University of California Press.

Regan, T., 2001. Defending Animal Rights. Urbana and Chicago: University of Illinois Press. ISBN 0-252-02611-X.

Rollin, B.E., 1992. Animal Rights and Human Morality. Canada: Prometheus Books.

Rushen, J., dePassillé, A.M., Muhnksgard, L., 1999. Domestic animals fear of humans and its effect on their welfare. Applied Animal Behaviour Science 65, 285–303.

Seamer, J.H., 1998. Human stewardship and animal welfare. Applied Animal Behaviour Science 59, 201–205.

Short, C.E., 1998. Fundamentals of pain perception in animals. Applied Animal Behaviour Science 59, 125–133.

Singer, P., 1989. All animals are equal. In: Tom Regan, T., Singer, P. (Eds.), Animal Rights and Human Obligations, second ed. New Jersey: Prentice Hall, pp. 148–162.

Spedding, C.R.W., 1995. Sustainability in animal production systems. Animal Science 61, 1–8.

Steinbock, B., 1978. Speciesism and the Idea of Equality. Albany, NY: University of New York.

Stull, C.L., Reed, B.A., Berry, S.L., 2005. A comparison of three animal welfare assessment programs on California dairies. Journal of Dairy Science 88 (4), 1595–1600.

Zulkifi, I., Gilbert, J., Liew, P.K., Ginsos, J., 2002. The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. Applied Animal Behaviour Science 79, 103–112.

Relevant Website

http://www.fawc.org.uk/fawc-index.htm
Farm Animal Welfare Council.

PROCESSING EQUIPMENT

Contents

Battering and Breading Equipment

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Introduction

Overview

Applying coatings to food substrates has been a common practice for centuries. Most foods are coated just before pan-frying to add texture and flavor. Some of the most popular items in the market at present include chicken nuggets, fish sticks, and cheese sticks, all of which come in various sizes and shapes and are found on menus around the world. They are produced on high-volume fully/semiautomated lines, which basically mimic the traditional hand-coating procedures. Manual coating is still done at home and at some restaurants, but it is too slow, inefficient, and inconsistent for today's high-volume production runs. Battering and breading equipment was first introduced in the 1950s and started the trend of producing low-cost, uniform, and high-quality coated products on dedicated production lines (Figure 1). At present, coated meat, vegetable, and cheese products are very popular throughout the world. This article reviews the range of coating equipment, coating materials, and coating operations found throughout the food industry in the beginning of the twenty-first century.

Why Substrates Are Coated?

Commercial battered and breaded products are considered as convenience foods, which require minimal preparation on the part of the consumer or restaurant. Consumers today expect more and better attributes in the food they purchase. They want food safety, value for their money, and certain preferred sensory properties (texture, taste, color, and smell). Processors have learned to cultivate preferences in their customer base. Processors also extend the yield of their substrates by adding coatings, and can enjoy economic gain by producing differentiated, value-added products.

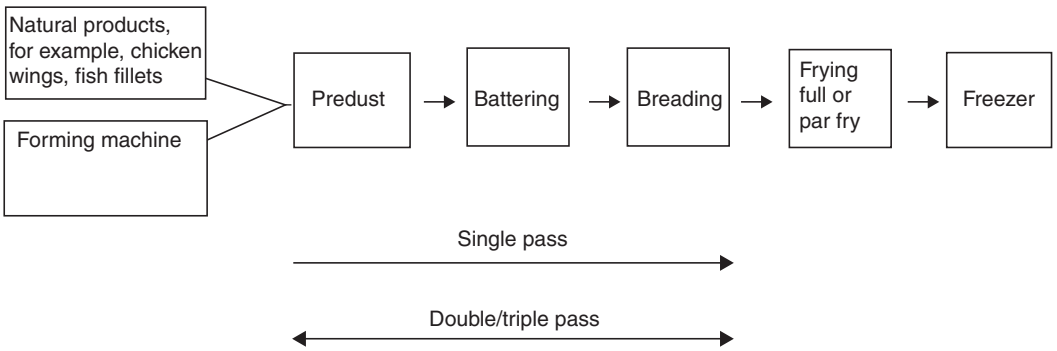

Figure 1 A schematic diagram of a dedicated battering and breading production line for making uniform shaped products such as chicken/pork nuggets, fish sticks, or irregular-shaped products such as coated drumsticks and cordon bleu.

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Early History and Process Goals

Some of the early pioneering work on coating equipment was carried out in the early 1950s at Syracuse University by Dick Johnson under the guidance of Professor John Hart. Johnson developed a prototype breading applicator for Jean's Beans to use in their outlet stores. As Johnson approached graduation, he was contacted by Sam Stein to bring that breading equipment concept to be used by Grill Meats. That venture proved successful, and in 1953 Sam Stein associates was established to continue the development and marketing of coating equipment. At approximately the same time, the Best Products Company in Chicago, IL, USA, developed a breading machine that claimed to perform the work of 17 people applying coatings by manual methods (Figure 2). Equipment manufacturers and the coating material manufacturers have maintained a mutually supportive relationship with one another throughout their histories. A case in point involves Japanese crumb, a breading that is composed of relatively large, crust-free bread crumbs. This delicate product breaks down quickly in traditional breading machines. New breading applicator designs were needed to successfully bring these crumbs to market. Of course, these new designs would have no demand if these crumbs were not available. So what came first – the new style breading or the new style breading machine? The equipment manufacturers worked closely with the coating suppliers to deliver the total processing solution and thereby ensure that the food processor would have the equipment needed to apply these new Japanese crumbs. Such equipment is now in commonplace.

Processors seek to maintain consistency from one piece of product to the next and from one day to the next. They want to add the targeted amount of coating materials onto each piece of product. They also want to achieve complete coverage, with no voids or bare spots. They demand consistent color and highlights. Their goal is to achieve a product that satisfies consumer demand. They then seek to continue producing identical product. This must all be accomplished in a coating system that provides economic gain for the processor.

Examples of Commonly Coated Substrates

Poultry	Boneless breast fillets, patties, nuggets; bone-in eight cuts (i.e., special splitting of the carcass for fast food vendors), wings, drums
Fish and seafood	Fish sticks, patties, shrimp, fillets, scallops
Red meat	Nuggets, cutlets
Vegetables	Onion rings; mushrooms (poppers), zucchini, French fries
Dairy	Cheese sticks
Combination	Cordon bleu, Chicken Kiev, poppers (breaded mushrooms)

Common Terms

- Batter (n): a suspension of dry solids in liquid that forms either the complete outer coating for a substrate or the binding layer between the food base and the subsequent layer(s) of breading.
- Batter (v): the act of transporting a substrate through a puddle of batter material or carrying the substrate through curtain(s) of the batter material.

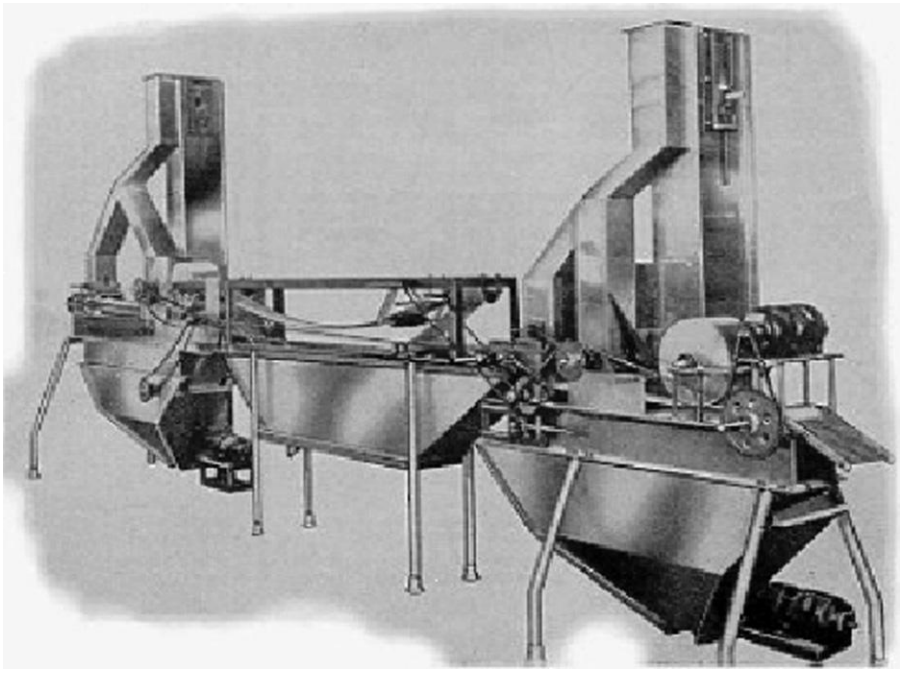

Figure 2 An original 1953 machine from Best Products Co. Chicago, which was claimed to coat shrimp, fish, and chicken up to 17 times faster than hand breading methods.

- Breading (n): a cereal-based, generally thermally processed, ground coating that is seasoned or unseasoned. Breadings are typically applied to moistened or battered food substrates to influence their flavor, color, texture, and appearance.
- Breading (v): the process of applying coating material to a moistened substrate.
- Home style breading: resemble a type of crumb, consumers can prepare at home. The crumb has a distinct crust and provides good highlighting during the frying operation.
- Japanese-style bread crumb (J-crumb): an elongated, typically crust-free crumb with outstanding textural and appearance properties. Generally used as an outside coating in full fry, oven, and microwave applications.
- Pass: the combination of a batter step followed by a breading step. Some products require a double-pass, whereas cheese sticks, for example, commonly require a triple-pass to ensure coating integrity.
- Pickup: a measure of the amount of material added to a product in a given step or in a series of steps, expressed as a percentage of the final coated weight. Pickup percentage is defined as added weight divided by final weight.
- Predust: when the first step in a coating sequence is the application of a dry coating, this is referred to as predusting. This step can improve adhesion and pickup, and also provides a place to bury flavors to protect them from degradation during frying.
- Substrate: the food material that is being coated. Common substrates are poultry, fish, seafood, meat, vegetables, cheeses, and fruit.
- Tempura (puff batter): leavened batters, used as an outer coating.

Coating Materials

Batters

Batters are made of blends of wheat flour, corn flour, starch, gums, browning agents, proteins, flavorings, and leavening agents. They can be divided into the following:

- Adhesion batters, which act as a 'glue' to bind subsequent layers to the substrate.
- Cohesion batters, which form an envelope, or shell, around the food product.
- Tempura batters, which are generally leavened cohesion batters used as the puffed coating.

Breadings

Breadings comprise a range of products from fine flour to granular coatings, including delicate Japanese crumbs. They have various colors, browning characteristics, absorption abilities, and textures. Spices and other ingredients are frequently added to customize taste and performance. Breadings can be divided into four main groups:

- Basic flour.
- Home style/American bread crumb.
- Traditional/cracker type crumb.
- Japanese-style crumb.

The Role of Frying in Coating Systems

Although a few products are chilled or frozen immediately after coating, the majority of coated products are parfried or fully fried before freezing. Two realities support this decision. First, the mechanical strength of the coating is increased by frying, and the crumbs adhere better during handling, transportation (e.g., vibrations on a moving truck), and preparation, as a result of frying. Second, as much as consumers may claim to avoid fried foods, the cooking oil absorbed during frying plays a major role in defining the highly desirable taste profile and the texture, including crispiness characteristics of coated food products. Unfried items that are reconstituted without frying, such as in an oven, can be sticky in the mouth, and less palatable.

Common Line Configurations

Natural and Formed Products

The first step in considering batter and breading applications is to decide about the food product to be coated. This can include a natural cut such as a piece of cauliflower, bone-in chicken wing, boneless pork Schnitzel, fish fillet, or a formed product such as a nugget, a meat patty, or a cheese stick; all of which can come in different sizes and shapes. Each category has its own challenges when it comes to coating. For example, a chicken wing/drum does not have even surfaces, therefore achieving a uniform dry coating layer is not possible by using a conventional belt type preduster. In such a case one needs a drum type preduster, as discussed below.

Forming machines, which are used to produce nuggets and patties can be divided into high- and low-pressure formers. Both machines mimic what people will do with their hands when forming a hamburger patty. However, a machine with multiple cavities can produce hundreds of identical patties every minute. The traditional approach uses a high-pressure former, where meat is pumped into the cavities (usually cut into a metal/plastic plate) and then the patties are 'punched out' by plungers with surfaces very similar to the cavity's shape. The patties are discharged onto a conveyor belt that takes them to the first predusting operation. Low-pressure formers have recently been developed in which cavities are filled with vegetable puree (spinach), meat, or their mixtures, and then gently pushed out by a stream of air from behind. This is typically done using a drum configuration (Figure 3(b)) where porous metal is used to allow air flow over the entire patty area inside the cavity. The advantage of using this technology is the reduced pressure applied to the patty, which reduces the quantity of water squeezed out of the product (i.e., lean meat is 75% water and most vegetables are 95% water) and the ability to form detailed 3D structures.

Nonleavened Systems

The most common coating configuration is a single pass with a preduster followed by battering and breading (Figure 1), which consists of dry–wet–dry applications. The predust

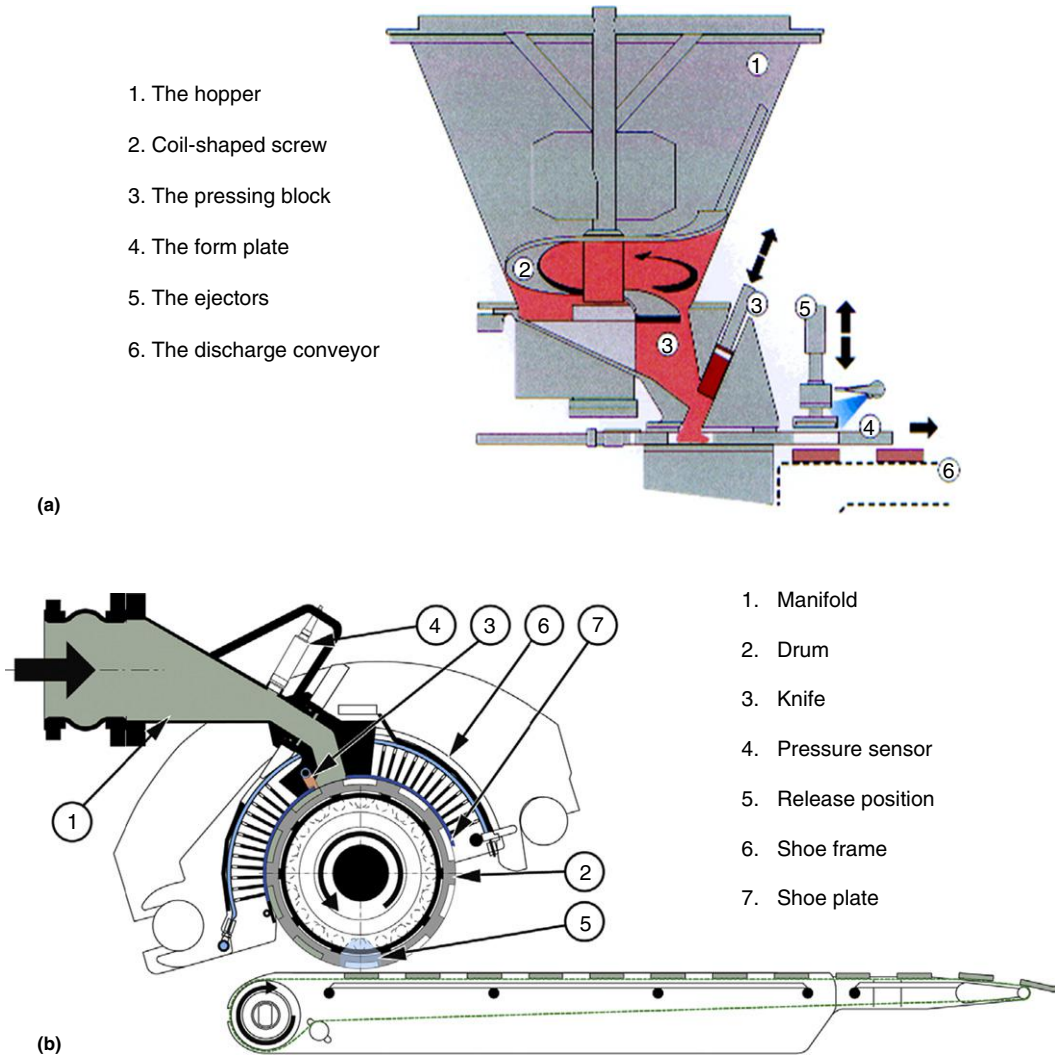

Figure 3 Forming equipment: (a) high-pressure; (b) low-pressure. Reproduced with permission from Marel and Townsend.

material is usually a flour-type coating, sometimes a dry batter mix. Figure 4(a) shows a general configuration of the equipment used to apply this coating. As illustrated, the product is dropped onto a bed of flour, and is moved toward a curtain of flour falling from above to cover the whole product. Then, the product is moved under adjustable pressure rollers that compress the flour onto the product. This is followed by air knives, which blow off excess flour while the product is transferred to a wire belt. When dealing with an uneven product such as chicken wing, a drum applicator (Figure 4(b)) is used, where the product is tumbled for a few revolutions while covered with predust material. The flour that is not picked up by the product is recirculated. This is followed by a batter applicator that in turn feeds the substrate into the final breading applicator as will be discussed in Section Batter Mixers. Many products use a two-pass system to achieve greater pickup levels. Cheese sticks, for example, commonly require a triple-pass arrangement to ensure that the substrate is thoroughly coated and will not leak cheese during the frying phase.

Leavened Systems

Various machine arrangements are included in the category of leavened systems. They all have the application of the wet leavened batter in common as the last step before frying.

There are special challenges in setting up and running these systems. For example, the leavened batter is often viscous, much like pancake batter. It clings to the flat flex belting moving through the puddle in the applicator, and then slowly drains from the belt. This characteristic can limit the system's ability to run properly at higher speeds where batter remains on the belt at the discharge zone near the fryer. Even if the submerger-style batter applicator runs acceptably at a certain speed, it may fail to run at a higher speed because the batter will tend to 'walk' out of the applicator as its available drain time from the belt has been reduced. In addition, the transfer of coated product into the fryer can be another problematic and related issue. Various schemes utilizing wiper bars, 'star' rollers, or 'porcupine' rollers are employed to carry the product the last few centimeters in the tempura applicator and then

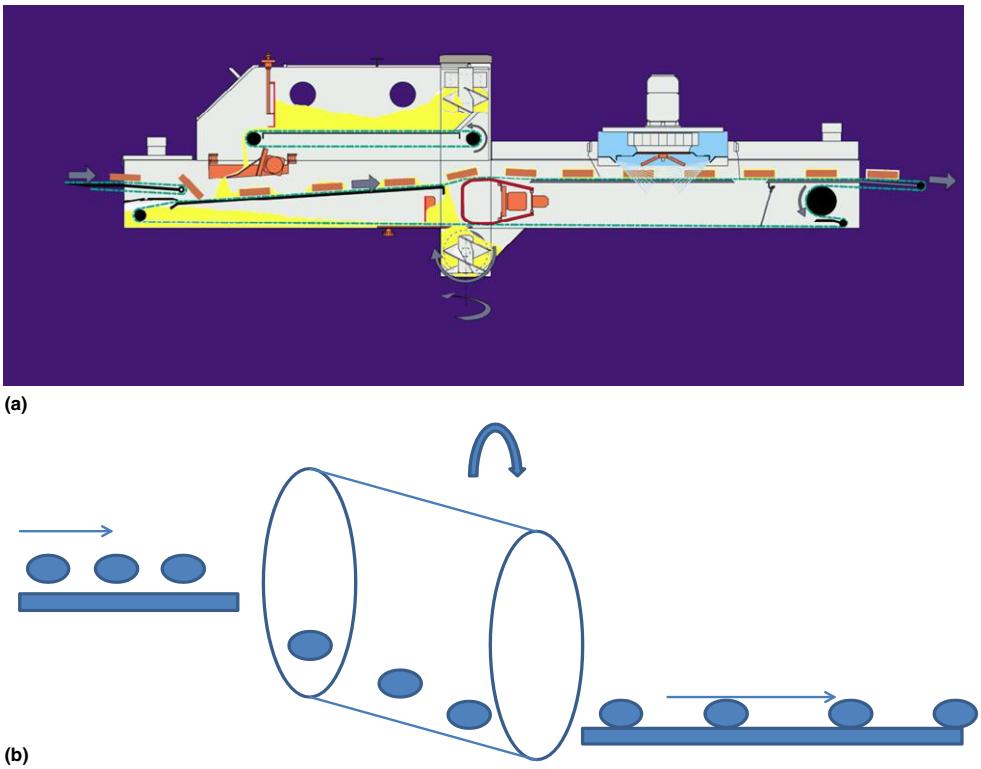

Figure 4 Predust equipment: (a) flatbed and (b) drum. Flatbed – Reproduced with permission from Townsend Further Processing.

to carefully release the coated substrate to drop into the hot oil. The heat coming from the nearby frying oil slowly cooks some of the excess batter to the applicators' components that are hanging over the frying zone, thus necessitating periodic stoppages for manual removal of crusted batter buildup. The goal is to transfer the undamaged product into the hot frying oil, letting it settle for a few seconds on a special Teflon slat conveyor to 'skin' over. After a few seconds, the coated product expands, and then floats off the bottom conveyor in the fryer and floats up against the underside of the hold-down conveyor.

Types of Coating Equipment

Batter Applicators

There are two principal modes of applying batter to a substrate: overflow and submerger.

The 'overflow' method has the batter continually pumped to maintain a curtain of batter falling on the product passing beneath (Figure 5). This system is usually used for nonleavened batters and commonly employs two or four curtains of batter. The product paths through these applicators are fairly flat, making them the preferred choice for high-speed coating lines. Note that the batter is continually being pumped, thus exposing the batter to shearing action and making these machines unsuitable for leavened batters.

The second method is the 'submerger' approach, wherein the substrate is conveyed down into and then up out of a puddle of batter. This is the viable choice for a leavened batter because the batter is not subjected to excessive pumping and shearing, actions that can drive the gases from the batter and render it flat. Some circulation, however, may be required when the submerger method is used for nonleavened batters that are subject to a settling out of their solids. The submerger method is very effective in delivering batter to all surfaces of even complex shapes, making it ideal for products such as bone-in poultry parts and shrimps.

Batter viscosity and batter temperature each play a key role in determining coating line performance. Batter viscosity relates directly to coating pickup whereas batter temperature impacts issues of food safety as well as the required volume of dry batter mix. A given level of batter viscosity can be maintained with less dry mix at lower temperatures than at warmer temperatures. Some batter machines feature cooling coils to help maintain cool batter temperatures, but viscosity can vary when batter is supplied incrementally as is the case in smaller capacity systems. As the product passes through the batter applicator, some of the coating particles may wash off the product and serve as a thickening agent in the batter pool, so monitoring and adjusting viscosity is very important.

High-volume coating lines normally employ batter mixers that continually mix new material, adjust viscosity, and circulate the batter between the mixer and the batter applicator. While the batter is in the mixer it can be chilled as needed, and it can be thickened or thinned to maintain target viscosity levels. The batter is sent to the applicator for a short period before it returns again to the mixer for reconditioning. This batter management system is intended for nonleavened batters only.

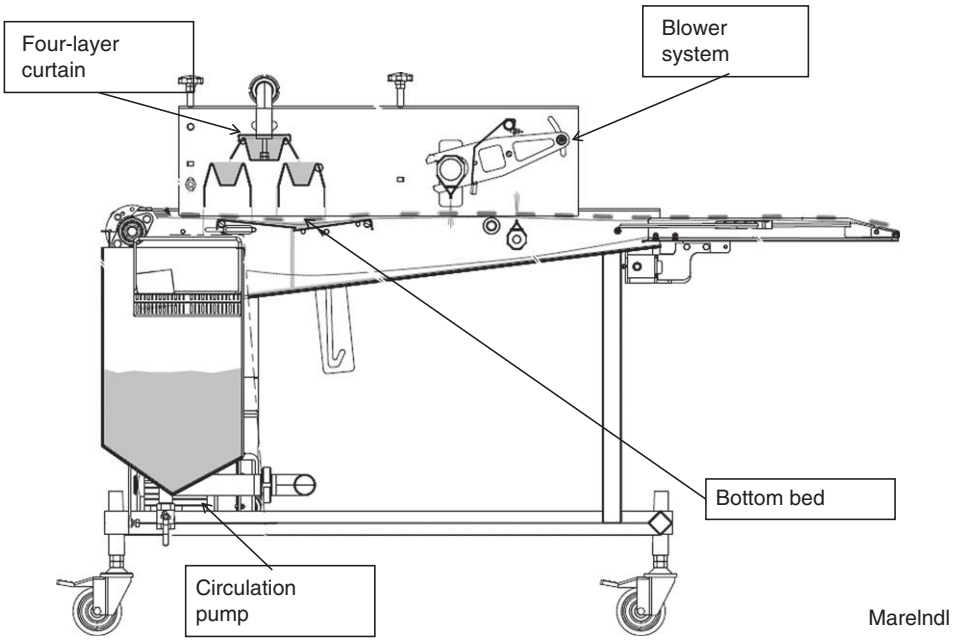

Figure 5 Equipment to apply batter to food products using the overflow configuration. Reproduced with permission from Marel and Townsend.

Batter Mixers

The choice of batter mixer reflects the volume of batter required per hour and the type of batter being produced. Mixers for leavened batters are specialized in that they blend very gently. They mix the dry batter material with water in relatively small quantities, for a short time, and then pump the batter to the applicator. There is no recirculation of the batter after it leaves the mixer. The batter must be used in a short time (minutes after mixing) or it will go flat. Conventional batter mixers for nonleavened products range from simple, low-volume machines to moderate-volume mixers, up to 1000 kg h^{-1} automated mixers that are coupled to the batter applicators and maintain a preset level of temperature and viscosity.

Breading Applicators

Breading can range from very fine particles (flour) to relatively large and brittle bread crumbs (Japanese crumbs). It is interesting to note that with the current economic situation, flour is becoming a popular coating material in places like North America. Overall, the breading material provides a unique texture to the coated product and can also be used to carry spices and flavorings to the product. A typical schematic diagram of a breading machine is shown in Figure 6. The battered product, which is wet on the surface is dropped onto a bed of dry breading material while more breading is sprinkled from the top. This is followed by pressure rollers, which help to push the material onto the product. This configuration provides a challenge for the breading applicator, however, in that breading/flour does not flow well and can easily clog and jam the equipment. Properly designed applicators use powered methods (augers or belting) to move the flour/breading through the machine. Augers, for example, move the flour laterally to feed other augers that lift the flour for returning to the main hopper. Flat flex belting is used to drag the flour out of the hopper and onto the product. This same belting also carries unused flour back to the start of the auger (Figure 6).

Japanese-style crumbs are delicate and cannot withstand the abuse of being powered through their applicators. These crumbs quickly break down in the equipment that uses excessive forces to move the crumb through the machine. Gravity is employed in place of power to move the delicate Japanese-style crumbs. The hopper has sloping surfaces and sifting screens to guide and sort the material wherever possible. Some mechanical powering is still required, however. Minimal use of power in the machine extends the crumb life so that crumbs survive long enough to be applied to the substrate without breaking down significantly.

Some substrates are difficult to lay out on the processing belt and are also difficult to transfer from one machine to the next unless they have picked up some coating material and become less sticky. Chicken tenders and clam strips are two good examples. For these products, a drum breader (Figure 4(b)) allows coating to adhere to the product surface while the product tumbles in breading material. Four to six rotations of the tumbler usually suffice for coating. Unless spreading conveyors are used after the drum breader, the processor can expect to have to assign people to spread the product across the belt, because the product stream emerges from the drum breader in a narrow path.

There are different variations in coating machines that can help to create different effects. One of these is the triple flip at the end of the breading machine, which is used to create a random texture on the surface giving what is referred to as a 'home style' look. This example can be used to understand what happens within a breading applicator. The product is loaded into the machine from the batter applicator. The batter applicator's discharge conveyor reaches into the breader and deposits the product on the lower layer of breading material, which is moving

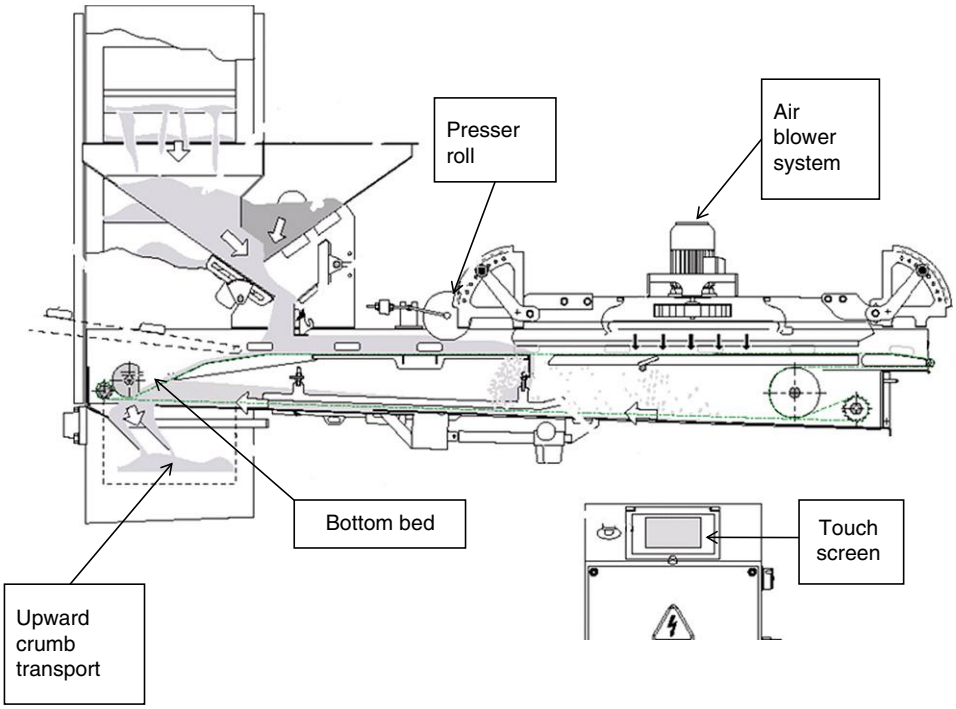

Figure 6 Breading equipment used for different types of crumbs. Reproduced with permission from Marel and Townsend.

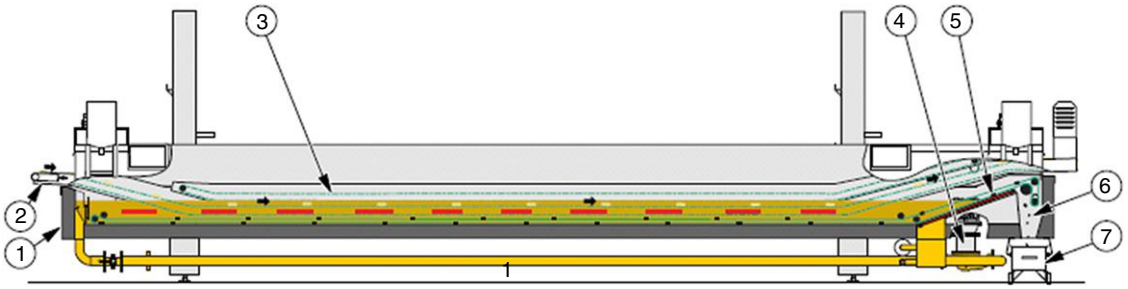

Figure 7 A continuous deep fat fryer, which can be used for a short par fry or fully cooking the product. Reproduced with permission from Marel and Townsend.

with the belting through the machine. Then the top layer of coating material is powered out of the hopper, cascading onto the product as it moves through the machine (Figure 6). The covered product moves under some optional soft rollers to help press and set the coating on the product. The product then travels to the discharge side of the machine, and the excess flour is shaken off as the product is dropped and flipped down through a series of short reversing conveyors, finally discharging from the machine. The product's journey through the flip section normally serves to create the random look of the outside layer.

Frying/Cooking

The coating material on the product at this stage is very soft and prone to deformations if not quickly hardened or 'fixed' to preserve its shape. Most of the products are therefore fried to 'cement' the texture on the surface. The frying operation can vary between flash/parfry (a 30–90 s immersion in 180–190 °C oil) and full fry in oil (time depends on product thickness). At this point the parfried product can also go into a forced air oven to complete the cooking. Most of the time this process is followed by freezing, and in the case of parfry, final cooking is done by the consumer/restaurant. An example of a continuous linear fryer is seen in Figure 7, where the product usually travels on a Teflon belt or a wire mesh belt so it moves at a constant speed (i.e., spends only the required time inside the fryer). An oven (Figure 8) can be used to finish the cook cycle and still provide fried product characteristics. After the initial parfry, the core temperature is still close to 0 °C (i.e., working with cold meat to minimize microbial growth and

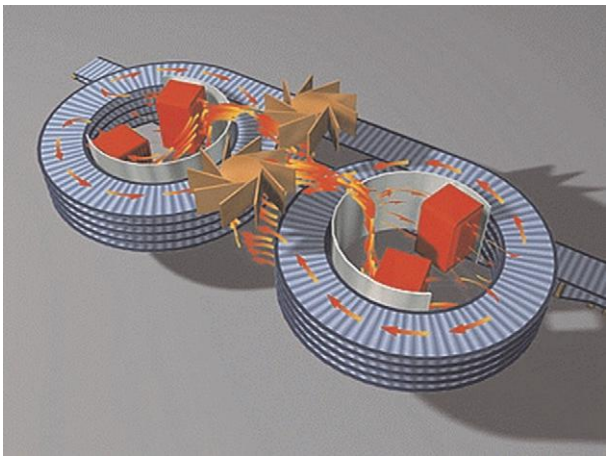

Figure 8 Forced air continuous oven with two separate cooking zones/towers. Reproduced with permission from Marel and Townsend.

maintain texture). Using a forced air oven allows complete cooking without absorbing any additional oil. Currently, there are various oven designs available in the market, mainly linear and spiral ovens (Figure 7), which allow temperature control in different zones. Some also include impingement at the end of the belt to provide a short, high-temperature exposure.

Conveyors

Conveyors may be found in a coating line in any of three places. First, at the beginning, conveyors deliver the product to the first coating applicator. Loading conveyors are used after forming machines and slicers, and are also used in hand-loading operations. Second, conveyors may be located within the line to provide the product some soak (hydration) time between processing steps when this will improve the coating's performance. Third, fryer-feed conveyors are used after the last breading applicator but before the fryer in virtually all nonleavened applications. These conveyors allow for some belt speed adjustments and also allow for some of the loose crumbs to fall free of the product before it goes into the frying oil.

Common Design Goals

The design engineer is charged with delivering a machine that meets several design goals:

- It must support food safety efforts by being easily and effectively cleanable, and by not harboring bacteria or foreign materials.
- It must be easily maintained.
- It must work harmoniously with other equipment in the processing line; addressing issues of belt height, belt width, belt transfers, belt speed, and capacity.
- It must be easy for the operator to learn to operate safely without posing hazards to personnel.
- It must be made from durable food-grade materials that can survive the challenging environment of a processing line.
- It must setup, change over, and tear-down quickly.
- It must handle the product gently, causing minimal damage and rejects.
- It must be easily cleaned, sanitized, and inspected.

Measures of Coating Line Performance

The capacity of a processing line is limited by the machine in the line that has the least capacity. Note that a continuous processing line is made up of short conveyor segments, each working harmoniously within the total system. For any uniformly loaded conveyor running at steady speed, its hourly capacity is defined in eqn [1].

$$\text{Capacity (kg h}^{-1}) = \text{BLD (kg m}^{-2}) \times \text{width (m)} \times \text{length (m)} \times [60/\text{time (min)}] \times \text{yield (\%)} \quad [1]$$

where BLD is the belt loading density, time is the process time for that conveyor's length, and yield reflects weight changes within the process segment (fryer, oven, freezer, etc.).

The reject rate measures the fraction of the production stream that is set aside for any reason: physical damage, falling from the conveyor, or failing to meet safety and quality specifications. Some items are rejected on the basis of value judgments by the line operating staff.

Key Control Points in the Performance of a Coating Line

The quality and safety of the final food product produced on a processing line is related to several conditions and settings throughout the line. When these are maintained as specified, the final product will conform to specifications. Several examples are listed below.

- Substrate temperature: the temperature of the substrate entering the processing system needs to remain constant from piece to piece and from time to time throughout the processing shift. Allowing the temperature to fluctuate will lead to variation in batter performance and coating pickup. Temperature variations can also have a dramatic impact on subsequent cooking operations, perhaps leading to undercooked product. Temperature variations near and below the freezing point are especially challenging because latent heating for a phase change of ice to water may be required in addition to sensible heating of the water. The energy required for this latent heating is typically quite large.
- Belt speeds: the components of a processing system are intended to work harmoniously with one another. Belt speeds for each machine should increase gradually across transfers as the product moves through the system. The basic recommendation is that the belt moving away from a transfer should exceed the speed of the belt approaching the transfer by approximately 0.5 m min^{-1}. This serves to maintain or improve alignment and helps to keep the product separated end-to-end.
- Transfer point setups: the product needs to flow smoothly from the belting of one machine onto the belting of

the following machine. Care needs to be taken to prevent the product from dropping through the belt gap and falling to the floor, or from colliding with the next machine's belting and causing edge damage. The ideal arrangement for belting at a transfer point should take into account the product size and shape, the style and pitch of the belting, and the speed of the line. Commonly, the gap between the machines should be 6–9 mm, and the elevation of the take-away belt should be 3–6 mm lower than the feeder belt.

- Batter temperature: there are several notable advantages to colder batters (5–10 °C). First, cold batter denies bacteria the ideal environment for rapid growth. Second, less dry batter mix is needed when making cold batter than when making warm batter of the same viscosity.
- Batter viscosity: viscosity is the term used in this context to describe a batter's ability to flow. It can be measured precisely with laboratory-quality instruments or quick proxy measures can be made in the processing area. The quickest measurements use either Zahn cups or Stein cups. A Stein cup is a clear plastic cup, approximately the size of a soup can, having a precisely sized drain hole in the bottom and a swiveling wire handle. The first step in using a Stein cup is to immerse the cup in the batter. The operator then starts a stopwatch as the cup is lifted up from the pool of batter. The draining action is allowed to continue until there are breaks in the batter stream coming from the hole in the bottom of the cup. At that moment, the stopwatch is stopped. The drain time is recorded and compared with previous readings (control charted) to provide a measure of the current batter viscosity. Pure water drains in approximately 8 s. Most adhesion batters drain in 10 s; cohesion batters in approximately 30 s; some tempura batters in approximately 45 s or more. Some of the thick leavened batters require other instruments to test their viscosities.
- Batter levels: batter levels in submerger-style batter applicators must be monitored and maintained above a minimum level to avoid failure to apply batter to the top of some products. There are gates to maintain puddle depth that function effectively as long as operators maintain adequate reserves in the machine.
- Breading levels: breading levels must be maintained to a prescribed depth to prevent bare spots on the products and to assure side coverage. At present automated gauges can help or a skilled operator needs to learn the pace for adding breading before the levels become too low.
- Hydration times: the coating and the pickup on some products are improved by providing some 'soak' (hydration) time between coating steps. This can be accomplished by inserting conveyor sections that delay the delivery of the product to the next machine.

See also: Chemical and Physical Characteristics of Meat: Water-Holding Capacity. Chemistry and Physics of Comminuted Products: Spices and Flavorings. Processing Equipment: Mixing and Cutting Equipment

Further Reading

Anonymous, 1998. The Complete Guide to Coating Systems. Chicago, IL: Griffith Laboratories.

Barbut, S., 2002. Battering and breading. Poultry Products Processing. New York, NY: CRC Press.

Barbut, S., 2012. Producing Battered and Breaded Meat Products. Meat Processing Technology Series. Champaign, IL: American Meat Science Association.

Kulp, K., Loewe, R. (Eds.), 1990. Batters and Breadings in Food Processing. St. Paul, MN: American Association of Cereal Chemists, pp. 250–260.

Suderman, D.R., Cunningham, F.E., 1983. Batter and Breading Technology. Westport, CT: AVI Publishing Com.

Xue, J., Ngadi, M.O., 2006. Rheological properties of batter systems formulated using different flour combinations. Journal of Food Engineering 77, 334–341.

Relevant Websites

http://www.kerry.com/americas/technology/?taxid=1145
Kerry Foods – Batter and Breading Ingredients.

http://marel.com/meat-processing/systems-and-equipment/beef/food-service/further-processing/coating
Marel BV – Batter and Breading Coating Equipment.

Brine Injectors

RE Rust, Iowa State University, Ames, IA, USA
CL Knipe, Ohio State University, Columbus, OH, USA

Glossary

Accumulator tank A receiver tank for injection solution that is not retained in the meat product and is returned to the system.
Brine A solution of salt and other soluble materials that does not contain curing ingredients.
Curing pickle A solution containing nitrate or nitrite as curing ingredients.
Marinade A solution or suspension of materials primarily for flavoring.
Walking beam A series of mechanical arms that advance the material in repetitive steps.

Introduction

Brine injectors are used to incorporate curing pickle, brine solution, or marinade into meat pieces to affect product color, texture, flavor, meat safety, plus protein, and water-binding abilities. In their simplest form, these may consist of a single 'hypodermic' type needle attached to a syringe, or some type of liquid pump capable of delivering the pickle, brine, or marinade under pressure. Another version might be a 'spray' type hollow needle that has a series of holes along its axis that allow the solution to be distributed throughout the product. Sometimes, these needles are in multiples, resembling fingers on a hand. This configuration enables the operator to distribute the curing pickle, brine, or marinade over a larger area.

A single hypodermic-type needle was once used to inject curing pickle into the femoral artery of hams. This practice of artery injecting has fallen into disuse owing to high labor intensity and the potential for problems with uniformity in solution distribution. Even and consistent distribution of these solutions throughout the muscle pieces should be the ultimate goal; proper injection can maximize the uniformity of the solution distribution. Good slicing yields and uniform cured color are two results of uniform distribution of injected solutions. Color defects, poor slicing yields, plus inconsistent texture, and cooking yields are all results of uneven distribution of injected solutions.

Continuous Injectors

There are two types of continuous injectors, those designed for boneless product and those designed for bone-in product. The bone-in injectors have needles that retract if they encounter a bone (Figure 1). The meat to be injected is carried through the machine on some type of stainless-steel or plastic belt (Figure 2) or on a 'walking beam' arrangement (Figure 3). Either of these conveying systems is designed to stop when the needles are in the product. Normally, the needles stop within approximately 5 mm of the conveyor. This needs to be taken into consideration when placing the product in the injector, because the last few millimeters of product will not receive any pickle or marinade.

When injecting bone-in products, it is necessary to be cognizant of the 'bone shadow' that occurs when the needles strike a bone and the material below the bone does not receive any of the injected liquid. In some cases it is necessary to turn the product over, and essentially double pump it, to eliminate this bone shadow.

Figure 1 A bone-in injector with the cover open to show the multiple-needle injection head.

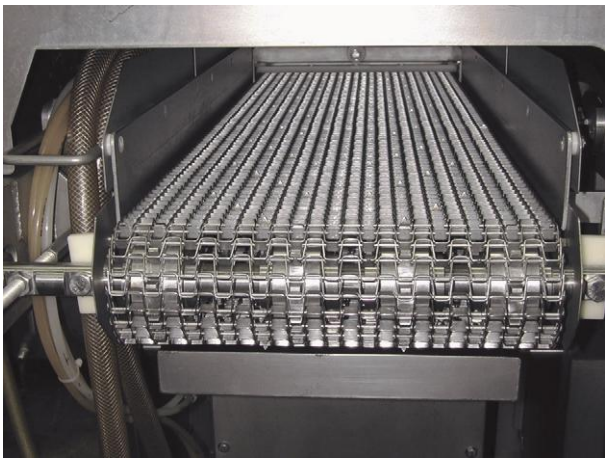

Figure 2 A stainless-steel injector conveyor belt.

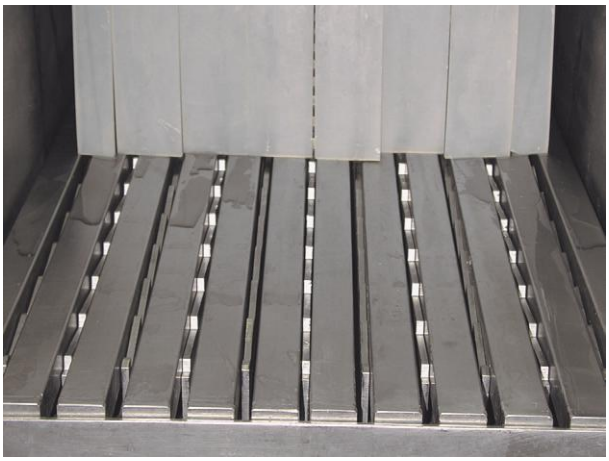

Figure 3 A 'walking beam' injector conveyor.

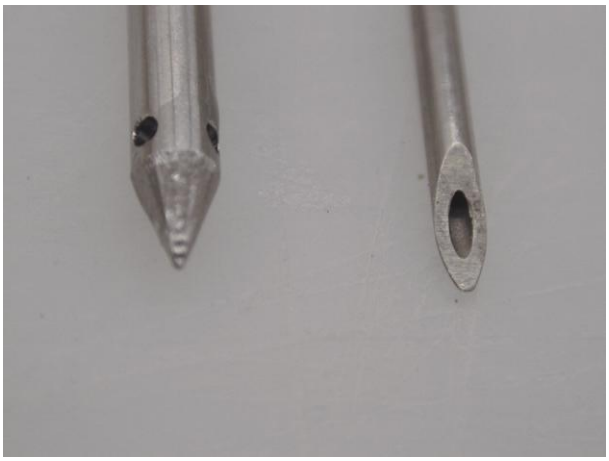

Figure 4 Injector needles. Left, a side-port needle; right, a hypodermic-type needle.

The needles may either be the hypodermic-type or the spray-type needles with side openings (Figure 4). The hypodermic-type needle is widely used in the US for bacon injectors. When using a needle with a bottom opening, care is needed to ensure that cores of fat do not clog the needle. For that reason, together with the fact that the last few millimeters of the product will not receive any injection, it is recommended that meat be placed in the injector fat side down.

Solution Uptake Targets

Meeting injection targets for solution uptake into meat pieces is a result of balancing pump pressure, injection time, and needle design.

Pump Pressure

Balancing injection pressure can be a challenge. Increasing pump pressure has been used in the past as a means to increase injection level. However, injecting at very high pressure may cause quality problems for products. For example, injecting pork bellies at very high pressure will result in solution accumulation in the fat streaks and seams, causing pickle pockets. However, injecting at very low pressure may result in under cured spots because of uneven distribution of the curing solution. It requires some experience in working with the equipment and the product to be injected to determine the optimum pumping pressure.

Injection Time

Injection time involves speed of the conveyor or walking beam, injection method (downstroke versus both down- and upstroke injection), number of passes through an injector or injection heads, injecting on both the up- and downstroke, versus only injecting on the downstroke, etc.

Higher injection levels can be accomplished with lower pump pressures by increasing injection time by methods, such as increasing needle numbers (i.e., closer needle spacing), multiple passes of product through the injector, and reducing the conveyor/walking beam speed. Some injectors are designed with multiple injection heads to simulate multiple passes through an injector. Multiple passes through an injector simulates the effect of closer needle spacing. Other injectors are designed to inject both in the down- and upstroke to increase solution retention. Reducing the speed that meat moves under the injector head will increase the number of times that the meat is injected, allowing for lower pump pressures.

Needle Design

Most injector needles for bacon have holes at the end of the needles. However, needles designed for other whole muscle products have holes along the sides of the needles. Side-port needles direct the solution in multiple directions away from the needle, which improves the solution distribution and retention in larger whole muscle products.

One method of dealing with products requiring a lower level of solution uptake would be to use smaller diameter needles with smaller holes. Larger diameter needles would be used for higher injection level products.

It is advisable to use needles with the smallest possible outside diameters to reduce needle marks in the product being pumped. However, the inside diameter should be sufficient to preclude the need for excessive pumping pressures to deliver the appropriate volume of pickle or marinade.

Pumps

The pickle, brine, or marinade is delivered to the injector needles by some type of pump. Pumping pressure can usually be varied. It is important to maintain the pumping pressure high enough to ensure good distribution, but not so high as to cause 'pickle pockets' to develop in the seams between muscle groups. Most injectors provide some mechanism for bleeding air from the injector head. If air accumulates in the injector, it can cause false pumping pressure readings and result in a lack

Figure 5 An accumulator tank. Note the filter screens and the pickle agitator.

of pumping uniformity. Pumps need to be suited to the type of material being pumped. If the pickle is viscous or contains particulate materials, the pump must have the appropriate capacity.

Accumulator Tanks

Injectors are normally equipped with an accumulator tank (Figure 5) that receives the excess pumping liquid and maintains the supply of liquid for the injector. These tanks have some type of filtration system to keep particles of meat from being recirculated in the pumping liquid. If the material being pumped contains suspended solids, such as soy proteins, it is necessary that the filtration system will not filter these out.

Agitation in the accumulator tank is normally provided by the overflow of the pumping liquid through a bypass from the injector pump. A mechanical agitator may supplement the overflow. Sufficient agitation must be maintained to keep the liquid thoroughly mixed, but it should not be so vigorous as to incorporate air into the pumping liquid.

A sufficient liquid level in the accumulator tank must be maintained to prevent the pump from sucking air when the level drops to near or below the tank outlet. As the accumulator tank can be a source of contamination from materials and microorganisms flushed off the surface of the meat, it is necessary to empty the tank and purge the system periodically. However, the return solution stream can be exposed to ultraviolet light to help reduce microbial contamination.

Product Temperatures

To maximize protein extraction and minimize microbial growth during subsequent processing steps, maximum product temperatures should be 4.4 °C (40 °F) during injection and a more optimal product temperature would be 1.6 °C (35 °F) to maximize protein extraction. However, warmer product temperatures have been shown to result in better uptake and retention of solutions, and in the case of pork bellies higher cooking yields.

Maintenance and Sanitation

Proper maintenance of injectors is critical. A preventive maintenance program should include checks for plugged, broken, or bent needles. All lines leading to and from the pump should be checked to make sure that seals are intact and that air is not being sucked into the brine or marinade. Injectors can provide reservoirs of spoilage and pathogenic microorganisms and a good sanitation program should be part of the daily maintenance schedule and the Sanitation Standard Operating Procedure.

See also: Bacon Production: Bacon; Wiltshire Sides. Curing: Brine Curing of Meat; Production Procedures

Relevant Website

http://meatsci.osu.edu
The Ohio State University.

Mixing and Cutting Equipment

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Glossary

Grinder This term is preferred in the USA when referencing a device using a rotating knife and stationary plate to reduce meat particles. Synonymous with mincer.
Meat emulsions Finely comminuted fat particles disbursed in a salt-soluble protein matrix.
Mincer This term is preferred in Europe and the UK when referencing a device using a rotating knife and stationary plate to reduce meat particles. It is synonymous with grinder.
Salt-soluble proteins Myofibrilar proteins soluble in a salt brine.
Vacuumize To remove entrapped air from a meat mixture.

Mixing Equipment

The terms 'mixer' and 'blender' are often interchanged and from an engineering standpoint there seems to be no well-defined distinction. In the meat industry, a blender is usually a device that imparts more mechanical action to the product for the purpose of solubilizing and extracting the salt-soluble proteins. A mixer, however, is used to incorporate and uniformly distribute components of the processed meat formulation.

When using any type of mixing and blending equipment, care must be taken to choose the correct piece of equipment for the particular product being manufactured. For example, the mixer that one would choose for a dry sausage would not be the type that one would choose in a blender/emulsion mill operation for the manufacture of an emulsion-type sausage. In production, care must be taken to ensure that the temperatures of the product are optimum for both the type of equipment and the product being produced.

Paddle Mixers

Paddle-type mixers are most often used where a more gentle handling of the meat materials is desired (Figure 1). These devices consist of a tank or trough with either a single shaft or two intermeshing shafts running through the longitudinal axis. Paddles of varying configurations are attached to these shafts. Rotation speeds are either fixed or variable depending on the type of drive configuration. The drive mechanism can be supplied by either an electric or a hydraulic motor.

Paddle mixers would be the equipment of choice for processed meats such as dry and semidry sausages or coarse ground fresh and cooked sausages where particle distinction is critical. They are also used where the main purpose is to distribute ingredients with a minimum of mechanical action to extract the salt-soluble proteins.

Paddle mixers are more desirable for most meat-mixing applications. Paddle mixers are more efficient in uniformly mixing two or more ingredients. Compared with ribbon agitators, paddle agitators provide more lift and less push to product, which results in less compaction of ground meat, and paddle agitators are preferred when mixing high-viscosity mixtures, such as very cold meat or drier meat blends, such as ground beef, fresh sausage, and dry sausage.

Paddles are typically self-cleaning during the mixing process, so fat buildup is less of a problem. Intermeshing of paddle agitators speeds up the mixing process, reducing the mixing time needed to produce a uniform mixture, which is particularly important when trying to introduce a number of different nonmeat ingredients into meat. This reduced mixing time should also reduce fat smearing for products in which particle definition is important.

There is some debate about this issue; many believe that paddle mixers would be more effective for extracting protein for mixing lean meat in preblends or for mixing blends before emulsification. However, there is little scientific data to support either argument. If the surface area of the paddles moving through the product is increased, by using either larger paddles or even smaller paddles, the mechanical action of meat working against meat is increased, which increases protein extraction.

Paddle mixers run at reduced revolutions per minute (rpms), which can be useful for applying mechanical action to whole-muscle pieces or softer muscle like poultry to produce a

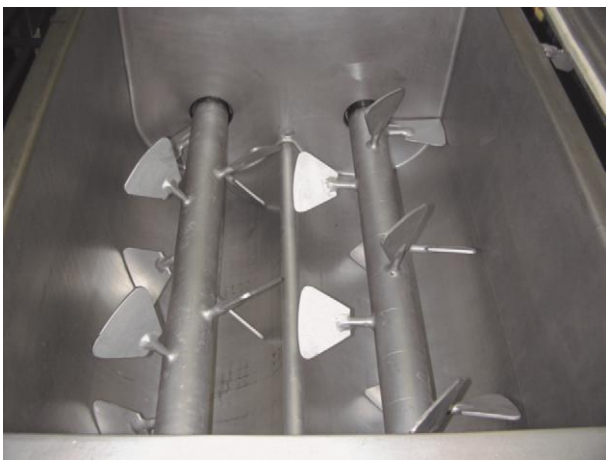

Figure 1 A view of a twin-paddle mixer.

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surface protein exudate without damaging muscle integrity. Single-shaft paddle agitators are also used in massagers to produce protein exudate and increase water-holding capacity of muscle pieces.

Ribbon Blenders

Ribbon blenders have a similar configuration and drive mechanism to the twin intermeshing paddle mixers (Figure 2). In a ribbon blender, a screw-type configuration is substituted for the paddles. The configuration of these twin screws is such that the meat is conveyed back and forth through the length of the blender tank to enhance the mechanical action being applied to the meat mixture. Ribbon blenders are used where solubilization and extraction of salt-soluble meat proteins is desired. One such application would be preceding an emulsion mill in producing an emulsion-type sausage. Ribbon blenders are used where solubilization and extraction of salt-soluble meat proteins is desired. One such application would be preceding an emulsion mill in producing an emulsion-type sausage.

Ribbon blenders are less effective than paddle mixers in evenly distributing different ingredients that are added to the mixer, unless paddles are added between the shaft and the ribbon. Regarding the protein extraction debate in blends intended for cooked or emulsified sausage, some claim that ribbon agitators are more effective than paddles due to the friction produced.

Ribbon blenders can cause compaction of drier blends, such as ground beef, fresh sausage, and dry sausage. Ribbons can also cause 'log' or 'barrel rolling,' which results in meat packing around the shaft and turning around the shaft with no mixing action. This is particularly a problem when water is added to frozen meat in a blender. Fat buildup is also a bigger problem on ribbon agitators than on paddles.

Fat smearing can be a problem with ribbon agitators because of the meat being slid along the sides of the blender and pinch points between the ends of the ribbons, particularly if product temperature is too warm.

Figure 2 The same mixer as shown in Figure 1 fitted with twin ribbons to function as a ribbon blender.

General Considerations

In addition to the type of agitator, mixing times and temperatures are also very important in getting proper protein extraction, particle definition, and final product texture. Mixing times for hamburger and sausage patties, as well as for fermented and dried products, would be shorter than for cooked sausages, because protein extraction is not important in making these products. Also, for hamburger and sausage patties, overmixing will result in an undesirable, rubbery texture and possibly fat smearing. For all of these products, colder meat temperatures will help to minimize melting of the fat and therefore fat smearing.

For cooked sausage products, where protein extraction is important, the lean portion should be mixed as long as is feasible at the coldest temperatures possible, with the salt (and phosphate, if used), to optimize protein extraction. After the fatter ingredients are added, mixing time should be minimized and product temperatures kept as cold as possible to minimize fat smearing.

When determining the capacity of a mixer, at a minimum, the volume of meat should be at least up to the center shaft of the agitators. Meat should not extend past the tip of the agitators. Bridging can occur with overfilling of mixers, and proper mixing will not occur with underfilling.

The sequence of final grinding versus mixing will also impact product texture and particle definition. Products, where good particle definition is important, should be mixed after the final grinding. This also applies to products that contain cheese, peppercorns, encapsulated acid, etc. For products where protein extraction is important (cooked sausage, emulsion products, etc.), mixing after final grinding is more effective.

Cooking and Cooling

Both mixers and blenders can be equipped with steam or hot-water jackets to heat the meat mixture during this step in processing. The most usual configuration for converting the ribbon blender to a cooker would be to insert scraper paddles between the ribbon flights to prevent the cooked meats from sticking to the heating surface. These jackets can also be used with chilled liquids to help maintain product temperatures.

More effective cooling can be obtained, however, by equipping the mixer/blender with a hood for applying carbon dioxide snow. The use of carbon dioxide as a chilling device is effective because of the large temperature differential between the product and the coolant, plus the fact that nothing is added to the finished product, as would be the case if ice were added. Mixer/blenders can also be fitted with a perforated pipe or bottom injection nozzles for the incorporation of carbon dioxide snow or liquid nitrogen as a coolant. In these cases, the blender needs to be fitted with a hood and an exhaust to vent the carbon dioxide or nitrogen gas.

Vacuum

A lid can be added to mixer/blenders to enable mixing and blending under vacuum. For most cooked products as well as

dry and semidry sausages, vacuum mixing is important, but vacuum mixing would not be recommended for looser textured products, such as ground beef and fresh sausage patties. Vacuum mixing removes air bubbles and pockets, which improves texture and appearance of cooked sausage products. Vacuum mixing is only applicable where it is applied in the final step before stuffing.

Other Types of Mixer/Blenders

Some smaller mixer/blenders use a single sweep arm as the mixing device. This produces a 'kneading' effect on the meat product. Sometimes this single sweep arm is coupled with counter-rotating paddles.

Combination mixer/mincers are available. These are designed to mix the product before the final mincing step. They are most useful in operations such as hamburger mincing, where the coarse-minced lean and fatter meats are being incorporated into a final blend before the final mincing.

Mincers/Grinders

Mincers/grinders are the most widely used particle reduction equipment for meat processing. These comminute the meat by forcing it through a plate with holes of varying sizes and then cutting off the extruded particles by means of a rotating knife. The most common configuration consists of a variable-pitch screw, which serves to deliver the meat to the mincer/grinder plate. Figure 3 shows a diagram of a typical mincer/grinder configuration. Because the screw drives the rotating knife, the knife rotation speed is fixed and the length of the particle being extruded can only be varied by changing the number of blades on the knife. The most common configuration is four knives, although two or six knives are sometimes used. The knives may be solid steel, which require periodic sharpening, or there may be a knife holder with insertable blades that can be replaced when worn.

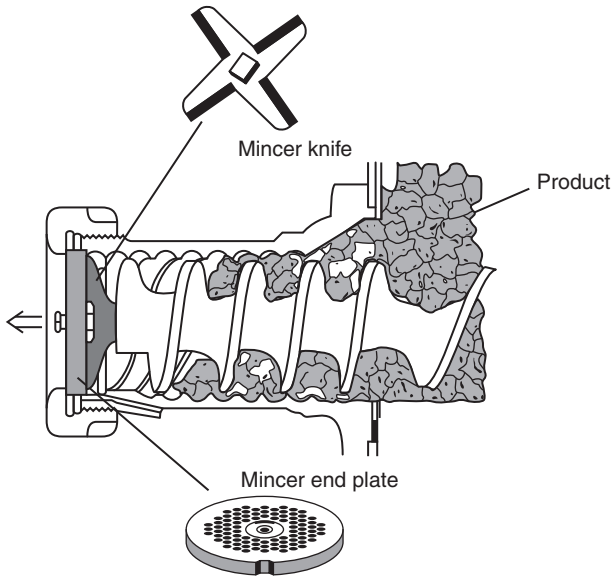

Figure 3 A diagram of a typical mincer/grinder. Reproduced from Aberle, E.D., Forrest, J.C., Gerrald, D.E., Mills, E.W., 2001. Principles of Meat Science, fourth ed. Dubuque, IA, USA: Kendall/Hunt Publishing Co., p. 127.

The particle diameter is controlled by the size of the holes in the mincer/grinder plate. They can vary from very large, the so-called kidney plate (because of the shape of the holes) that produces fist-sized chunks, down to 3 mm.

Some mincers/grinders are designed with a double plate that consists of a double-edged knife or knife holder sandwiched between a coarse plate of, say, 13 mm and a fine plate of 3 mm. The objective here is to complete a coarse mincing/grinding and a fine or final mincing/grinding in one operation. It is common to use a coarse mincing/grinding followed by a fine mincing/grinding to ensure more uniform distribution of fat and lean particles. There are also triple-plate configurations.

It is critical in operating a mincer/grinder that the knives and plates are sharp and carefully matched, so that a clean cut is obtained. The knives should make close contact with the plate for the entire length of each knife. Because the knife and plate are in close contact, the mincer/grinder should never be run empty as this will result in burning of the knife and plate and loss of sharpness. It could also result in metal fragments being incorporated into the product.

To obtain the proper consistency and appearance of the final product, it is important to pay attention not only to the sharpness of the knives and plates but also to the temperature of the meat being ground/minced. Each product has very specific temperature requirements. Large-capacity mincers/grinders can be equipped with screws that are designed to give optimum performance with a specific type of meat. Although there are 'general-purpose' screws, it is best to use a screw designed for a specific purpose – frozen meat, fat, lean, etc.

In general, plates with the largest hole size possible should be used. The first grinding/mincing step in which large chunks or whole muscles are to be reduced, plate hole sizes of 0.5–0.75 inch (13–19 mm) are recommended. Larger plate hole sizes in the first mincing/grinding step improves the uniformity of fat distribution and also increases the efficiency of bone or hard tissue separation in the final grinding process. The final plate hole size affects the texture of the finished product and should be selected accordingly.

Bone Separators

Bone or hard cartilage separators can be fitted to most mincer/grinders. These devices involve designing the knife and plate so that hard particles will migrate to either the center or the outer edge of the mincer/grinder head and there be eliminated. Because some soft tissue is forced out as well, the user has to be prepared to sacrifice some yield in order to eliminate the hazards and annoyance of bone or hard cartilage pieces in the finished meat product. Regulating the discharge rate usually controls the degree of bone removal. At best, bone removal systems can only be expected to eliminate 80% of the hazard. They are most effective when used on final mincing/grinding of 0.125–0.25 inch (3–7 mm). Figure 4 shows a typical mincer/grinder screw, plate, and knife assembly. The plate is designed

Figure 4 A typical mincer/grinder screw, plate, and knife assembly. The plate is designed to allow the bone or hard cartilage to move to the center of the plate where it is discharged under controlled back pressure.

Figure 5 A bowl chopper fitted with a vacuum hood. The knife hood is open to show the six-blade knife assembly. The unloading scoop is to the left of the knife assembly.

to allow the bone or hard cartilage to move to the center of the plate where it is discharged under controlled back pressure.

In-Line or Pump Mincer/Grinder

One of the more recent designs is the in-line or pump mincer/grinder. In these systems, the meat is delivered to the mincer head by a stuffing pump, usually a piston or vane type. A separate drive motor controls the knife rotation so that the relationship of the meat being delivered through the plate to the speed of the knife can be varied infinitely. This allows varying the particle length. Using a pump rather than an auger to deliver the meat mixture to the plate and knife further reduces damage to the fat particles. In addition, these systems can operate as closed systems, reducing contamination risks. These in-line systems can also be vacuumized, a further advantage as it will help to eliminate air particles in the meat mixture. In many cases, these in-line mincer/grinders are connected directly to stuffing and linking devices, making for a continuous in-line, closed system.

Bowl Choppers

These devices have been popular for batch operations for the production of coarse-cut sausages and meat emulsions (Figure 5). They offer the advantage over other systems that the mixing and comminution step can be accomplished in one operation. A disadvantage of bowl choppers is that they are best suited to batch operations as opposed to high-speed continuous operations. Bowl choppers are versatile, permitting a wide range of variability that is operator controlled. They have the disadvantage that particle size is totally operator dependent, making uniformity from batch to batch quite difficult. As contrasted with mincer/grinders, they give better particle distinction and less smearing but produce more variability in particle size. Bowl choppers cannot be fitted with bone removal systems as can mincer/grinders.

The bowl chopper consists of a rotating bowl with a series of rotating knives running in a vertical plane in the trough of the bowl. The knife head speed can be varied, as can the rotation speed of the bowl. Knife heads can vary from 2 to 12 knives, and chopper capacity can range from a few kilograms of meat to well more than 1000 kg. Each chopper will have an optimum capacity range for effective chopping. Overloading and underloading can decrease the effectiveness of the chopper.

In operation, the knives must be kept sharp and uniformly balanced. The knives should be set with minimum bowl clearance to produce effective chopping. As with the mincer/grinders, all types of comminution equipment produce frictional heat. This heating effect must be considered in arriving at the optimum final batch temperature.

Most larger choppers have an unloading device that scoops the finished meat batter out of the chopper as the bowl rotates. They may also be equipped with temperature-measuring devices to monitor the meat temperature during chopping and may be equipped with bowl rotation counters and timers. Monitoring the condition of the meat by number of minutes or number of revolutions of the bowl has severe drawbacks because it does not take into account variations in meat texture.

Other Features

Choppers can be equipped with a vacuum hood to enable the vacuumizing of an emulsion or meat batter during chopping (Figure 5). They can also be equipped with a carbon dioxide snow hood to enable cooling.

Choppers can be equipped with a steam jacket to allow cooking while chopping. This feature is useful in the manufacture of some liver sausages and patés.

Vertical Cutter/Mixers

There are some vertical mixer/cutters that resemble large food processors. In these cases, the knife head rotates in a

horizontal plane. These are usually of relatively small capacity for production use. They are quite useful, however, for preparing samples for laboratory analysis.

Emulsion Mills

These devices are used to convert a meat mixture to a fine batter or emulsion. Although they act to disperse the fat particles in the meat and other protein matrix, they must be preceded by a blender to solubilize the myofibrillar proteins. This is the reason that it is usually referred to as a blender/emulsion mill system.

Emulsion mills accomplish the same basic task as bowl choppers, but they are part of a continuous system. They also have the advantage of producing a uniform particle size, as opposed to a bowl chopper in which the particle size is operator dependent. Emulsion mills can range in capacity, and it is to be expected that the capacities of a continuous blender/emulsion mill system will exceed that of a bowl chopper. For this reason, most high-capacity continuous systems employ a blender/emulsion mill combination. Meats are usually preminced or ground through a conventional mincer/grinder before being introduced into this type of system.

There are two general types of emulsion mills. The most common type uses a plate and knife or multiple plates and knives, not unlike a mincer/grinder. The meat is forced through this plate/knife combination by some type of pump. Because this is a closed system, a vacuumizing device can be included in the system to vacuumize the emulsion. The frictional heat rise must be taken into account before the meat mixture is introduced into the emulsion mill. If additional heating is needed to attain the desired final temperature of the emulsion, this can be achieved by controlling the back pressure on the finished emulsion side. The particle size is controlled by the size of the holes in the emulsion mill plates. As with the mincer/grinders, it is critical to maintain sharp knife and plate sets and the same cautions apply to running the equipment empty.

Another type of emulsion mill uses an impeller to force the meat mixture against stationary blades. This design does not have metal-to-metal contact, so it can be operated without meat in the system. However, it does not have provisions for vacuumizing the emulsion. This must be accomplished later with a separate piece of equipment in the system.

Flaking Equipment

There are various types of equipment designed to cut meat particles into very fine flakes. Such equipment is primarily used where the meat is to be reformed into a solid piece under pressure. The popularity of this technology has diminished in recent years.

Frozen Meat Breakers

There are various types of frozen meat breakers that are used to reduce particle size for chunks of frozen meat. It is advisable to temper the frozen block of meat before breaking it up. This saves energy and prevents the destruction of the texture of the meat. A temperature of −7 °C is advisable. The most common types employ a guillotine-type knife that shaves off a layer of meat. Another type uses a rotating drum with knives attached that chip the meat. A third type is a specially designed mincer/grinder that forces the meat through large holes.

Slicers

Slicers are used in the final processing step. They can range from the small meat slicer in a retail operation to the high-speed slicers used for slicing and portioning ready-to-eat (RTE) meat. The meat is presented to a rotating circular or elliptical knife. The thickness of the slice is controlled by the distance the meat advances before it contacts the knife. Slicers can be linked to portioning devices as well as devices that shingle or stack in exact weight units. In the more sophisticated slicers, the weight of a unit coming off the slicer can regulate the thickness of slices in the next unit to achieve exact weights. The type of product being sliced as well as the configuration of the slicer determines the optimum temperature for slicing. This can range from partially frozen, in the case of bacon, to near the freezing point in the case of RTE luncheon meat.

A relatively new entry into the array of slicers is used to preslice the meat on a boneless or bone-in cooked and smoked ham in a spiral pattern, making it easier for the consumer to carve.

Dicers

Precooked meats and some fresh meats are often diced for further processing or for final use by the consumer. Dicers come in various configurations. Usually the meat is forced through a grid made up of knives or a plate with holes of various sizes. A guillotine or elliptical rotating knife then cuts off the pieces. Each particular dicer has rather specific requirements as to temperature of the material being diced and the adjustments to the machine, such as knife clearance.

See also: Chemistry and Physics of Comminuted Products: Emulsions and Batters. Minced Meats. Sausages, Types of: Emulsion

Relevant Website

http://meatsci.osu.edu
Meat Science, The Ohio State University.

Smoking and Cooking Equipment

RE Hanson, HansonTech LLC, Hudson, WI, USA

Glossary

Conduction Particle-to-particle heat transfer within the product. No bulk movement occurs.

Convection Heat transfer from steam, air, or water to the surface of the product. Convective heat transfer results from the bulk movement of air or water due to natural temperature gradients (free convection) or forced agitation using a fan or pump (forced convection).

Dry-sensor temperature The temperature of air measured using a clean, dry temperature probe.

Forced convection An external source (e.g., fan or stirrer) to aid heat transfer from the heating medium to the product surface. Agitation reduces the thickness of the boundary layer around the product, thus producing higher rates of heat transfer.

Free convection Heat transfer from the heating medium to the product surface from natural bulk movement of air or water.

Impinge To strike, dash, or collide against something. Used in the term 'impingement oven' to describe an oven design where fan-driven air is delivered perpendicular to the product surfaces using air supply ducts located directly above and below the product placed on a wire conveyor belt.

Wet-sensor temperature The temperature of air measured using a temperature probe fitted with a moisture-wicking cloth. Moisture evaporation from the cloth cools the sensor to the wet-bulb temperature. In a steam environment, the dry- and wet-sensor temperatures are equal, but if any drying is occurring, the evaporation will cool the wet-sensor probe to a temperature that is lower than the dry-sensor temperature.

Cooking Equipment

Industrial cooking equipment for meat and poultry processing can be separated into two basic categories – batch and continuous. In batch equipment, the product is loaded, cooked, and unloaded as a single batch (Figures 1–3). Batch oven capacities range widely from small units that hold 100 kg of boneless hams per batch to large ovens that hold 25 000 kg of bone-in hams. Batch ovens are commonly known as smokehouses, even though smoke is not always added. Most batch ovens can be used for both steam cooking and forced-air convection cooking, whereas some are designed as dedicated steam cookers (Figure 2). Kettles or tanks are used for batch cooking using hot water (Figure 3).

In large-scale factories, high-volume continuous systems are often used for cooking and smoking. In these systems, the cooking, smoking, and cooling operations are usually integrated into a single large unit with multiple zones or multiple units integrated into a single production line. The product is loaded onto a conveyor that transports it through one or more cooking zones followed by a cooling zone or a freezing system. Common continuous system designs use chain, a walking beam, or belt conveyors to move the product through the zones.

Chain-conveyor systems may be designed as straight-line tunnels (Figure 4), horizontal serpentines (Figures 5 and 6), or vertical serpentines (Figure 7). In straight-line and horizontal-serpentine systems, strands of sausages are hung on

Figure 1 Large meat-processing batch ovens. Reproduced with permission from Brandt Meats, Ltd, Canada.

Figure 2 Batch steam cook cabinet. Reproduced with permission from Butterball LLC, USA.

Figure 3 Steam-jacketed hot-water kettle. Reproduced with permission from Groen, a division of Lee Industries, USA.

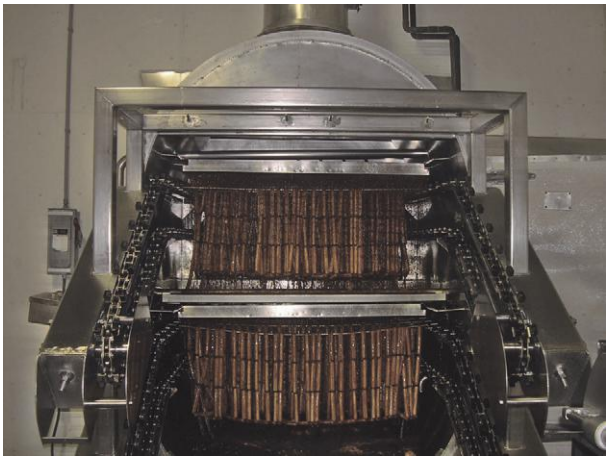

Figure 4 Straight-line chain conveyor – continuous frankfurter cook/chill system. Linked sausages are looped onto sticks and sticks are manually placed on a dual-chain conveyor. The sticks span the gap between the two chains. Reproduced with permission of Cargill, Inc., USA.

sticks or hooks that are conveyed through the system on a slow-moving chain. For vertical serpentine systems, products are often stuffed into casings as individual links, logs, or chubs that are then loaded into trays for cooking and chilling.

Chain-conveyor systems are commonly used for production of small sausages (18–28 mm diameter) such as frankfurters and smoked sausage. Output capacities typically range from 3000 to 8000 kg h^{-1}. For these systems, sausages are stuffed into long cellulose casings and automatically twist-linked into strands. The strands of sausages are either looped onto metal sticks that are placed on a chain conveyor (Figures 4 and 5) or are looped directly onto a J-hook chain conveyor

Figure 5 Horizontal serpentine chain-and-yoke conveyor – continuous small sausage cook/chill system. Linked sausages are looped on sticks and then sticks are manually hung on yokes attached to a mono-rail chain conveyor. Reproduced with permission of Maple Leaf Foods, Inc, Canada.

Figure 6 Horizontal serpentine chain-and-hook conveyor – continuous small sausage cook/chill system. Linked sausages are automatically looped onto hooks attached to a chain. Reproduced with permission of Marathon Enterprises, Inc., USA.

(Figure 6). The conveyors slowly move the sausage strands through a multizone forced-air convection oven and a brine- or water-shower chiller. After cooking and chilling, the sausages are unloaded, stripped, and packaged. Cooking times for these small sausages are typically 30–75 min.

In coextrusion sausage systems, continuous strands of sausage are coextruded with collagen casings made from collagen dough. The continuous strands are automatically cut to length and loaded into trays on a serpentine conveyor (Figure 8). In these systems, the sausages are discharged from the trays after being smoked but only partially cooked to a core temperature of approximately 45–60 °C. The warm sausages are then packaged in plastic film and loaded into a continuous hot-water cooker for final cooking.

Walking beam systems are designed to index product racks through a cook/chill system at preset time intervals (Figure 9).

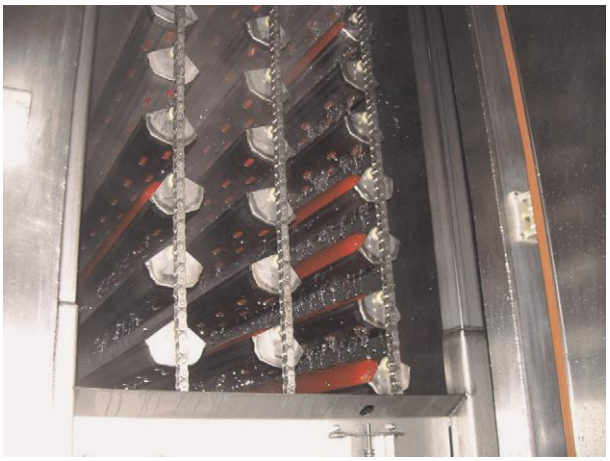

Figure 7 Vertical serpentine chain-and-tray conveyor – continuous large sausage cook/chill system. Stuffed sausage logs are automatically stuffed and loaded into trays that span the gap between two chains. Reproduced with permission of Smithfield Foods, Inc., USA.

Figure 8 Serpentine chain-and-tray conveyor – continuous coextruded sausage system. Blended sausage meat is coextruded with collagen dough to form continuous ropes of sausage encased in a film of collagen dough. Ropes are automatically cut to length, and cut sausages are loaded into trays that span the gap between two chains. Reproduced with permission of Salm Partners LLC, USA.

These systems are commonly used for larger products such as bacon or ham and have output capacities ranging from 5000 to 10 000 kg h^{-1}. These systems can use forced-air or steam cooking, and typical cooking times are 4–6 h.

High-temperature continuous ovens are used to cook a wide range of thin meat and poultry products using spiral-tower or linear (straight-line) belt conveyors (Figures 10–11). Products cooked in these ovens may be either coated or noncoated. Batter, breading, and sauces are commonly used as coatings added before cooking. Typical products include chicken pieces (wings, breast fillets, etc.), formed chicken meat (nuggets, patties, etc.), turkey pieces, sausage links, pizza toppings, meatballs, pork patties, and ground-beef patties. In linear- and spiral-belt ovens, a single layer of product is placed

Figure 9 Floor-mounted walking beam conveyor – continuous steam-cook/brine-chill system for molded hams. Hydraulic cylinders are used to index the loaded racks through the system. Reproduced with permission of Butterball LLC, USA.

Figure 10 Spiral-belt continuous oven. Product is conveyed on a belt that is configured as one or more spiral towers. Airflow is horizontal across the belt and parallel to the product. Reproduced with permission of Marel Townsend Further Processing, USA.

on a conveyor belt that carries the product through the oven. Fan-driven air is heated to high temperatures (150–275 °C) and driven through the process zone at high velocities (3–7 m s^{-1}), thus promoting rapid cooking and browning. Typical cooking times are very short at 3–12 min. These products are often conveyed directly from the belt oven into a freezer and then to a packaging machine.

Cooking Processes

Meat-processing ovens are used to cook products to moderate endpoint temperatures of 60–80 °C, thus destroying vegetative pathogens but not spores. As such, these cooking processes are best defined as pasteurization processes because they do not fully sterilize the products.

Although the designs for cooking equipment vary widely, only five heating media are commonly used either alone or in

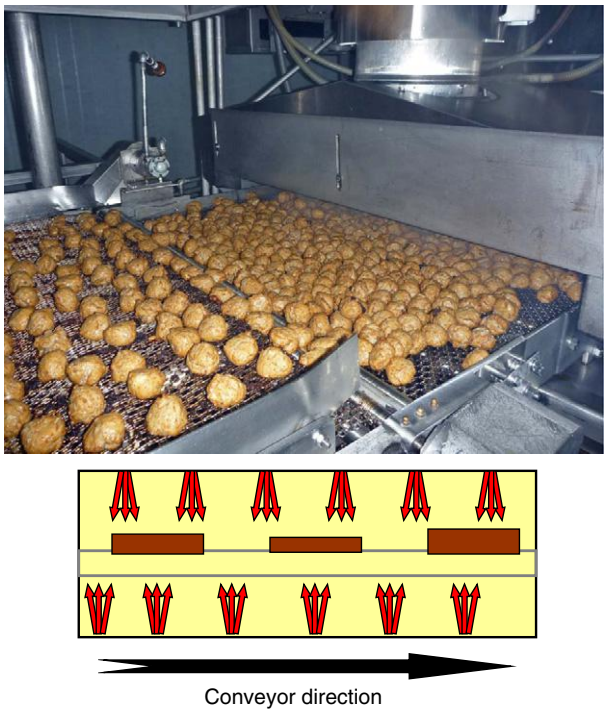

Figure 11 Linear-belt impingement oven. Product is conveyed on a linear-belt straight through a tunnel oven. The airflow is directed perpendicular to the product during cooking from forced-air impingement slots located above and below the belt. Reproduced with permission of Sugar Creek Packing Company, USA.

combination – (1) hot air (free or forced convection), (2) steam, (3) hot water, (4) infrared, and (5) microwave.

Forced-air convection ovens, steam cabinets, and hot-water tanks are widely used across the industry for cooking and smoking countless types of meat products. Infrared heating is used in specialized applications for rapid cooking and browning of thin products such as beef patties. Microwave ovens are also used in specialized heating systems, and have been widely adopted for continuous cooking of precooked bacon slices.

Steam and Hot Water Cookers

Steam and hot water cookers are used to process products that are not browned. These products are usually encased in plastic film and sometimes placed in stainless steel molds, and then cooked in steam cabinets, hot water tanks, or recirculated hot-water showers. The plastic casings may be coated with liquid smoke to add smoke color and flavor.

Large batch cooking tanks may be individually loaded using manually operated hoists or use automated hoist systems to automatically load and unload the tanks. The hot water may be pumped out of the tanks and replaced with chilled water for cooling. In continuous systems, the product is conveyed through either a steam atmosphere or a hot water shower, and then cooled using a chilled-liquid shower (fresh water, salt water, or propylene-glycol water). Steam and hot water temperatures within batch or continuous cookers are typically uniform, and therefore finished product temperatures are also uniform.

Steam and hot water cooking are simple processes that have only two variables – time and temperature. Condensing steam and hot water both have extremely high surface heat transfer coefficients and are thus highly effective at transferring heat to the product surface. Because the surface heat transfer coefficients are extremely high for both steam and hot water, products cooked at the same temperature in either medium will have approximately the same cooking time.

The basic principles of steam and hot-water cooking are easy to understand. If the steam or hot-water temperature is increased, the product surface temperature immediately increases and – after a lag time for heat conduction to the core – the core temperature cooks faster. If the steam or hot-water temperature is lowered, the surface temperature immediately decreases and the core cooks more slowly. When designing a steam or hot-water cooking process, it is important to consider that the product surface temperature closely tracks the temperature of the heating medium. Meat proteins denature strongly at approximately 55–60 °C. If the steam temperature in the first step of a process is substantially higher than 60 °C, the surface proteins will rapidly denature and cooking yields will suffer. To maximize cooking yields, the first step of a steam or hot-water cooking process should have a temperature set-point of 55–60 °C. This 55–60 °C set-point should be held long enough to slowly denature the surface proteins before increasing the steam or hot-water temperature. The slow denaturation of the surface proteins will improve water-binding ability and yields for the product.

Forced-Air Convection Ovens

Batch, continuous-chain, spiral-belt, and linear-belt ovens that use fan-driven air to cook product are classified as forced-air convection ovens. Regardless of the design, size, shape, or age of an oven, all forced-air convection ovens are designed to control the same four variables: (1) dry-sensor temperature, (2) moisture level in the air (wet-sensor or dew-point temperature), (3) air velocity, and (4) cooking time.

Precooked and smoked meats are often cooked in either batch or continuous-chain forced-air convection ovens. As previously described (Figures 4–6), continuous-chain systems convey the product through a series of smoking, cooking, and chilling zones. Most smoked meat products are cooked using dry-sensor temperatures of less than 100 °C, and therefore the maximum operating temperature for these ovens is usually approximately 110 °C. An exception is that some batch ovens are specially designed for high-temperature cooking or browning at dry-sensor temperatures of up to 260 °C.

Cooking times and air velocities are distinctly different for batch and continuous smokehouses, spiral-belt ovens, and linear-belt ovens. A comparison of typical air velocities through the product zone for various types of forced-convection ovens is shown in Table 1.

Batch ovens are remarkably versatile, and are used to cook products having cooking times ranging from 45 min to over 15 h (Figures 1 and 12). If the products are smoked, the cooking processes will have steps for either liquid or

traditional smoke application. Many smoked products are stuffed in presmoked fibrous casings or nets that are pretreated with liquid smoke, and therefore do not require an external smoke application.

Spiral-belt ovens are used to cook products such as meatballs, links, whole-muscle pieces, formed pieces, and patties that generally have short cooking times of 6–20 min, although longer cooking times are possible. Spiral ovens operate at high temperatures of up to 275 °C. The combination of high temperatures and fan-driven air induces rapid browning of the product surfaces. Airflow is generally horizontal across the belt – parallel to the product surfaces – and therefore the surfaces brown more slowly for a spiral oven than an impingement oven. However, the longer process times allow for better-optimized and thus higher-yielding processes for spiral ovens than for impingement ovens. Because of the horizontal airflow, the edges of the product facing the airflow tend to brown faster than the horizontal surfaces – a phenomenon known as 'edge-browning.'

Table 1 Comparison of typical air velocities through the product zone for various forced-air convection oven designs

Oven design	*Air velocity ($m\ s^{-1}$)*
Batch and continuous smokehouses	1.0–2.0
Spiral-belt tower oven	3.0–5.0
Linear-belt horizontal-airflow oven	2.5–3.0
Linear-belt impingement oven	4.0–7.0

Source: Reproduced with permission from HansonTech LLC, USA.

Linear-belt ovens are generally designed for operating temperatures similar to those for spiral-belt ovens but usually have shorter cooking times of 3–10 min. Many linear-belt ovens use an impingement-airflow design that uses supply-air slots above and below the belt to drive (or impinge) the air directly into the perpendicular surfaces of the product on the belt (Figure 11). The perpendicular impinging action of the air on the product surfaces accelerates the drying and browning of product surfaces in impingement ovens.

High-temperature belt ovens are sometimes used for the surface roasting of large precooked products such as turkey breast, roast beef, and ham to create a roasted appearance. For this application, molded or plastic-casing products are steam or hot water cooked and then cooled. To create the roasted appearance, the casings are stripped off and the product is run through a belt oven for a rapid, high-temperature browning of the surface. Depending on the desired appearance, this rapid-roasting process may take 10–20 min.

In a similar application, belt ovens have been adapted to use high-temperature and high-velocity air to dramatically accelerate the development of smoke color. Molded or plastic-casing products are steam or hot water cooked and then cooled. The product is then stripped, drenched in liquid smoke, and run through a belt oven using high-temperature,

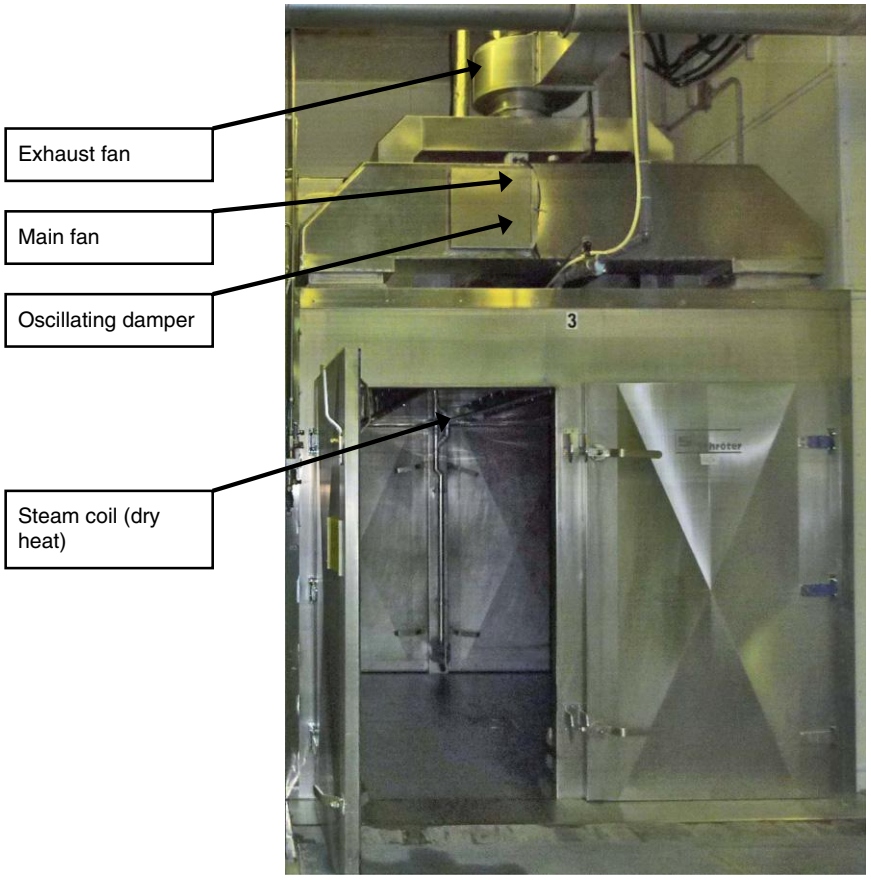

Figure 12 Typical components of a batch oven. Reproduced with permission of Maple Leaf Foods, Inc, Canada.

Table 2 Cooking process for traditional-smoked bone-in hams

Step	*Step type*	*Step time*	*Dry-sensor temperature (°C)*	*Wet-sensor temperature (°C)*	*Intake/exhaust dampers*	*Smoke generator*
1	Condition	10 min	50	45	Closed	
2	Predry	2 h	70	0	Open	
3	Smoke	2 h	70	0	Closed	On
4	Cook	1 h	75	60	Closed	
5	Finish	To 67 °C core	80	68	Closed	

Source: Reproduced with permission from HansonTech LLC, USA.

high-velocity forced air for rapid smoke color development. This rapid-smoking process replaces slower conventional batch oven processes, reducing smoking times from 3–6 h to only 15–20 min.

Controls

Electronic controls are used to measure and control the process variables in batch and continuous ovens. These control systems use either a computer or a programmable logical controller to create and store cooking programs and process data. The required oven settings are entered into the control system software and then the cooking programs are saved for repeated use. Depending on how the oven is equipped, the cooking programs may include step times, dry-sensor temperature, wet-sensor or dew-point temperature, intake and exhaust damper position (automatic, open, or closed), main fan speed (one, variable, or multispeed), exhaust fan (on or off), and smoke generator (on or off). An example of a typical cooking program for smoked bone-in hams cooked in a batch smokehouse is shown in Table 2.

Cooking and Smoking Processes

Cooking processes for precooked and smoked meats must strike a balance between dry and wet conditions. Dry conditions promote the development of smoke color, browning, aroma, and firm surface texture. Wet conditions promote smoke absorption, tender surface texture, tender casings, light color, improved yields, good peelability, uniform temperatures, and reduced cooking times.

Air Temperature Measurement

A clean, dry temperature probe measures the dry heat in the oven, known as the dry-sensor or dry-bulb temperature (Figure 13). A wet-sensor probe or dew-point sensor is used to measure and control the moisture level in the oven air. A large temperature difference between the dry- and wet-sensor temperatures indicates that the air is very dry, whereas a small temperature difference indicates that the air is high in moisture.

The wet-sensor probe measures the temperature of evaporating water in the oven air – known as the wet-sensor or wet-bulb temperature. One standard method of measuring the wet-sensor temperature is to fit a wet, moisture-wicking cloth over an ordinary temperature probe, and then drape the cloth

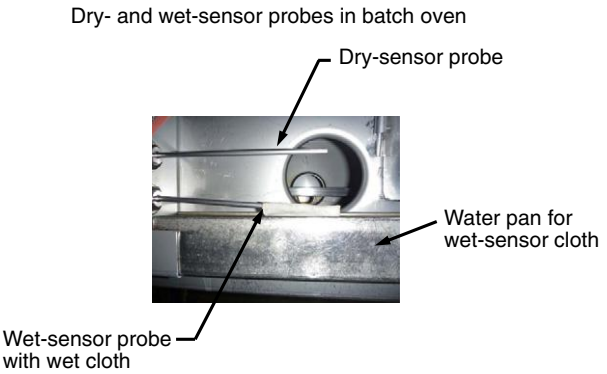

Figure 13 Dry- and wet-sensor probes in mounting bracket. Reproduced with permission of HansonTech LLC, USA.

in a pan of water to keep it wet (Figure 13). Water evaporating from the wet cloth cools the probe to the wet-sensor temperature. The wet-sensor temperature may be equal to but not higher than the dry-sensor temperature. For example, if the air is saturated during steam cooking, no evaporation will occur and therefore the dry- and wet-sensor temperatures will be the same. If the relative humidity decreases to 99% or less, however, moisture evaporating from the wet cloth will cool the probe to the wet-sensor temperature, which is lower than the dry-sensor temperature. Instead of a wet cloth, some ovens spray a mist of water over a probe located in the oven air stream to measure the wet-sensor temperature. This misting method is more reliable than the wet-cloth method because there is no fouling of the cloth from smoke or grease. Some ovens use a dew-point sensor instead of a wet-sensor probe to measure the moisture level in the air.

Relative Humidity

The relative humidity is a measure of the actual moisture content of the air compared to the moisture content of saturated air at the same temperature. It is useful as an indicator of the drying capacity of the air.

The dry-sensor temperature, wet-sensor temperature, dew-point, and relative humidity are all directly related. If any two of these variables are known, then the others can be looked up using a psychrometric chart or psychrometric software. In meat-processing ovens, the dry-sensor and the wet-sensor or dew-point temperatures are usually measured directly, and the control system uses the two values to calculate and display the relative humidity.

Effect of Dry- and Wet-Sensor Temperatures on Drying and Heating Rates

In forced-air convection ovens, evaporative cooling has a strong effect on the product surface temperature and resulting heating rates. Changes in the wet-sensor or dew-point temperatures will strongly affect product drying and heating rates.

An increase in the oven wet-sensor or dew-point temperature will cause an immediate increase in the product surface temperature, thus cooking the product faster. If the dry-sensor temperature is kept the same and the wet-sensor temperature is increased, the product surfaces will dry more slowly, resulting in higher cooking yields but slower surface color development. For smoked meats, if the wet-sensor temperature is increased too soon before the smoke color is fully developed, the result may be a blotchy or pale smoke color. To correct this problem, the product should simply be dried longer to further develop the smoke color before the wet-sensor temperature is increased.

If the wet-sensor temperature is kept the same and the dry-sensor temperature is increased, the higher dry-sensor temperature will dry the product surface more rapidly, resulting in an initially slower increase in the surface temperature that becomes a gradually faster heating rate as the product surface becomes drier. More importantly, the hot, dry conditions will promote Maillard browning, and thus a higher dry-sensor temperature promotes faster browning and smoked color development.

Forced-air convection cooking processes must balance dry and wet conditions to optimize quality characteristics, production efficiencies, and food safety. Dry conditions promote desirable quality and functional characteristics such as faster color development, reduced color variation, improved surface firmness, and casing adhesion. However, too much drying causes undesirable characteristics such as low yields, increased temperature variation, hard-to-peel casings, tough casings, and over-dry texture. Wet conditions generally promote higher production through higher yields, faster cooking times, and reduced temperature variation. Wet conditions also increase destruction of bacteria on the product surfaces and reduce the protective effect of dehydration for vegetative bacteria, thus increasing process lethality. As such, cooking programs must be written to balance and optimize dry and wet conditions. Given the powerful forces at work during forced-convection cooking, even small changes in oven variables may have a large effect on quality, throughput, and food safety.

Heating Systems

To control the dry-sensor temperature to the set-point, the oven control system regulates the heat source to increase or decrease the input of dry heat until the set-point is achieved. The three most common dry-heat sources for meat-processing ovens are direct-fired gas burners, heated coils, and electric heating elements.

- Direct gas: In a direct-gas fired oven, a gas flame is fired directly into the oven air stream to supply dry heat. Direct-gas heat is highly efficient and generally has the lowest cost of operation where natural gas is readily available. It can be used as a heat source for high-temperature operation up to 275 °C. Natural gas and liquid propane are the two most common fuels for direct-gas burners. A negative side effect of direct-gas heat is that the combustion gases are contained in the process air-stream, and these combustion gases often cause pinking in uncured meats.
- Heated coil: In an indirect-heated oven, a heat source such as steam, a gas flame, or thermal oil is used to heat a heat exchanger. The oven air is passed over the hot coil to provide dry heat. Steam coils are inherently limited to ovens that operate at temperatures of 105 °C or less. For higher-temperature ovens, an indirect-gas and thermal-oil coil must be used to achieve high temperatures of 230–275 °C.
- Electric heating elements: In an electrically heated oven, tubular heating elements are used to heat the air. Electric heat is extremely efficient and generally has a low maintenance cost, but it still has a high cost of operation because of the high cost of electricity. For this reason, electric heat is most often used only in small batch ovens (1–4 racks).

Pinking

Combustion gases from direct-gas fired burners will often cause a pink or purple ring to form on the outside edges of uncured meat products such as roast beef, chicken, or turkey. This pink ring is only a problem for uncured products: cured products are already pink and therefore the combustion gases have no noticeable effect. To prevent pink ring in uncured products, an indirect heat source such as a steam coil, thermal-oil coil, or indirect-gas must be used. Alternatively, the product can be encased in an impermeable plastic film to prevent exposure to the combustion gases.

Research has shown that nitrogen dioxide (NO_2) is the combustion gas that results in the pinking reaction. Although natural gas burns very cleanly, low levels of combustion by-products, including NO_2, are contained in the process air-stream during cooking. Nitrogen dioxide is highly soluble in water and thus readily absorbed into the meat surfaces. Nitrogen dioxide results in pinking even at very low concentrations – 0.4 ppm for turkey breast and 2.5 ppm for beef.

Moisture Control

To regulate the moisture level in the process air, oven control systems use a steam humidity valve or a combination of a steam humidity valve and the intake/exhaust dampers to adjust the actual wet-sensor or dew-point temperature to match the controller set-point. In a combination control system, the controller modulates the intake/exhaust dampers to control the amount of evaporated product moisture that is retained in the oven. If the retained moisture with fully closed dampers is not enough to achieve the wet-sensor set-point, then a humidity valve is opened to inject steam into the oven until the set-point is achieved. The humidity valve will then be cycled or modulated to maintain the actual temperature at the set-point. If the oven does not use dampers for moisture control, then the steam humidity valve alone is modulated to control the actual wet-sensor or dew-point temperature to match the

set-point. On-off or proportional on-off controls are sometimes used instead of modulating controls.

In most ovens, saturated steam is used as a humidity source. However, if the oven must maintain a relatively low dry-sensor temperature of, for instance, 42 °C or lower (e.g., for a fermentation process), atomized water is sometimes used instead of steam to prevent overshoot. Atomized water is also used as a humidity source in facilities where steam is not available.

Air Velocity and Airflow Patterns

In most batch ovens and some continuous ovens, the main fan and oscillating dampers are used to control the air velocity in the oven cabinet. The main fan speed can be adjusted using either a multiple-speed fan motor or a variable-speed motor control. The oscillating dampers sweep the air from side-to-side in the oven, thus creating localized changes in air velocity (Figure 14).

In a typical batch oven design, the main fan, heat source, and humidity source are mounted either on the roof of the oven (Figure 12) or on the back of the oven. The main fan supplies air to the supply ducts, where it is delivered to the process cabinet through supply cones or slots (Figure 14). The air is forced down along the side walls and across the floor where the two airstreams meet at what is termed the breakpoint. The air is then drawn up through the product to the return duct. The return duct is on the suction side of the fan and draws (returns) the air back to the fan cabinet to be reheated and rehumidified. Although many variations of this design are used, this basic air recirculation pattern is common to most batch ovens and many continuous ovens.

The point where the two opposing airstreams meet is known as the breakpoint. In Figure 14, the breakpoint is shown at the bottom-center and upper corners of the oven. The airflow at the breakpoint is highly turbulent and is the highest-velocity air in the cabinet. Most ovens use an oscillating damper system to slowly sweep the breakpoint from side-to-side in the oven. Some ovens use dual main fans equipped with variable-speed drives instead of rotating dampers to oscillate the air, but the effect is the same – the air slowly sweeps from side to side in the oven. If the oscillating damper system fails or is out of adjustment, the result will be severe side-to-side color and temperature variation in the oven.

Linear and spiral-belt ovens typically come equipped with variable-frequency drives on the main fans that are used to control fan speeds and air velocities. The fans can be set at any speed between the low and high limits of the drive. In some

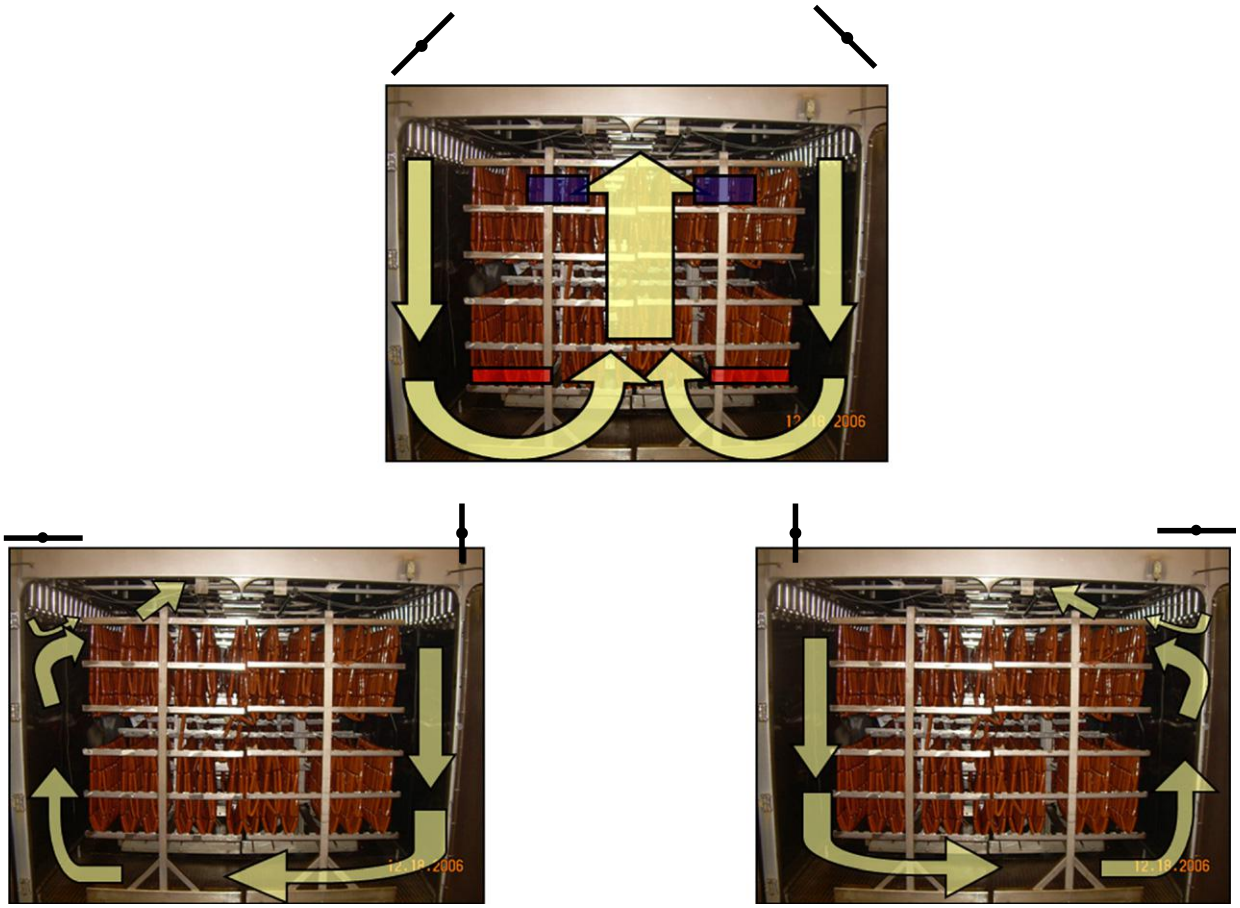

Figure 14 Typical airflow pattern in a batch oven. The point where the two airstreams meet is known as the breakpoint. An oscillating airflow system sweeps the air from side to side during operation. Reproduced with permission of Johnsonville Sausage LLC, USA.

impingement ovens, separate upper and lower fans are used to independently control the air velocities directed at the product from the upper and lower supply ducts. In other impingement designs, a single main fan and drive are used to control the volume of air delivered to a common plenum. A manually set diverter damper then splits the air between individual upper and lower plenums to control the air velocities directed at the product from those plenums.

Cooking Time

The cooking times in batch ovens are typically controlled using an established cooking program that includes several preset cooking and smoking steps (Table 2). The time required to achieve the target endpoint temperature determines the total cooking time for a product. The control system can be programmed to automatically shut down the oven when the target endpoint temperature is achieved. However, most processors use hand-held digital thermometers to manually confirm that the endpoint temperatures are achieved.

Some batch oven controls can be programmed to use 'ramp'-controlled step changes. Using a ramped step change, the come-up rate is controlled or 'ramped' from one set-point to the next instead of the conventional approach of an immediate and rapid temperature change between steps. Ramp-controlled temperature changes are designed to slowly increase the oven temperatures between steps, thereby slowing the denaturation rate of the surface proteins during cooking. Muscle proteins are known to strongly denature between 55 and 60 °C. Research has shown that if proteins are slowly denatured through this critical zone, the proteins will bind water better than proteins that are rapidly denatured in this zone. Ramp-controlled temperature changes in a cooking program, then, can be used to slowly denature surface proteins through this denaturation zone, thereby increasing cooking yields.

The conveyor speed is used to control the cooking times in continuous ovens. Process temperatures and conveyor speed are calibrated to optimize the cooking times in the various zones – balancing the desired quality characteristics with production throughputs while achieving the target endpoint temperature. Endpoint temperatures are manually measured at the outlet of the last cooking zone, and this temperature data is used along with yield and quality data to adjust process variables and conveyor speeds as needed throughout the day.

Temperature Variation

The oven design, product load density, inter product contact, product shape, and cooking program will all affect the temperature variation within the oven.

Oven design

In batch ovens, the oscillating airflow system continually sweeps the air from side to side in the oven cabinet, but the general direction of the airflow is still vertical from bottom to top. The dry-sensor temperature decreases as the hot air passes through the cold product, and thus the hottest air is located along the sides and bottom of the cabinet whereas the coolest air is at the top-center. For densely loaded, small-diameter sausages that have a lot of exposed surface area, this supply-to-return air temperature difference is commonly 8–9 °C and can be as high as 12–14 °C at the beginning of the process when the product is cold and wet. Larger-diameter products such as bone-in hams have less surface area per kilogram, and therefore the supply-to-return air temperature difference is narrower – typically 3–5 °C at the beginning of the process. At the end of the process when the product is warmer and drier, the supply-to-return air temperature difference for most products is typically only 1–3 °C. During steam cooking there is no dry heat and no evaporative cooling, and thus the supply-to-return air temperature difference is negligible.

The supply-to-return air temperature difference in batch ovens causes product at the bottom of the racks to cook faster than the product near the top-center. Therefore, as a good processing practice, endpoint temperature checks should be measured in product in the cold zone near the top-center of the racks. In addition, to minimize temperature variation, the finishing step(s) of a cooking program should use steam or high-humidity conditions whenever possible.

In chain conveyor tunnel and horizontal-serpentine continuous lines, the product is conveyed from front to back in the oven, thus eliminating the front-to-back variation. However, the air-handling systems in these ovens are similar to batch ovens, and therefore a significant top-to-bottom variation will still exist – and the cold zone will still tend to be located at the top-center of the oven. In vertical serpentine chain-conveyor systems, the product is loaded into trays that travel vertically up and down as well as front to back, leaving only side-to-side temperature variation that must be measured.

For belt conveyor ovens, the product is conveyed through the oven as a single layer, and thus top-to-bottom and front-to-back variation are eliminated, but the side-to-side variation is still significant. Furthermore, given the fast, high-temperature cooking processes that are used, even small differences in product thicknesses can cause large differences in endpoint temperatures. For this reason, belt ovens require frequent cross-belt endpoint temperature checks to ensure that final target temperatures are met for all products.

Product loading

For all forced-air convection ovens, product should be evenly spaced leaving adequate gaps for airflow around the product. Touching product (touchers) results in color defects at the touch spots and also tend to come out at lower temperatures than nontouchers. Touchers are an acute food safety and quality problem for belt ovens using processes that depend on dry heat for cooking – pieces that are touching or overlapped dry more slowly than nontouching pieces, resulting in much colder temperatures and distinct color defects for the touchers.

In batch ovens, a minimum clearance of 30 cm should be maintained between the product and the oven floor and side walls to allow free flow of air around the racks. This 30 cm clearance should also be maintained between the rack structural members and the oven floor. Any rack structure that is closer than 30 cm to the floor should be positioned to be parallel with the airflow (i.e., perpendicular to the ovens walls) so that the structure does not impede air circulation around the product. For partial loads, the product racks should be

evenly spaced from front to back in the oven to maintain a balanced airflow from front to back.

Product shape

If the product shape varies significantly from piece to piece, the core temperatures will also vary. Any variation of the product diameter or thickness will cause a corresponding variation in product core temperatures, even if all the pieces are exactly the same weight. In other words, it is the product diameter or thickness that determines the cooking time, not the piece weight. Heat conduction from surface to core takes longer for thicker or larger-diameter pieces. Products that are naturally shaped such as chicken fillets, turkey breast, bacon, bone-in hams and natural-casing sausages have inherently more temperature variation than products with tightly controlled dimensions such as cellulose-casing sausages, fibrous-casing sausages, formed products, or molded hams. Variable product thicknesses are a big concern for fast-cooking processes such as those used in belt ovens. Fast cooking times leave no extra time to equilibrate the temperature differences between the thinner and thicker products, leaving the thicker products more at risk for undercooking. To compensate, belt oven operators typically cook products to high endpoint temperatures and are extremely vigilant about having line workers conduct frequent cross-belt temperature checks.

Cooking processes

Cooking processes that use dry conditions induce more temperature variation than high-humidity or steam-cooking conditions. Higher wet-sensor temperatures during the finishing steps of a process will reduce product temperature variation. As a good processing practice for minimizing temperature variation, in the final step of a cooking process in a batch oven or in the final zone of a continuous line, the wet-sensor temperature should be set equal to or higher than the target core temperature. For example, if the target core temperature for a product is 72 °C, then the wet-sensor temperature in the finishing step or the final zone should be set at 72 °C or higher. This practice will reduce temperature variation, decrease cooking times, and increase yields.

Smoking Equipment

Smoke is added to meat products for the desirable color, flavor, aroma, and preservative effects. Two types of smoke are used extensively throughout the industry: traditional smoke and liquid smoke.

Traditional smoke is produced using one of several different types of smoke generators (Figure 15). Smoke generators use heat to smolder dry or wet woodchips or logs to produce smoke. Various generator designs use different heating methods including electric-heated hot-plates, super-heated steam, direct-gas flames, and friction. The smoke is piped from the smoke generator into the oven where it is absorbed into the moist product surfaces. After the smoke is absorbed, the product is heated and dried to evaporate surface moisture and promote the smoke color reaction.

Using traditional smoke, it is important to measure the amount of smoke exposure for the product. One simple method to indirectly measure the smoke exposure is to hang a permeable, water-filled cellulose casing in the oven with the product during smoking. After smoking, the casing is removed and the titratable acid content of the water is measured. Because traditional smoke contains acid, the acid level in the water will be directly proportional to the level of smoke exposure. Using this method, the smoke exposure can be compared among different products and processes regardless of the size of the oven or number of smoke generators attached.

Figure 15 Dry-sawdust hot-plate smoke generator. Reproduced with permission of Villari Brothers Foods LLC, USA.

Many meat processors have successfully converted from traditional smoke into liquid smoke. Liquid smoke can be applied using several different methods, including atomization, showering, immersion, direct addition, and presmoked casings (or nets). Atomization is a common method for batch ovens (Figure 16). The liquid smoke is atomized into the oven to create a cloud of atomized smoke that is then absorbed into the product surfaces. Much like traditional smoke, the product is then heated and dried to develop the smoke color.

Liquid smoke showers are commonly used in continuous systems (Figure 17). For example, in continuous frankfurter systems, the product is often conveyed under a shower of diluted liquid smoke before entering the first cooking zone where the product is heated and dried to develop the smoke color.

For presmoked casings, the product is stuffed into liquid smoke-treated fibrous casings or nets. After stuffing, the liquid smoke is absorbed from the casings or nets into the product

Figure 16 Liquid smoke atomization nozzle. Reproduced with permission of Red Arrow Products Company LLC, USA.

Figure 17 Liquid smoke shower system. Reproduced with permission of Red Arrow Products Company LLC, USA.

surface. The first steps of the cooking process use hot and dry conditions to promote surface drying and smoke color development on the product.

For direct addition, liquid smoke can be either mixed into injection brines or incorporated into the product during tumbling or massaging. Liquid smokes used for direct addition are specially formulated for this method. Direct addition smoke adds smoke flavor to the entire product, not just the surface, but usually does not contribute significantly to surface color.

For most smoking processes, it is easier to produce consistent smoke color and flavor using liquid smoke than using traditional smoke. Particularly in batch ovens, it is difficult to produce consistent absorption and uniform drying of traditional smoke within an entire load and also from load to load. The use of liquid smoke showers or presmoked casings takes much of the guesswork out of the smoking process. Other operational advantages of liquid smoke include elimination of the smoke generator and lower cleaning and pollution control costs.

Conclusion

Everyday across the meat industry, thousands of different processes are used to cook and smoke a countless variety of meat products. These myriad cooking processes, though, share a few principles of heat and mass transfer that are common to all. Cooking equipment should be designed to take full advantage of our knowledge of the effects of these variables on products during cooking, thus enabling processors to fully optimize processes to ensure safe meat products that meet quality specifications while maximizing throughput. It is crucial, too, that processors gain a thorough understanding of the functions and limitations of their cooking equipment for controlling each key variable, so that the equipment can be optimized to its full potential.

See also: Bacon Production: Bacon; Wiltshire Sides. Cooking of Meat: Heat Processing Methods; Physics and Chemistry; Warmed-Over Flavor. Cutting and Boning: Traditional. Physical Measurements: Other Physical Measurements; Temperature Measurement. Processing Equipment: Battering and Breading Equipment. Sausages, Types of: Cooked. Smoking: Liquid Smoke (Smoke Condensate) Application. Thermophysical Properties

Further Reading

Fellows, P.J., 2009. Food Processing Technology, third ed. Cambridge, UK: Woodhead Publishing.

Foegeding, E.A., 1988. Gelation in meat batters. Proceedings of the 41st Annual Reciprocal Meat Conference, pp. 44-47. Kansas City, KS: American Meat Science Association.

Hallström, B., Skjöldebrand, C., Trägårdh, C., 1988. Heat Transfer and Food Products. London: Elsevier Applied Science.

Hanson, R.E., 1990. Cooking technology. Proceedings of the 43rd Annual Reciprocal Meat Conference, pp. 109–115. Kansas City, KS: American Meat Science Association.

Hanson, R.E., 1997. Reducing process variation in the cooking and smoking process. Proceedings of the 50th Annual Reciprocal Meat Conference, pp. 33–42. Kansas City, KS: American Meat Science Association.

Maga, J.A., 1988. Smoke in Food Processing. Boca Raton, FL: CRC Press.

Pearson, A.M., Gillett, A.M., 1996. Processed Meats, third ed. New York: Chapman and Hall.

Singh, R.P., Heldman, D.R., 2009. Introduction to Food Engineering, fourth ed. London: Elsevier Academic Press.

Watson, E.L., Harper, J.C., 1988. Elements of Food Engineering, second ed. New York: Van Nostrand Reinhold.

Relevant Websites

http://www.meathaccp.wisc.edu/
Center for Meat Process Validation.
www.combase.cc
Combase.
www.frperc.com
Food Refrigeration and Process Engineering Research Centre.
https://itunes.apple.com/gb/app/psycalc/id420172439?mt=8
iOS App for Psychrometric Calculations. PsyCalc App on Apple iOS AppStore, USA.
www.linric.com/PsyCalc.htm
Linric Company.
www.rpaulsingh.com
R. Paul Singh. University of California-Davis, USA.
www.engineeringtoolbox.com
The Engineering Toolbox.

Tumblers and Massagers

CL Knipe, Ohio State University, Columbus, OH, USA

Glossary

Binding strength The ability of meat pieces to bind together during the cooking process.
Cure migration The migration of curing ingredients (salt, nitrite, phosphates, etc.) throughout the pieces of meat.
Massaging Involves frictional energy generated from meat pieces being rubbed and massaged against each other by rotating paddles.
Pickle A solution containing nitrite that is used to cure hams and other processed meat products.
Protein extraction The process of dissolving proteins from meat by mixing a salt and phosphate solution with the meat.
Protein exudate A protein mixture that accumulates on the surface of meat pieces, during mixing or tumbling processes.
Sliceability The ability to produce slices of meat products that hold together and have good eye appeal during the packaging process.
Stuffing The process of filling a casing.
Tumbling Involves a rigorous physical treatment with impact energy achieved from meat pieces being lifted and falling against each other.
Water-holding capacity The ability of meat proteins to hold water during the application of stresses, such as grinding, cutting, cooking, etc.

Introduction

The mechanical action required for producing high quality, sectioned and formed, whole muscle meat products may be achieved in a variety of ways but most commonly involves the processes of tumbling and massaging. Tumbling and massaging are different physical treatments. Massaging involves frictional energy that is generated from pieces being rubbed and massaged against each other by rotating paddles. There are basically two types of massagers: one with horizontal paddles and the other with vertical paddles (Figure 1). The vertical paddles are reported to give a better, faster massage treatment and cause less damage to meat (or bone) than the horizontal paddles. A massager should have paddles that can be reversed so as to prevent lodging of meat pieces in the corners of the massaging vat and to free pieces that are caught on the paddles. Furthermore, a variable speed drive allows the speed of the paddles to be varied in an attempt to find the optimal treatment for each product.

Tumblers, however, provide a more rigorous physical treatment and involve the impact energy achieved from meat pieces being lifted by baffles along the sides of a rotating drum and falling from the baffles onto the drum or rolling over other pieces of meat as the drum rotates (Figure 2). It has been suggested that the meat needs to drop at least 3 ft (90 cm) in a rotating tumbler to achieve the maximum effect. If a tumbler is filled too full, the meat will only roll around as the tumbler rotates, reducing the physical treatment to the meat. The speed with which the barrel rotates also affects the impact of the falling meat and needs to be adjusted according to the type of meat, size of meat pieces, etc. in order to optimize the effect without damaging the meat.

Rigorous tumbling action causes foaming of the protein exudate that is produced from this physical treatment. Foamy

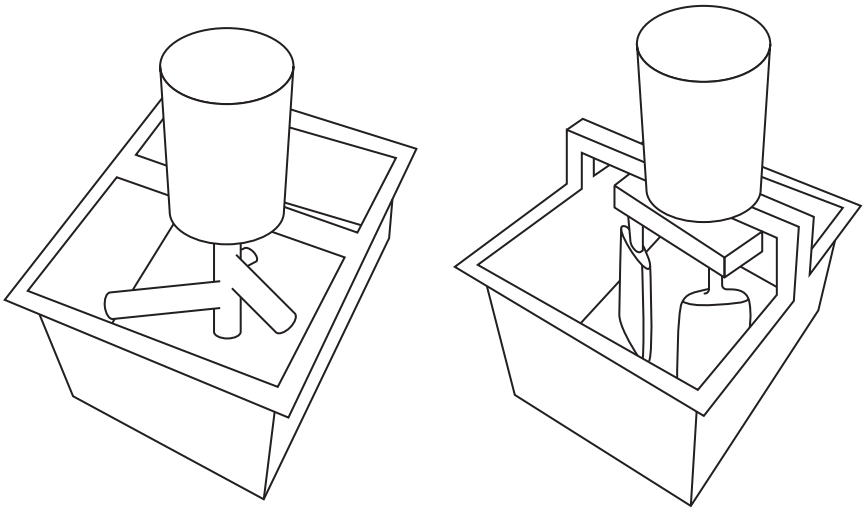

Figure 1 Massagers with horizontal (left) and vertical (right) paddles.

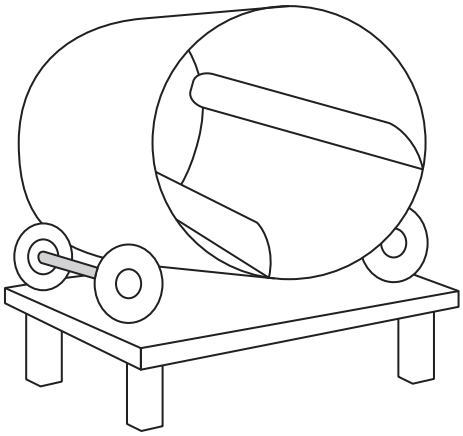

Figure 2 End view of a tumbler.

exudate is not desirable, resulting in the need to vacuumize the lidded drum tumbler before the start of the tumbling treatment. Reducing the speed of the tumbler may also reduce the foaming of the surface proteins.

There are two basic types of tumblers: the rotating drum and the end-over-end tumbler. The rotating drum is relatively gentle in treatment and uses less space per volume of capacity. Although massaging was more commonly used in the early days of this technology, vacuum tumbling is now the most commonly used method.

Mixers can also be used for this process; however, as the mixing action is typically much more damaging to meat pieces (particularly for softer muscles, such as that of chicken and turkey) than tumblers or massagers, care must be taken to adjust the mixers or the total time of the mixing action to avoid overworking the meat pieces. Paddle mixers can be run at reduced rpms to apply sufficient mechanical action to whole muscle pieces or softer poultry muscles in order to produce a surface protein exudate without damaging the muscle integrity. Smaller paddles, fewer paddles, or single-shaft paddle agitators could be used in mixers to produce protein exudate and increase water-holding capacity of muscle pieces without severely damaging the meat pieces.

Owing to the wide variety of equipment that is used to tumble, massage, or mix meat products, it is helpful to refer to the overall process as mechanical conditioning.

Mechanical Conditioning Cycles

The main idea behind mechanical conditioning is to apply a physical treatment to enhance the migration of the curing ingredients (salt, nitrite, phosphates, etc.) that have been either injected into the meat or absorbed into the meat during marination process. This improved migration of ingredients results in increased extraction of proteins from the meat as well as improved color development, if cured. Increased protein extraction increases bind in meat products.

There are two types of binds to consider: one is a protein–protein bind, which is a heat-initiated surface bind or adhesion of pieces, resulting from the protein exudate that is produced. It accumulates on the surface of meat pieces. Larger quantities of exudate are produced when starting with smaller meat pieces in which surface membranes of muscles have been cut. Surface maceration of larger intact muscles may be required to increase the quantity of exudate that is produced during tumbling or massaging.

The other type of bind is the internal protein–water bind that impacts juiciness, texture, and cooking yield. This latter type of bind is produced by cell wall and membrane damage in the center of meat pieces that allow faster migration of water and ingredients, such as salt and phosphates.

The mechanical action of tumbling or massaging involves a time–work intensity relationship. Many different theories exist about the optimal length and aggressiveness of the tumbling and massaging treatments. The length of time required depends on the intensity of the physical treatment imparted to the pieces of meat and is balanced against the desired final product quality. In general, the more intense the mechanical action, the less tumbling or massaging time required. Care must be taken not to overwork the meat, as this will result in a product with a dry surface that may result in meat pieces which are more difficult to stuff into casings or can result in extensive physical damage to the meat.

Although massaging is less destructive to the meat, the resultant protein extraction is typically slower than with tumbling. Binding strength will increase with increased massaging or tumbling time, due to increased exudate formation on the surface of the meat.

Sufficient adhesion of meat pieces may be achieved after 2–3 h of mechanical action, but a longer mechanical treatment may be necessary to obtain a significant yield and quality advantage. To maximize, the internal protein–water bind takes at least 18–24 h, which includes significant rest periods. The use of vacuum while tumbling further decreases the tumbling time required to produce a good bind by preventing the production of foam. Periodic rest periods during the tumbling or massaging processes are important to allow time for adequate cure migration while preventing excessive muscle destruction that could result from a long continuous process and may allow for dissipation of foam in the exudate in the case of nonvacuum tumblers. Holding periods longer than 24 h have been shown to further increase water-holding capacity and internal color development of cured products.

An alternative approach to the periodic rest periods of massagers and tumblers would be to tumble or mix continuously for 1–2 h, followed by a rest period of 18–24 h in a separate combo bin, and ending with a final mixing until sufficient exudate is produced. This approach may give sufficient mechanical action while allowing adequate time for distribution and action of brine ingredients without tying up tumblers and massagers for long periods of time.

Mechanical conditioning may also raise the temperature of the meat. The distribution of ingredients and the curing reaction will occur faster at warmer temperatures; if the product temperature increases above 4.4 °C (40 °F), the time at that temperature should be minimized as much as possible. Colder temperatures, 0 °C (32 °F) or lower, are better for extracting and solubilizing proteins from meat to maximize bind.

The cycle and total treatment times will vary between different pieces of equipment, so every processor needs to

experiment with the equipment available to determine an optimum processing cycle for the desired product. For boneless meat products that are to be sliced, high-speed slicers, more aggressive tumbling, or massaging would be needed; however, the texture of this product would be expected to be more rubbery. For higher quality products, with a more traditional texture, less aggressive tumbling or massaging processes is recommended.

Binding strength also depends on the meat temperature during tumbling or massaging. Salt-soluble proteins are extracted over a wide range of temperatures, but they are most readily extracted from lean meat at 2.2–3.3 °C (36–38 °F). This supports the findings that the bind strength of ham was much better when massaged at −0.9 and 4.4 °C (30 and 40 °F) than at 10 °C (50 °F). In one of the original US patents dealing with mechanical conditioning of meat, the mechanical action was observed to be enhanced by chilling the pieces of cured meat to as low as −3 °C (25 °F), then mixing the product until it reached a temperature of approximately 1.7 °C (35 °F). For protein extraction and functionality, as well as food safety, tumblers and massagers should be operated in refrigerated rooms (4.4 °C/40 °F or colder). This could also be accomplished by using refrigerated tumblers or by direct addition of carbon dioxide to the meat inside the tumblers and massagers. Refrigerated tumblers have circulating refrigerant in jacketed walls of the barrels, which chills the meat as it makes contact with the walls of the tumbler.

Vacuum tumbling or massaging improves bind strength by reducing protein foaming. The development of foam denatures proteins and subsequently decreases bind strength. Vacuum tumbling and massaging have been credited with increasing the amount of protein extracted and decreasing the time required to produce the optimal product. If foaming occurs, when using vacuum tumblers or massagers, faulty seals allow air to leak into the container.

Advantages of Mechanical Conditioning

The advantage of mechanical conditioning that is of the greatest interest to most meat processors is the increased cooking yield and the decreased shrinkage of the final product. The disruption of internal meat tissues due to the mechanical action of tumbling or massaging combined with the addition of salt (NaCl) and alkaline phosphates (most commonly a 90:10 mixture of tripoly- and hexametaphosphates) causes increased protein solubilization. Solubilized proteins in the meat tissues enhance water absorption before heat processing. On heating, the gel formed by the solubilized proteins holds additional water and decreases cooking loss. Typically, proper tumbling decreases cooking shrinkage by 2–3%. Some equipment manufacturers claim that massaging increases cooking yields by at least 4%. Because the amount of added moisture is regulated in many countries for both sectioned and formed whole muscle meat products, the most significant effect of reduced cooking loss is the reduction in injection levels, which makes it easier to achieve the desired injection level.

If the water-holding capacity of meat is improved during the mechanical conditioning process, smoke color development is faster, because drying of the product surface is faster. This results in a more desirable smoked product color, and because the smoking process is faster, the total cooking time should be less than when the product is not tumbled or massaged.

The mechanical action of tumbling and massaging also provides a simple mixing action on meat products. The mixing action causes any loose, available moisture to be absorbed into the muscle. Marination of meat products can be accomplished using tumbling and massaging. The extent to which the marinade is absorbed into the product is based on the time of application and the thickness of the meat. Dry ingredients can also be applied to whole muscle surfaces by the use of a tumbler.

Mechanical conditioning also increases sliceability of sectioned and formed meat products, because of the improved cohesiveness of muscle pieces. This allows smaller, lower value meat pieces to be bound together to produce a more appealing and saleable final product.

Improved pickle penetration also reduces curing time to much less than the 3–7 days, which is traditionally considered necessary to obtain uniform cure distribution by conventional stitch pump curing, especially in the case of dark, firm, and dry hams. This results in a much higher production and turnover rate. Furthermore, improved pickle penetration causes a better, more uniform cured color. Use of vacuum during mechanical conditioning reduces the exposure of lean tissue to oxygen, which results in a brighter, more stable cured color and eliminates pinholes and air pockets in formed products.

Also, mechanical conditioning includes the advantage of more complete and efficient use of available raw meat materials. Imagination is the only limitation to the wide variety of raw meat materials that can be incorporated into sectioned and formed meat products. Some examples include the following: the use of high-quality fresh meat trimmings resulting from meat preparation in food service establishments; the addition of minced or emulsified, cured shank meat to hams during mechanical conditioning; the use of whole hams, with bruises or broken bones removed; the use of heavier primal cuts, which without mechanical conditioning would tend to be tough; the use of meat from poultry carcasses to produce a boneless product of more desirable shape and size; the sectioning of sow loins of various sizes to produce products with less bone and fat and of more desirable and uniform shape; and the use of soy isolates and similar extenders to produce 'combination hams.'

Mechanically conditioned meat also results in greater ease in stuffing. Because the meat is more pliable, it is easier to pump this product through stuffing horns and to completely fill casings or molds of any shape to predetermined weights while eliminating undesirable voids between pieces. This is a temporary softening effect, so if meat products are held very long without any physical action, the meat may need to be retumbled or mixed to make the meat more pliable for stuffing.

Mechanical conditioning has also been found to increase tenderness of cured meats. This is due to the disruption of cellular connective tissue and increased moisture retention after cooking. This tenderizing effect can be applied to heavier primal cuts to produce a more desirable product.

Finally, mechanical conditioning has even been credited with improving the flavor and aroma of the final product. This is best explained by reduced drip loss, which could contain water-soluble flavor compounds and the improved distribution of nitrite and alkaline phosphates. Nitrite and phosphates are both credited with having antioxidant properties and are more likely to reduce off-flavor development.

Disadvantages of Mechanical Conditioning

An obvious major disadvantage of mechanical conditioning is the initial cost of equipment. The equipment is relatively expensive, and one product batch may occupy the unit for 18–24 h. Although the actual expenditure for an individual tumbler is much greater than for a massager, the greater capacity per tumbler can generally make it the more favorable option.

Another disadvantage is the labor required for boning, skinning, trimming, and sorting. The extent of this disadvantage depends on the desired quality of the product. This process does not work miracles. Fat and connective tissue in particular, and muscles of contrasting colors to a lesser degree, can decrease visual attractiveness and esthetic value of the product. The highest quality products demand the time of skilled labor to remove silver skin as much external and seam fat as possible and (in the case of ham) sorting muscles by color.

Finally, the procedure may not work optimally or may even cause excessive muscle destruction if the treatment time and intensity are not carefully monitored. Excessive mechanical conditioning could yield a softer and yet more rubbery finished product, whereas mechanical conditioning cycles that are too short could result in a dense, dry product, which lacks uniformity of pickle distribution.

See also: Chemical Analysis for Specific Components: Curing Agents. Curing: Brine Curing of Meat; Production Procedures. Ham Production: Cooked Ham. Processing Equipment: Brine Injectors; Mixing and Cutting Equipment; Smoking and Cooking Equipment

Further Reading

Jabaay, R., 2007. Manufacturing Processes: Hams, Meat Processing Technology Series. Savoy, IL: American Meat Science Association.

PROFESSIONAL ORGANIZATIONS

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Introduction

The initiative for developing organizations originates from the efforts of dedicated individuals to meet responsibilities, accomplish goals, and become socially involved through sharing, as provided by group interactions and resolutions. For some circumstances (such as food safety), it is appropriate that problems should be solved through group actions.

As long as records have existed, there have been organizational structures of varying designs and complexities for social, political, economic, and scientific groups (and surely others), from elementary and small ones to sophisticated and large ones. Records show that until well into the twentieth century, there were no organizations that formally complimented meat scientists and meat industry groups. The growth of such organizations has more likely resulted because of general prosperity and through technical advancements, such as air travel and electronic communications. This article identifies some of the major meat-related groups (e.g., the International Congress of Meat Science and Technology;

Figure 1 Cover page of the final announcement for the 58th International Congress of Meat Science and Technology, Montreal, Canada, 2012. Reproduced with permission from the Chairman of the Scientific Committee of 58th ICoMST.

Table 1 Twelve international professional organizations

Name	*Abbreviation*	*Address and/or web site*	*Year began*	*Meeting cycle*	*Characteristics*
American Meat Science Association	AMSA	1800 South Oak Street, Suite 100, Champaign, IL 61820, USA www.meatscience.org	1964	Annual (3 days)	Formal structure and dues required for members worldwide; Reciprocal Meat Conference and proceedings at different universities; Meat Industry Research Conference; and publishes bulletins, brochures, and newsletters
American Society of Animal Science	ASAS	P.O. Box 7410 Champaign, IL 61826-7410, USA www.asas.org	1908	Annual (4 days)	Formal structure and dues required for members; regional meetings; includes a Meat Science and Muscle Biology Section at national and regional meetings; and publishes *Journal of Animal Science*
Central American and Caribbean Symposium on Meat Processing		EARTH Apartado 4442-1000, San José, Costa Rica	1981	Biennial	Conducted by Costa Rican Association of Meat Processors; educates producers, processors, and individuals in meat business about science and technology
European Livestock and Meat Trading Union	UECBV	81 A, Rue de la Loi, (box 9), B-1040 Brussels, Belgium www.uebcv.eu	1952	Annual	Promotes international livestock and meat trade and represents and defends members' interests before European Economic Commission and internationally
European Natural Sausage Casings Association	ENSCA	Gotenstrasse 21, D-20097 Hamburg, Germany	1956	Semiannual	Defends interests of the sausage casings trade
Institute of Food Technology	IFT	525 West Van Buren Street, Suite 1000, Chicago, IL 60607, USA www.ift.org	1939	Annual (5 days)	Formal structure and dues required for its members worldwide; trade show at national meeting; regional meetings; includes all foods and has a muscle foods section; and publishes *Journal of Food Technology and Journal of Food Science*
International Association for Food Protection	IAFP	620 Auroa Avenue Suite 200 W Des Moines, IA 50322-2864, USA www.foodprotection.org	1911		An organization of 3600 food safety professionals from 50+ countries committed to advancing food safety worldwide by providing a forum to exchange information on protecting the global food supply
International Butchers Confederation	IBC	Rue Jacques de Lalaing 4-box 10, B-1040 Brussels, Belgium	1907	Semiannual	Comprised of 14 national butchers' associations and meat traders' federations representing approximately 150 000 companies; defends interests of meat trading and catering through social and economic policy, knowledge, and legislation on meat products; and vocational training
International Congress of Meat Science and Technology	ICoMST	University of Helsinki, Box 66, Viikki EE, FI-00014, Helsinki, Finland www.icomst.helsinki.fi	1955	Annual (5 days)	Informal structure; meetings rotate to countries worldwide by invitation; host country establishes program and fee required for conference and proceedings
International Meat Secretariat	IMS	6 Rue de la Victoire, 75009, Paris, France www.meat-ims.org	1974	Biennial	Represents, promotes, and serves meat and livestock industry worldwide; conducts research and educational programs; provides International Meat Research Award; and meets in different countries by invitation
International Natural Sausage Casing Association	INSCA	18 Zaki Ragab High Class One (1205), Smouha, Alexandria, Egypt www.insca.org	1965	Annual	Importers and processors of natural sausage casings in 40+ countries including 200+ companies and publishes INSCA newsletter and National Link
Liaison Centre of the Meat Processing Industry in the EU	CLITRAVI	Blvd. Baudouin, 18 Box 4, B-1000 Brussels, Belgium www.clitravi.eu	1958	Annual	Represents 16+ countries of the meat processing industry and protects and promotes industry's interests

Table 2 Thirty-seven national professional organizations

Country	*Name*	*Abbreviation*	*Address and/or website*	*Year began*	*Meeting cycle*	*Characteristics*
Australia	Beef Improvement Association of Australia		96 Harbours Road Yenden, VIC 3352, Australia www.beefline.org.au	1968	Annual	Seeks to genetically improve quality of beef in Australia
Australia	Meat and Livestock Australia, Ltd.	MLA	Locked Bag 991, North Sydney 2060, Level 1, 165 Walker Street, North Sydney, NSW 2060, Australia www.mla.com.au	1998	Annual	Provides marketing information and receives funds from livestock sale levies and represents approximately 30 000 livestock producers and has four state offices
Australia	National Meat Association of Australia	AMIC	2nd Floor, 460 Pacific Highway, St. Leonards, NSW 2065, Australia www.amic.org.au	1928		Meat processors, wholesalers, small goods manufacturers, and retailers; promotes increased demand for meat products; gathers and disseminates industry information; and publishes Australian Meat Industry Bulletin
Belgium	National Verbond van Slachthuizen in Vleesuitsnijderijen	NVS	Rue des Deux Eglises 2 G–B4, B-1000 Brussels, Belgium www.unizo.be			Meat processing companies and slaughterhouses in Belgium and publishes monthly newsletter
Canada	Canada Beef, Inc.		#235, 6715-8th Street NE, Calgary, AB T2E 7H7, Canada www.canadabeef.ca	2011		Producers and exporters of beef and related products; promotes international demand for Canadian beef; and represents members' interests before agricultural and industrial organizations, government agencies, and international trade associations
Canada	Canada Pork International	CPI	220 Laurier Avenue, Suite 900, Ottawa, ON K1P 5Z7, Canada www.canadapork.com	1991	Annual	Exporters of pork products; promotes exportation of Canadian pork; facilitates establishment of international business relations; and conducts promotional campaigns
Canada	Canadian Meat Council	CMC	Dow's Lake Court, 1545 Carling Avenue, Suite 407, Ottawa, ON K1Z 8P9, Canada www.cmc-cvc.com	1919		Meat packers and distributors; promotes growth and development of meat industries; and conducts promotional campaigns
Canada	Canadian Meat Science Association	CMSA	4–10 Agriculture Centre, U. of Alberta, Edmonton, AB T6G ZP5, Canada www.cmsa-ascv.ca	1985	Annual	Formal structure of dues-paying members; provides directory, newsletter, and annual technical symposium. Involves support for research, education, and government relations
Denmark	Danske Slagterier – Danish Bacon and Meat Council	DS	Axeltorv 3, DK-1609, Copenhagen V, Denmark www.danskeslagterier.dk	1897	Annual	Pig slaughterhouses and meat processing plants and coordinates joint activities and represents industry (including pig farmers) nationally and internationally

(*Continued*)

Table 2 Continued

Country	*Name*	*Abbreviation*	*Address and/or website*	*Year began*	*Meeting cycle*	*Characteristics*
Denmark	Danish Livestock and Meat Board	KF	Vesterbrogade 6D, PO Box 438, DK-1505, Copenhagen V, Denmark www.meatboard.dk	1972		Cattle slaughterhouses and represents industry toward governments and EU
France	MHR Group		LeChillenge 17000, La Rochelle, France			Represents all meat groups
Germany	Deutscher Fleischer–Verband (Metzger–Immung)	DFV	Kennedyallee 53, D-60596 Frankfurt, Germany www.Fleischerhandwerk.de	1875	Annual	Gains influence on the economy and government to reduce or block disadvantageous measures
Germany	Federation of Meat Industry and Trade		Postfach 2566, D-53015 Bonn, Germany	1924		Wholesalers of livestock and meat; advises members on foreign trade, customs, and tax issues; and publishes a journal
Germany	National Association of Wholesale Butchers and Meat Merchants		Adenauerallee 176, D-53113 Bonn, Germany www.b-b-b.de	1925	Annual	Wholesalers of meat in Germany; promotes meat industry; and informs members of changes in laws and issues concerning meat industry
Germany	Renderer's Association		Kaisestrasse 9, D-53113 Bonn, Germany www.Fleischmehlindustrie.de	1920		Promotes and protects economic and hygienic interests of meat meal industry and publishes *Die Fleischmehl-Industrie*
Germany	Verband der Fleischwirtschaft	VFD	Sekretariat des 1. Bürgermeisters von Wang, Untere Hauptstraße 17c, 85368 Wang/Volkmannsdorf, Germany www.v-d-f.de	1953	Annual	Represents members of all areas from the meat industry. It considers itself a binding link between the public, the officials, and the 200 companies that belong to this organization
Germany	Biofleischhandwerk		Sekretariat des 1. Bürgermeisters von Wang, Untere Hauptstraße 17c, 85368 Wang/Volkmannsdorf, Germany www.v-d-f.de	2010	Annual	Strengthens environmentally sustainable meat production with high transparency and no or little transportation of the animals in order to produce a healthier product
Germany	Fleischprufring Bayern e. V.		Sekretariat des 1. Bürgermeisters von Wang, Untere Hauptstraße 17c, 85368 Wang/Volkmannsdorf, Germany www.v-d-f.de	1990		Monitors weights, registers, and classifies the butcheries and is under the control of the Bavarian government and a service provider for more than 90% of the butchers in Bavaria
India	All India Meat and Livestock Exporters Association		World Trade Centre, 11th Floor, Center 1, Cuffe Parade, Mumbai, 400005, Maharashtra, India	1987		Wholesalers and exporters of meat and livestock in India
The Netherlands	Koninklijhe Nederlandse Slagersorganischie	KNS	Postbus 1234, NL-2280 CE Rijswyk, The Netherlands			Master butchers and firms selling meat and increases production, consumption, and trade of meat
New Zealand	Meat New Zealand		Box 121, Wellington, New Zealand www.nzmeat.co.nz	2002		Advances domestic meat industry for meat producers and facilitates communication and cooperation among members and represents their interests

New Zealand	New Zealand Animal By-Products Exporters' Association		11 Longchurch Terrace, Box 12–222, Christchurch, New Zealand	1938		Exporters of animal hides, skins, tallow, and other products; seeks to ensure a domestic and international political and economic climate conducive to trade; facilitates communication and cooperation among members and represents their interests; and gathers and disseminates information
Norway	Norwegian Independent Meat Association		Karoline Kristiansens vei 2, Box 6272, Etterstad N-0603 Oslo, Norway			Slaughterhouses and cutting and packing plants engaged in meat industry; represents members' interests before government agencies, international organizations, and the public; and publishes Kjottbransjen
Spain	National Association of Cold Storage Meats and Cutting Rooms		Gran via de les Corts Catalanes 631, 6, E08010 Barcelona, Spain www.anafric.es	1988	Annual	Promotes growth and development of slaughterhouses and cold storage facilities handling meat and meat products; makes available legal and technical services; assists members in conforming to sanitary and other regulations; facilitates establishment of overseas business contacts; and represents members before national and international regulatory bodies, labor organizations, and the public
UK	British Contract Packers Association	BCPA	Syonsby Lodge, Nottingham Road, Melton Mowbray LE13 0NU, UK	2000		Promotes technical, trade, and commercial interests of British contract manufacturers and packers
UK	British Meat Federation		12 Cock Lane, London EC1A 9BU, UK	1934	Annual	Slaughterers and wholesalers of red meat within England and Wales; provides information service; represents membership in government; and publishes monthly newsletter
UK	Butchers' Company		Butchers' Hall, 87 Bartholomew Close, London EC1A 7EB, UK www.butchershall.com	1975		Applies professional code of conduct in meat trading; promotes education and training throughout meat industry; and publishes newsletter
UK	National Association of Catering Butchers	NACB	217 Central Markets, Smithfield, London EC1A 9LH, UK www.Haighs.com/nacb.htm	1983		Catering butchers whose premises have been inspected and approved by NACB Plant Evaluation Committee; raises standards; promotion; and protects interests through negotiations with authorities
UK	National Federation of Meat and Food Traders		1 Belgrove, Tunbridge Wells TN1 1 YW, UK www.Butchers-online.net	1888		Members include independent retail butchers, slaughterhouse operators, bacon curers, meat manufacturers, and

(*Continued*)

Table 2 Continued

Country	*Name*	*Abbreviation*	*Address and/or website*	*Year began*	*Meeting cycle*	*Characteristics*
						wholesale distributors; publishes monthly, Food Trader for Butchers
UK	Northern Ireland Meat Exporters Association	NIMEA	24 Ballydown Road, Banbridge, Belfast BT32 3RP, UK www.nimea.co.uk			Major beef- and lamb-approved slaughtering and cutting companies; promotes interests of meat processors and exporters in Northern Ireland
USA	American Association of Meat Processors	AAMP	Box 269, Elizabethtown, PA 17022, USA www.aamp.com	1939	Annual	Represents small packers, processors, wholesalers, home food service businesses, meat retailers, deli, mail order businesses, and catering operators and their suppliers; government relations; provides education, insurance options, and business management assistance; and publishes AAMPlifier semimonthly
USA	American Meat Institute	AMI	1700 North Moore Street, Suite 1600, Arlington, VA 22209, USA www.meatami.com	1906	Annual	Represents interests of packers and processors of beef, pork, lamb, veal, and turkey products and their suppliers throughout North America; provides legislative, regulatory, and public relations services; funds scientific research; and offers marketing and technical assistance and sponsors educational programs
USA	National Cattlemen's Beef Association	NCBA	9110 East Nichols Avenue, Suite 300, Centennial, CO 80112, USA www.beef.org		Annual	Represents dues-paying US beef cattle producers via check-off levies; addresses marketing, production, health, advertising, quality, consumer concerns, and government relations and regulations; provides funds for beef research; and absorbed portion of National Livestock and Meat Board
USA	National Pork Producers Council	NPPC	10664 Justine Drive, Urbandale, IA 50322, USA	1966	Annual	Represents dues-paying US pork producers and receives funds from checkoff levies; addresses marketing, production, health, advertising, quality, consumer concerns, and government relations and regulations; provides funds for pork research, advertising, and education; 40 state associations; and absorbed portion of National Livestock and Meat Board

USA	National Turkey Federation	NTF	1225 New York Avenue NW, Suite 400, Washington DC, USA, www.turkeyfed.org			
USA	North American Meat Association	NAMA	1910 Association Drive Reston, VA 20191, USA 1970 Broadway, Suite 825 Oakland, CA 94612, USA	2012	Annual	A merger of North American Meat Processors Association and National Meat Association to represent 700 +companies primarily in North American, also to include other countries. Provides conferences and is concerned with education, government issues, and economic policies
USA	US Meat Export Federation	USMEF	1050 17th Street, Suite 2200, Denver, CO 80265-2073, USA www.usmef.org	1976	Semiannual	Marketing meat products internationally; government relations and regulations; safety; advertising; participates in overseas trade shows and foreign market survey trips; disseminates information and coordinates product demonstrations and menu promotions; and publishes Directory of US Meat Suppliers

Figure 1) that have developed and currently play significant roles in the advancement of scientific knowledge, and applied technology, economic strategies, and governmental regulations. Regrettably, there will be unintentional omissions. The organizations have been categorized as either international or national in scope and are alphabetized by organization in Table 1 and by country and organization in Table 2. Where they are known, mailing addresses and websites, years begun, frequency of meetings, and some brief characteristics are included.

See also: Meat Research Institutions

Further Reading

American Meat Science Association, 1998. Reciprocal Meat Conference 50th Anniversary 1948–1986. Savoy, IL: American Meat Science Association, pp. 1–68.

American Meat Science Association, 2012. Who's Who in Meat Science. Membership Directory. Savoy, IL: American Meat Science Association.

Bray, R.W., 1997. History of meat science. Proceedings of the Reciprocal Meat Conference. Champaign, IL: AMSA.

Hedblad, A. (Ed.), 2003. Encyclopedia of Associations: International Organizations, thirty-ninth ed. Farmington Hills, MI: Thomson-Gale Group. Part 1, Descriptive Listings, Sections 1–5, Entries 1–11, 727.

Hedblad, A. (Ed.), 2003. Encyclopedia of Associations: International Organizations, fortieth ed. Farmington Hills, MI: Thomson-Gale Group. Part 3, Name and Keyword Index.

Honikel, K.O., 2003. Weblinks for Meat Research. Kulmbach, Germany: BAFF. Available at: http://www.bfa-fleisch.de/weblinks.htm (accessed 03.02.2004).

Kinsman, D.M., Niinivaara, F.P., Jul, M., 1994. History of the European meetings of meat research workers 1955–1986 (ICoMST). Proceedings of the 40th International Congress of Meat Science and Technology. The Hague, The Netherlands: ICoMST.

PROTEOMIC TECHNOLOGIES AND THEIR APPLICATIONS IN THE MEAT INDUSTRY

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Introduction

Proteome refers to the set of all proteins expressed in a cell, tissue, or organism at a specific point of time. Essentially, proteome is the protein complement expressed by the genome. Although the genome of an organism is static in nature, the proteome is dynamic and continuously changes over time. Proteomics is the systematic and methodological analysis of a proteome for identification, quantification, and functional characterization of proteins. Because most biological functions in an organism are carried out by the proteins transcribed from the genome, the application of proteomics is necessary to understand the functioning of an organism. The genome of meat animals contains several thousands of genes, and each of them can produce multiple messenger RNA species, which in turn are translated into individual proteins. The proteins, thus synthesized, can further undergo various posttranslational modifications. This, in turn, results in the expression of more than a million different proteins from a genome. Some of these proteins are highly abundant in a cell, whereas others exist only in a few copies.

Tools in Proteomics

Purification, detection, and identification of proteins, usually from a complex mixture, are the major strategies in proteomics. In this perspective, two-dimensional gel electrophoresis (2-DE) is the classical technique widely used for protein purification. In 2-DE, the proteins are separated based on isoelectric points in the first dimension and then separated in the second dimension based on molecular weights. The individual proteins, appearing as spots in the 2-DE gels, are matched on the basis of isoelectric point and molecular weight by imaging software. In addition to 2-DE, new techniques such as difference gel electrophoresis (DIGE) and diagonal polyacrylamide gel electrophoresis (diagonal PAGE) are also becoming common tools in gel-based protein purification. In addition, high performance liquid chromatography (HPLC) renders a valuable tool to separate and purify low molecular weight peptides for proteomic studies. The purified proteins/peptides of interest are then detected by mass spectrometry (MS) and are identified using complex algorithms from the protein databases. Advances in the seamless integration of 2-DE and HPLC with MS, as well as ever expanding protein databases, have significantly contributed to efficiently purify, identify, and quantify several thousands of proteins in relatively short timeframe with high accuracy.

The postgenomic era has witnessed the emergence of MS from an analytical tool for volatile compounds to an application for characterizing macromolecules such as proteins. MS positioned itself as a superior tool to conventional ones in protein biochemistry because it can rapidly and accurately determine the molecular mass of proteins in low abundance and sequence peptides in a proteolytic digest mixture. Developments in ionization techniques, namely matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI), contributed profusely to the use of MS in characterizing proteins. MALDI and ESI made it possible to create gas-phase ions from peptides and proteins, and thus became indispensable to the modern day MS. Nevertheless, MS is primarily exploited as a qualitative tool rather than quantitative one due to the unpredictable ionization properties of proteins and peptides.

The proteomic approach in agriculture represents an important cornerstone to characterize and improve the quality of muscle foods harvested from animals. Compared to the conventional techniques in protein chemistry focusing on one or two major proteins in a sample, modern-day proteomic tools enable simultaneous examination of a multitude of proteins and their modifications at a reasonable time and with superior accuracy. The application of proteomics in meat science and industry is in an early stage compared to some other disciplines in life sciences where these tools are extensively exploited. Nevertheless, their application in the meat industry is continuously growing.

Role of Proteome in the Properties of Muscle as Food

Meat, being a protein-rich food, has several attributes influenced by the quantity and quality of the proteins in skeletal muscle proteome. Furthermore, skeletal muscle proteome exhibits dramatic changes during various phases of meat production, harvest, and processing. Proteome of muscle continuously undergoes changes antemortem (different stages of animal growth), perimortem (conversion of muscle to

meat), and postmortem (postharvest storage). This necessitates the use of proteomics to advance our understanding on meat quality and to engineer novel strategies to improve quality and quantity of meat production.

In the meat industry, proteomics can be applied to the pre- and postharvest aspects of meat quality. Preharvest applications encompass growth, metabolism, muscle biology, and genetics of meat-producing animals. From this point of view, proteomic investigations have attempted to explain the molecular basis of variations in meat quality due to diet, gender, genetics, and management. While this approach highlighted the robustness and usefulness of proteomic tools, the primary focus of these investigations was animal production. In this article, application of proteomics in postharvest aspects of meat production is emphasized.

Conversion of Muscle to Meat

Immediately after the harvest, blood supply to various organs stops leading to an anoxic condition. The biochemistry and metabolism of skeletal muscles immediately change in response to the anoxic conditions. These changes continue in postmortem muscles and result in alteration of biochemical and physicochemical properties, ultimately leading to the conversion of muscle (live tissue) to meat (food). Proteins play a major role in the biological functions of skeletal muscles as well as in the conversion of muscle into meat, and proteomic tools are utilized to characterize the cellular changes immediate postmortem. The robustness of 2-DE and MS to interpret changes in muscle proteome became evident more than a decade ago leading to several discoveries characterizing the fundamental basis of conversion of muscle into meat in beef and pork.

Early proteomic investigations in this area demonstrated structural changes in several sarcoplasmic and myofibrillar proteins during conversion of muscle to meat attributing to action of endogenous enzymes. Furthermore, the expression and/or abundance of endogenous enzymes also exhibit changes during this period confirming the shift in the metabolism of muscles. Examination of proteolytic changes revealed the existence of breed-specific mechanism in pigs. The biochemical basis of pale, soft, exudative (PSE) condition in pork, caused by ante-mortem stress, was also studied using proteomics, and the low abundance of heat shock protein 27 kDa was linked to the occurrence of PSE. Heat shock proteins are molecular chaperones that protect cellular proteins from stress-induced denaturation, aggregation, and oxidative damages. While muscle biochemistry was the focus of the majority of these investigations, application of proteomics identified several candidate proteins for meat quality attributes as well as quality defects. The information gathered from these studies can potentially be applied to predict the quality of meat prior to carcass chilling and fabrication, and thus appropriately utilize such meat in suitable products.

Meat Tenderization

Meat tenderness is influenced by the biochemical properties of muscle fibers as well as connective tissue matrix and is improved by aging – primarily due to degradation of cytoskeletal proteins. Tenderness is the major factor contributing to eating satisfaction and consumer acceptance and, therefore, is considered an important trait influencing repurchase decisions. It has been estimated that the inconsistencies in tenderness lead to US$217 million annual revenue loss to the US meat industry. Postmortem degradation of several structural proteins is the major reason for improvement in meat tenderness. Nevertheless, the fundamental mechanisms through which these biochemical changes govern meat tenderization are yet to be completely elucidated.

Improving and predicting tenderness are two factors critical to profitability of the meat industry. Although improvements in tenderness can be made by aging, predicting this attribute is not straight forward primarily due to muscle- and species-specific variations in meat biochemistry. The improvement in tenderness is dependent on the activity of endogenous proteolytic enzymes in postmortem muscles. The interactions between the enzymes and their substrates, primarily cytoskeletal proteins, are complex and are often influenced by different intrinsic (protein oxidation, calcium and vitamin D concentrations) and extrinsic (packaging system, aging condition, brine injection, antioxidant, and electrical stimulation) factors. The fact that the substrates for these enzymes are also proteins makes proteomics an invaluable tool to interpret the biochemistry of tenderness.

Meat tenderness is a muscle-dependent attribute, and significant amount of research has been undertaken to explain the muscle-specific biochemistry of beef tenderness. Use of DIGE to examine the proteolytic changes in beef adductor (tough muscle) and longissimus (moderately tender muscle) revealed that proteins such as actin, myosin heavy chain 1 fragment, myomesin-2, and α-actinin-3 undergo muscle-specific changes during aging; the abundance of these proteins in sarcoplasmic and myofibrillar fractions was different in adductor and longissimus indicating the possibility of using them as potential candidates for further investigations in muscle-dependent mechanism of tenderization. Of specific interest is the fragment of myosin light chain 1, which was more abundant in the sarcoplasmic extracts of tender beef than in the tough beef.

The calpain system is a major enzymatic system responsible for postmortem tenderization of meat and has been extensively studied. Conventional protein chemistry has characterized various calpain isoforms and their biochemical modifications, whereas the use of proteomic tools revealed that μ-calpain undergoes oxidation by forming an intermolecular disulfide bond and that the oxidation results in the loss of proteolytic activity. This finding highlighted the adverse influence of protein oxidation on meat tenderness.

Analyses of muscle proteome identified several proteins potentially associated with the calpains that can influence tenderness in a multitude of ways. These identified proteins included structural proteins, mitochondrial proteins, and proteins associated with calcium and glucose metabolism. The potential association between calcium-regulating proteins and calcium-dependent calpains suggest a complex nature of the mechanisms governing postmortem proteolysis.

Although the role of oxidation in loss of protein functionality has been known for several years, use of diagonal PAGE

and MS demonstrated that myosin heavy chains in postmortem muscles are susceptible to oxidation and that the exposure to an oxidizing environment promotes cross-linking between titin and myosin; both phenomena lead to protein aggregation that subsequently results in an increase in toughness of meat.

Meat Color Stability

Color of meat is the most important quality influencing purchase decisions. Discolored meats are often sold at discounted price leading to revenue loss of more than US$1 billion per year in the US beef industry. Proteomic tools have been utilized extensively to examine the mechanistic interactions between myoglobin and other small biomolecules governing meat color.

The ability of MS to determine the exact mass of proteins has been used to differentiate the myoglobins from various meat animals and for meat species identification. Although conventional gel electrophoresis identifies myoglobins from different species as 17-kDa bands in the gels, MS-based studies revealed that the myoglobins from birds are approximately 300 Da heavier than their mammalian counterparts, offering partial explanation for why certain color phenomena are observed only in poultry whereas some others are reported primarily in red meats.

Lipid oxidation compromises flavor as well as color of meat. MS and proteomic tools demonstrated that the adduction of reactive lipid oxidation products (such as aldehydes) to histidines in myoglobin, especially the proximal (position 93) and distal (position 64) histidines, is responsible for compromising the stability of the heme group and thus favoring formation of metmyoglobin in meat surfaces, eventually leading to brown discoloration. Furthermore, lipid oxidation-induced meat discoloration is species-specific in nature; MS analyses indicated that beef myoglobin is more susceptible to lipid oxidation than pork myoglobin supporting the observation that the color stabilizing effect of dietary vitamin E is more pronounced in beef than in pork. The greater number of histidines in beef myoglobin renders it more susceptible to the nucleophilic attack by reactive aldehydes and subsequent oxidation than its pork counterpart. Recent studies in this area suggested that the susceptibility of myoglobins to lipid oxidation-induced oxidation is governed by the number and location of histidines in the primary structure.

Beef muscles exhibit differences in color stability attributes during retail display, and this has been utilized to categorize some muscles as color-stable and some as color-labile. Studies employing MS and 2-DE revealed differential abundance of antioxidant and chaperone proteins in color-stable (Longissimus lumborum) and color-labile (Psoas major) muscles and concluded that the over abundance of the antioxidant proteins is likely the major contributing factor to the superior color stability in beef Longissimus lumborum by minimizing myoglobin oxidation.

Dry-Cured Meats

Dry-cured meats are traditional products popular in different parts of the world. Degradation of muscle proteins in dry-cured meats generate peptides, which contribute to various product- and region-specific attributes. The degree and nature of proteolysis are influenced by the region's climate, temperature, humidity, and duration of ripening. Furthermore, the chemistry of the peptides is influenced by the conditions of aging and ultimately contributes to the unique savory attributes of products developed in a specific geographical region. Proteomic studies of dry-cured meats demonstrated that myofibrillar and sarcoplasmic proteins are extensively degraded during aging process. In addition, the peptides generated are product-specific and may be utilized as biomarkers for product identity and geographical specification.

Conclusions

With the emergence of proteomics as a robust and high throughput analytical tool in life sciences, its application in meat quality also has grown exponentially. Integration of gel electrophoresis, HPLC, MS, and data-mining algorithms, empowered scientists to explain biomolecular interactions influencing the properties of skeletal muscle as food, to interpret molecular mechanisms governing meat quality, and to solve protein-based concerns in muscle foods.

See also: Chemical and Physical Characteristics of Meat: Chemical Composition; Color and Pigment; Protein Functionality. Conversion of Muscle to Meat: Color and Texture Deviations; Glycolysis. Curing: Dry; Natural and Organic Cured Meat Products in the United States. Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Ham Production: Dry-Cured Ham. Muscle Fiber Types and Meat Quality. Tenderizing Mechanisms: Enzymatic

Further Reading

Bendixen, E., 2005. The use of proteomics in meat science. Meat Science 71, 138–149.

Hollung, K., Veiseth, E., Jia, X., Faergestad, E.M., Hildrum, K.I., 2007. Application of proteomics to understand the molecular mechanisms behind meat quality. Meat Science 77, 97–104.

Huff-Lonergan, E., Anderson, M.J., Kim, Y., Lonergan, S.M., 2011. Application of proteomic tools to define contributions of protein to variation in meat tenderness. In: Proceedings of American Meat Science Association Annual Reciprocal Meat Conference. Manhattan, KS: American Meat Science Association.

Joseph, P., Suman, S.P., Rentfrow, G., Li, S., Beach, C.M., 2012. Proteomics of muscle-specific beef color stability. Journal of Agricultural and Food Chemistry 60, 3196–3203.

Lametsch, R., 2011. Proteomics in muscle-to-meat conversion. In: Proceedings of American Meat Science Association Annual Reciprocal Meat Conference. Manhattan, KS: American Meat Science Association.

Mullen, A.M., Stapleton, P.C., Corcoran, D., Hamill, R.M., White, A., 2006. Understanding meat quality through the application of genomic and proteomic approaches. Meat Science 74, 3–16.

Suman, S.P., 2012. Application of proteomics to understand meat quality. In: Hui, Y. H. (Ed.), Handbook of Meat and Meat Processing. Boca Raton, FL: Taylor & Francis, pp. 287–299.

Suman, S.P., Joseph, P., 2011. Proteomics of meat color. In: Proceedings of American Meat Science Association Annual Reciprocal Meat Conference. Manhattan, KS: American Meat Science Association.

Relevant Websites

http://www.matrixscience.com
Matrix Science Inc.

http://www.ncbi.nlm.nih.gov/protein
National Center for Biotechnology Information.

http://www.pdb.org
Research Collaboratory for Structural Bioinformatics.

http://www.expasy.org
Swiss Institute of Bioinformatics.

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QUALITY MANAGEMENT

Contents

Abattoirs and Processing Plants

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Glossary

Certification Confirmation that certain characteristics are met by a timely limited certificate.
ISO 9000 series Quality management standards (ISO 9000:2005; ISO 9001:2008; ISO 9004:2009; ISO 19011:2011) providing guidance and tools in order to ensure that customers' requirements are met.
Process control The measures to ensure that processes result in the desired result.
Product testing Analytical procedures for examining products.
Quality assurance Engineering activities, such as measurement and monitoring activities, to fulfill requirements in a quality system.
Quality control Revision activities directed toward the quality traits of the product during production.
System control Control measures comprising several steps within a production chain.
Taylorism A scientific management theory developed by Frederick Winslow Taylor (1856–1915) using process control measures, i.e., analysis and synthesis of workflows in order to improve labor productivity.

Introduction

Meat and meat products are valuable sources of concentrated, easily digestible protein, vitamins, minerals, and micronutrients contributing to a human's balanced diet. Per capita meat consumption varies widely depending on the world's region, but a general trend is that more meat is consumed with increasing personal income. The availability of meat depends also on local agricultural conditions, as animal production may be undertaken in landscapes that are unsuited for crop production.

A great number of trade flows have developed in the global food market that enable the supply of populous and urban regions with food from rural areas all over the world. However, increased transportation distances of easily perishable foods like meat require improved production and handling techniques in order to prevent excessive product losses as well as the risk of foodborne diseases. Systems to effectively measure, manage, and improve product's safety and quality enjoy widespread use. When product losses are reduced, yields and profits may increase; yet, on the other hand, such systems help to assume responsibility for the products that is clearly assigned to the manufacturer by legal standards. As a consequence, the participants of the meat production chain are highly interested in the introduction and maintenance of quality management systems in their processing establishments.

Quality Attributes of Meat and Meat Products

According to the International Standardization Organization (ISO), 'quality' is defined as "the ability of a set of intrinsic characteristics to satisfy requirements" (ISO 9000:2005). With respect to meat and meat products, the intrinsic characteristics are described by several quality attributes. Even though objective quality attributes are analytically detectable, resulting in quantitative or categorized values, subjective quality attributes express the consumer's individual esteem for special product traits.

Objective quality attributes of meat and meat products may be grouped into sensory traits, nutritive values, processing properties, and hygienic-toxicological aspects (Table 1). In subjective terms, the acceptance of the product is also determined by the consumer's attitude toward psychological (nutritional habits, nausea-causing experiences, etc.), ethical (e.g., animal welfare and religious customs), ecological, political, social, and sustainability factors.

Quality Strategies in the Meat Production Chain

Manufacturers must decide which quality traits they wish to attach to their products in both qualitative and quantitative terms. In this decision, they will consider the desires of the target group and market trends, as well as legislative requirements. During production, compliance with these defined quality features, as well as maximal homogeneity of the products, must be guaranteed. For this purpose, quality assurance and quality control measures were introduced in order to fulfill quality requirements. Although the former refers to systematic monitoring, measuring, and threshold comparison techniques for error prevention, the latter comprises observation and steering activities of processes.

Generally, three overall strategies may be distinguished depending on the manner and extent of the quality assurance measures taken: (1) product testing, (2) process control, and (3) system control (Figure 1).

Product Testing

Established in the era of mass production and Taylorism, end-product testing was the accepted concept to guarantee defined product quality features. In other words, products were manufactured by unskilled workers as fast as possible and nonconforming products were sorted out by quality inspectors at the end of the production line. If it is not possible to test each single product (i.e., when complex or destroying testing methods have to be applied), statistically valid sampling plans have to be used to judge the entire production batch.

However, application of valid sampling plans in the meat trade is neither economically nor ethically acceptable. For example, pathogenic microorganisms are most often present in very low numbers and are mostly inhomogeneously distributed in the food matrix. If 5 packages out of 100 contain this hazard, at least 60 packages must be tested in order to detect it with a probability of 95%. If only 5 packages are tested, the probability of finding this health risk is only 23%.

Additionally, the financial implications of the end-product testing concept may be serious. Reprocessing of fresh meat items in order to eliminate risks is not applicable without changing the physical appearance of the product. Thus, serious financial losses up to total destruction of nonconforming batches may have to be borne. Another problem arises when only time-consuming and cumbersome testing methods are available. In particular, this occurs when highly perishable foods, such as fresh meats, have left the producer's site before analytical results become available. If the quality criterion is not reliably met, a cost-intensive and reputation-damaging product recall may be inevitable.

Although product testing will always be necessary in food production, more preventive concepts are required to avoid the occurrence of nonconforming products. Such concepts are primarily based on the control of food production processes.

Process Control

Process control is the second conceptual approach for safeguarding defined quality features. To fulfill the quality criteria, preventive, supervisory, and corrective measures are established in the course of the processes. In this context, a process is described as a "set of interrelated or interacting activities which transforms inputs into outputs" (ISO 9000:2005), or raw material into end products. Process control measures may be restricted to certain quality attributes (e.g., hygienic-toxicological characteristics), may include all quality features of a particular product or manufacturing process, or may cover all internal processes of an enterprise (management, procurement, production, sales, etc.), as in the case of quality management according to ISO 9001.

In recent decades, internal process control has been increasingly requested by food surveillance authorities and food retailers, especially if private labels are manufactured. Operators of meat processing plants worldwide are obliged to safeguard safety and quality of processes and products by identification of critical steps in their processing lines and to introduce effective monitoring and corrective action systems at these critical processing steps. Additionally, documentation of all significant product traits is mandatory.

To support process control, several 'quality management tools' have been developed. This very heterogeneous group of procedures, methods, and techniques are useful for the description, analysis, assessment, regulation, prevention, and visualization of processes. Some quality management tools are rather simple (daily tally sheets), whereas others are quite complex systematic approaches (hazard analysis critical control point (HACCP) concepts). A brief survey of quality management tools throughout different steps in a product's life cycle, beginning with the product idea and ending with maintenance service, are presented in Figure 2.

System Control

Effective quality control measures must also cover preceding and subsequent production steps in order to achieve and maintain the intended product quality characteristics. Although contractual agreed raw material specifications already have a long tradition, the longitudinal integration of quality

Table 1 Quality attributes for meat and meat products

Sensory traits	Appearance ←	Color ←	Taint
			Brightness
		Form (cutting style)	
		Marbling (distribution visible fat)	
	Aroma ←	Smell	
		Taste	
	Texture ←	Juiciness	
		Tenderness	
		Firmness (consistency)	
		Mellowness	
		Stringiness → Structure	
		Granularity	
		Smoothness, sliding ability	
Nutritional value	Chemical analysis ←	Protein content ←	Muscle protein
			Collageneous protein
			Foreign protein
		Fat content	
		Carbohydrate content	
		Vitamin content	
		Ash content ←	Minerals
			Trace elements
	Physiology ←	Utilization capacity	
		Digestibility	
		Biological value	
Hygienic-toxicological aspects	Microorganisms ←	Bacteria	
		Spores	
		Yeasts and fungi	
	Storability impacts ←	pH-value	
		a_w-value	
		E_h-value	
	Food additives ←	Nitrate and nitrite curing salt	
		Others	
	Residues and coantaminants ←	Antibiotics	
		Hormones	
		Thyreostatics	
		Pesticides	
		Herbicides	
		Fungicides	
		Toxins	
		Heavy metals	
		Nitrate, nitrite, and nitrosamines	
Processing properties	Water-binding capacity		
	Protein →	Content	
		Condition	
	Fat →	Content	
		Condition	
	Connective tissue, tendon content		
	Firmness (toughness)		
	Structure		
	pH-value		
	Color		

Source: Reproduced from Hofmann, K., 1987. Der Begriff Fleischqualität. Definition und Anwendung. Fleischwirtschaft 67, 44–49.

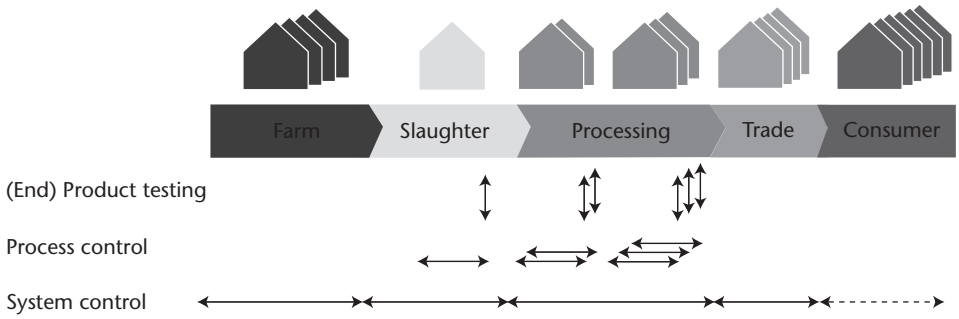

Figure 1 Conceptual approaches to quality assurance in the meat production chain.

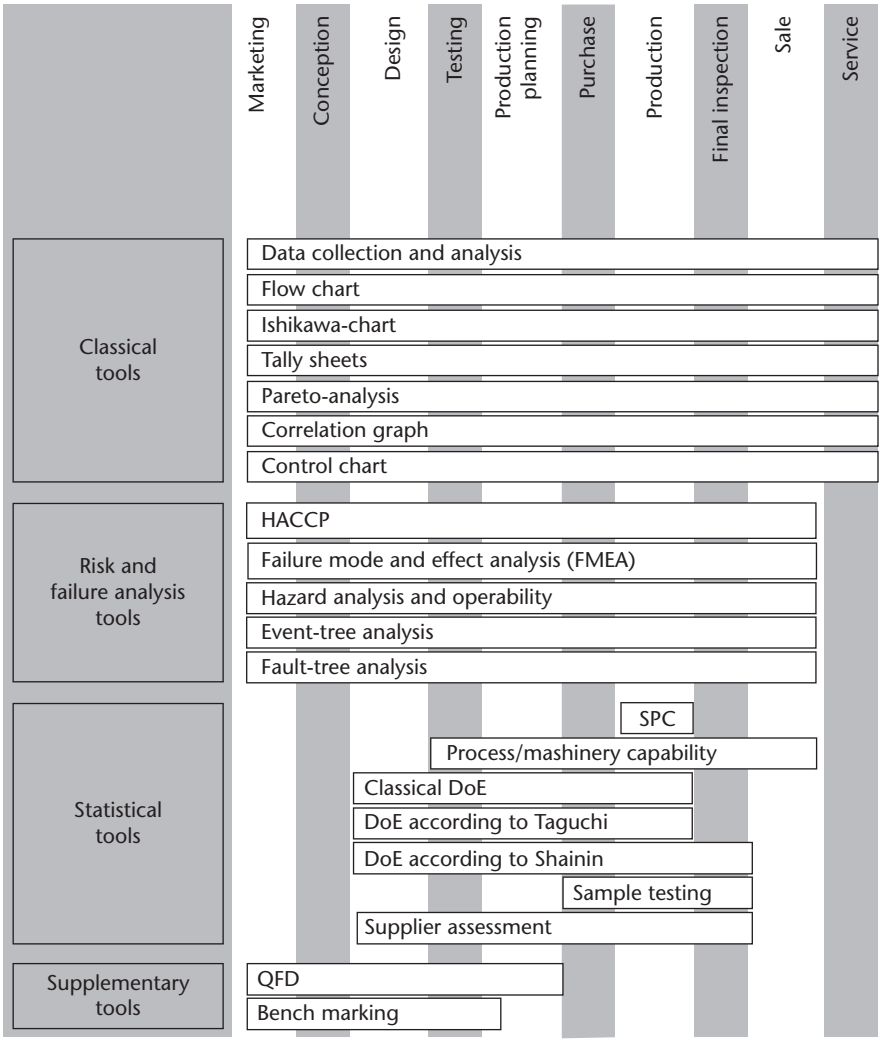

Figure 2 Quality management tools and their application in a product's life cycle (DoE: design of experiments; QFD: quality function deployment; SPC: statistical process control). Reproduced with permission from Deutsche Gesellschaft für Qualität e.V. (DGQ), 1998. Qualitätslenkung in der Lebensmittelwirtschaft, vol. 21, issue 12. Berlin: Beuth.

assurance activities into a holistic approach was largely developed in the 1980s. Over the intervening years, meat production and marketing chains were established that guarantee certain quality traits and production properties.

Promoted by the rapid developments in the electronic data-processing sector, such integrated quality control systems (longitudinally integrated quality and safety assurance) use interlinked documentation systems that ensure traceability of

the products from the primary production to the consumer's household, concisely depicted by expressions such as 'from farm to fork,' 'from stable to table,' or 'from conception to consumption.'

Standardized Quality Management and Certification

Historical Development

The aim of each business enterprise is for customers to give preference to its products over the products of its competitors. Accordingly, its products must be more favorable regarding price, availability, and/or desired quality features.

At the beginning of the twentieth century, quality features were guaranteed by product inspection. However, in the late 1930s and 1940s, industrial producers realized that preventive process control would allow them to produce better, faster, and with less waste, and this realization was prompted mainly by the needs of the weapons industry. Again in the 1960s, military requirements resulted in the first quality management standards (e.g., the US Mil-Q-9858 and NATO's AQAP 1, 4, and 9). During the 1970s and 1980s, multiple quality standards were developed in many countries for different needs, particularly the nuclear, automobile, and aerospace industries. In 1987, this resulted in the generally applicable quality management standards of the ISO 9000 series. These basic standards have been revised several times, leading to actual version of ISO 9001:2008. In the meat industry, however, these standards were applied only in a reserved way. In France, for example, the first slaughterhouse was not certified before 1995.

Management Systems

The basic rules for introducing directing systems for quality traits are laid down in the ISO 9000 standard series. Herein, quality management systems are defined as "…that part of the organization's management system that focuses on the achievement of results, in relation to the quality objectives, to satisfy the needs, expectations and requirements of interested parties…" (ISO 9000:2005). The ISO 9000 requirements are universally applicable for all kinds of 'organizations' that stand for "…a group of people and facilities with an arrangement of responsibilities, authorities and relationships …," such as companies, corporations, institutions, associations, etc. Requirements for the products themselves are not established because the products' quality features should be stipulated by customers and organizations.

Although ISO 9000 standards are primarily directed toward the satisfaction of customers' needs and expectations, several additional benefits result from the introduction of a quality management system. For costumers, decisive economic advantages arise, thus products reduce the need for retesting. For the organization itself, processes within the organization grow more transparent and responsibilities become more clearly assigned. If there are personnel changes, the know-how remains within the organization and the quality awareness of the employees will be enhanced. The control of the (production) processes will serve as a sound basis for their continual improvement and as proof of due diligence with regard to product liability. Last but not least, positive marketing effects may occur.

The development of a quality management system within an organization takes approximately 6–12 months, depending on the size and complexity of the organization and on the available human and financial resources. At the beginning, a survey is conducted comparing the actual processes and documentation procedures with the requirements of the standards to be applied. To meet the requirements, a hierarchically organized documentation system will be generated. This consists of a statement concerning the quality policy and quality objectives; a quality manual; certain documented procedures as required by ISO 9001; and the documentation needed to ensure the effective planning, operation, and control of the organization's processes. It is indispensable to include the employees in the compilation of the documentation. This helps the acceptance of the quality management system in the organization. An important part of the documentation consists of the records that provide objective evidence of the activities performed or the results achieved. After completion of the documentation, it is officially brought into the organization. The functioning of the quality management system is verified by means of internal audits, being a 'systematic, independent, and documented process' (ISO 9000:2005) for the evaluation of the suitability, adequacy, effectiveness, and efficiency of the quality measures conducted. A description of procedures and requirements to carry out internal audits are found in ISO 19011:2011.

Using common elements, the quality management system will reason for integration into an organization's overall management system, which considers objectives such as economic, social, environmental, and other aims.

Certification Process

If a quality management system is implemented and its functioning has been subjected to several internal audits regarding its usefulness and function, it is possible to have an external survey and certification of the system. Certification (from Latin *certum facere* = to make sure) is a procedure in which third-party experts confirm (in writing) that a product or a process agrees with a certain standard. In case of ISO 9001, it is certified that an organization's quality management system complies with the ISO 9001 requirements – it does not, however, specifically evaluate the technical competence of an organization.

First of all, an external independent organization, a state-approved or accredited certification body, is needed to carry out the certification on the basis of a private law contract. Care and diligence are necessary when choosing a certification body, as the contract period lasts usually several years. Criteria for the choice of certification bodies are, among others, their reputation, their international business activities, their proximity to each other, their competence in the business branch in question, customers' requests, or financial considerations. For example, opting for an international certification body is indicated if the organization itself works internationally. Websites similar to the International Accreditation Forum may be helpful when looking for certification services.

After conclusion of the contract, the quality manual and the relevant documentation are given to the certification body, which chooses an expert audit team. At least one of the audit team should come from the line of business in question. The experts read and review the submitted documentation against an appropriate check list and look for compliance with the requirements of the standard in question. If no deviations are identified, a date for the certification audit will be fixed; otherwise, the organization first has to rework its procedures and documents.

The certification audit is by far the most substantial audit. Its duration and extent depend on the size of the organization (number of employees and locations). At the fixed date, the audit team comes into the organization to conduct a systematic on-site survey of the quality management system. Usually, after an opening session, the quality manager accompanies the auditors through the organization. It is the aim of the audit to find out whether the specifications of the quality manual and the other documentation conform with the day-to-day life within the organization. Additionally, the auditors have to check whether the quality management system has become part of the organization's culture and also whether the stated quality policy and quality objectives are feasible in terms of the processes described. For this purpose, all divisions of the organization are visited, employees are interviewed, activities are observed, and on-site documents (records) are reviewed. It goes without saying that the auditors are bound to professional discretion concerning all of the internal affairs of the organization. At a closing session, the auditors will present the most important audit findings and will draw an audit conclusion. Nonfulfillment of audit criteria will result in written nonconformity reports that are part of the audit report. The auditors and the quality manager will agree on certain corrective and preventive actions, as well as on dates by which the actions have to be established. Depending on the nature and severity of nonconformity, the issue of the certificate may be postponed or linked to an additional audit that deals only with the deviations.

A written audit report will deal with all audit findings. It will include a comparison between the regulations of the quality management system laid down in writing versus the actual quality procedures of the organization, as well as all indications of flaws, weak points, and potential for further improvement. Subsequently, the certificate will be issued and, as a rule, yearly follow-up audits will be performed for renewal of the certificate.

Special Certification Programs

In recent years, globalized trade, legal requirements, and increased liability demands of the food supply chain participants have led to the development of multiple certification standards focusing on different quality traits, products, processes, production plants, and production chain stages within the meat production chain. Owing to its outstanding importance, management systems directed toward food safety aspects enjoy widespread use, especially the British Retail Consortium (BRC) Global Standard, International Featured Standard (IFS), and Food Safety System Certification 22000.

In 1998, the BRC introduced the BRC Food Technical Standard. Because retailers and brand owners are (under the EU food law) legally responsible for their private labels, the evaluation of food manufacturers according to this standard assists private label owners, first to receive food products of consistent safety and quality and second to prove their due diligence in case of a prosecution by the enforcement authorities. The rapid spread and development of this standard has demonstrated its value as the benchmark for best practice in the food industry. During the past decade, the standard evolved into the BRC Global Standards series comprising requirements for the production, packaging, storage, and distribution of food and consumer products. Each of these standards is regularly reviewed, revised, and updated.

A similar standard was launched in 2003 by the French and German retailer federations. Developed for the assessment of food suppliers' safety and quality systems at postfarm gate stages, the IFS have been recently developed to provide uniform safety and quality requirements for food and nonfood products, as well as for related services. As in the BRC standards, the responsibility of the brand owner was the rationale for the development of uniform, common, and internationally accepted audit standards originally meant for products and processes of manufacturers producing those private label products. In the meantime, certification according to the IFS standard requirements is expected from nearly all food manufactures delivering products to wholesalers and large retailers. The latest IFS Food Standard (version 6) has been developed also for certification bodies, food industry, and food service companies from all over the world.

In 2005, the ISO published the ISO 22000 standard. This general derivative of ISO 9001 specifies requirements for food safety management systems to control safety hazards in order to ensure that food is safe at the time of human consumption. For this purpose, the management system requirements of ISO 9001 were merged with food safety requirements of HACCP, good manufacturing practice (GMP), and preventive programs. A certification scheme for such systems was developed by the Foundation for Food Safety Certification and called FSSC 22000. It comprises requirements for management systems to ensure the safety and suitability of food throughout the supply chain.

Although product safety still remains the most important quality trait in the meat supply chain, several other certification schemes have been widely introduced into the meat market. The reasons for the introduction of such schemes were growing quality demands and expanded quality perceptions of the customers. Requirements concerning animal welfare; animal feedstuff composition; environmental aspects like carbon dioxide footprint; organic farming; traceability; sustainability comprising economical, ecological, and social criteria; and/or regional provenance were considered and organized as certifiable private or public quality labels. After successful passing of the certification audit, the issued certificate communicates to the customer that the products comprise the attributes that the certification scheme seeks to affect. This helps the enterprise to be distinguishable from its competitor. According to Bredahl *et al.* such certification schemes can also act as a coordinating mechanism in the supply chain where different levels of the chain are under different ownership. Most of these schemes are related to preceding and subsequent stages of the

production chain; thus, constituting an integrated system control approach. One of the earliest systems in this field was the Dutch 'Integrale Keten Beheersing (IKB)' program (an overview on current European certification systems was provided by Theuvsen *et al.*). Although not yet certifiable, Upmann *et al.* recently presented a standard to evaluate the sustainability status of pig abattoirs and pork processing establishments; hence, the introduction of a sustainability certification programs can be expected within the next few years.

Quality Management in the Meat Production Chain

Aims

In addition to basic meat safety and quality traits presented in Table 1, meat enterprises must consider quality traits, such as animal welfare, organic farming, and sustainability, according to their customers' demands. Thus, quality management activities of abattoirs and meat processing plants nowadays contain a vast variety of quality attributes deriving from different quality programs. Most of them are equally related to preceding and subsequent stages of the production chain, requiring the integration of external data and setting standards for suppliers. The backbone of the activities is, therefore, a sophisticated electronic data-processing system that enables the collection and analysis of data, the traceability of all products on all stages of the production chain, and a detailed documentation of all quality relevant activities.

Activities

First of all, the enterprise must install and maintain a management system. To integrate the quality demands into this management system, all requirements have to be broken down over the whole manufacturing process into single measures at each step of the processing line in order to guarantee that the intended quality traits are maintained throughout the whole process. For this purpose, additional technical production guidelines are followed, supplemental product analyses are performed, additional control points are established, and extra inspection, survey, and monitoring systems are implemented.

Meat plants possess elaborated, often computer generated, product sampling and testing plans that may apply to each single quality characteristic mentioned previously. Sampling for *Salmonella*-monitoring programs may serve as an example. If feasible, the sampled products should be retained from market until the test results are available (sample/test and hold system). Process control measures integrate the technical steering activities during processing that are mandatory in HACCP systems, FMEA, or GMP concepts. Computer-aided survey of chilling or heating processes or online control of exsanguination with respect to animal welfare should also be mentioned. System control measures are equally well introduced in order to integrate all participating enterprises, processes, and products. For instance, abattoirs implement supplier assessment strategies in order to categorize slaughter animals according to their quality deviation risk. Also, individual animal identification systems are maintained to safeguard traceability, and early information transfer between farm and abattoir is mandatory to install logistic slaughter systems. Further, multiple measures are taken during animal transport and arrival, slaughter, meat handling, processing, and meat shipment. As demonstrated in Figure 3, safeguarding upstream and downstream information flows is crucial for the establishment and maintenance of the quality systems, as well as for the transparency and traceability of products.

Last but not least, independent and competent third-party certification services are required to assure compliance with the previously mentioned certification programs. This will increase the number of audits in which the personnel have to participate considerably.

Documentation

Quality management systems require an extensive documentation system. Generally, it is arranged hierarchically following, for example, the structure of the basic certification standard. Some information on the ownership, nature and extent of the production, and organizational structure should be given. A basic description of the available resources (e.g., plant infrastructure, internal/external personnel, etc.), production processes, and supporting processes (such as cleaning and disinfection), as well as the product portfolio should also be provided. The descriptions should be short and precise. For example, a number of resources, such as processing machinery, water supplies, material flows, personnel routes, temperature zones, or clean and unclean areas, can be best represented by a set of ground plans. Description of production processes may clearly be done by process flow charts, and it is recommended to number all processing steps for references.

Specifications must be elaborated for all processing steps laying down the target parameters in order to meet the quality demands at this specific point. Similar specifications must be elaborated for supporting processes, such as personnel hygiene, cleaning and disinfection, pest control, waste removal, and others that indirectly may have an impact on the product quality.

Finally, records must be maintained on the quality relevant activities according to the principle: "Activities that are not documented were not carried out."

Prospect

Definitely, the future will bring further longitudinal integration of the different segments of the agrifood chain. Intelligent information exchange systems, electronic communication, and intense data transfer are prerequisites for the optimization of the different quality management activities.

Multiple concepts and tools can be used to develop an organization's quality management system. Which concepts and tools are employed will depend on the field to cover, the organization's structure and aim, legal requirements, and customers' expectations. Principally, it should be kept in mind that the quality management system should serve the organization and not vice versa. Therefore, implementation of special concepts or tools should always be checked toward their simplicity and suitability for the desired purpose.

Quality system elements	Steps of production		Safety	Quality	Environment	Traceability	Sustainability	CSR	Animal welfare
Breeding/fattening/genetics	Agricultral production	<- information / HACCP monitoring / QA Checks-->		•	•	•	•		•
Control of medicaments			•	•	•	•	•		•
Consulting program			•	•		•	•		•
Origin control			•	•	•	•			•
Feed control			•	•	•	•	•		•
Salmonella monitoring			•	•	•	•	•		•
Results of veterinary control and data exchange			•	•		•			•
Company audits			•	•				•	•
Research and development			•	•	•	•	•	•	•
Acquisition of pigs	Lifestock and slaughtering			•		•	•		•
Plausibility checks			•	•		•			
Video documentation			•	•		•	•		•
Barn management and stunning			•	•					•
Postmortem inspection			•	•		•	•	•	•
Salmonella monitoring			•	•		•	•		•
Cooling facilities	Cooling and cutting		•	•	•		•		
Cooling management			•	•	•		•		
Selecting and slots of pigs			•	•		•	•		
Automatization			•	•	•	•	•	•	
Personal hygienics			•	•			•	•	
Maturing system			•	•			•		
WMS (warehouse management system)			•			•	•	•	
Finished product testing	Preparing / packaging	Continous chain of cooling	•	•	•	•	•		
Automatization			•	•		•			
Sensory testing			•	•		•			
Identity check			•	•		•			
In process control			•	•		•		•	
Online checkweighering			•	•		•			
Identifying of lots			•	•		•			
Room control system			•	•	•		•		
ERP system from line to dispatch*			•	•		•			
ERP system from dispatching to logistic*	Dispatch and logistics		•	•		•			
Commissioning				•		•	•	•	
Cooling facilities and management			•	•	•	•	•		
GPS controlled chain of cooling			•	•	•	•	•		
Temperature gates			•	•					
Tracking and tracing	Feed-back		•	•		•		•	
Management of production records			•	•		•			
Complaints management			•	•	•	•		•	
Customer service			•	•	•	•		•	

* ERP = Enterprise resource planning

Figure 3 Elements of a meat plant quality system with respect to different meat quality traits during meat processing. Necessary upstream and downstream information flows for steering activities are included. CSR, corporate social responsibility; GPS, global positioning system. Modified from Trilling, J., 2013. Inline production Tönnies group. Rheda: Tönnies.

Additional certification programs directed toward new or increasingly important quality features can be expected. Their incorporation into the companies' quality system seems possible as long as the basic definitions of the certification systems are compatible.

See also: Hazard Analysis Critical Control Point and Self-Regulation. Quality Management: Farm Level: Pork Quality; Farm Level: Safety and Quality of Beef. Risk Analysis and Quantitative Risk Management

Further Reading

Baourakis, G., Kalaitzis, P., Mattas, K., 2013. Food Chains: Quality, Safety and Efficiency in a Challenging World. Abingdon, UK: Routledge.

Bender, A., 1992. Meat and meat products in human nutrition in developing countries. FAO Food and Nutrition Paper 53. Rome: Food and Agriculture Organization of the United Nations.

BRC, 2013. BRC Global Standards for Food Safety. London: BRC Trading Ltd. Available at: www.brcglobalstandards.com (accessed 22.01.13).

Bredahl, M.E., Northen, J.R., Boecker, A., Normile, M.A., 2001. Consumer demand sparks the growth of quality assurance schemes in the European food sector. In: Regmi, A. (Ed.), Changing Structure of Global Food Consumption and Trade. USDA, Agriculture and Trade Report. WRS-01-1. Available at: http://www.ers.usda.gov/media/293645/wrs011_1_.pdf (accessed 07.11.13).

FSSC, 2013. Food Safety System Certification 22000. Foundation for Food Safety Certification. The Netherlands: AR Gorichem. Available at: www.fssc22000.com (accessed 22.01.13).

Hofmann, K., 1987. Der Begriff Fleischqualität: Definition und Anwendung. Fleischwirtschaft 67, 44–49.

IFS, 2013. International Featured Standards. Berlin: IFS Management GmbH. Available at: www.ifs-certification.com (accessed 22.01.13).

ISO 9000:2005. Quality Management System – Fundamentals and Vocabulary.

ISO 9001:2008. Quality Management System – Requirements.

ISO 19011:2011. Guidelines for Quality and/or Environmental Systems Auditing.

Luning, P.A., Marcelis, W.J., 2009. Food Quality Management: Technological and Managerial Principles and Practices. Wageningen, NL: Wageningen Academic Publishers.

Nollet, L., 2007. Handbook of Meat, Poultry and Seafood Quality. Ames, IA: Blackwell Publishing Professional.

Pearson, A.M., Dutson, T.R., 1995. Quality Attributes and Their Measurement in Meat, Poultry and Fish Products. Dordrecht, NL: Kluwer.

Petersen, B., Nüssel, M., 2013. Qualitätsmanagement in der Agrar- und Ernährungswirtschaft. Düsseldorf: Symposion Publishing.

Theuvsen, L., Plumeyer, C.-H., Gawron, J.-C., 2007. Certification systems in the meat industry: Overview and consequences for chain-wide communication. Polish Journal of Food and Nutrition Sciences 57 (4(C)), 563–569.

Theuvsen, L., Spiller, A., Peupert, M., Jahn, G., 2006. Quality Management in Food Chains. Wageningen, NL: Wageningen Academic Publishers.

Upmann, M., Hölscher, M., Kelliger, H., Müller, T., Stiebing, A., 2012. Nachhaltigskeitsstandards für die Schweineschlachtung, Zerlegung und Verarbeitung von Schweinefleisch. In: Proc. 35. Lemgo: Lemgoer Arbeitstagung Fleisch und Feinkost, 5.11.2012.

Relevant Websites

http://asq.org/
: American Society for Quality.

www.brcglobalstandards.com
: BRC Global Standards for Food Safety.

http://www.cen.eu/cen/Pages/default.aspx
: European Committee for Standardization (CEN).

http://www.european-accreditation.org/content/home/home.htm
: European Co-operation for Accreditation.

http://www.efqm.org/
: European Foundation for Quality Management.

http://www.eoq.org/
: European Organization for Quality.

www.fssc22000.com
: Food Safety System Certification 22000.

http://www.iaf.nu
: International Accreditation Forum.

http://www.ipcaweb.org/
: International Auditor and Training Certification Association (IATCA).

www.ifs-certification.com
: International Featured Standards.

http://www.iso.org/
: International Organization for Standardization.

http://www.juse.or.jp/
: Union of Japanese Scientists and Engineers.

http://waq.asq.org/
: World Alliance for Quality.

Farm Level: Pork Quality

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Glossary

Current Good Manufacturing Practices A set of guidelines for processing nonmedicated and medicated feed.

Good Production Practices A set of guidelines for safe, healthy, efficient and humane production of pork.

Hazard Analysis Critical Control Point A production control system for the food industry designed to prevent rather than catch potential hazards; identifies where potential contamination can occur (the critical control points) and strictly manages and monitors these points as a way of ensuring the process is under control and that the safest product possible is being produced.

Pork Quality Assurance® A producer-driven program they can use to ensure US pork products are of the highest quality and safe, and animals raised for food are cared for in a way ensuring their well-being.

Transport Quality Assurance® A program that helps swine transporters, producers, and handlers understand how to handle, move, and transport pigs and the potential impacts of those actions on pig well-being and pork quality. Anyone who handles or transports pigs, or sets protocols for handling pigs, is a potential influencer of animal well-being and pork quality.

Veterinary-Client-Patient Relationship A relationship that exists between a client and a veterinarian where the veterinarian has assumed the responsibility for making medical judgments regarding the health of the animals, has sufficient knowledge of the animals, and is readily available for follow-up consultation.

Withdrawal time Length of time between final administration of an animal-health product and animal harvest.

Introduction

Defining the parameters that encompass the topic of pork quality at the farm level can be a challenging task. Pork quality starts at the farm level and is carried through the food supply continuum. The pork industry maintains its responsibility to support animal well-being and provide consumers with a safe, abundant, and high-quality food. In modern pork production systems, pork producers, and other industry stakeholders have made great strides in improving pork quality at the farm level with attention on such factors as animal health, nutrition, welfare, and proper transportation. Major advances in swine sciences over the past several decades have allowed the pork industry to focus on factors of importance with intensity and the ability to create change. The goal of achieving efficient, safe, and sustainable production of a wholesome pork product has been a focal point for many years and will continue to be critical to pork as a global protein source for a growing world population. This article considers (1) factors of pork quality, (2) how pork quality assurance (Pork Quality Assurance Plus® (PQA Plus®)) in the United States emphasizes quality and integrity, and (3) responsibility of producers to maintain high standards of pork quality.

Pork Quality

To understand the on-farm parameters that can affect pork quality, it is important to define pork quality. Pork quality can be defined as the series of attributes and traits that have a direct influence on the purchasing decision, food safety, and palatability attributes as measured by the consumer. Although pork quality is traditionally thought of as measurements of muscle color, pH, water holding capacity, tenderness, juiciness and flavor, today's consumer also characterizes animal welfare, health, nutrition, and production claims such as 'natural' or 'antibiotic free' as quality attributes.

Because many factors of pork quality begin at the farm level, establishing robust quality assurance programs is critical to ensuring good pork quality for the global pork industry for years to come. A good example that will be outlined throughout this article is the PQA Plus® program in the United States. Many other countries across the world, such as Canada, Germany, the Netherlands, and Denmark also have pork quality assurance programs. These programs all share similar program goals and objectives that help define on-farm pork quality.

Pork Quality Assurance

Developed by the United States National Pork Board in 2007, the PQA Plus® program was designed for continuous improvement and used to identify and educate Good Production Practices (GPPs) that positively affect food safety, animal well-being, and pork quality. The PQA Plus® program combines scientific research with best production practices to educate pork producers about administering proper swine management throughout the lifecycle of the hog. Food safety and animal well-being are the cornerstones of the PQA Plus® program and the pork industry. The PQA Plus® program achieves its goals by educating pork industry professionals during certification sessions. An objective on-farm assessment is also conducted, which, when combined with the individual education certification, results in the production site being granted PQA Plus® site status. This is a very important

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Table 1 Pork Quality Assurance® Plus 10 Good Production Practices (GPPs)

GPP #1	• Use an appropriate Veterinarian-Client-Patient Relationship as the basis for medication decision making
GPP #2	• Establish and implement an efficient and effective health management plan
GPP #3	• Use antibiotics responsibly
GPP #4	• Properly store and administer animal health products
GPP #5	• Follow proper feed processing protocols
GPP #6	• Establish effective swine identification, medication records and withdrawal times
GPP #7	• Practice good environmental stewardship
GPP #8	• Maintain proper workplace safety
GPP #9	• Provide proper swine care to improve swine well-being
GPP #10	• Utilize tools for continuous improvement

Source: Reproduced with permission from the National Pork Board, Clive, IA, United States.

designation for the production site because site certification is required for swine procurement by most pork processors. On-farm reviews and audits are becoming commonplace throughout the pork industry and are intended to build credibility, trust, and integrity.

Table 1 outlines the 10 GPPs of the PQA Plus® program. According to the PQA Plus® Education Handbook "the GPP's, when implemented, will help ensure pork is free from chemical and physical hazards; that the pigs are raised in a caring, humane manner; that our natural resources are protected by pork producers; and that employees on pig farms have a safe place to work." The 10 GPPs are based on the following objectives:

1. Hazard Analysis and Critical Control Point principles are the standard for controlling hazards in foods produced and processed in the United States and many foreign countries.
2. The Food and Drug Administration's Compliance Policy Guide (CPG) 7125.37 – "Proper Drug Use and Residue Avoidance by Non-veterinarians."
3. The Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994.
4. Science-based animal care and well-being guidelines.

Production Factors Affecting Pork Quality

There are many factors for a producer to consider when implementing a pork quality assurance program within a production system. This article details some of the most important factors in pork production that contribute to pork quality on the farm level. Many of these factors are major topics included in quality assurance programs. The following list of factors describes a variety of elements necessary to be considered for optimal pork quality.

Barn Management

The most basic aspect of proper swine management comes through the understanding of production systems. Farm setup and design are key components to ensuring both an efficient production system and one that produces a quality product. The production system encompasses barn design, pen size and layout, proper handling facilities, and proper ventilation. Controlling barn environment is a key component of minimizing stress and optimizing animal performance. Barn parameters will vary by region and desired level of biosecurity. Examples of these specific adaptations include the incorporation of cooling cells to maintain comfortable ambient temperature during the summer months and use of body heat and air flow strategies in the winter time to maintain temperatures in the pig's thermal neutral zone without diminishing air quality or increasing energy cost. Even though the system represents a fixed cost in production, proper barn design and setup is the first step in ensuring optimum production.

Genetics

The selection of breeding stock that fit both the rearing environment and the market to which hogs will be sold is critically important. Today's pork industry is very diverse in the genetics available, the production systems where swine are reared, as well as the avenues in which the pork is bought and sold. Production claims such as 'naturally raised,' 'gestation stall free,' 'organic,' 'antibiotic free,' and/or claims of improved meat quality will impact the type of breeding stock used in a production system. The pork industry has observed the development of many high-producing commercial lines of gilts that are designed to maximize the number of pigs weaned and their respective weaning weight. Unfortunately, some of these same females tend to see less parities in a sow herd due to reproductive or body condition challenges. Therefore, it is important to the long-term success of a swine operation that producers find the optimal sow to fit the system.

Modern swine production now utilizes more than 95% artificial insemination. The systematic use of boar lines to improve heterosis for growth, efficiency, and meat quality has been beneficial. Utilization of swine genetics has led to huge gains in production parameters. For example, utilizing Duroc and Hampshire boars on Yorkshire, Landrace, and Chester White base females has led to improved farrowing rates, feed efficiency, and carcass composition while also reducing days to market and overall cost of production. The utilization of the purebred Berkshire breed in niche markets is also very popular. Known for superior marbling capability, higher pH, dark pork color, and recognized palatability attributes, the Berkshire breed retains a reputation for a premium eating experience.

Swine Husbandry

Proper animal husbandry skills are a major component in successful swine operations. A thorough understanding of the production system and ability to recognize challenges are critical to swine welfare and pork quality parameters. Examples of management challenges affecting pork quality could include monitoring animal health, recognizing nutritional deficiencies, changes in air quality or temperature, and animal handling.

Feed and Nutrition

With rising feed costs affecting all livestock sectors, much focus has been turned toward feed efficiency and the utilization of alternative feeds to reduce cost of production in all phases. Producers need to ensure that Current Good Manufacturing Practices are used as guidelines on formulating and feeding both nonmedicated and medicated feed. The importance of high-quality feed to swine operations is essential for growth and maintenance, as well as swine well-being.

Changes in diet formulation can have adverse effects on pork quality parameters. One example of a common feed ingredient used in swine diets to reduce overall cost is dried distillers grains with solubles (DDGS). The use of DDGS in gestation, lactation, nursery, and grow/finish diets has dramatically increased in recent years. This spike in utilization has led to a parallel increase in research in this area. Although cost is reduced, several pork quality challenges have also been observed. Owing to the higher proportion of unsaturated fatty acids in DDGS compared to the traditional corn/soy diet, market hogs fed higher than 20% DDGS have shown significant changes in pork quality and, more specifically, fat quality. An alteration in the fat composition of meat also leads to processing challenges and changes in shelf life and consumer acceptance. Pork with higher concentrations of unsaturated fatty acids undergoes a higher rate of fatty acid oxidation, resulting in off flavor if gone unchecked.

Swine Health

An efficient and effective herd health management plan, coupled with a working relationship with a swine veterinarian, is an essential component of farm management. This relationship is known as the Veterinary-Client-Patient Relationship (VCPR). Without a VCPR, swine herds are put at an increased and unnecessary risk for animal disease and potentially compromised food safety. Herd health is a key to food safety. Animals in good health are more efficient and produce a better end product. The healthier an animal is, the less need for pharmaceutical use, reducing the risk of antibiotic residues while also reducing the associated cost of treating sick animals. A proper herd health protocol should contain regularly scheduled herd evaluation by a veterinarian, as well as biosecurity measures in place to ensure animal safety and well-being.

Regular veterinarian inspections of swine operations are critical in maintaining a healthy herd. Visits by a trained professional grant producers the ability to review their vaccination protocol, potentially observe symptoms previously over looked, and also view the production system from a different vantage point. Many times veterinarians are consulted to address health problems negatively impacting carcass value and quality. These defects are typically brought to the attention of the producer by the pork processor, and in most cases the producer needs to quickly address these issues to improve performance, decrease financial losses, and ensure continued acceptance from the processor.

Sometimes overlooked is the negative impact these health issues have on meat quality. Some examples of common health issues that negatively affect carcass value include abscesses, pneumonia, and arthritis. For example, the presence of pneumonia in swine reduces breathing capacity. Swine can adapt to a more restricted oxygen intake in their normal environment, and, even though a reduction in performance is observed, the animal is still able to cope with its surroundings. However, when swine are exposed to mixing, transportation, lairage, and movement to harvest, the reduced capacity to take in oxygen can cause extreme stress on their system, i.e., not only stress on the circulatory system, but also the musculoskeletal system. The reduction in oxygen consumption and capability to remove by-products from adenosine triphosphate (ATP) hydrolysis out of the muscle creates a challenging metabolic situation. The decrease in muscle pH can lead to fatigue, inferior pork quality, and possibly death.

Biosecurity

Biosecurity is a combination of management practices and protocols designed to prevent the transmission of diseases and disease-causing agents. In general, biosecurity involves restricting the movement of anything capable of carrying disease or disease-causing agents, including people, pigs, birds, other animals, and water. Biosecurity measures can be applied both internally and externally to the facility. For example, an internal biosecurity practice could include an all-in all-out finishing system where any disease cycle present in the facility is confined to the specific group of pigs and the facility is sanitized before another group of pigs enter. This strategy is also observed in the farrowing rooms, where following weaning, all farrowing rooms are washed, disinfected, and have a period of down time before reloading the next farrowing group.

Although internal biosecurity practices are utilized, external measures are more commonly recognized. Some external biosecurity measures include isolation of new animals prior to entering the herd, control of wildlife and pests, air filtration systems, limiting visitors, supplying clean clothes to all guests, shower-in/shower-out, and managing vehicle traffic.

Animal Welfare

Animal welfare is increasingly becoming a focal point of swine production worldwide. There is a wide range of public opinion within and across different countries regarding what constitutes proper animal welfare and humane production practices. Major topics include gestation sow housing, tail docking, physical castration, and immunocastration. Regardless of opinions, providing proper care to improve swine well-being should be the foundation of any livestock producers operation. Swine raised in the proper environment are healthier, perform better, and produce quality pork products. The pork quality assurance programs offered around the world address many factors that directly affect swine well-being at the farm level. Some of these factors are described in more detail.

Recordkeeping

Keeping accurate and consistent records of all procedures and processes within an operation is an essential part of preserving producer/processor relationships and earning the consumer's trust. Documenting the VCPR relationship along with all

medication and treatment records provide a health history on all pigs and help ensure food safety. This documentation process also affords the opportunity to identify trends in animal treatments, helping to identify possible improvement strategies in herd health protocols. All treatment records should be maintained for a minimum of 12 months after marketing of the animal.

Daily Observation

All swine production facilities should be observed daily. Daily observation allows for prompt recognition and delivery of necessary care, as well as detects any facility or management issues present. A daily walk through can also monitor effectiveness of health protocols, current plane of nutrition, and overall animal care. Making sure all feed and water delivery systems and air flow systems are functioning will ensure the utmost care of the hogs.

Daily monitoring should also be used to identify and separate any ill or nonambulatory pigs to an alternative pen. Treatable pigs should be cared for promptly in order for them to fully recover. Any seriously ill pigs should be humanely euthanized following farm protocols.

Animal Evaluation

Evaluating animals on a regular basis will verify that well-being programs are positively impacting the animals. The response of the pigs to human presence alone is an indication of animal well-being. Pigs that are calm and relaxed when someone enters a facility are more than likely beneficiaries of pleasant handling and care. However, pigs that appear afraid and fearful of people may be neglected or may not be in a facility that is meeting their needs.

Specific traits of evaluation include overall performance, physical appearance including lameness, lesions, abscesses, wounds, and prolapses. One of the best indicators of well-being can be measured by pig performance. Changes in average daily gain, feed efficiency, mortality rate, or even farrowing rate are noticeable signs of problems. The ability to benchmark these performance traits and comparing to previous values is essential in monitoring herd performance. All changes in performance should have an explanation as to why, and if not, producers should move swiftly to explore the causes.

Visual appraisal of the animals also serves as a strong indicator of well-being. Lameness, lesions, and wounds can result from many different things. Some of these causes are fighting, biting, and facility problems which can all negatively impact well-being and quality. For example, lameness could be a result of bacterial infections, nutrition deficiency, and (or) genetics.

The presence and location of lesions can be very telling with regard to the problem. Lesions on the main part of the body (shoulder, back, flanks, and legs) are commonly the result of fighting with pen mates. Flooring problems, such as cracks, missing pieces, or raised sections, can lead to lesions primarily on the pig's hooves. Faulty feeding and watering equipment can result in cuts around the head and ears, whereas tail and genital biting is more likely the cause of nutrient deficiencies and overcrowding.

Barn environment challenges can also lead to rectal prolapses in swine. The presence of pneumonia and other respiratory tract infections can lead to excessive coughing, one of the primary causes of prolapses. Piling of pigs to stay warm can also result in prolapse, or it could even be the result of a genetic predisposition to prolapse. Quick isolation and treatment is necessary to prevent further injury and enhance the chance of the pig making a full recovery.

Body Condition Score

Body condition scoring (BCS) is useful in assessing the adequacy of a nutrition program. Literature also suggests that body condition score gives insight to the effectiveness of a barn's heating and cooling strategies. The most accepted BCS system is on a scale of 1–5, with 1 representing a thin, emaciated animal, and 5 being obese. Animals with a BCS less than 2 should receive immediate attention. Poor BCS commonly result from poor or inadequate nutrition or health challenges. Treatment plans may be antibiotic treatment, an increase in caloric intake or total intake, or if not treatable, the animal should be euthanized.

Over-conditioned pigs are also a disadvantage to the producer. On the grow/finish side, high body condition score pigs have reduced growth performance, are less efficient in converting feed to pounds of lean, and have a higher overall cost of production. From a carcass standpoint, these hogs also produce a less desirable and less valuable carcass due to lower lean-to-fat ratio. Breeding stock that deviates from the ideal BCS of 3 also have reduced performance. With regard to welfare, these females also create management challenges with regard to increased incidence of lesions, shoulder and leg sores, lameness, and general welfare concerns. Furthermore, low and high BCS females have reduced conception rates, reduced piglet survivability, and lower weaning weights. There is also evidence that these pigs born from challenged litters have a reduced production performance throughout their lives.

Proper Handling

The proper movement of swine throughout their lives will be important to the overall performance, health, and eventual meat quality of the animal. Having adequate handling facilities along with proven pig-handling equipment and proper handler training will ensure good well-being of the animal and safety for both the pig and the workers. Poorly moved pigs can become stressed, leading to physical injury, increased cases of nonambulatory animals, reduced performance, and increased load and unload times of market animals. These stressors can also lead to increased carcass shrink, increased incidence of carcass bruises and subsequent trim, and decreased pork quality. Improper handling and transport of pigs is one of the largest profit-reducing issues facing the pork industry today. Estimates show that bruises alone cost the US pork industry millions of dollars each year, with overall pork quality defects totaling several hundred million dollars annually. As a general rule, calm, slow, and quiet handling is the best bet for the pig and the handler.

Flight Zone

Flight zone is the imaginary space that surrounds an animal and is considered the animal's 'comfort zone.' Understanding an animal's flight zone and predicting their response to the handler's presence can be very beneficial in moving livestock. When a handler enters the pig's flight zone, the pig will attempt to move in the opposite direction. For example, if a handler approaches a pig from behind it will move forward and if the handler approaches from the right, the pig will try to move left. However, if no escape route is available, the pig will attempt to go back to where it came from. When handling pigs, it is best to use the edge of the flight zone to move them, as this will result in the least amount of stress possible.

Herding Instinct

Pigs are instinctively herd animals. This predictable behavior causes pigs to want to follow each other in order to maintain contact. Understanding this behavior can make it easier to move pigs up and down ramps, through hallways, and into or out of pens and rooms. Many facility designs today use this concept to their advantage by using double alley raceways with a see-through gate in the middle. This allows pigs to see others in front of them as well as next to them, reducing the stress of moving into a new environment.

Using the herding instinct can greatly reduce the potential for stress and injury in pigs. When pigs feel isolated, they will try to escape. This response can lead to injuries caused by nearby gates, feeders, chutes, or other objects. The pig may also try to escape by jumping, increasing the risk of injury to joints, muscles, and bones.

Transport

Loading, transport, and unloading have been identified as high stress events for hogs. These preharvest stressors significantly increase metabolic factors commonly known for quantifying stress and reducing pork quality. Research and anecdotal evidence suggest that transport within different seasons can have a significant effect on animal welfare, survivability of swine, and pork quality. In the United States it is very common for swine to be transported over great distances through vastly different geographic regions. Trailer type, bedding material, and trailer design can influence these transport factors. The National Pork Board manages the Transport Quality Assurance® program in the United States to help those involved in swine transportation understand and manage the factors involved in reducing losses associated with transport, improve animal welfare, and reduce risk of deteriorating pork quality.

Conclusion

There are many farm level factors that affect pork quality. Pork producers can find significant value in quality assurance through quality assurance programs offered by producer groups around the world, such as PQA Plus® in the United States. These programs enable pork producers to participate and engage in programs that benefit the welfare of pigs, reduce costs associated with management, and provide documentation for continuous improvement. As protein consumers become further removed from production agriculture and basic knowledge about food production, it will be critical that swine producers provide customers with factual, science-based information about pork production, pork quality, and food safety. Doing so can add a tremendous amount of value to the entire food chain.

See also: Classification of Carcasses: Pig Carcass Classification. Conversion of Muscle to Meat: Slaughter-Line Operation and Pig Meat Quality. Hazard Analysis Critical Control Point and Self-Regulation. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Modeling in Meat Science: Meat Quality. Nutrition of Meat Animals: Pigs. On-Line Measurement of Meat Quality. Preslaughter Handling: Preslaughter Handling; Welfare Including Housing Conditions; Welfare of Animals. Quality Management: Abattoirs and Processing Plants; Farm Level: Safety and Quality of Beef. Species of Meat Animals: Pigs

Further Reading

NPB, 2013. Pork quality assurance education manual (version 2.0). Clive, IA: National Pork Board. Available at: http://www.pork.org/filelibrary/PQAPlus/V2.0/TrainingAdults/PQAPlusEducationHandbookVersion2.0.pdf (accessed 03.04.14).

Relevant Websites

www.cqa-aqc.ca
Canadian Pork Council.
www.cqa-aqc.ca
Canadian Quality Assurance.
http://www.agricultureandfood.co.uk/Quality_assurance.aspx#.UhVqSBtkN8E
Danish Agriculture & Food Council.
http://www.danishcrown.com/B2B/Pork.aspx
Danish Crown.
http://www.pdv.nl/Imbinaries/brochure_pdv_vv_uk.pdf
Dutch Quality Assurance.
http://www.q-s.de/qs__a_mark_of_approval_that_stands_for_food_you_ca.html
German QS System.
http://www.pork.org
National Pork Board.
http://www.porkgateway.org/FileLibrary/PIGLibrary/References/Pork%20Quality%20Assurance%20Plus%20(PQA%20Plus)%20AS%20REFERENCE.pdf.pdf
Pork Information Gateway.
http://www.q-porkchains.org/news/Conference/meat_week/quality/stoier.aspx
q-porkchains.
http://www.tulipltd.co.uk/index.php/quality-assurance
Tulip Ltd.
http://www.fda.gov/AnimalVeterinary/default.htm
US Food and Drug Administration.

Farm Level: Safety and Quality of Beef

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Glossary

Antimicrobial A substance that kills or slows the growth of microbes, like bacteria (antibacterial activity), fungi (antifungal activity), viruses (antiviral activity), or parasites (antiparasitic activity).
Contamination Something that causes a product to be infected, corrupted, or polluted after contact with it.
Hazard analysis critical control point A production control system for the food industry designed to prevent rather than catch potential hazards; it identifies where potential contamination can occur (the critical control points) and strictly manages and monitors these points as a way of insuring that the process is in control and that the safest product possible is being produced.
Resistant determinants Having exact limits that give the ability to ward off bacteria.
Violative residues Residues resulting from the use of animal drugs and pesticides or from incidents involving environmental contaminants that are outside the tolerances established by the Food and Drug Administration, Environmental Protection Agency, and Food Safety Inspection Service.

Introduction

Addressing beef safety and quality on farm has always been a sensitive topic because of the number of producers who are typically involved in the production of animals from conception and birth all the way to harvest. Depending on the production systems involved, cattle might be in as few as one production unit/facility or in as many as three or four during their lifetimes. Regardless, the goal of beef production is to produce a safe, wholesome, quality product that consumers will purchase again and again. In achieving this feat, producers must understand consumer's definition of beef safety and quality and then use production systems that produce a final product which meets that definition.

Definition of Beef Quality

If general consumers are asked to define beef safety and quality, likely responses could be tender, juicy, flavorful, free of defects (residues, lesions, etc.), nutritious, and safe. Although producers might not include safety as a quality measure, consumers commonly do, and hence it needs to be considered. Given such a definition, the beef quality assurance (BQA) program was developed on a national level to help producers identify production practices that impact the factors included in the definition of quality.

Structure and Impact of Beef Quality Assurance Programs on Product Safety and Quality

The BQA program's mission is to maximize consumer confidence and acceptance of beef by focusing cattle producers' attention on daily production practices that influence beef. Ultimately, BQA has the intention of assuring consumer confidence that beef produced by BQA-certified producers and under the program guidelines meets their demands. The BQA program guidelines are simply practices that revolve around five major aspects of production: feedstuffs, feed additives and medications, processing/treatment and records, injectable animal health products, and care and husbandry practices.

The BQA program is implemented on a state-by-state basis; however, the National Cattlemen's Beef Association (NCBA) in the United States provides national leadership and oversight of the program. More than 45 states in the United States have implemented a BQA program. Although the NCBA provides such a leadership, it is important to realize that each state's effort is producer driven, voluntary, and educational in scope. States can vary in terms of their emphasis on 'certification' of educational efforts, but nearly all programs have such a component in them. It is believed that, although voluntary, this effort by the NCBA and each state program will help to avoid government regulation of production practices in the United States.

History

History of BQA dates back to the late 1970s and the early 1980s, when programs such as the Beef Safety Assurance program and the Residue Avoidance Program existed. These programs, although voluntary, focused on one major issue – elimination of residues in the meat supply. This focus was understandable at the time, given the concern of antibiotic residues in beef by the United States Department of Agriculture (USDA) and specifically the USDA Food Safety and Inspection Service. The concepts found in the BQA program are based on the same concepts found in total quality management or hazard analysis critical control point (HACCP) programs – identify potential problems, outline where they can occur, prevent such issues from occurring, and if they do occur, solve the problem.

Goals

According to the national BQA program training manual, the goal of the program is "To ensure consumers that all cattle shipped from a beef production unit are healthy; wholesome and safe; their management has met Food and Drug Administration (FDA), USDA, and Environmental Protection Agency (EPA) standards; they meet quality requirements throughout the production system; and are produced with environmentally-sound production practices." Further, objectives of the BQA program are:

1. Set production standards for quality and safety that are appropriate to an operation and that can be met or exceeded. Key elements that influence production of defect-free food include biosecurity, animal health and well-being, production performance, and environmental stewardship.
2. Establish data retention and recordkeeping systems that satisfy the FDA/USDA/EPA guidelines to help allow validation of management activities and fulfill program goals.
3. Provide hands-on training and education to help participants meet or exceed the BQA program guidelines and help to realize the benefits of the program.
4. Provide technical assistance through BQA program staff, veterinarians, extension specialists, and other qualified individuals working with the BQA program.

Guidelines

The BQA guidelines were developed and evaluated by the national BQA advisory board that consisted of veterinarians, producers, researchers, extension personnel, and beef industry leaders committed to producing safe, quality products for consumers. This advisory board continually reviews and updates guidelines as needed to meet the ever-changing production and regulatory climate of the beef industry. The guidelines, presented by their topic area, of the BQA program can be found in Table 1 and the following paragraphs outline a summary of each category.

Feedstuffs

Although utilization of feedstuffs changes as economic conditions change in the industry, the premise of the guidelines remains the same – maintain a safe feed supply free of contamination by pesticides, herbicides, other feed ingredients, or banned substances and be sure the feeding of such feedstuffs is supported by sound science. An example of this is making sure that producers follow the ruminant-derived protein source feed ban.

Feed Additives and Medications

The emphasis on the category of feed additives and medications is to assure that producers utilize medicated feed additives according to the strict guidelines set forth by the FDA's Good Manufacturing Practices – using them only in accordance with label directions for dose, species, duration, etc. This category also points out that even though producers can work with their veterinarians to use antimicrobials in an extra-label drug use manner, this is not allowed for medicated feed additives.

Processing/Treatment and Records

The emphasis in processing/treatment and records is for producers to understand that records of product – herbicide, pesticide, antimicrobials, etc. – use can benefit them in their production system. Keeping accurate and reliable records help to assure that cattle sent to harvest are free of residues, that feeds are free from contamination, and that ultimately producers meet consumer demands for a safe, wholesome product. Documentation could also help producers to identify trends that might be happening in their own production unit.

Injectable Animal Health Products

Administration of antimicrobials has been the main stronghold of the BQA program since its inception. Although the original focus was on residues, a subsequent focus was on injection site lesions. Early results from the Injection Site Lesion Audits revealed the amount of product loss the industry was suffering as a result of placing injections in the top sirloin butt or the round of cattle and the loss suffered from giving injections in the muscle tissue. The focus in this category is proper placement and administration of antimicrobials. This proper administration includes proper route (giving injection subcutaneous (SQ) instead of intramuscular (IM) if that is a label option), proper location (giving all injections regardless of route in the neck region), and giving low-dosage products (or limiting administration to 10 cc per site) to control the severity of injection site lesions.

Care and Husbandry Practices

The NCBA has taken measures to assure that everyone involved in care, husbandry, and handling of cattle is educated on handling and caring for cattle in a humane manner, inspecting facilities to insure the ease of cattle handling and movement, and to keep equipment clean and sanitary. However, the education in this category goes beyond producers. The NCBA has also developed educational materials for truckers and auction market employees, for example, to make sure that everyone in the production chain from birth to harvest knows the impact they can have on product safety and quality.

Impact of Beef Quality Assurance on Product Quality

Beef quality has been measured over the years through research projects, such as the National Beef Quality Audit (NBQA) and the National Market Cow and Bull Beef Quality Audit. The NBQA was first conducted in 1991 and was followed by audits in 1995, 2000, 2005, and 2011. The focus of the NBQA is to measure and monitor producer-related defects, and then disseminate results through the BQA program to producers on ways management can be improved to decrease the incidence of defects in fed steers and heifers and cull cows,

Table 1 Beef quality assurance guideline categories and guidelines within each category

1. Feedstuffs
   - Maintain records of any pesticide/herbicide use on pasture or crops that could potentially lead to violative residues in grazing cattle or feedlot cattle
   - Implement adequate quality control program(s) for incoming feedstuffs. Program(s) should be designed to eliminate contamination from mold, mycotoxins, or chemicals of incoming feed ingredients. Supplier assurance of feed ingredient quality is recommended
   - Suspect feedstuffs should be analyzed before use
   - Ruminant-derived protein sources cannot be fed as per the Food and Drug Administration (FDA) regulations
   - Feeding by-product ingredients should be supported with sound science
2. Feed additives and medications
   - Only FDA-approved medicated feed additives shall be used in rations
   - Medicated feed additives shall be used in accordance with the FDA Good Manufacturing Practices regulation
   - Follow Judicious Antibiotic Use Guidelines
   - Extralabel use of feed additives is illegal and strictly prohibited
   - To avoid violative residues, withdrawal times must be strictly adhered to
   - Where applicable, complete records must be kept when formulating or feeding medicated-feed rations
   - Records are to be kept for a minimum of 2 years
   - Operator shall assure that all additives are withdrawn at the proper time to avoid violative residues
3. Processing/treatment and records
   - Follow all the FDA, United States Department of Agriculture, and Environmental Protection Agency guidelines for product(s) utilized
   - All products are to be used as per label directions
   - Extralabel drug use shall be used only when prescribed by a veterinarian working under a valid veterinary–client–patient relationship
   - Extralabel drug use of aminoglycosides (a group of antibiotics effective against certain bacteria) is strictly prohibited
   - Employ strict adherence to extended withdrawal periods
   - Individual treatment records shall be maintained. Record the following:
     - Individual animal or group identification
     - Date treated
     - Product administered and manufacturer's lot/serial number
     - Dosage used
     - Route, location, and person administering the product
     - Earliest date animal shall have cleared withdrawal period
   - When cattle are processed as a group, record the following information:
     - Group or lot identification
     - Date treated
     - Product(s) administered and manufacturer's lot/serial number
     - Dosage used
     - Route, location, and person administering the product
     - Earliest date animals shall have cleared withdrawal period
   - All cattle shipped to harvest shall be checked by appropriate personnel to assure that all treated animals meet, or exceed, label or prescription withdrawal times for all animal health products administered
   - Transfer all processing and treatment records with the cattle to the next production level. Prospective buyers must be informed of cattle that have not met withdrawal times
   - Keep records for a minimum of 3 years. Examples would include processing and pesticide application records
4. Injectable animal health products
   - Products labeled for subcutaneous (SQ) administration should be administered SQ in the neck region (ahead of the shoulders)
   - All products labeled for intramuscular (IM) use shall be given only in the neck region (no exceptions, regardless of age)
   - All products cause tissue damage when injected IM; therefore, all IM use should be avoided if possible
   - Products cleared for SQ, intravenous, or oral administration are recommended
   - Products with low dosage rates are recommended and proper spacing (at least 2 in between injection locations) should be followed
   - No more than 10 cc of product is administered per IM injection site
5. Care and husbandry practices
   - Follow the Quality Assurance Herd Health Plan that conforms to good veterinary and husbandry practices
   - All cattle shall be handled/transported in such a fashion to minimize stress, injury, or bruising
   - Facilities (fences, corrals, load-outs, etc.) should be inspected regularly to insure proper care and ease of handling
   - Strive to keep feed and water-handling equipment clean
   - Provide appropriate nutritional and feedstuffs management
   - Strive to maintain an environment appropriate to the production setting
   - Biosecurity should be evaluated
   - Records should be kept for a minimum of 2 years (3 years for restricted use pesticides)

their carcasses, and cuts obtained from their carcasses. In 1991, 22% of top sirloin butts had injection site lesions, whereas less than 2.0% of top sirloin butts had injection site lesions in 2000. In 2000, 100% of federally inspected beef-packing plants had implemented a HACCP system and 85% of all fed cattle was harvested in plants that use a multiple-hurdle carcass decontamination (MHCD) system (both HACCP and MHCD helped to assure that the United States had the safest beef in the world). Cattlemen in all 50 states had access to a BQA program in 2000.

Producers' Role in Beef Quality and Safety

The role of producers seems easy – get involved, attend a training meeting, and implement BQA practices into the production system. Although that might be easier said than done, the NCBA has developed tools to help producers in this manner.

Continual Improvement

Using the HACCP program as a basis, finding improvements in the beef production system requires taking a look at control points throughout the production process. These control points are common management steps, such as calving, purchasing feedstuffs, weaning calves, and transporting cattle, as part of an overall management scheme. It is during these control points that BQA practices should be incorporated in order to limit any potential hazards from occurring to food safety and quality. Table 2 provides some examples of control points impacting the BQA program.

Self Assessments

Three assessments (cow/calf, stocker, and feedyard) are available to producers via the BQA program as an on-site tool for assessment and benchmarking of key indicators of animal care and well-being as well as operational conditions. This tool may be used as a self-assessment or may be conducted by a third-party assessor. The key, regardless of who conducts the assessment, is that the assessment be repeated on a periodic basis so that comparisons can be made, trends observed, and management actions be taken to maximize animal care and well-being and operational efficiency. This assessment is about continuous improvement. However, it can help to identify items and create benchmark points that might need to be improved, including animal handling, facility/equipment maintenance, and recordkeeping/best management practices (BMPs), among other items. Repeating the assessment on a regular basis can help an operation to identify trends and take appropriate management action as necessary. Generally, the assessments focus on three main areas – animals, records and BMPs, and facilities and equipment. The Cow/Calf Assessment consists of multiple assessment points grouped into several categories, or tiers, that are most easily defined by management level and effort. Remember, the assessment tools are available for producers to use to benchmark their production units relative to implementing guideline categories and actual guidelines into practice but not to judge the production techniques in relation to other producers.

Producer Code of Cattle Care

In an effort for producers to be considered accountable for accepting their responsibility to provide proper care to cattle, the Code of Cattle Care was created by the BQA program. Most states require that producers who complete the BQA certification exam also sign a document with the following general recommendations for care and handling of cattle.

- Provide necessary food, water, and care to protect the health and well-being of animals.
- Provide disease prevention practices to protect herd health, including access to veterinary care.
- Provide facilities that allow safe, humane, and efficient movement and restraint of cattle.
- Use appropriate methods to humanly euthanize terminally sick or injured livestock and dispose of them properly.
- Provide personnel with training/experience to properly handle and care for cattle.

Table 2 Use of the hazard analysis critical control points program during on-farm and on-ranch processes to avoid potential hazards that negatively influence the quality and safety of beef

Process	*Control point*	*Potential hazard*
Feeding/supplementation	Purchasing	Antibiotic residues
	Receiving	Chemical residues
	Storage	Feed toxins
	Feeding livestock	
Prevention and treatment of health disorders	Calving	Injection site blemishes
	Weaning calves	Antibiotic residues
	Receiving breeding or stocker cattle	
Processing and cattle handling	Working cows and calves	Injection lesions
	Weaning calves	Bruises
	Shipping calves	Hide damage
		Hide defects
		Poor health
Pasture chemical use	Herbicide/pesticide applications	Water quality
		Soil contamination
	Container disposal	Residues

- Make timely observations of cattle to insure that basic needs are being met.
- Minimize stress when transporting cattle.
- Keep updated on advancements and changes in the industry to make decisions based on sound production practices and consideration for animal well-being.
- Persons who willfully mistreat animals shall not be tolerated.

Unfortunately, participation in BQA programs by cattle producers across the United States has been less than ideal. In recent years, the national BQA program has been evaluated as to its effectiveness at influencing producer behaviors related to beef safety and quality as well as the level of participation among cattle producers in this voluntary program. One of the first evaluations of the program occurred in 2006, in which the Joint Evaluation Advisory Committee (of the Cattlemen's Beef Board and NCBA) requested an external review of the beef checkoff-funded national BQA program by two university faculty members. The authors commended the BQA program for its extensive use of collaboration among diverse groups, a substantial reduction in injection site lesions from 1991 to 2001, existence of BQA programs locally in nearly every state, the BQA certification of approximately 65 000 producers, and the 'ability to educate a large number of producers about BQA in a relatively short time.' However, the authors also indicated that the BQA program could not continue to rely on past accomplishments and redundant training materials and that more standardization in core materials as well as an outline for a future strategy for BQA was needed.

One of the first objective evaluations of the BQA program, including the quantification of beef cattle producer knowledge about BQA and BQA-related issues, was conducted by the USDA National Animal Health Monitoring Service in 2007. When asked about their knowledge of BQA, 51.3% of beef cow/calf respondents indicated that they had heard of BQA, but only 22.2% of those that had heard of BQA had attended a BQA meeting or training session. Moreover, only 57.2% of producers who attended a meeting were actually BQA certified. Ultimately, less than 5% of cow/calf producers were BQA certified – a necessity to insure that producers are aware of BQA guidelines and recommendations. Another evaluation of BQA was conducted in 2011 on a state-by-state basis by surveying state BQA program coordinators. This effort was intended to identify BQA participation levels, characteristics of successful state programs, and future challenges associated with BQA. Results of that survey showed that considerable state-to-state variation exists among BQA programs, including oversight, funding sources, communication methods, and certification requirements. Furthermore, large opportunities existed by increasing the 'challenge' associated with certification (and recertification) tests and offering more BQA-related aspects, including additional levels and an auditing system, as well as dairy-, feedyard-, and youth-oriented BQA programs. It was documented that 709 BQA trainers existed nationwide to offer BQA certification. However, when estimates provided by state coordinators were summarized, just 50 832 producers were actively BQA certified as of 2011 (which equates to approximately 7% of cow/calf producers in the United States). Feedback from state BQA coordinators suggested that successful programs resulted from local trainings, university extension involvement, industry support, and training methods. However, challenges hindering success were related to inadequate funding, competing priorities among trainers, lack of producer participation and buy-in, absent financial incentives, and inconsistent programs across states.

Conclusion

The voluntary BQA program enables producers to improve product safety and quality and, subsequently, acceptance of their beef products by consumers. The guidelines that are outlined in the national BQA program are simply good management practices that will not only help producers to meet consumer demands but also help producers to be more economically viable by maintaining animal health and well-being in the entire production system. In reality, progressive cattlemen have gone beyond certification to actually verify that they are following BQA standards and are using that verification as a marketing tool to add extra value to their production system.

See also: Environmental Contaminants. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Beta-Agonists; Red Meat Animals. Nutrition of Meat Animals: Ruminants. Preslaughter Handling: Welfare Including Housing Conditions. Residues in Meat and Meat Products: Feed and Drug Residues; Residues Associated with Meat Production. Species of Meat Animals: Cattle

Further Reading

NCBA, 2010. Beef Quality Assurance National Manual. Centennial, CO: National Cattlemen's Beef Association. Available at: http://www.bqa.org/CMDocs/bqa/NationalManual.pdf (accessed 05.01.14).

Neto, F.A.C., Ahola, J.K., Archibeeque, S.L., Paulino, P.V.R., Belk, K.E., 2013. Beef production and the demands of modern society. In: Lana, R.P. (Ed.), Sustentabilidade da pecuária bovina brasileira (Sustainability of Brazilian Cattle Production). Brazil: Universidade Federal de Viçosa, Vicosa, pp. 129–145.

Smith, G.C., Savell, J.W., Morgan, J.B., Lawrence, T.E., 2006. National Beef Quality Audit – 2005. A New Benchmark for the U.S. Beef Industry – Staying on Track. Final Report of the National Beef Quality Audit – 2005. Centennial, CO: The National Cattlemen's Beef Association.

VanOverbeke, D.L., 2007. Handbook of Beef Safety and Quality. New York, NY: Haworth Food and Agricultural Products Press.

Relevant Website

www.bqa.org
NCBA Beef Quality Assurance.

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REFRIGERATION AND FREEZING TECHNOLOGY

Contents

Applications

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Glossary

Chilling Cooling an object to a temperature above its freezing temperature.
Freezing A cooling process that results in at least some of the water content of a material becoming solid in the form of ice crystals.
Freezing temperature The temperature at which a material starts to freeze.
Plate freezing Freezing an object by putting it into direct contact with metal plates that are, in turn, cooled by refrigerant.
Refrigeration The process of removing heat from an object.
Refrigeration application A specific use of refrigeration, such as chilling meat, freezing meat, air-conditioning, etc.

Introduction

The main application of refrigeration in meat processing is to cool meat, thereby reducing the rate at which meat deteriorates as a result of microbiological and chemical processes. This process is generally described as either 'chilling' or 'freezing,' depending on whether the aim is to reduce the product to a temperature above or below its freezing point, respectively. Refrigeration is also applied in meat plants as air-conditioning, in order to keep working areas at a comfortable temperature for staff and to limit microbial growth during cutting, boning, or further processing.

The effect that air-conditioning has on meat products is very similar to that of other forms of chilling. Although there are engineering differences between chilling and air-conditioning systems, they are considered together in this article. Freezing, however, has effects on meat products that are quite different from those of chilling, hence that is described in a separate section of this article. The final section discusses some of the important issues that must be considered in designing refrigeration applications.

The Chilling Process

For moisture-rich materials, such as meat, chilling is the process of cooling the material from some starting temperature down to some temperature at or above the freezing temperature of meat. The starting temperature is typically near live animal's temperature of approximately 38 °C in the case of chilling after harvest or near the maximum temperature of 60–80 °C in the case of chilling after cooking. The initial freezing

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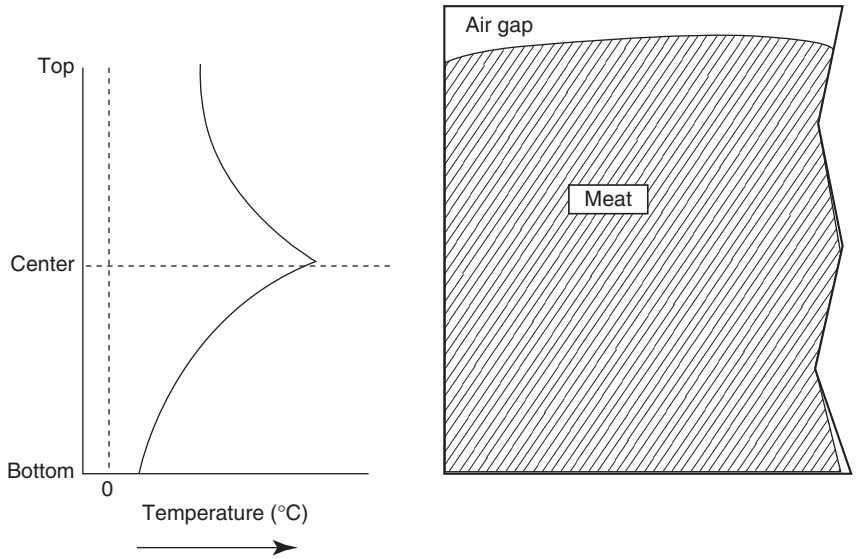

Figure 1 Typical temperature profile through the thickness of a meat carton during chilling. The static air layer in the top of the carton on the cooling acts as a thermal resistance, with the result that the warmest point in the carton is typically above the geometric center.

temperature is typically approximately $-1\,^{\circ}C$ for fresh meat or lower for processed products with some salt added.

To chill any object, the temperature outside of the object must be reduced below its current surface temperature. This will result in heat flowing away from the surface by conduction, convection, or radiation (depending on the chilling method used), causing the temperature of the surface to decrease. When the surface temperature has dropped below the temperature of the deeper parts of objects, those parts will also start to cool by conduction of heat to the surface. The objects will, therefore, develop a temperature profile in which some deep parts of the objects are the warmest, with the temperature gradually decreasing toward the surface, where the objects are the coldest. For a given thermal conductivity, large objects with high rates of heat transfer at the surface have a surface temperature that is proportionately much cooler than the warmest point, whereas small objects with low rates of heat transfer at the surface have surface temperatures that are proportionately only a little cooler than the warmest point.

Temperature Profiles and the Thermal Center

It is useful to define the point in a food material that will remain the warmest at any time during the cooling process or, alternatively, will be the last to freeze in a freezing process. This point is known as the thermal center and, if the temperature of food products is to be measured or predicted, the thermal center is usually the most important point where this should be done.

For homogeneous objects with a simple, regular shape and the same rate of heat transfer over the whole surface, the thermal center corresponds to its geometric center. For complex shapes, or if the rate of surface heat transfer varies from location to location, the thermal center can be at some distance from the geometric center. An example of this is seen in chilling or freezing cartons of meat, where an air gap is often left at the top of the carton and the rate of heat transfer through the top surface of the carton is, therefore, reduced by the insulating layer of static air. In this situation (illustrated in Figure 1) the warmest point during chilling is only a small distance under the top surface of the meat.

The geometry of carcasses or sides and the interplay of the surface heat transfer coefficient with that geometry are both complex and the thermal center must, therefore, be identified from the experience of making many temperature measurements during chilling processes. For example, the thermal center of beef sides is typically in a location known as the 'deep butt,' which can be found near the head of the thigh bone. In lamb carcasses, the thermal center has traditionally been described as being at the 'deep leg,' which is also against the thigh bone but perhaps a quarter of the way down from the pelvis. In practice, however, the low rate of cooling inside the body cavity of lamb carcasses can mean that a point between the thickest part of the shoulder and the inside of the cavity (the deep shoulder), and another point between the deepest part of the loin and the inside surface, can take almost as long to cool as the deep leg. Although the deep shoulder can often be the slowest cooling point in lamb carcasses in some chilling configurations, the deep leg or loin temperatures are measured most often because they correspond with the locations of higher valued cuts.

Chilling Methods

Objects exposed to a fluid that is cooler than objects' surfaces cool slowly by natural convection. This method is uncomplicated and has minimal equipment requirements, so natural convection air cooling was the original way in which meat was chilled – generally in the form of carcasses (for poultry and sheep meat), split or partially split into sides (for pork and beef), or quarters. The cold air itself was typically cooled by coils of tube filled with evaporating refrigerant, generally hanging from the ceiling of the chiller room.

Unfortunately, natural convection air chilling is both slow and relatively uncontrollable, because the rate of chilling is determined only by the thickness of meat and temperature of the air. For a given meat thickness, the rate of chilling can be accelerated by reducing the air temperature. The extent to which this can be achieved is limited because if the air temperature is more than a little below the initial freezing temperature of the meat, the meat surface could start to freeze. This would have detrimental consequences for product quality. As a consequence, the rate of chilling is relatively difficult to control in a natural convection process.

Probably the most common method used to chill food products is forced convection air chilling. In this method, fans are used to blow air over finned tubes filled with evaporating refrigerant. This cools the air, which passes over the food product, cooling the food and warming the air as heat is transferred from the food to the air. The air then passes back to the refrigerant evaporator to be cooled again. Using fans to move the air across the product allows the rate of chilling to be controlled by changing the velocity of the air, which makes a forced convection chilling process much more controllable than a natural convection chilling process.

Although the relationship between fluid velocity and heat transfer coefficient differs between fluids and different heat transfer situations, it is often possible to correlate heat transfer coefficient with velocity using a relationship with a form such as eqn [1].

$$h = av^b \qquad [1]$$

where, h is the surface heat transfer coefficient in W m^{-2} K^{-1}, v is the fluid velocity in m s^{-1}, and a and b are constants. Values of b typically range from 0.5 to 0.8, so (all other things being equal) doubling the fluid velocity in a given situation would typically increase the heat transfer coefficient by between 40% and 75%.

It is important to direct the air flow in a forced convection chiller so that it passes over products as evenly as possible to avoid large variations in chilling rate between different parts of the chiller. This can be achieved by the use of vanes or slotted ceilings that spread the air flow evenly across the product stow.

When moisture-rich food products, such as meat, are not wrapped in waterproof packaging during chilling, or while being processed in an air-conditioned area, the moisture on the surface and within the surface layer of the food can evaporate into the air. Because the air is cooled by contact with the refrigeration system's evaporator coils, its humidity is typically the saturation humidity at the temperature of the air leaving the evaporator. This means that the absolute humidity of the air is usually somewhat lower than that at the surface of the food. The driving force for moisture evaporation is the difference between the absolute humidity at the surface of foods (which is the product of the water activity and the saturation absolute humidity at the food surface temperature) and the absolute humidity of the air passing over the meat surface, so moisture evaporation will frequently occur while chilling unwrapped products. The rate of moisture loss can, therefore, be defined by eqn [2].

$$M = k_Y A(Y_{surf} - Y_{amb}) \qquad [2]$$

where, M is the rate of moisture loss in kg s^{-1}, k_Y is the surface mass transfer coefficient in kg m^{-2} s^{-1} (kg water/kg dry air), A is the exposed surface area of products in m^2, and Y_{surf} and Y_{amb} are the absolute humidities at the surfaces of objects and in the surrounding air, respectively, in kg water per kg dry air. Surface mass transfer coefficients are difficult to measure and few measurements have been made for meat processing situations. Fortunately, for the evaporation of water into air, the mass transfer coefficient can be related approximately to the surface heat transfer coefficient using the Lewis relation, shown in eqn [3], where C_{air} is the humid heat capacity of the air per unit of dry air mass in J kg^{-1} K^{-1}.

$$k_Y \approx \frac{h}{C_{air}} \qquad [3]$$

Moisture evaporation has three key effects on the chilling process:

1. The surfaces of meat will become dry and appearances will be undesirable.
2. The mass of meat products will be reduced by the moisture that evaporates. Because meat is often sold on a weight basis, a reduced mass of meat will result in reduced revenue when the meat is sold.
3. Moisture evaporation increases the effective surface heat transfer coefficient compared with pure convection, thereby increasing the rate of chilling.

Although it is frequently desirable to increase the rate of chilling, substantial increases in chilling rate while chilling in air can usually only be achieved through moisture evaporation. This increase in chilling rate is, therefore, at the expense of significant weight loss and degradation of products' surface appearance – both of which are undesirable. These difficulties can be avoided by spraying surfaces of meat with water intermittently during chilling. Most countries and customers have rules for most meat products that prevent weight from being added to meat before sale, so the amount and frequency of the water sprays are usually arranged to ensure that the product loses a little weight during the process, in order to provide a safety margin that avoids infringing such rules. Exceptions to these rules often include salted products, such as ham.

Spray chilling can eliminate weight losses found in air chilling of carcasses or sides, but experiences with respect to the effect of spray chilling on product surface appearances have been mixed. In some cases, appearances have been reported to be largely indistinguishable from that of air-chilled products, but cases have been reported where meat surfaces developed a streaked appearance and somewhat increased microbial growth. It might be best to conclude that considerable care and perhaps some trial and error may be necessary to achieve the best results with spray chilling.

The last common chilling method is liquid immersion chilling, used for red meats in waterproof wrappings, wrapped cooked products, unwrapped salted products, and (sometimes) bare poultry carcasses. In this method, meat products are immersed in cold liquid (generally water or brine) and cooled quickly as a result of the high heat transfer coefficient that is found between liquids and solids. As with spray chilling, when bare products are immersed in water, it is usually

important to ensure that products contain no more water at the end of the chilling process than they did at the start.

The Freezing Process

The freezing process is similar in most respects to the chilling process described in the Section The Chilling Process, except that the temperature to which the water-rich food products are exposed must be lower than the initial freezing temperature of foods. This leads to the growth of ice crystals in foods during the cooling process.

Ice Crystal Growth

When meat is cooled below its initial freezing temperature, temperature of the meat surfaces must drop somewhat below that temperature before there is sufficient temperature driving force to overcome the energy barrier associated with ice crystal nucleation. This phenomenon is known as 'undercooling' or 'supercooling' and its extent depends on the availability of suitable seed sites for the nucleation process. The extent of supercooling tends to be greater for greater rates of surface cooling, but the abundance of nucleation sites usually present in meat means that the effects of supercooling usually have little practical impact on the rate of freezing or on meat quality in typical meat processing situations.

Once ice crystals start to grow in meat, a freeze concentration process takes place in which the first part of the ice to freeze is almost pure water and solutes diffuse away from the growing ice crystals. As the solute concentration in the remaining unfrozen water becomes higher, the freezing point of the solution is progressively depressed, with the result that ice crystals forming later contain increasing concentrations of solutes. As a result, meat (unlike a pure substance) freezes progressively over a range of temperatures. Indeed, several percent of the moisture content in meat never freezes, even at $-40\,^{\circ}C$.

After freezing starts, it can progress at any given instant either by growing an existing ice crystal or by nucleating a new crystal. The former case tends to occur preferentially when freezing is relatively slow, whereas the latter case occurs preferentially when freezing is fast, with the result that smaller numbers of larger ice crystals are formed by slow freezing and larger numbers of smaller crystals are formed by fast freezing. Both freeze concentration and mechanical damage to cells caused by ice crystal growth can cause some quality degradation in meat, but the macroscale quality changes caused by well-designed freezing processes do not necessarily have demonstrable effects on eating quality.

Whatever the initial size of the ice crystals formed by freezing is, the crystals are relatively unstable and subsequent frozen storage will result in recrystallization, in which large ice crystals grow at the expense of smaller ones. Recrystallization is enhanced by temperature fluctuations, so it is preferable to maintain stable temperatures during frozen storage.

Temperature Profiles in Freezing

A sufficiently high rate of heat extraction at surfaces could make it possible for the surfaces of food products to start freezing while the thermal center is significantly warmer than the freezing temperature of foods. However, in most food freezing situations the rate of heat extraction at surfaces is low enough that the thermal center temperature reaches the freezing temperature almost as soon as the surface starts to freeze. As a result, the whole of food products between the point where freezing commences (known as the freezing front) and the thermal center typically spends much of the freezing process with its temperature equal to the initial freezing temperature. This means that the temperature of the thermal center can remain constant for much of the process and measuring that temperature will give no indication of the extent to which products are frozen. However, once the freezing front reaches the thermal center, the temperature at that point will drop very quickly. A typical thermal center temperature profile is shown in Figure 2.

Freezing Methods

Except for spray chilling, the cooling methods available for chilling processes can all be considered for freezing processes. The fact that the cooling medium in a freezing process must be colder than the freezing temperature of the meat means that some additional methods are available for freezing that are not appropriate for chilling.

The need to transfer heat from products to air or liquid, and then from the air to the refrigerant evaporator, is a drawback of any air- or water-based cooling process. This double heat transfer step can be replaced by a single step if meat can be put in direct contact with the refrigerant evaporator. Plate freezing processes achieve this by clamping slabs of meat (either bare or in packaging) between metal plates filled with evaporating refrigerant as well as by providing the high rate of heat transfer that exists when good solid-to-solid contact is achieved. Eliminating one heat transfer step means that, for a given refrigerant evaporating temperature, the temperature of the environment around the meat product is colder in a plate-freezing system than in an air or immersion freezing system. One drawback to this process is that the product must

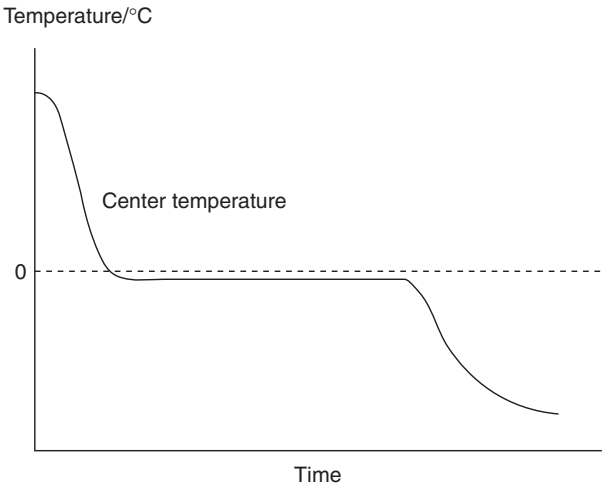

Figure 2 Typical temperature profile at the thermal center during a freezing process.

have flat, parallel surfaces with which the plates can make contact.

Cryogenic freezing, achieved by spraying meat with liquid nitrogen or carbon dioxide, or (occasionally) immersing the meat in liquid cryogen, also results in much shorter freezing times than that of air freezing, both due to the large initial temperature difference between the cryogen and meat products and to the high rate of surface heat transfer resulting from the boiling cryogen. As with plate freezing, the benefits of cryogenic freezing relative to other approaches become smaller as thicknesses of products increase, but the cryogenic freezing has an added advantage that it does not require any mechanical refrigeration equipment – requires only a tank to contain the cryogen and a suitable spraying arrangement. However, the cost of purchasing cryogenic liquid is relatively high in most locations, with the result that it is often prohibitively costly to freeze large quantities of meat in this way.

Designing Refrigeration Applications

In selecting the product temperature profile that should result from chilling or freezing after slaughter, the key quality criteria are that:

- Chilling must reduce the temperature of any meat surface, where spoilage or pathogenic microorganisms might be present, sufficiently quickly so that the number of those organisms does not grow to an undesirable level by the end of the chilling process.
- Chilling must reduce temperature slowly enough so that the biochemical process that converts muscle to meat reaches completion. This process proceeds much more quickly at higher temperatures than at lower temperatures.

For chilling after cooking, muscle has already been converted into meat, so the second criterion is not important, but the higher temperatures involved mean the microbiological growth criterion is even more important. In either case, microorganisms would normally be expected to be present on the outer surfaces of meat, but for meat products or packages that are put together from several pieces of meat, microorganism-bearing surfaces might also exist deep in the combined package. In that situation, microorganisms can be exposed to the thermal center temperature profile rather than the surface temperature profile. During cooling, the center is generally warmer than the surface so this can provide more scope for microbial growth at the center than at the surface for composite meat products.

In some situations, other criteria must be considered in designing a chilling or freezing process, including the health and safety of staff carrying out subsequent processing. For example:

- In carcass chilling processes before cutting or boning operations, it is desirable not to chill carcasses in such a way that the surface fat becomes hard and therefore difficult to cut. Some processes have deliberately reheated surfaces of carcasses so that the fat is warmer and therefore softer, or they have allowed carcass temperatures to equilibrate for some time after chilling and thereby allowed surface temperatures to rise as the thermal center temperatures fell.
- In processes where meat is to be handled afterward, it is desirable for the surfaces of meats to be dried during chilling to make them easier to manipulate. This must be balanced with the desire to retain the maximum amount of moisture in meat.
- Some freezing processes can result in unattractive surface appearances and it is helpful to design the processes to correct this problem. In one case, for example, it was reported that the surface appearance of an immersion-frozen vacuum-packed offal product was unsatisfactory as it emerged from the liquid bath, but this was corrected by spraying the offal packs with water, which briefly thawed the meat surface and then allowed it to refreeze more slowly. The slower refreezing appeared to generate larger ice crystals that reflected light better and provided a more attractive surface.

Many refrigeration processes have traditionally been designed very satisfactorily by designers who use their judgment and experience of many similar processes to ensure that the processes perform as required. However, with the increasing number of different variables that must be taken into account, designers increasingly use mathematical models to complement their judgment. A range of mathematical models from simple (but approximate) to complex (but relatively accurate) exists to calculate temperature profiles of a food product over time during cooling processes. From the temperature profile and other data about the process, mathematical models can be used to predict microbial growth and meat quality at the end of the processes. Models are also available to predict heat loads that must be removed by the refrigeration system, including the products' heat content, heat loads due to equipment in the chiller or freezer (e.g., fans), and heat infiltrating into the room through the walls, ceiling, floor, and doorways. If predicted temperatures and heat load profiles, hygiene, and meat quality resulting from a process are found to be satisfactory to achieve product specifications, equipment can then be selected to meet those requirements. If not, a range of different chilling conditions may be tried until a satisfactory set can be found. The details of models that may be used for refrigeration process design are beyond the scope of this article, but some may be found in other articles and in the Further Reading section.

See also: Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycolysis. Cooking of Meat: Heat Processing Methods. Curing: Production Procedures. Drying. Modeling in Meat Science: Meat Quality; Microbiology; Refrigeration. Refrigeration and Freezing Technology: Equipment; Principles. Smoking: Liquid Smoke (Smoke Condensate) Application; Traditional. Thermophysical Properties

Further Reading

ASHRAE, 2009. ASHRAE Handbook: Fundamentals, SI ed. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers.
ASHRAE, 2010. ASHRAE Handbook – Refrigeration, SI ed. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers.
Benítez, J., 1984. Principles and Modern Applications of Mass Transfer Operations, second ed. Hoboken, NJ: John Wiley & Sons.

Cleland, A.C., Ozilgen, S., 1998. Thermal design calculations for food freezing equipment – Past, present and future. International Journal of Refrigeration 21, 359–371.
Dossat, R.J., Horan, T.J., 2001. Principles of Refrigeration, fifth ed. Upper Saddle River, NJ: Prentice-Hall.
Lovatt S.J., Merts I., 1999. Recommended methods for food refrigeration process design. In: Proceedings of the 20th International Congress of Refrigeration, IV, Paper 350. Sydney, NSW: International Institute of Refrigeration.
McCabe, W.L., Smith, J.C., Harriott, P., 2004. Unit Operations of Chemical Engineering, seventh ed. New York: McGraw-Hill.
Santos-Yap, E.M., 1995. Fish and seafood. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York, NY: Marcel Dekker.
Savell, J.W., Mueller, S.L., Baird, B.E., 2005. The chilling of carcasses. Meat Science 70, 449–459.
Smith, J.M., Van Ness, H., Abbot, M., 2004. Introduction to Chemical Engineering Thermodynamics, seventh ed. New York, NY: McGraw-Hill.
Willix, J., Harris, M.B., Carson, J.K., 2006. Local surface heat transfer coefficients on a model beef side. Journal of Food Engineering 74, 561–567.

Relevant Websites

https://www.ashrae.org/
ASHRAE.
http://www.iifiir.org/
International Institute of Refrigeration.

Equipment

C James and SJ James, The Grimsby Institute of Further & Higher Education (GIFHE), North East Lincolnshire, UK

Glossary

Conduction, thermal Mechanism for heat transfer. The process of heat transfer through a solid material/medium in which kinetic energy is transmitted by the particles of the material from particle to particle without gross displacement of the particles.

Convection, thermal Mechanism for heat transfer. The transfer of heat from one place to another by the movement of fluids and is usually the dominant form of heat transfer in liquids and gases.

Heat transfer coefficient Coefficient used in thermodynamics to calculate heat transfer, typically by convection or phase change, between a fluid and a solid.

Pasteurization A form of heat treatment that kills certain vegetative bacteria and/or spoilage organisms in milk and other foods. Temperatures less than 100 °C are used.

Radiation, thermal Mechanism for heat transfer. Electromagnetic radiation generated by the thermal motion of charged particles in matter. All matter with a temperature greater than absolute zero emits thermal radiation.

Refrigeration May be defined as the process of removing heat from any substance to: (1) render colder – reduce temperature, (2) change its state – for example, water to ice, and (3) maintain its state – preserving foods, storing ice.

Water activity (a_w) A measure of the available water in a substance. 'High a_w' foods support bacterial growth, whereas 'low a_w' do not. This is not the same as water content. Some foods with a high water content have a relatively low a_w because the water is bound up with dissolved salts or sugar, for example, jam.

Introduction

Refrigeration equipment is required to initially chill or freeze meat and then maintain its temperature throughout the cold chain. After harvest, meat is normally refrigerated in the form of whole carcasses, sides, or quarters. Although some meat is distributed in these forms, a growing proportion is boned out into primal cuts and transported, frozen, or chilled, in 1 t pallets of 25 kg cartons. After further processing, meat and meat products are often stored, transported, and displayed in consumer packs. These can be as small as 50 g vacuum packs, for sliced cooked meat, to modified atmosphere packs containing 3–4 kg meat joints.

Basic Refrigeration System

Mechanical refrigeration systems operate using the same basic refrigeration cycle (Figure 1). A low-pressure liquid refrigerant is allowed to evaporate to a gas within a coil. This process requires heat, which is extracted from (thus cooling) any medium surrounding the 'evaporator' coil. The gas from the evaporator is compressed in the 'compressor' to a high-pressure hot gas. This high-pressure hot gas is then passed through another coil where it condenses, which releases heat into any medium surrounding the 'condenser' coil. This high-pressure, cold, liquid refrigerant then passes though an 'expansion valve,' back to the evaporator.

In a direct expansion system, the evaporator coil is either in contact with the meat to be refrigerated or the media, i.e., air, brine, etc. surrounding the meat. In a secondary refrigeration system, a liquid, i.e., water, brine, etc. is cooled by passing it over the evaporator coil. This cooled liquid is pumped through cooling coils into different parts of the meat plant where it is used to cool air that is subsequently used to cool the meat.

Primary Chilling

Immediately after harvest, red meat and poultry carcasses are at a temperature close to that for the optimal growth of many pathogenic and spoilage organisms. Primary chilling systems rapidly reduce the carcass temperature to a temperature where microbial growth is minimal or does not occur at all.

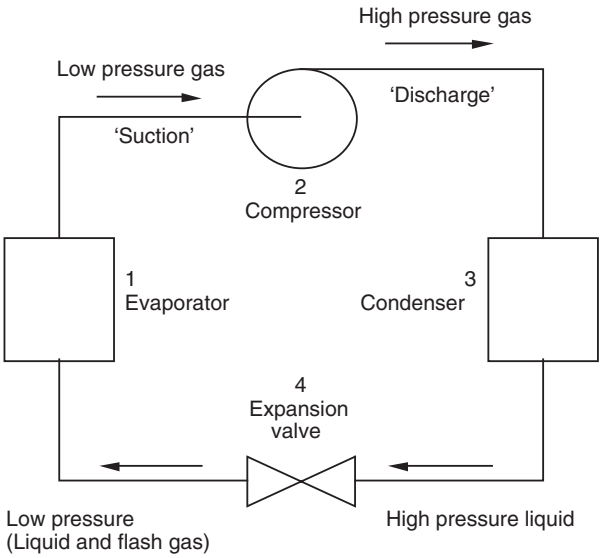

Figure 1 Basic refrigeration system.

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Red Meat

Most red meat is chilled in large insulated rooms. A steel structure in the roof supports banks of rails from which carcasses or sides are hung. The rooms are usually manually loaded by pushing the suspended carcasses along connecting rails in the abattoir onto the chill room rails. Sets of manually operated points are used to direct the carcasses onto the required rail. When the room is full, the doors are closed and the batch of carcasses is left for a predetermined time before removal. The rooms are then cleaned and the next batch loaded.

Chill rooms are designed in a way that there is free space between the carcasses or sides when they are fully loaded. In practice, they are sometimes badly and/or overloaded such that carcasses are in contact with each other or the walls of the room, which restricts the passage of refrigerated air over the surfaces to be cooled and allows cross-contamination between carcass surfaces.

The cold refrigerated air is produced by evaporator coils that are usually positioned at the top of the chill room above the steel support structure. Fans mounted on the face of the evaporator coils draw or push air through the coils, where it is cooled, and then distribute it over the carcasses to be cooled. As it passes over the warm meat, the air rises in temperature before it is returned to the coil to be recooled.

Simple batch air cooling systems are commonly used because they are economical, hygienic, easy to operate, and versatile. Relatively low rates of heat transfer are attained from product surfaces in air systems and chilling times are consequently long. They are also very dependent on the localized air temperature and velocity over the surface of the carcasses. In simple, in single-stage batch chilling systems, the risk of surface freezing limits the lowest air temperature that can be used.

In high-throughput abattoirs, the carcasses/sides are conveyed through a chilling tunnel or refrigerated room, usually by an overhead conveyor or on a belt. This overcomes the problem of uneven air distribution because each item is subjected to the same velocity/time profile and the carcasses/sides are kept separated so that all the surfaces are exposed. Different air conditions can also be maintained throughout the system. Normally, for pork, the air temperature in the first stage of the process will be less than 0 °C – sometimes as low as −30 °C. This will rapidly reduce the surface temperature of the carcasses/sides, slowing both the rate of weight loss and bacterial growth and increasing the rate of heat loss. In later stages, the temperature is progressively raised to avoid surface freezing. Temperatures around 0 °C are more typical in lamb chilling. In large US beef plants, carcasses are conveyed through a 'hot box' where they are sprayed with cold, often chlorinated, water before being placed in batch chillers.

In the initial stages of chilling, the rates of heat and moisture loss are very high. Consequently, the evaporators have to be very large to remove the heat, and the spacing of the 'fins' on the coils is wide. Moisture from the meat condenses and freezes on the evaporator coil and the ice may block the passage of air through the coil if the spacing is too small.

In some carcasse/side chilling systems for beef and lamb, a spray chilling system similar in principle to that described below for poultry is sometimes used.

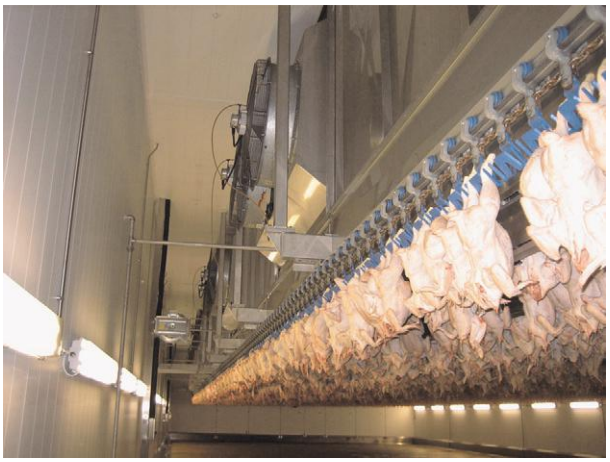

Figure 2 Continuous air chilling system for whole poultry.

Poultry Carcasses

Air, spray/evaporative, and immersion systems are the three most common methods of chilling dressed poultry.

Air

As with red meat, poultry carcasses are often chilled in large insulated rooms. The basic refrigeration equipment is identical to that used in red meat plants. Owing to the scale of production in most poultry abattoirs, the dressed carcasses are normally conveyed on rails through the room (Figure 2) or tunnel.

Spray/Evaporative Chilling

With spray chilling systems, the carcasses are transferred after soft scalding to an air chiller where sprays of potable water are applied at intervals, for example, at 5 and 15 min after the start of air chilling and on four or five more occasions during the whole chilling process. The principle of the process is to increase the rate of evaporative heat loss and, by replacing the water lost during air chilling, reduce the overall weight loss.

Immersion

Most poultry destined to be frozen are initially chilled by being immersed in chilled water or ice/water mixtures. In the US, immersion chilling is also used for poultry destined for sale as chilled birds. The process is normally a counter flow system in which the birds are conveyed in the opposite direction to the water flow to minimize cross-contamination (Figure 3).

Primary Freezing Equipment

Freezing of red meat and poultry in carcass, side, quarter, or boned primal form is normally carried out in an air blast freezer. However, plate freezers are often used for offal and low-value materials.

Air Blast

Systems range from the most basic, in which a fan draws air through a refrigerated coil and blows the cooled air around in an insulated room, to purpose-built conveyorized blast freezing chilling tunnels. The big advantage of air systems is their versatility, especially when there is a requirement to freeze a variety of irregularly shaped products.

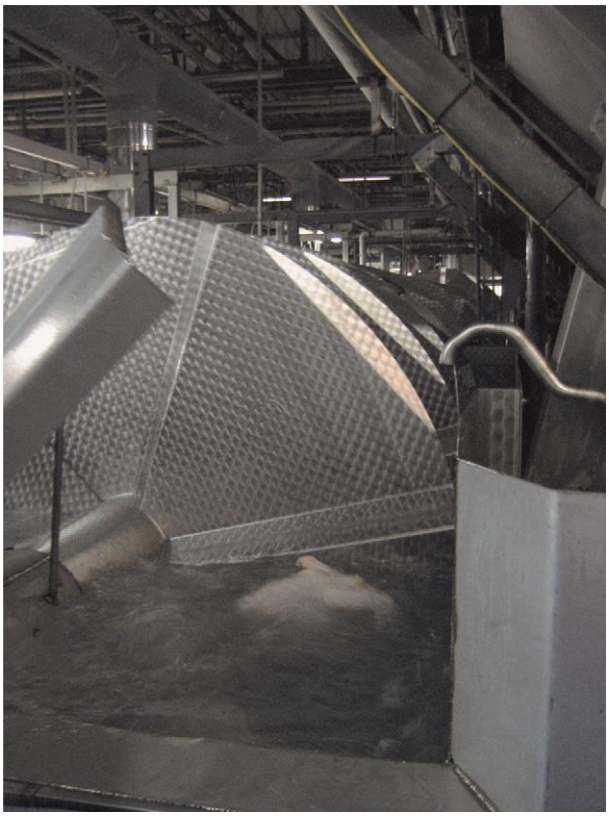

Figure 3 Spin chiller for whole poultry.

Carcass meat is usually hung on rails in order to be frozen and boxed products are carried on racks or trolleys. Freezing times for beef quarters, pork and lamb carcasses and cartons of primals, poultry carcasses, or joints are long, typically 10 h to 3 days. Continuous freezing equipment is, therefore, only used in very high-throughput plants.

In a typical continuous air blast freezer, a shelf is automatically filled with a row of 25 kg cartons. The shelf is hydraulically pushed into the freezing chamber and a second shelf takes its place. A chain system then slowly moves the shelves of cartons through the freezing chamber. Banks of very large evaporator coils with high-powered fans situated inside the chamber circulate air, typically at −35 °C, 3 ms^{-1}, over the surface of the cartons. At the end of the desired freezing period, the shelf automatically emerges from the chamber and is unloaded ready to start the cycle again.

Plate Freezing

Modern plate freezing systems differ little in principle from the first contact freezer patented in 1929 by Clarence Birdseye. Essentially, the product is pressed between hollow metal plates containing a circulating refrigerant (Figure 4). A hydraulic cylinder is used to bring the freezing plates into pressure contact with the product. These plates can be either horizontal or vertical. Contact freezing offers several advantages over air cooling, including much better heat transfer and significant energy savings. However, the need for good contact between the plates and the product necessitates regularly shaped products with large flat surfaces.

Good heat transfer is also dependent on product's thickness and the conductivity of the product. Plate freezers are often limited to a maximum thickness of 50–70 mm to gain the most benefit from the high rate of surface heat transfer. Air spaces in packaging and fouling/contamination of the plates can have a significant effect on cooling time. For example, a water droplet frozen on the plate can lengthen the freezing time of the product in contact with that plate by as much as 30–60%.

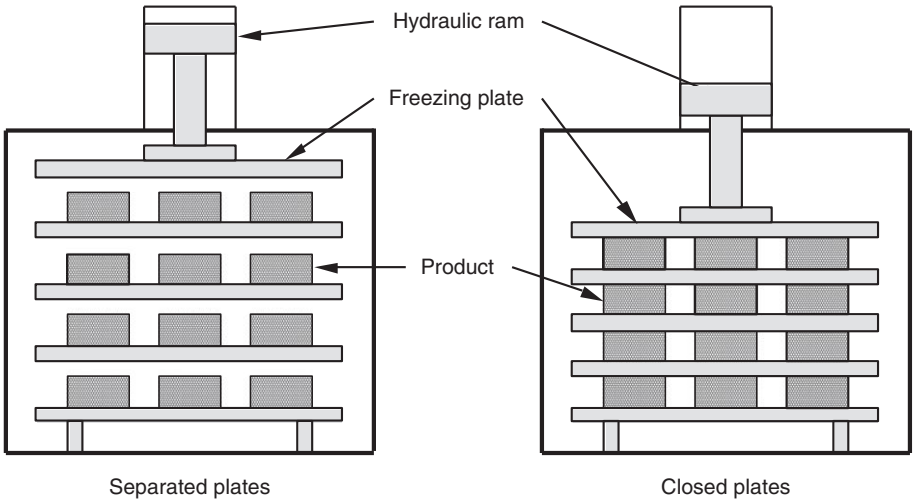

Figure 4 Example of a horizontal plate freezer.

Secondary Chilling and Freezing Systems

After secondary processing, i.e., cooking, portioning, or packing, meat cuts and products require further chilling either to remove the heat gained during the processing, or to be frozen before storage and distribution.

Secondary chilling and freezing equipment tends to be different from primary equipment because the individual items tend to be much smaller and the chilling/freezing times are consequently much shorter. Again, air-based refrigeration systems are the most common, but spray, vacuum, and cryogenic systems may also be used.

Air

In many small operations, cooked products are batch chilled on, or in, racks of trays (2.5–5 m high) that are manually loaded into a chilling tunnel (Figure 5). Very similar systems are often used to freeze batches of small products, the only difference being the temperature employed and the processing time. These systems, however, involve double handling, and it is difficult to achieve even air distribution through the layers. For larger operations, it is more satisfactory to use linear tunnels or spiral chillers/freezers. Linear tunnels are of simpler construction than spirals but are restricted by the length of belt necessary to achieve the cooling time required and the space available in the factories. Spiral chillers are, therefore, often a more viable alternative.

Recently, the use of impingement technology to increase the surface heat transfer in air chilling/freezing systems has received attention. Impingement is the process of directing a jet or jets of air at a solid surface to effect a change. The very high velocity (20–30 ms^{-1}) impingement air jets 'break up' the static surface boundary layer of gas that surrounds a food product. The resulting medium around the product is more turbulent and the heat exchange through this zone becomes much more effective. Impingement chilling/freezing is best suited for products with high surface area to weight ratios (i.e., hamburger patties or products with one small dimension). Testing has shown that products with a thickness less than 20 mm freeze most effectively in an impingement heat transfer environment. The process is also very attractive for products that require very rapid surface freezing and chilling.

Spray

Spraying with ambient or chilled water is an effective method of initially cooling cooked products that can withstand wetting, for example, hams, sausages, chubs, etc. Spray bars can either be fitted into the top of the cooking vessel itself or the hot product transferred to a separate spray-cooling booth.

Vacuum

Vacuum cooling is mainly applied to solid products having a large surface area to volume ratio and an ability to readily release internal water and to liquid or solid/liquid mixtures. Pie and pastry fillings and components of ready meals are commonly cooked in large heated vats under high pressure, and then cooled (often in the same vats) under low pressure. Products are placed in a sealable chamber, which is then evacuated to a pressure of typically 500–700 Nm^{-2} (atmospheric pressure is 101 kNm^{-2}). At reduced pressure, water readily evaporates from the surface of the products, and as it does so, the latent heat of evaporation is drawn from within the products and hence rapid cooling rates are achieved. In general terms, a 5 °C reduction in product temperature is achieved for every 1% of water that is evaporated. As this is a batch process, it requires greater production flexibility and has a high capital cost; moreover, if the pressure is reduced too fast, internal boiling may damage the product.

Cryogenic

Cryogenic cooling uses refrigerants, such as liquid nitrogen (LN) or solid carbon dioxide (CO_2), directly. The method of

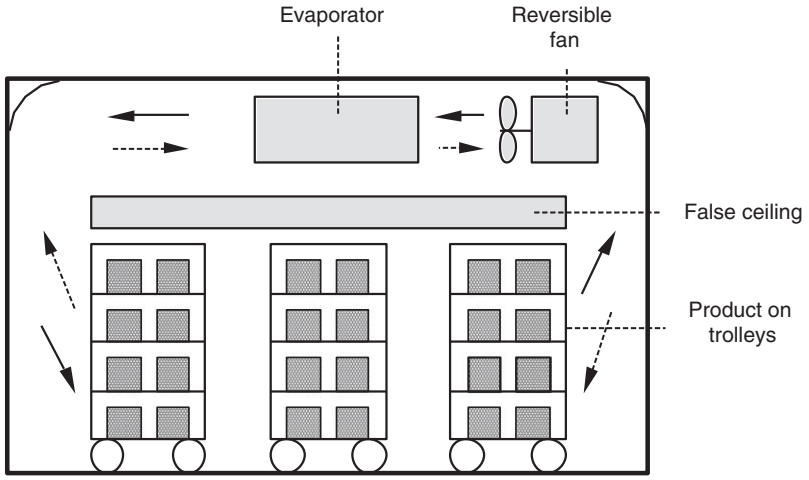

Figure 5 Batch chilling tunnel for cooked products.

cooling is essentially similar to water-based evaporative cooling, i.e., cooling is brought about by boiling off the refrigerant; the essential difference is in the temperature required for boiling. Also, using the latent heat absorbed by the boiling liquid, sensible heat is absorbed by the resulting cold gas.

Owing to very low operating temperatures and high surface heat transfer coefficients between product and medium, cooling rates in cryogenic systems are often substantially higher than other refrigeration systems.

This process is mainly used for small products such as burgers, ready meals, etc. The most common method is by direct spraying of LN onto a food product while it is conveyed through an insulated tunnel. Avoiding surface freezing of the product is the main problem in using cryogens for chilling, particularly when direct spraying; hence, LN is occasionally used indirectly in order to produce cold air that is then blown onto the product.

Cooling of solids and solid/liquid mixtures during cutting and mixing is increasingly common to prevent heating of products due to mechanical movement of the mixing and cutting blades. LN and liquid, or solid CO_2, are commonly employed for such processes.

High Pressure

High-pressure freezing and 'pressure shift' freezing in particular are attracting considerable scientific interest. The meat is cooled under high pressure to subzero temperatures but does not undergo a phase change and freeze until the pressure is released. Rapid nucleation occurs, which results in small, even ice crystals. However, studies on pork and beef have failed to show any real commercial quality advantages, and an increase in toughness was found in one study.

Storage

Most unwrapped meat and poultry and all types of wrapped foods are stored in large air-circulated rooms. To minimize weight loss and appearance changes associated with desiccation, air movement around unwrapped product should be the minimum in order to maintain a constant temperature. With wrapped products, low air velocities are also desirable to minimize energy consumption.

Using a false ceiling or other form of ducting to distribute the air throughout the storage room can substantially reduce variations in velocity and temperature. Using air socks, an even air distribution can be maintained with localized velocities not exceeding 0.2 ms^{-1}.

Transportation

Most International Standard Organization containers for food transport are either 6 or 12 m long, hold up to 26 ton of product, and can be 'insulated' or 'refrigerated.' The refrigerated containers incorporate insulation and have refrigeration units built into their structure. The units operate electrically, either from an external power supply on board the ship or dock, or from a generator on a road vehicle. Insulated containers either utilize plug-type refrigeration units or may be connected directly to an air-handling system in a ship's hold or at the docks. Close temperature control is most easily achieved in containers that are placed in insulated holds and connected to the ship's refrigeration system. When the containers are fully loaded and the cooled air is forced uniformly through the spaces between cartons, the maximum difference between delivery and return air can be less than 0.8 °C. The entire product load in a container can be maintained to within ±1.0 °C of the set point.

Refrigerated containers are easier to transport overland than the insulated types but often have to be carried on deck when shipped because of problems in operating the refrigeration units within closed holds.

For bulk transportation of frozen meat, refrigerated cargo ships are commonly used. Frozen meat is generally stored and transported at −18 °C or below. Unlike chilled meat, small temperature changes during loading and unloading can be tolerated with frozen meat.

The majority of current road transport vehicles for chilled foods are refrigerated using mechanical, eutectic plates, or LN cooling systems.

Mechanical Units

Many types of independent engine and/or electric motor-driven mechanical refrigeration units are available for lorries or trailers. One of the most common is a self-contained 'plug' unit that mounts in an opening provided in the front wall of the vehicle. The condensing section is on the outside and the evaporator on the inside of the unit, separated by an insulated section that fits into the gap in the wall. Units have one or two compressors. Depending on their capacity, these can be belt driven from the vehicle but are more usually driven direct from an auxiliary engine. This engine may use petrol, either from the vehicles' supply or from an independent tank, or may use liquid petroleum gas. Many are equipped with an additional electric motor for standby use or for quiet running, for example, when parked or on a ferry.

Eutectic Plates

Eutectic plate cooling systems are used in refrigerated vehicles serving local distribution chains. The eutectic plate consists of a coil, through which a primary refrigerant can be passed, mounted inside a thin tank filled with a eutectic solution. Standard eutectic solutions freeze at temperatures between −3 and −50 °C. A number of these plates are mounted on the walls and ceilings or used as shelves or compartment dividers in the vehicles. Two methods are commonly used for charging up the plates: (1) when the vehicle is in the depot, the solutions are frozen by coupling the plates to stationary refrigeration plants via flexible pipes, and (2) a condensing unit on the vehicle is driven by an auxiliary drive when the vehicle is in use or an electric motor when stationary. Eutectic systems are chosen for the simplicity, low maintenance, and quietness of their operation but can suffer from poor temperature control.

Liquid Nitrogen

A typical LN system consists of an insulated LN storage tank connected to a spray bar that runs along the ceiling of the transport vehicle. LN is released into the spray bar via a thermostatically controlled valve and vaporizes instantly as it enters the body of the vehicle. The air is then cooled directly utilizing the change in the latent and sensible heat of the LN. Once the required air temperature has been reached, the valve shuts off the flow of LN and the temperature is subsequently controlled by intermittent injections of LN.

Retail Display

In general, display cabinets have to accommodate three types of meat and meat products: (1) chilled wrapped, (2) unwrapped, and (3) frozen wrapped products. Cabinets with a big display area and an absence of doors are generally preferred, but this leads to a higher heat gain and higher operating costs to maintain correct temperatures. Retail display consumes almost 50% of the total refrigeration energy used in the cold chain.

Chilled Wrapped

A typical cabinet has a refrigeration unit behind the display area. Chilled air from the refrigeration unit is blown by a fan and delivered to the relevant area by duct work behind the display area (Figure 6). After the air has been delivered to the display area, it is then drawn back into the duct through a grille and is refrigerated again to continue the cycle.

The duct provides two functions: provides cold air through the holes in the rear panel and provides an air curtain at the front of the cabinet. The holes in the rear panel direct chilled air over the food and the air curtain provides a thermal barrier between the chilled display area and the store.

Chilled Unwrapped

Display cabinets for delicatessen products are available with gravity or forced convection coils and the glass fronts may be nearly vertical or angled up to 20°. Sections through three of the commonest types of delicatessen cabinets are shown in Figure 7. In the gravity cabinet (Figure 7(a)), cooled air from the raised rear-mounted evaporator coil descends into the display well by natural convection and the warm air rises back to the evaporator. In the forced circulation cabinets (Figure 7(b) and (c)), air is drawn through an evaporator coil by a fan and then ducted into the rear of the display, returning to the coil after passing directly over the products (Figure 7(b)), or forming an air curtain (Figure 7(c)), via a slot in the front of the cabinet and a duct under the display shelf.

Frozen Wrapped

There are a number of different types of display cabinets for frozen meat.

Chest cabinets, similar to domestic freezers with products stored generally on one horizontal shelf. Well cabinets have access from only one side of the cabinet, whereas Island cabinets have access to products from all sides. Cabinets can be open or lidded. Air distribution can be forced or gravity.

Multideck cabinets, similar to the chilled cabinets shown in Figure 6, incorporate a number of tiered shelves (graduated or horizontal) with open or glass door front access. Air distribution is often forced.

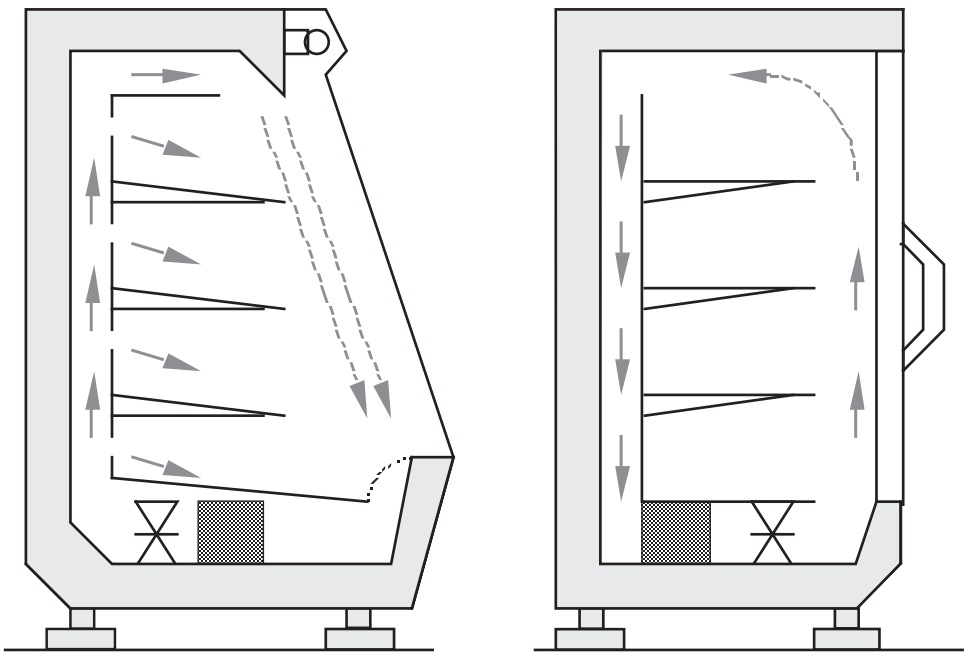

Figure 6 Multideck display cabinet for wrapped products.

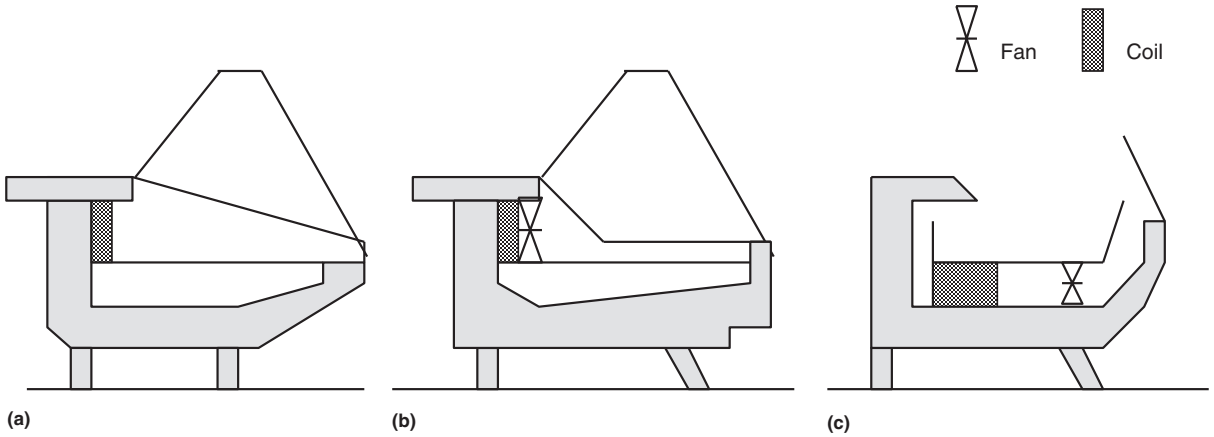

Figure 7 Three types of retail display cabinet for unwrapped products.

Domestic Storage

Consumers commonly store meat and meat products in refrigerators, freezers, or fridge–freezers. These are insulated boxes ranging in size from 0.04 to more than 1 m^3 and they are sold in a range of configurations, which depend on the orientation and type of evaporator coil. Traditional refrigerators with the coil forming an ice box at the top are increasingly being replaced by 'larder' type systems with the coil built into the back of the device.

Freezers are typically the top opening 'chest' design or are 'upright' with one or more doors on the front and internal trays or compartments.

Combination fridge–freezers with a refrigerator mounted above a freezer compartment have taken a majority share of the European market. The large combination appliances with a refrigerator one side and a freezer on the other are common in the USA and are increasingly being sold in other countries.

See also: Meat Marketing: Transport of Meat and Meat Products. Modeling in Meat Science: Refrigeration. Physical Measurements: Temperature Measurement. Refrigeration and Freezing Technology: Freezing and Product Quality; Thawing

Further Reading

Ciobanu, A., Lascu, G., Bercescu, V., Niculescu, L., 1976. Cooling Technology in the Food Industry. Tunbridge Wells, UK: Abacus Press. ISBN 0 85626 018 5.

Evans, J.A., 2008. Frozen Food Science and Technology. Oxford, UK: Wiley-Blackwell.

James, S.J., Evans, J.E., James, C., 2008. A review of the performance of domestic refrigerators. Journal of Food Engineering 87, 2–10.

James, S.J., James, C., 2002. Meat Refrigeration. Cambridge, UK: Woodhead Publishing Limited. ISBN 1 85573 442 7.

James, S.J., James, C., 2010. The food cold-chain and climate change. Food Research International 43 (7), 1944–1956.

James, C., Vincent, C., de Andrade Lima, T.I., James, S.J., 2006. The primary chilling of poultry carcasses – a review. International Journal of Refrigeration 29 (6), 847–862.

Kennedy, C.J., 2000. Managing Frozen Foods. Cambridge, UK: Woodhead Publishing Limited. ISBN 1 85573 412 5.

Kerry, J.P., Kerry, J.F. (Eds.), 2011. Processed Meats: Improving Safety, Nutrition and Quality. Cambridge, UK: Woodhead Publishing Ltd.

Mallett, C.P., 1993. Frozen Food Technology. London, UK: Blackie Academic & Professional. ISBN 0 7514 00726.

Mena, C., Stevens, G., 2010. Delivering Performance in Food Supply Chains. Cambridge, UK: Woodhead Publishing Ltd.

Sun, D., 2012. Handbook of Frozen Food Processing and Packaging. Boca Raton, FL: CRC Press/Taylor & Francis.

Relevant Websites

http://www.ecff.net/
European Chilled Food Federation.

http://www.fao.org/
Food and Agriculture Organization of the United Nations.

http://www.iifiir.org/
International Institute of Refrigeration.

http://www.chilledfood.org/
UK Chilled Food Association.

Freezing and Product Quality

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Glossary

Freezer burn A condition that occurs when frozen meat has been damaged by dehydration and oxidation, due to air reaching the product.

Freezing rate The time to transverse a given temperature range, i.e., freezing time (recommended by the International Institute of Refrigeration). Freezing time for meat is often defined as the time taken to pass from 1 °C (approximately the beginning of freezing in the case of meat) to −7 °C, at which point approximately 80% of the water is frozen.

Lipid oxidation The oxidative degradation of lipids; can result in rancid and other undesirable off-flavors. It may also influence the texture and color of meat products.

Storage life The length of time a food product can be stored under specified environmental conditions, for example, frozen, without being sensorially unacceptable or constituting a health risk.

Introduction

The fundamental reason for freezing meat and other food products is preservation. Traditionally, animals slaughtered for meat were consumed immediately. Preservation was, therefore, not necessary. However, as surplus meat began to be produced, or production peaked at certain times of the year, preservation methods were required, so that excess product could be stored and used later, or – as today – distributed, stored, and sold in a different location, sometimes on the other side of the world.

One of the earliest preservation methods for meat, along with other methods such as salting, curing, and smoking, was through reducing product temperature by packaging products in ice, i.e., essentially refrigeration. The advantage of refrigeration over other early preservation methods was the 'relatively' small change in product quality attributes. Freezing was a natural progression from refrigeration, as technologies evolved that permitted the reduction of product temperatures below the freezing point. This logical progression significantly extended the storage life of meat products.

Today, a substantial portion of the meat consumed around the world has been frozen for storage and/or distribution, particularly in areas where local demand exceeds production. However, for a long time, frozen meat has suffered from a reputation of inferior quality compared with that of fresh 'chilled' meat, particularly in the eye of the general public. This most likely occurred in the first instance because frozen meat was generally frozen without a prior storage period at refrigerated temperatures to allow the meat to tenderize. However, consumers are happy to purchase chilled meat and freeze it at home; possibly because they feel that they are in control of the situation. Notwithstanding this fact, freezing remains the most viable solution for long storage life of meat and meat products. The hotel and restaurant trade is aware of the advantages of frozen product with its associated benefits for inventory control.

As meat is composed of a complex of soluble and structural proteins, fat, and electrolytes, its freezing properties are more complex than a single-phase water-based system. However, many aspects of the physics and freezing of such simple systems are important in freezing meat, and the preceding articles on principles and application of refrigeration and freezing technology as well as 'Further Reading' should be consulted to understand the physics involved. The dynamic changes from the producer through the slaughter procedure, carcass processing, and the temperature control through the whole marketing chain, including freezing, all interact with the unique composition of meat. One must keep in mind how the entire supply chain affects the final quality attributes of the meat.

The areas of the freezing process that have a major impact on product quality consist of three steps: (1) freezing, i.e., reducing the product temperature to the temperature at which the meat will be stored; (2) frozen storage time and temperature characteristics; and finally (3) how the product is thawed before further processing or cooking. The three steps have to be controlled and applied correctly so that they do not negatively affect the quality attributes. However, it should be kept in mind that the quality of frozen meat is also governed by the supply chain preceding the freezing process; in other words, freezing must been seen in the context of the entire supply chain, not merely as an add-on. Ultimate product quality can only be optimized by careful control of the totality of the value chain, rather than any particular operations or steps in the chain.

Packaging Before Freezing

Packaging is crucial in maintaining quality during frozen storage, particularly for cooked products, and performs two basic functions. First, packaging is often the marketing vehicle by which the product is delivered to customers, which means that the package must effectively communicate its content in an attractive way. Second, the package protects its contents from external contamination during shipping and handling and from the environmental factors that cause chemical and

physical changes in the product during storage. The most important factors in the latter group are exposure to oxygen and to low-humidity surroundings. As a result, packages must provide a good barrier to oxygen to minimize oxidation and hence off-flavor development and to moisture loss to avoid dehydration or freezer burn.

To provide the greatest protection, a package must be well evacuated of air (oxygen) using either vacuum or gas flushing and the package must provide an adequate barrier to both oxygen and moisture. For frozen products, particularly cooked products, a complete vacuum coupled with an adequate barrier package that adheres well to the product surface is the most common choice. Avoiding headspace between the product surface and package aids in preventing ice recrystallization and frost formation inside the package during frozen storage that can damage the muscle cells and modify the way the muscle thaws.

'Freezer burn' is the main appearance problem that traditionally affected the appearance of meat in frozen storage. Desiccation from the surface tissues produces a dry, spongy layer that is unattractive and does not recover after thawing. Freezer burn can also lead to accelerated lipid oxidation. It occurs in unwrapped or poorly wrapped meat. The problem is accentuated in areas exposed to low-humidity air at high velocities and by poor temperature control. With improved packaging technologies and temperature control, the issue with freezer burnt meat has been significantly reduced, although it is still prevalent when meat that is bought fresh and is inadequately packaged before freezing is stored in domestic freezers.

Freezing Process

The freezing process starts once the meat reaches temperatures below its initial freezing temperature when ice crystal nucleation will take place. From there, freezing can progress through growth of an existing ice crystal or nucleating a new crystal. Growth of existing crystals tends to dominate when freezing is relative slow, whereas new crystals are more likely to form when freezing is fast. Hence, slow freezing initially results in fewer but larger ice crystals, whereas fast freezing results in more but smaller ice crystals. However, the ice crystals are unstable and, during subsequent frozen storage, recrystallization takes place. In this phenomenon, large ice crystals grow at the expense of small ice crystals – a process that is enhanced by temperature fluctuations during frozen storage.

Freezing itself does not overtly result in poor quality, although undesirable changes can take place in meat during freezing that are associated with the formation of large ice crystals in extracellular locations, mechanical damage by the ice crystals to cellular structures through distortion and volume changes, and chemical damage arising from changes in concentrations of solutes. These changes are related to the rate at which the meat is frozen.

Freezing rate has been evaluated using many different methods. However, a convenient way of expressing freezing rate, which is also recommended by the International Institute of Refrigeration for foods, is the time to transverse a given temperature range, i.e., freezing time. Freezing time for meat has in several studies been defined as the time taken to pass from −1 (approximately the beginning of freezing in the case of meat) to −7 °C, at which point approximately 80% of the water is frozen. At characteristic freezing rates shorter than 10 min, the formation of intracellular ice takes place (several ice crystals in each fiber), whereas at characteristic freezing times longer than 20 min, most ice crystals are extracellular.

Frozen Storage

Meat is not inert during frozen storage; chemical and biochemical reactions continue to take place in frozen meat but at a much slower rate than in fresh and chilled meat. Thus, the physical and chemical properties of meat slowly change with time, and, with extended storage periods, these will ultimately result in detectable quality changes. Ice recrystallization and more pronounced ice crystal growth can occur at higher temperature or due to drastic and repeated temperature fluctuations. This can result in some structural damage and relatively faster quality deterioration. Thus, any perceived benefits of rapid freezing can be easily reversed during frozen storage at high and unstable freezer temperatures.

Chemical and enzymatic reactions during frozen storage can also cause loss of quality, usually through lipid oxidation, which causes rancid odors and flavors to develop, 'warmed-over' flavor in previously cooked meats, and changes in color due to either oxidation of the color pigments or freezer burn (see Section Appearance). In general, these quality-deteriorating reactions occur faster at higher temperatures, especially more than −5 °C. However, some reactions, such as myoglobin oxidation, have maximum rates between −5 and −15 °C.

Thawing

The thawing process is more time consuming and difficult to control than freezing because temperatures more than 0 °C increase the risk of microbial growth; hence, the thawing temperature must, therefore, be kept low. The quality factors most likely to be affected by thawing are microbial growth, drip loss, and appearance and palatability of the product; these changes will be added to the changes initiated before and during freezing.

Appearance

The water-holding capacity of thawed meat is dependent on the rate at which the meat was frozen. The effect of freezing rate on water-holding capacity (measured as centrifuge drip) measured over a wide range of local freezing times is shown in Figure 1. The highest water-holding capacity is obtained with very short freezing times, whereas the lowest is obtained with freezing times of approximately 17–20 min, which coincides with a single ice crystal inside the muscle fibers. Beyond freezing times of 20 min, the water-holding capacity improves again until it plateaus at freezing times more than 30 min,

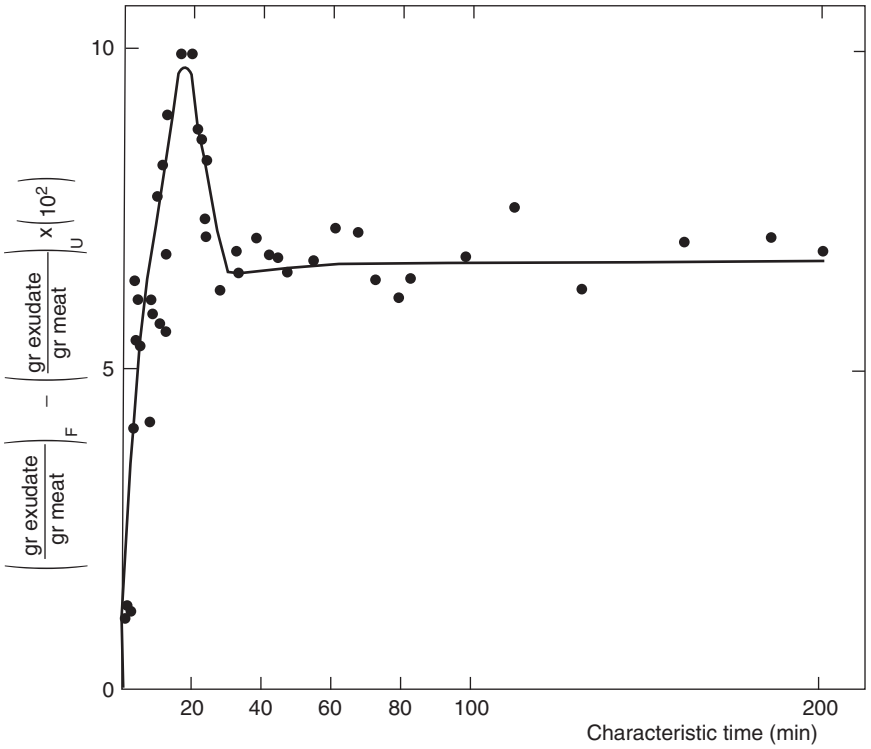

Figure 1 Changes in exudates from thawed meat in relation to the characteristic freezing time. The exudate was obtained by centrifugation. Reproduced from Mascheroni, R.H., Añón, M.C., Calvelo, A., 1980–81. Basis for a method of characterization for quick frozen beef. Meat Science 5, 457–472.

beyond which no further improvement in water-holding capacity is achieved.

A slow freezing rate results in the formation of relatively large ice crystals, which create tissue damage. Temperature fluctuations during frozen storage often result in recrystallization, which influences subsequent thaw-drip losses. Thawing inevitably produces drip, particularly from cut muscle surfaces. However, some uptake of this drip occurs when thawed meat is held at refrigeration temperatures and most of the drip would ultimately be lost during cooking, primarily through evaporation, if it had not already been lost during thawing.

Freezing induces alterations in optical properties and hence a change in meat color when the frozen meat is thawed. Previously frozen thawed meat has been found to exhibit a slower rate of blooming than meat that had not been frozen, because the mitochondrial respiratory enzyme still remains active. Thus, previously frozen meat appears darker in color than nonfrozen meat. Frozen and thawed meat is also likely to be more susceptible to myoglobin oxidation, resulting in a faster conversion into metmyoglobin and hence shorter color display life compared with chilled meat. In addition, the longer the meat is stored frozen, the shorter its color stability is after thawing. Recent studies have found that aging meat before freezing can provide equivalent color and better lipid oxidation stability compared with aged nonfrozen meat. This indicates that aging meat for a certain limited period before freezing can allow oxygenation conditions similar to aged nonfrozen meat, improving meat color and color stability of the frozen/thawed meat.

Eating Quality

Although tenderness has been found to increase following freezing and thawing, this cannot be contributed to proteolysis, because the proteolytic enzymes responsible for the postmortem tenderization process are inactive at commercial freezing temperatures ($<-12\,^{\circ}C$) and proteolysis does not contribute to tenderization to any large extent during frozen storage. For instance, if samples for shear force measurements are cooked from frozen, these will have higher shear force values compared with samples that are thawed before cooking. Rather, the tenderness improvement is ascribed to the loss of structural integrity caused by ice crystal formation. It should be noted that thawed red meat continues to age (i.e., become more tender) unless it is already fully aged before being frozen.

Lipid oxidation occurs not only in fat deposits but also in lean meat tissue through phospholipid oxidation. Lipid oxidation can result in rancid and other undesirable off-flavors and can also influence the texture and color of meat products. Development of off-flavors as a result of oxidation, including rancidity, is common and is a major hurdle to be overcome when producing frozen meat with an acceptable quality. The susceptibility of meat to lipid oxidation and rancidity is related to the fatty acid composition of the muscles, with the polyunsatured fatty acids in the phospholipids being liable to oxidative breakdown. Thus, meat cuts containing more polyunsaturated fatty acids will be more susceptible to oxidation, i.e., when stored under the same conditions, pork, which has more unsaturated fat, will have a shorter shelf life than beef,

which has more saturated fat. In contrast, meat containing antioxidants, such as vitamin E, i.e., meat from animals supplemented with vitamin E or from pastoral systems, will be less prone to oxidation. For detailed information, see those articles on oxidative, enzymatic, and warmed-over flavors.

Microbiological Quality and Safety

Microbial growth can make meat less pleasant to eat (spoilage) and can make the consumer ill if the numbers of pathogenic microorganisms are too high or if too many toxins are produced. Bacteria and molds have a temperature range in which they prefer to grow. As the temperature moves away from this range, their growth rate slows. Once the temperature gets too far away from the preferred range, growth stops completely and the microorganism may die. The growth rate at a given temperature varies between microorganisms, but the principle remains the same. Freezing meat is a very good way to slow or stop microbial growth and thereby reduce the rate of microbial spoilage or the growth of pathogens.

Freezing of Other Flesh Foods

The principles, including packaging, described for red meat generally apply to poultry and fish. However, a few specific characteristics of these foods related to freezing and product quality are described in the Sections Poultry and Fish and Seafood.

Poultry

Similar to red meats, poultry requires a freezing process that is rapid enough to minimize surface dehydration during freezing and decreases drip loss during thawing. With respect to appearance, a light surface color of the frozen product is considered important. This can be achieved with a rapid surface-freezing temperature because supercooling the product nucleates a high number of small ice crystals, which reflect light and appear white in color. Further, young birds are prone to bone darkening following freezing, which is caused by hemoglobin leaching from bone marrow to adjacent muscle as a result of the freeze/thaw treatment. Eating quality is not affected, but the dark bones negatively affect consumer acceptance.

Poultry tend to have fewer tenderness issues than red meat. This is related to the age of the animals (less than 6 weeks) and the fact that poultry are less susceptible to cold shortening and the associated toughening that can occur in prerigor muscle below 10 °C, because the postmortem pH decline is rapid in poultry and leads to a rapid onset of rigor (1–3 h postmortem). Poultry can, therefore, be frozen sooner after slaughter without compromising the tenderness of the product.

Fish and Seafood

In fish, aging is not required to ensure tenderness, and spoilage begins at the time of death, which is usually accompanied by gradual loss or development of compounds that affect fish quality. The most effective way of controlling the spoilage, and hence maintaining product quality, is to either chill or freeze the product soon after slaughter.

Changes in appearance can occur immediately after the fish is caught. Blood pigments become noticeably discolored to various degrees after some period of time. These pigments are subjected to considerable oxidation when the fish is frozen and stored, which results in dark meat color. This discoloration occurs especially when the fish is stored for an extended period of time or during thawing. However, it is highly dependent on storage temperature, with lower temperatures extending the storage period. In shrimp, black pigments can form within a few hours of death and this is enhanced by exposure to oxygen, but rapid freezing to less than −18 °C largely prevents this. In other shellfish, such as crab and lobster, the development of blue or black discoloration is one of the most troublesome quality issues.

A frozen cloudy liquid, known as thaw drip, can sometimes be found in packaged frozen fish. This quality defect is an indicator of inappropriate handling, prolonged storage before freezing, frozen storage at inappropriate cold storage temperatures, or improper thawing.

With respect to eating quality, frozen fish gradually loses its juiciness and texture. The changes are more pronounced in some species than in others. In cod and codfish, for instance, protein denaturation leads to formation of formaldehyde, cross-linking of formaldehyde to muscle proteins, and a 'cottony' or 'spongy' texture. Other fish species and crustaceans such as crab, shrimp, and lobster tend to toughen when stored for prolonged periods.

Changes in the delicate flavor of fish and seafood take place in three steps during frozen storage: first, a gradual loss or decrease in flavor compounds; then neutral, bland, or flat flavors are detected; and finally, off-flavors develop due to the presence of acids and carbonyl compounds as a result of lipid oxidation. Similarly, changes in odor take place in two phases: first, a loss of the characteristic odor and then development of undesirable odors. Generally, fish and seafood have a fresh, seaweedy odor that can be retained even after freezing and frozen storage. Gradually, such odors are lost and unpleasant odors can develop that are often related with inappropriate storage temperatures.

Storage Life of Frozen Foods

However, despite the potential impact of freezing, frozen storage, and thawing on product quality, red meats, poultry, and fish and seafood can be stored in the frozen state for several months or more without appreciable changes in quality, provided the food products are kept under appropriate conditions.

Storage life expectations have been defined by the International Institute of Refrigeration as "the period of frozen storage after freezing during which the product retains its characteristic properties and remains suitable for consumption or the intended process." For frozen meat, the recommended length of storage remains controversial because of the influence of packaging, storage temperature, relative humidity, moisture loss during freezing, and inherent variation in the

Table 1 Practical storage life in months at three storage temperatures

Products	*− 12 °C*	*− 18 °C*	*− 24 °C*
Beef carcass (unpackaged)	8	15	24
Beef steaks/cuts	8	18	24
Minced (ground) beef	6	10	15
Veal carcasses (unpackaged)[a]	6	12	15
Veal steaks/cuts	6	12	15
Lamb carcass, grass fed (unpackaged)[a]	18	24	>24
Lamb steaks	12	18	24
Pork carcass (unpackaged)[a]	6	10	15
Pork steaks/cuts	6	10	15
Sliced bacon (vacuum packed)	12	12	12
Chicken, whole	9	18	>24
Chicken, parts/cuts	9	18	>24
Turkey, whole	8	15	>24
Liver	4	12	18

[a]carcass may be wrapped in stockinet.

Source: Reproduced from Bøgh-Sørensen, L., 2006. Recommendations for the Processing and Handling of Frozen Foods, fourth ed. Paris, France: International Institute of Refrigeration.

products themselves. In other words, 'one size does not fit all.' The ranges of indicative practical storage lives of frozen meat and meat product are given in Table 1.

See also: Chemical and Physical Characteristics of Meat: Color and Pigment. Conversion of Muscle to Meat: Rigor Mortis, Cold, and Rigor Shortening. Cooking of Meat: Warmed-Over Flavor. Packaging: Technology and Films. Refrigeration and Freezing Technology: Applications; Principles; Thawing. Spoilage, Factors Affecting: Oxidative and Enzymatic

Further Reading

Bøgh-Sørensen, L., 2006. Recommendations for the Processing and Handling of Frozen Foods, fourth ed. Paris, France: International Institute of Refrigeration.

Devine, C.E., Bell, R.G., Lovatt, B.B., Chrystall, B.B., Jeremiah, L.E., 1996. Red meat. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York, US: Marcel Dekker, pp. 51–84.

James, S.J., James, C., 2009. Chilling and freezing of meat and its effect on meat quality. In: Kerry, J.P., Ledward, D. (Eds.), Improving the Sensory and Nutritional Quality of Fresh Meat. Cambridge, England: Woodhead Publishing Limited, pp. 539–560.

Kim, Y.H.B., Hunt, M.C., 2012. Advanced technology to improve meat color. In: Joo, S.T. (Ed.), Control of Meat Quality. Kerala, India: Research Signpost, pp. 31–60.

North, M.F., Lovatt, S.J., 2012. Chilling and freezing meat. In: Hui, Y.H. (Ed.), Handbook of Meat and Meat Processing, second ed. Boca Raton, FL: CRC Press, pp. 357–380.

Santos-Yap, E.E.M., 1993. Fish and seafood. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York: Marcel Dekker, pp. 109–133.

Sebranek, J.G., 1993. Poultry and poultry products. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York: Marcel Dekker, pp. 85–108.

Relevant Website

www.iifiir.org
International Institute of Refrigeration.

Principles

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Glossary

Conduction The method by which heat (or another form of energy) is transferred through a solid material or a static layer of fluid.
Convection The method by which heat is transferred from one location to another by a moving fluid.
Heat transfer coefficient The ratio of a heat flux (e.g., the amount of heat flow per unit area of a surface) to the difference in temperatures between a surface and a fluid that drives that heat flux.
Insulation A material with a low thermal conductivity used to reduce the rate of heat transfer.
Radiation The method by which energy is transferred from one location to another as particles or waves.
Refrigeration The process of removing heat from an object.

Introduction

If animals were harvested and consumed almost immediately, there would be no need for any form of preservation. In practice, however, meat that is not preserved in some way will quickly deteriorate in quality and safety to the point where it is both unpalatable and dangerous to consume. Thus, most meat that is produced is subsequently preserved in one way or another.

Historically, meat has been preserved in a variety of ways, with the most common methods being drying, curing, smoking, heat processing, fermentation, irradiation, canning, packaging, and refrigeration.

Of all these alternatives, refrigeration has the key benefit, along with irradiation, that it leaves the form of the meat product almost unchanged and (when carried out appropriately) almost indistinguishable from the original fresh product. In comparison, most other preservation methods can be seen more as ways to change meat into different products that have longer storage lives rather than as genuine methods of preservation. Unlike any of the other preservation technologies, refrigeration is effective in reducing deterioration due to chemical reactions (fat oxidation, for instance) as well as deterioration due to the growth of microorganisms.

Refrigeration is often described as the process of removing heat from an object but, because energy is conserved (i.e., cannot be destroyed or created), it would be more accurate to say that refrigeration is the process of transferring heat from one object to another. Heat flows naturally from hotter objects to colder objects and no mechanism is required to achieve this. With the use of a refrigeration system, however, the object from which heat is removed can be colder than the object to which the heat is added.

At the same time that heat is transferred, masses of material are usually moved around by the refrigeration system. These can include air or liquid in which the heat is carried from the food product to the air or liquid cooler, water vapor and other gases carried in air, and the primary and secondary refrigerants in the refrigeration system itself.

Fundamentals of Heat Transfer

There are three fundamental mechanisms of heat transfer: conduction, convection, and radiation. Of these three, most refrigeration processes involve combinations of conduction and convection, whereas radiation is occasionally significant.

Conduction

The molecules of a solid, liquid, or gas interact with each other, either all the time (as in a solid) or intermittently (as in a liquid or a gas). When two molecules interact, the one with the higher energy level transfers some of its energy to the one with the lower energy level. The relative energy levels are indicated by temperature, with materials at higher temperatures having molecules with relatively higher energy levels and vice versa. Energy (in the form of heat), therefore, flows through a material from areas of higher temperature to areas of lower temperature. In pure metallic solids, heat can also be transferred via 'free' electrons but, again, the direction of heat flow is from higher to lower temperature areas of the material.

The relationship between the characteristics of the conducting material and the rate of heat flow was described by Fourier in 1822. To simplify Fourier's description for a slab-shaped object, such as that shown in Figure 1, when the heat flow is steady over time, the rate of heat flow Q in W (Watts; energy flow per unit time), is given by eqn [1].

$$Q = \frac{kA}{x}(T_1 - T_2) \qquad [1]$$

Here k is the thermal conductivity in W m^{-1} K^{-1}, A is the area over which the heat is conducted in m^2, x is the distance through which the heat is conducted in m, and T_1 and T_2 are the temperatures on either side of the object in K (Kelvin).

Thus, to increase the rate of heat flow due to conduction, one could do one or more of the following things:

- increase the conductivity, k;
- increase the area, A;
- decrease the thickness, x; or
- increase the temperature difference between the two sides of the object, $(T_1–T_2)$.

Typical thermal conductivities for materials of interest in a food refrigeration process include air at 0.03 W m^{-1} K^{-1}, lean meat at approximately 0.5 W m^{-1} K^{-1} when unfrozen or 1.5 W m^{-1} K^{-1} when frozen, steel at approximately 43 W m^{-1} K^{-1}, and copper at approximately 380 W m^{-1} K^{-1}. Thus,

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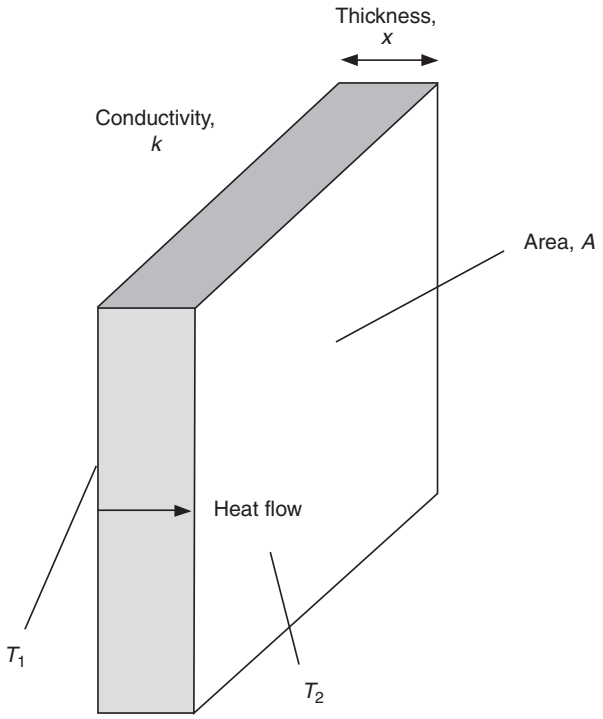

Figure 1 Heat conduction through a slab of material.

the air that frequently surrounds meat products when they are packaged is, when it is prevented from moving, a very good thermal insulator. This is an important consideration in refrigeration process design.

Convection

Although its low thermal conductivity makes immobile air a very effective thermal insulator, the movement of air or any other fluid over a surface is the second fundamental mechanism of heat transfer, known as convection. Convection is the most common practical way in which heat is transferred from a solid to a liquid or a gas (or vice versa) or from one part of a liquid or gas to another part. There are two forms of convection – forced and natural – and both occur as a result of fluid movement.

Forced convection occurs when the fluid is moved by an external force. Examples of forced convection include a fan blowing air across a surface or a pump forcing liquid along a pipe.

Natural convection, however, occurs naturally as a result of the heat transfer process itself. If a warm surface heats some air, the density of the warm air will be less than the density of the surrounding cold air. The warm air, therefore, rises due to its buoyancy in the denser cold air, and surrounding cold air sweeps in from the sides to replace it. This produces air movement around the warm surface without any external force being applied. The rate of this air movement is dependent on the difference between the temperature of the surface and the temperature of the air, so higher temperature differences generate higher rates of air movement.

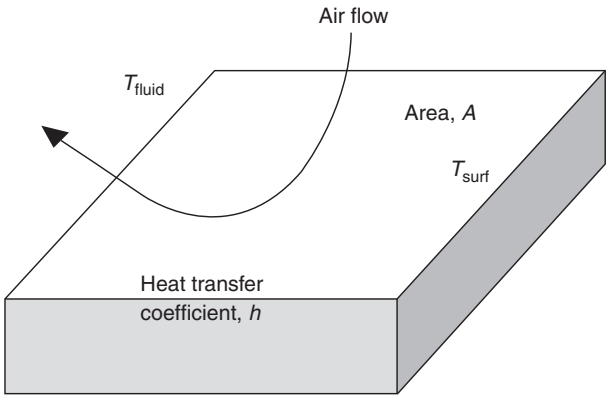

Figure 2 Convection heat transfer at a surface.

The rate of heat transfer due to either forced or natural convection was described by Sir Isaac Newton in 1701 according to eqn [2].

$$Q = hA(T_{\text{surface}} - T_{\text{fluid}}) \qquad [2]$$

here h is the surface heat transfer coefficient in W m^{-2} K^{-1}, and T_{surface} and T_{fluid} are the temperatures of the surface and fluid, respectively in K.

Figure 2 illustrates the conceptual situation. The rate of heat flow in convection is driven by the temperature difference and the surface area, as it is in conduction, and by the heat transfer coefficient, h. Also, h depends on a number of factors, including the characteristics of the fluid, the velocity of fluid flow past the surface, and the level of turbulence. In the case of natural convection, h also depends on the position of the surface (e.g., vertical or horizontal, above or below the fluid) and whether the surface is colder or warmer than the fluid.

Typical values for h in food refrigeration processes include approximately 5 W m^{-2} K^{-1} for natural convection cooling in air, 20–30 W m^{-2} K^{-1} for forced convection cooling in air, 200–500 W m^{-2} K^{-1} for cooling in water and much more than 1000 W m^{-2} K^{-1} if liquid is evaporating or gas is condensing. The value of h can be increased in a forced convection regime by increasing the velocity of flow across the surface. In practice, however, h increases less than linearly with flow velocity whereas the power (and hence the cost of energy and capital equipment) required to move the fluid increases much more than linearly with velocity. This means that it is rarely economic, in a food refrigeration process, to move the fluid fast enough to increase h to more than 5–10 times its natural convection value.

Radiation

Radiation is the last of the three fundamental heat transfer mechanisms. Like convection, thermal radiation transfers heat from the surface of an object but, unlike convection, radiation requires no transfer medium. Radiation actually transfers heat better if there is no intervening fluid. The amount of radiation heat transfer depends on the surface area, as with conduction and convection. Although rates of conduction and convection depend linearly on the difference in temperatures between two objects, radiation depends on the difference between their

absolute temperatures raised to the fourth power and the geometric relationship between the two objects.

Radiation is usually more important in heating processes than in cooling processes, particularly when the source of heating is at a high temperature. This can occasionally be a significant issue during cold storage when radiative heating from lights in the cold store may be sufficient to warm and even start to thaw the top surface of product stacked close to the lights. Radiative heating can also affect the accuracy of air temperature measurements. Radiation is usually not very important in practical chilling and freezing processes, however, so it will not be described further and the reader should refer to an engineering heat transfer text if more information is required.

Refrigeration Systems

As has been noted above in Section Introduction, heat does not naturally flow from a cold object to a warmer object. This means that to cool an object below the surrounding ambient temperature, it is necessary to use some sort of refrigeration system. The types of refrigeration systems that are used for cooling meat range from very simple to fairly complex but their essential principles are similar. The simplest practical system is, therefore, described first and more complex systems are described later in this article.

Cryogenic Refrigeration

When a material changes phase, from solid to liquid, from liquid to gas, or directly from solid to gas, heat must be absorbed in addition to the heat required to raise the temperature of the material. This additional heat is known as the latent heat of fusion (or melting), in the case of the transition from solid to liquid; the latent heat of evaporation, in the case of the transition from liquid to gas; or the latent heat of sublimation, in the case of the transition from solid to gas. Thus, if one has a material that melts, boils, or sublimes at a low temperature, that material will draw heat from warmer objects around it in order to change its phase. By putting such a material in contact with a meat product, it can be arranged that most of the required heat will be drawn from the meat, thereby cooling the meat. This is the principle of cryogenic refrigeration.

Materials that melt, boil, or sublime at temperatures well below normal room temperatures are known as cryogens. Although many different cryogens could potentially be used as refrigerants to cool meat products, the choice of cryogen for food cooling is restricted by food safety, food quality, and environmental acceptability criteria because the cryogen usually comes into direct contact with the food product and much of it is often released into the atmosphere. Two cryogens that meet these criteria, and are, therefore, used most often for food cooling, are carbon dioxide (in either solid form, as pellets of 'dry ice' or as a pressurized liquid) and liquid nitrogen.

Solid carbon dioxide at atmospheric pressure sublimes at $-78.5\,^{\circ}C$. It is, therefore, kept in a thermally insulated container to slow the sublimation process until it is put into physical contact with the food product to be cooled. Liquid carbon dioxide is kept under pressure (at least 6.7 MPa at $27\,^{\circ}C$) to prevent it from evaporating. Liquid carbon dioxide cannot exist in equilibrium at a pressure of less than 0.52 MPa so, when the liquid is released into an atmospheric pressure environment, some evaporates and carries away much of the heat content whereas the rest solidifies in the form of carbon dioxide 'snow.' This 'snow' then sublimes at $-78.5\,^{\circ}C$, drawing heat from its surroundings, until all the carbon dioxide has transformed into gas.

Liquid nitrogen boils at $-195.8\,^{\circ}C$ and its critical point is $-146.9\,^{\circ}C$, so it is kept in a thermally insulated container to slow down the evaporation process or as a pressurized supercritical gas. When liquid nitrogen is put in contact with a warm object, it boils vigorously, drawing heat from the warm object to do so, and thereby cools the object.

Both of these cryogens can be used to refrigerate food products but, whereas such a process requires very little equipment and so has a small capital cost, the cost of purchasing cryogen is typically high. This means that refrigeration with cryogens is mainly used for trial runs in which only small amounts of meat have to be frozen, for products that have very high value or to deal with emergencies when more conventional refrigeration systems are unavailable and there would be a food safety or quality risk if the meat was allowed to warm up.

Two issues make cryogenic refrigeration intrinsically expensive:

- Cryogens are only used once and are then lost into the atmosphere.
- Cryogens are manufactured using very low temperature processes that require a lot of energy.

These two issues can be resolved by a refrigeration system that reuses its refrigerant and that operates at temperatures no lower than it must reach to provide the required amount of cooling. The most common way to achieve these objectives is to use a mechanical vapor compression system.

Mechanical Vapor Compression Refrigeration

The principle of a mechanical vapor compression refrigeration system is to refine the process described above for cryogenic refrigeration in two ways. First, instead of putting the refrigerant into direct contact with the object to be cooled, it is retained inside the tubes of an evaporator, which is part of the refrigeration system. Second, three other pieces of equipment – the compressor, the condenser, and the expansion valve – are used in combination to convert the gaseous refrigerant back into a cold liquid that can be recycled and evaporated again in a continuous cycle. A diagram of a simple refrigeration system is shown in Figure 3.

Starting at the expansion valve, on the left of Figure 3, the warm refrigerant liquid passes through the expansion valve and suddenly reduces in pressure. This results in part of the liquid evaporating, thereby cooling both the vapor and the remaining liquid. This mixture of cold liquid and vapor then passes into the evaporator inside the cold room, where heat is drawn from the room to evaporate the rest of the cold liquid refrigerant. The resulting cold, low-pressure vapor is then sucked into the compressor where energy is applied to raise its pressure. As the pressure of the vapor is increased, its temperature also increases so that the vapor becomes hotter than

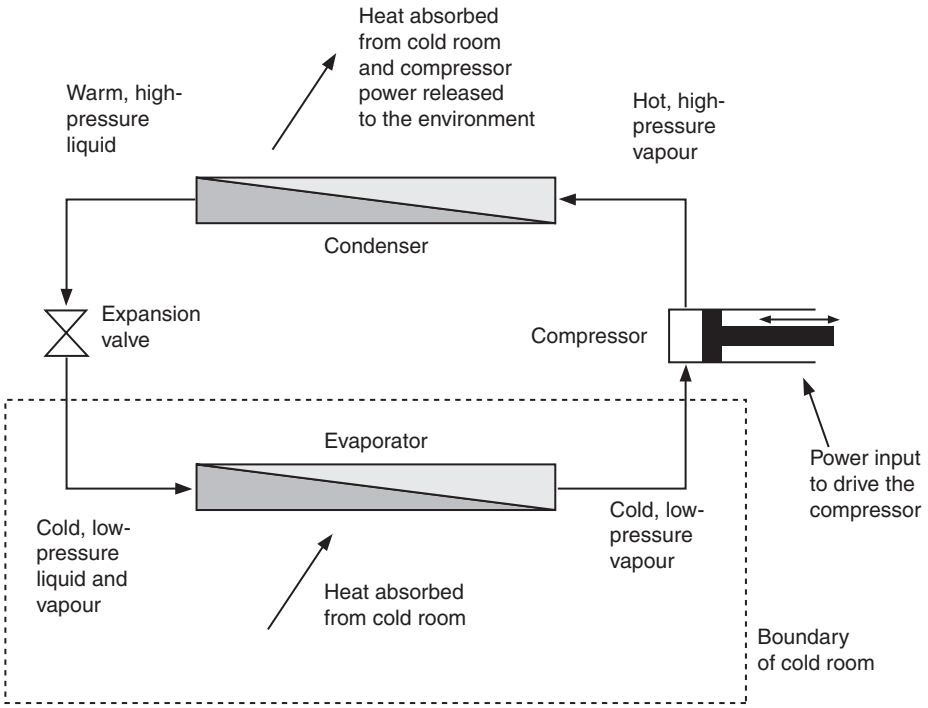

Figure 3 Diagram of a mechanical vapor compression refrigeration system. The arrows show the movement of refrigerant around the cycle, driven by the compressor, and the flows of heat and energy into and out of the system.

the ambient environment. When the hot vapor passes into the condenser, it cools until it reaches its saturation temperature at the pressure in the condenser, and then the vapor condenses, losing some sensible heat and its latent heat to the ambient environment. The warm liquid refrigerant then reenters the expansion valve and the cycle begins again.

By this method, the heat extracted from the cold room is expelled from the system through the condenser along with the energy added at the compressor and a cycle is created that extracts heat from the cold room continuously, rather than being limited by a finite supply of refrigerant. In practice, there are many refinements in real refrigeration systems that make mechanical vapor compression refrigeration efficient, cost-effective, safe, and reliable. For example, most meat plant refrigeration systems would have more than one of each of the components shown in the figure. Almost all mechanical vapor compression systems conform to the general concept shown in Figure 3, however.

The fluid used as a refrigerant must be selected carefully to meet a range of often conflicting criteria. The ideal refrigerant would, among other positive attributes:

- evaporate at the target evaporation temperature above atmospheric pressure, so that air does not leak inward if there are any leaks in the system;
- condense at the target condensing temperature under a relatively low pressure, so that the power required to compress it from the evaporating pressure to the condensing pressure is also relatively low;
- have a large latent heat capacity per unit of vapor volume, so that the compressor would be physically small (and therefore relatively inexpensive) for a given heat extraction rate;
- be compatible with a wide range of materials (e.g., metals, plastics, and lubricants), thus making it convenient and inexpensive to construct and run the refrigeration system;
- be nontoxic;
- be nonflammable; and
- be environmentally friendly.

It was initially thought that synthetic chlorofluorocarbon (CFC) compounds met all of these criteria, and were, therefore, ideal refrigerants. This led to a great many refrigeration systems – especially those with smaller capacities – being constructed to use these compounds. Over time, however, it became clear that CFCs were causing serious damage to the ozone layer, and the Montreal Protocol resulted in CFC production and consumption around the world being phased out by 2000. CFCs were temporarily replaced in many applications by hydrochlorofluorocarbons (HCFCs), which were less damaging to the ozone layer, and in the longer term by hydrofluorocarbons (HFCs) and hydrocarbons, which were not detrimental to the ozone layer.

CFCs, HCFCs, and HFCs are greenhouse gases, and their release into the environment, therefore, contributes to global warming. As was noted earlier, however, the objective of the refrigeration system is to contain the refrigerant and ensure that it is not released so, as long as leakage rates are low, the global warming potential (GWP) of these refrigerants is often considered to be acceptable. Indeed, the most important factor in determining the overall GWP of a refrigeration system is commonly the efficiency of the system. For example, if a

system contains a high-GWP refrigerant, but uses energy more efficiently than another system with a low-GWP refrigerant, the saving in global warming due to the smaller amount of carbon dioxide that would be produced in the electricity generating plant supplying power to the more efficient refrigeration system can often more than make up for the higher GWP of the small amount of refrigerant that will leak out over time.

Several effective refrigerants have little or no GWP. Ammonia is the most important of these in the modern meat industry because it is both a thermodynamically good refrigerant and inexpensive to purchase compared with HFCs. Ammonia is therefore often used in large refrigeration plants, such as meat freezing facilities and cold stores. In these large applications, ammonia's undesirable attributes, such as its toxicity and flammability, can be controlled safely and economically – something that is more difficult in smaller installations. Ammonia also has the advantage of boiling at $-33.3\,^{\circ}C$ under atmospheric pressure. This temperature is often satisfactory for a meat freezing system, so there is often no need to run the evaporator below atmospheric pressure and therefore risk air leaking into the system.

Hydrocarbons (e.g., propane) also have little or no GWP, compared to HCFCs and HFCs and they are often used as working fluids in small- or medium-sized refrigeration systems. Hydrocarbons are highly flammable, however; so careful design and maintenance are required to ensure they are vented safely when they leak and that there is no source of ignition available either in normal operation or in an accident.

Figure 3 shows a reciprocating compressor but many different types of compressor can be used in refrigeration systems. For smaller systems, and where the ratio between the condenser and evaporator pressures is high, reciprocating compressors containing one to eight cylinders are used most commonly. For systems with larger capacities, screw compressors or centrifugal compressors are used. Other types of compressor have advantages under particular circumstances.

For refrigeration systems with larger capacities, it is usual to install several small compressors rather than a single large unit. This allows a compressor to be taken out of service for maintenance without stopping the whole refrigeration system running and it can make the system more efficient when it is run at a fraction of its full capacity. Although many compressors can be controlled to operate with reduced gas throughput, most types of compressor operate less efficiently in that mode. It is, therefore, desirable to achieve most of the required capacity reduction by completely stopping one or more compressors when they are not required.

Although some types of compressor are more tolerant of high pressure ratios than others, the performance of a given refrigeration system always deteriorates as the pressure ratio increases. As a result, refrigeration systems are often designed to compress the refrigerant vapor in two or more stages, with the vapor being cooled between the compression stages. The number of stages is chosen during system design to optimize the added capital cost of additional compressors and the reduced energy cost of a multistage system. A further advantage of a multistage system is that refrigeration in a meat plant is often required at several evaporation temperatures. For example, refrigerant may be needed at $-33\,^{\circ}C$ to cool freezers and cold stores, $-10\,^{\circ}C$ to cool chillers and cool stores, and $0\,^{\circ}C$ to cool air-conditioned processing rooms. If refrigerant can be supplied at each of these temperatures then the energy cost for compression can be reduced when compared with the alternative of supplying all refrigerant at the lowest required temperature.

The condenser is designed to discharge the heat removed from the cold room together with the energy put into the system through the compressor. In small refrigeration systems, the heat released from the refrigerant as it cools and then condenses is released into the air but, for larger systems, the amount of air required for air cooling can be very great and condensers are, therefore, often designed to be cooled by water or by a combination of air and water spray. The latter design results in the water evaporating into the air stream outside the condenser tubes as the refrigerant condenses inside the tubes and it is known, somewhat confusingly, as an evaporative condenser.

The evaporator is the point at which heat is transferred from the object being cooled to the evaporating refrigerant. The simplest way to do this, while keeping the refrigerant sealed inside the refrigeration system, is to put the metal surface of the evaporator in direct contact with the object being cooled – a meat product, for example. This is the principle of the plate freezing system, where meat products are clamped between hollow metal plates that contain evaporating refrigerant.

For meat products that do not have flat, parallel sides suitable for plate freezing, or when the product is to be chilled rather than frozen, the evaporator is usually designed as a set of tubes containing the evaporating refrigerant, with fins mounted outside the tubes to increase their heat transfer area. Air is then blown by a set of fans across the evaporator tubes and fins (where the air is cooled), across the meat product (where the cold air warms and the meat product is cooled), and then back to the evaporator. Although air is the fluid used most often to transfer heat from the cooling object to the evaporator, there are advantages in using liquids such as water or brine to carry out the same function because of their much greater heat capacity per unit volume and the greater heat transfer coefficient between a liquid and a surface compared with that between a gas, like air, and a surface.

Other Types of Primary Refrigeration System

Although mechanical vapor compression is the most common design of refrigeration cycle, there are other alternatives. Absorption refrigeration, for example, uses a liquid solution to absorb the refrigerant vapor while removing heat, the pressure of the liquid is then raised and the vapor is released by applying heat. The vapor is condensed and the liquid refrigerant is evaporated, as in a mechanical vapor compression system, and then the cycle returns to the absorption stage. The advantage of this system is that most of the energy required to run an absorption refrigeration system can be provided in the form of heat rather than as mechanical power. This makes absorption systems ideal for making use of the waste heat that is often available in a meat processing plant. For information on other types of refrigeration system, the reader should refer to the Further Reading.

Secondary Refrigeration Systems

It is sometimes appropriate for safety or cost reasons to separate the evaporator from the point at which cooling is required. A common example occurs where an ammonia refrigeration system is used to cool a processing room in which there may be many staff working and where it is undesirable to risk an ammonia leak from the evaporator into the room. In this case, the evaporator can be used to cool a fluid (usually a liquid), which is pumped to the point where the cooling is needed and then through a heat exchanger to cool air before the fluid is returned to the evaporator for further cooling. A fluid circulated in this way is known as a secondary refrigerant.

Secondary refrigerants are often brines or glycol solutions, but many different fluids can be used, depending on the temperature at which the secondary refrigerant is required. Two-phase secondary refrigerant systems are more complex to design but they make use of the latent heat available when changing phase to effectively increase the secondary refrigerant's volumetric heat capacity. This makes the pumping power and pipe sizes required to circulate a two-phase secondary refrigerant substantially smaller than they would be for a brine or glycol solution with the same refrigeration capacity. One example is an ice slurry (i.e., a mixture of ice and water), in which the ice fraction of the slurry changes around the secondary refrigerant loop, allowing a volumetric heat capacity between four and six times greater than that of water. Another example is carbon dioxide, in which the system pressure is arranged so that the fluid changes phase between liquid and vapor during the cycle, similarly increasing its effective volumetric heat capacity.

Although secondary refrigerants are advantageous for several reasons, they all have an important disadvantage. In any heat transfer situation, the material gaining heat must be colder than the material that is losing heat. For example, the air flowing over a meat product must be colder than the meat product if that product is to be cooled. If a secondary refrigerant is used, the secondary refrigerant must be colder than the air and the evaporating primary refrigerant must be colder than the secondary refrigerant. In contrast, without the secondary refrigerant, the evaporation temperature would only have to be colder than the air. Based on eqn [2], to achieve a small temperature difference in any heat exchange step where a given amount of heat is to be transferred, it is necessary to have a large heat transfer area. A large heat transfer area requires a high cost of manufacture for the heat exchanger or evaporator, so, for economic reasons, the temperature difference at each heat transfer step is typically between 5 and 10 °C.

Using a secondary refrigerant, therefore, incurs additional operating costs because of the lower evaporating temperature required for the primary refrigerant. These costs must be balanced against the benefits of separating the primary refrigerant from the point where cooling is needed.

See also: Canning. Cooking of Meat: Heat Processing Methods. Modeling in Meat Science: Microbiology; Refrigeration. Refrigeration and Freezing Technology: Applications; Equipment

Further Reading

ASHRAE, 2009. ASHRAE Handbook I Fundamentals, SI ed. Atlanta, GA: American Society of Heating, Refrigerating and Air-conditioning Engineers.
ASHRAE, 2010. ASHRAE Handbook – Refrigeration, SI ed. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers.
Dossat, R.J., Horan, T.J., 2001. Principles of Refrigeration, fifth ed. Upper Saddle River, NJ: Prentice Hall.
Green, D.W., Perry, R.H. (Eds.), 2007. Perry's Chemical Engineers' Handbook, eighth ed. New York, NY: McGraw-Hill.
Jeremiah, L.E., 1995. Freezing Effects on Food Quality. New York, NY: Marcel Dekker.
Kreith, F., Manglik, R.M., Bohn, M.S., 2010. Principles of Heat Transfer, seventh ed. Stamford, CT: Cengage Learning.
Smith, J.M., Van Ness, H., Abbot, M., 2004. Introduction to Chemical Engineering Thermodynamics, seventh ed. New York, NY: McGraw-Hill.
Wang, K., Eisele, M., Hwang, Y., Radermacher, R., 2010. Review of secondary loop refrigeration systems. International Journal of Refrigeration 33, 212–234.

Relevant Websites

https://www.ashrae.org/
American Society of Heating, Refrigerating and Air-Conditioning Engineers.
http://www.iifiir.org/
International Institute of Refrigeration.

Thawing

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Glossary

Apparent specific heat The heat required to raise the temperature of a unit mass of product by one degree.
Dielectric heating The heating by high frequency alternating electric field inducing molecular rotation or ionic motion.
Drip The water lost from a product in liquid form during thawing.
Extracellular (ice) The ice formation outside (between) the cells.
Finite element method The method of calculating changes in temperature and other variables in a region of space by dividing it into small elements.
Freezing point depression The lowering of freezing point due to solutes in the water or increase in pressure.
Frozen fraction The fraction of freezable water that has solidified.
Heat transfer coefficient (HTC) The heat transfer rate per unit area per unit time with a temperature gradient between the product and surrounding temperature.
Hot spot A location in the product where the temperature is much higher than average, because of uneven heating.
Tempering The partial thawing achieved by raising the temperature of frozen food near to but still below the freezing point in order to reduce hardness and facilitate processing.

Introduction

Frozen meat must usually be thawed before cooking or further processing. Often only partial thawing (known as tempering) is carried out, as tempered meat is firmer than completely thawed meat and can be more easily sliced or flaked. This article covers both the thawing and tempering processes.

Thawing/tempering is a more difficult process to carry out safely than chilling or freezing because of the high danger of subjecting some parts of the food to high temperature and humidity, which favor microbial growth. A good thawing or tempering regime is one that minimizes process time, microbial growth risks, drip loss, and other quality losses. It is desirable that the product is uniformly treated, and exposure to high temperature is stopped as soon as the desirable endpoint has been reached.

Thawing and tempering may be carried out by industrial, commercial, or domestic users. Food plants may thaw or temper meat for further processing (boning, cutting, grinding, curing, mixing, etc). Shops and supermarkets may thaw frozen meat for retail display. Consumers usually thaw frozen meat before cooking. Although the general principles are the same in each case, different methods and guidelines may be recommended due to differences in circumstances.

This article will examine what happens to meat during thawing or tempering, review the available thawing/tempering methods, and make some recommendations on best practice.

Physical Aspects of Thawing and Tempering

Thawing is more time consuming and difficult to control than freezing. Above-zero environment temperatures must be used, which carries the risk of microbial growth. Because the environment temperature has to be kept low, the temperature gradient that drives the heat transfer is usually much lower during thawing than during freezing, leading to long process times. Furthermore, thawed food has a lower thermal conductivity than frozen food, which slows down the heat transfer even more. Internal heating methods such as microwave thawing alleviate the heat transfer problem but, if the heating is too intense, local hot spots will occur.

Food freezing and thawing do not take place at a sharp temperature, as for pure water, but occur gradually over a temperature range. The amount of residual unfrozen water at any given temperature below the freezing point can be calculated by the law of freezing point depression, and is approximately proportional to T_f/T, where T is the temperature and T_f the initial freezing point of the food, both in degrees C. Thus, for meat, which begins to freeze at approximately $T_f = -1$ °C, approximately half the water in the food (more precisely, half of the water that is not bound to food molecules) remains unfrozen at −2 °C, one-third at −3 °C, one-tenth at −10 °C, and so on (Figure 1). Hence that meat will require almost the same amount of heat to rise from −30 to −2 °C as from −2 to −1 °C. If that meat is being thawed in air, the second part of the process (from −2 to −1 °C) will take longer than the first part because the temperature difference between air and food will be less and less as time goes on. Hence, tempering a food takes much less time than complete thawing, even though the final tempering temperature may be quite close to the freezing point of the food.

The apparent specific heat (the amount of heat required to raise the food temperature by a degree) is greatest just under the initial freezing point T_f, because that is where most the ice thaws (Figure 2). Once all the ice has thawed, the specific heat falls back to a low value. The peak is known as the 'latent heat peak' and means that, while frozen food may take a long time to thaw completely. Those parts of the food that thaw earliest (such as the corners) will rapidly heat up further and stay at

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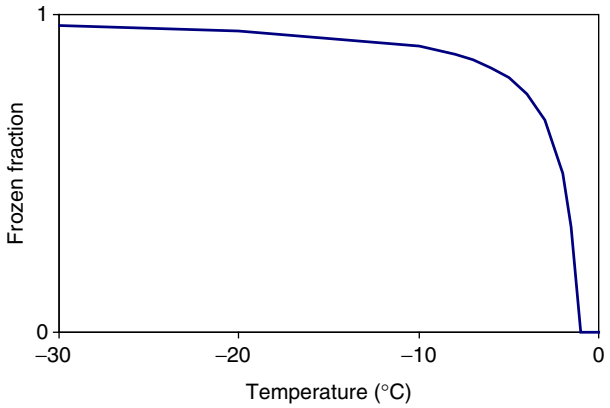

Figure 1 Frozen fraction vs. temperature.

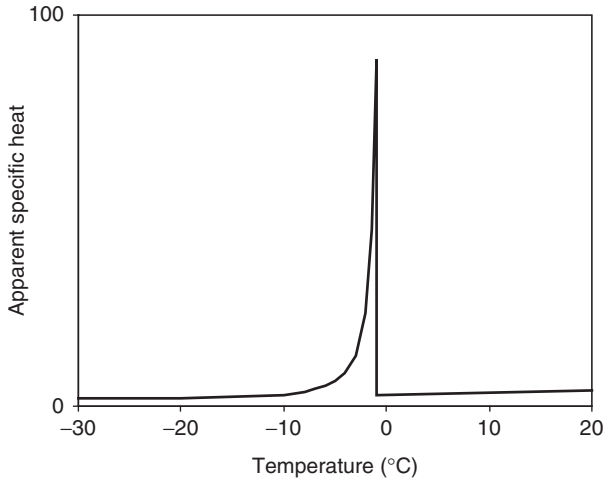

Figure 2 Apparent specific heat of food around the freezing point (shown in arbitrary units).

high temperatures for a long time, causing nonuniformity of treatment and increasing the risk of local microbial growth.

This nonuniformity is greatest for large products and when the heat transfer rate is fast. During external thawing (in air or water), the surface and corners of a carton or large piece of meat can stay at high temperatures for a long time, causing microbial and other quality problems. A compromise must be made between speed of thawing and uniformity of treatment.

The thawing time of a piece of food can be calculated by a variety of methods ranging from simple approximations to rigorous computer calculations. For most practical purposes, a simple formula proposed by Cleland *et al.* and reported in Pham (see Further Reading) is adequate for external thawing, if the food can be approximated by a simple shape: slab, long cylinder, or sphere. The thawing time depends mainly on the following factors:

- the surrounding temperature
- the heat transfer coefficient (HTC), a number that measures how easily heat is transferred from the surroundings to the product. High air velocity around the product, or using water instead of air will increase the HTC, while wrapping will reduce it
- the shape and thickness of the product, which determines how far the heat has to travel from the surface to the center
- the composition of the meat, especially its water and fat contents

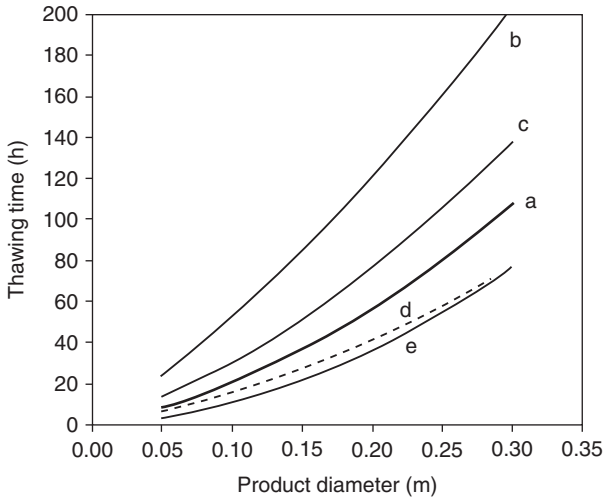

Figure 3 Effect of various factors on the thawing time of a cylinder of meat. (a) Base conditions: air at 1 m s^{-1}, 5 °C, no wrap. (b) 0 m s^{-1}, (c) polythene wrapped, (d) 10 °C, (e) water thawing.

Figure 3 illustrates how some of these factors affect the thawing time for a cylinder of meat. It can be seen that when the diameter is doubled, the thawing time increases by a factor of 3–4. Thawing in still air increases the thawing time by a factor of 2–3 compared to air moving at 1 m s^{-1}. A polythene wrap increases thawing time by approximately 30–50%, whereas raising the air temperature to 10 °C decreases it by 20–30%. Changing from air to water is more effective for small products.

During internal thawing (as in microwave thawing), heating concentrates in certain spots, depending on the shape, size, and composition of the product. There may even be some local cooking of the meat while the rest is still frozen. These problems will be looked at in more detail in the section on thawing methods.

Meat Quality Aspects

The quality factors that are of most concern in meat thawing are microbial growth, drip loss, and the appearance and palatability of the product.

Microbial Growth

Microbial growth is the most important factor because it affects the safety of the product. Microorganisms include molds, yeast, and bacteria, with the last being most important from a health and safety point of view. Microbial growth is affected by temperature, water activity (relative humidity of the meat), pH, and

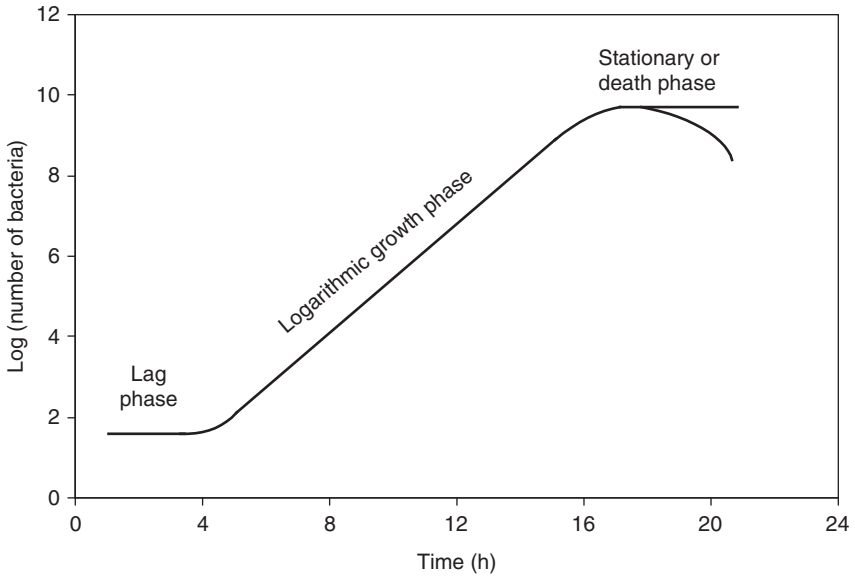

Figure 4 Typical bacterial growth curve at constant temperature.

the concentration of nutrients. During meat thawing, conditions for microbial growth are highly favorable (high water activity due to melted ice and/or water condensing on the surface, near-neutral pH, high availability of nutrients) and it is important to control growth by restricting temperature rise.

Below 5 °C, most pathogenic bacteria will not grow on meat and therefore it is often recommended (for example, by US Food and Drug Administration) that meat be kept at 5 °C or below at all times. However, there are several reasons for permitting the temperature to rise temporarily above this critical temperature. First, microbial growth normally occurs only on surfaces that have been exposed to contamination. Deep meat in whole cuts (not sliced, ground, diced, etc.) can therefore be allowed to rise temporarily above 5 °C in microwave thawing or other internal heating methods as long as it does not get cooked. Second, when temperature rises above the critical point, microorganisms must first pass through a lag phase that may last up to several hours (depending on temperature and other conditions) before growth starts (Figure 4). Therefore, when operating conditions are rigorously controlled and monitored, the 5 °C limit does not always need to be adhered to. Parts of the meat can be allowed to rise above it for some time, before being brought back down below the critical temperature. The extent of microbial growth can be calculated by a suitable model. An example is the Refrigeration Index used in the Australian meat industry, which is based on growth curves for *Escherichia coli*.

Computer software based on finite element methods are available commercially or as public domain (free) programs that will enable the temperature at various spots in the product to be predicted, and hence the potential for microbial growth can be calculated. This can help to design acceptable thawing regimes, although the results are often conservative because the programs are not yet capable of taking into account all the factors that may limit microbial growth.

In poorly controlled situations, such as in the home or when thawing is done commercially by untrained operators, it is best that thawing be carried out at 5 °C or below, i.e., in a refrigerator.

Water Loss

In air thawing, water loss may occur in two stages. First, some of the melted ice fails to be reabsorbed into the meat structure and is lost as drip. Second, in the later stages of air thawing, water may continue to be lost by evaporation. The extent of evaporation depends on air temperature and humidity, and can even be reversed (i.e., condensation can occur) if the air is at a higher temperature and humidity than the product. If thawing is carried out in water or humid air, water can be absorbed by the meat, causing weight gain.

Existing data on water loss during thawing are conflicting and inconclusive. Some experiments have shown that slow thawing causes higher drip losses, others that it reduces drip loss, and others have shown no definite influence. Various explanations have been proposed for the effect of thawing conditions on water loss, if any. On one hand, slow thawing is thought by some to cause meat proteins to denature as the meat spends long periods of time in a partly desiccated condition, with high solute concentration prevailing, at temperatures just below freezing, and the denatured proteins lose some of their water-binding capacity. On the other hand, fast thawing is thought to give water insufficient time to be reabsorbed into the cellular structure. It can easily be seen that these (hypothetical) effects act in opposite directions.

In addition, drip loss may be influenced by many factors before thawing: postmortem treatment, pH, cut surface to volume ratio, orientation of cut surface, rate of freezing, duration and temperature of frozen storage, and temperature fluctuations during frozen storage. The variability in these factors (which are never reported in complete detail) may have masked whatever effect of thawing conditions exists in the reported investigations.

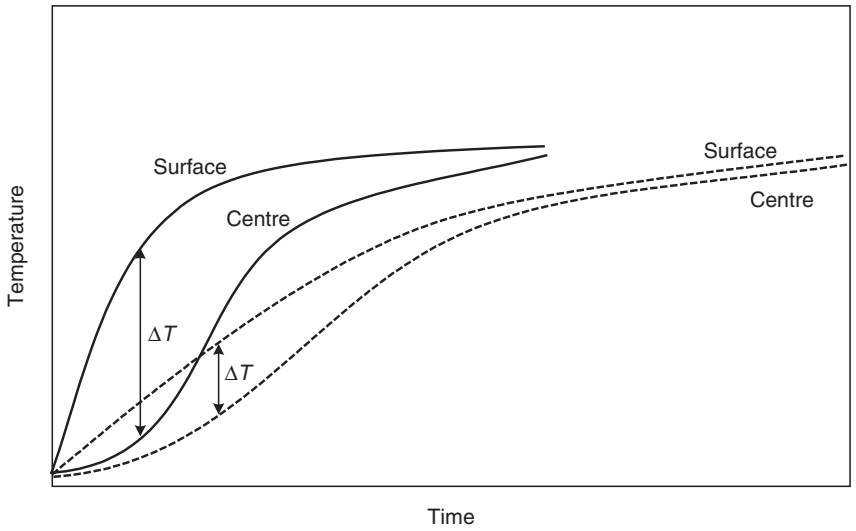

Figure 5 Temperature at center and surface of meat during thawing by external heating. ———— high surface heat transfer;- - - - - low surface heat transfer. ΔT: maximum temperature difference in product.

It is this author's opinion that, because in commercially frozen meat the ice is always extracellular, except perhaps for a thin surface layer in cryogenically frozen meat, the resorption effect would usually predominate and thus very fast thawing would not be desirable if drip loss is to be minimized. However, exposing thawed meat to air for longer than necessary will increase evaporative losses.

Appearance and Eating Quality

Various reports have shown that the appearance and eating quality (aroma, tenderness, and juiciness) of thawed meat do not seem to depend greatly on the thawing method, as long as the process does not run out of control. The exception is that meat thawed by direct contact with water may gain a bleached appearance, which is unsuitable for subsequent retail display.

In conclusion, because there is no conclusive evidence of the effect of thawing on drip loss, appearance, and eating quality (unless there is gross abuse, for example, when the product is left to thaw for far longer than needed), it seems reasonable to concentrate instead on optimizing thawing processes in terms of potential microbial growth. If meat is to be displayed after thawing, water thawing should be avoided because the meat surface may appear to be bleached. In all cases, the meat should be processed or returned to refrigeration as soon as thawing or tempering is completed.

Common Thawing Methods

Common thawing methods can be divided into external heating methods where heat flows from warm surroundings into the meat, such as thawing in air, flowing water, or under a spray; and internal heating methods, where heat is generated inside the product, as in microwave thawing or radio frequency (RF) thawing.

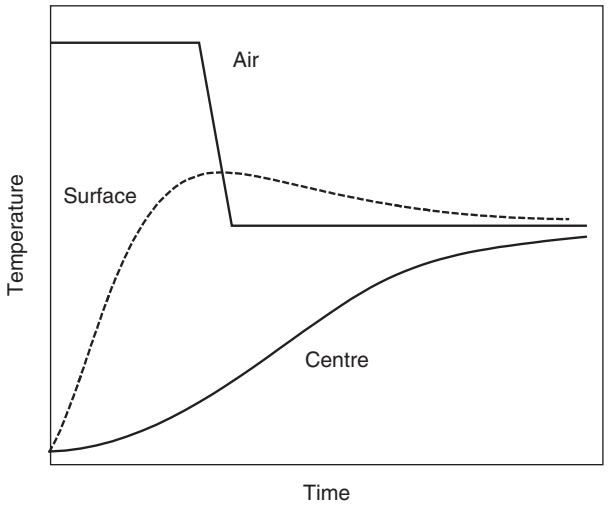

Figure 6 Product temperature history during two-stage thawing.

External Heating Methods

Air Thawing

Air thawing is quite common in industry and in the home, because it is easy to control and no special facility is needed. The drawback with air thawing is the low rate of heat transfer and hence long process time, especially when there is no forced air circulation. However, low surface heat transfer can be an advantage as it ensures that the product is heated relatively uniformly (see Figure 5). For this reason air heating is well suited to tempering, where a uniform final product temperature is desirable. Unwrapped meat thawed in air is more likely to lose water by evaporation, in addition to drip loss.

In industry, air thawing can be carried out in two stages: a high-temperature stage to inject heat quickly into the product, followed by a low-temperature stage (5 °C or below) to avoid microbial growth on the surface (Figure 6). The air velocity

may also be changed from high to low. Even though the surface of the product may briefly rise to temperatures that would allow microbial growth, microorganisms are not allowed to complete their lag phase, while thawing is accelerated. The optimum design of multistage thawing processes depends on the product shape, size, and composition, and it is unsafe to use a regime that has been developed for one product for a different product. Any thawing regime that involves air temperatures higher than 5 °C must be tailored to the product. For this reason no 'typical regime' can be given here. A number of regimes are listed by James and James (see Further Reading).

In the home or small, poorly equipped commercial premises, where there is practically no monitoring and control, the safest way is to thaw meat in the refrigerator, at a temperature of 5 °C or below, to prevent microbial growth. However, an hour or two in cool air will not pose significant risks, as long as the meat is put back into the refrigerator after that. After thawing is complete, the drip should be discarded to avoid an excessively moist surface.

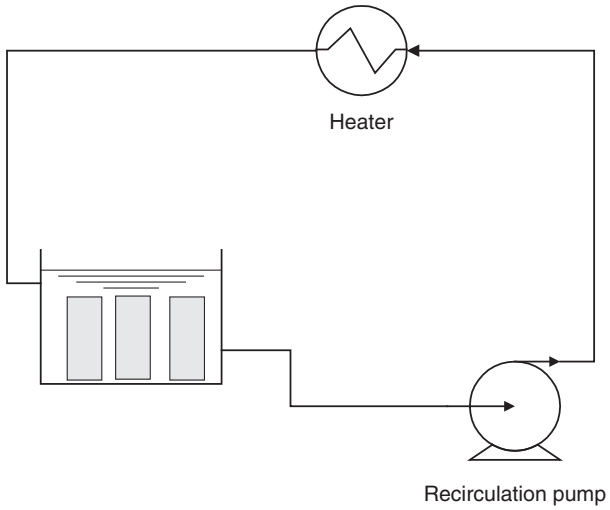

Figure 7 Immersion thawing.

Water Thawing

Water thawing can be carried out by immersing the product in a tank of water (Figure 7) or by spraying the product with water (Figure 8). The water should be reheated and circulated. The water should be changed frequently, preferably after each batch, together with thorough cleaning of the equipment, to prevent microbial growth. Water thawing is much faster than air thawing because water is a better carrier of heat than air (see Article 265: Refrigeration and Freezing Technology: Principles). The relative improvement in thawing time is greater for small products but is appreciable in all cases. Drip loss is reduced because the meat is in a moist environment (in fact, if the product is thawed by direct contact, a weight gain may be obtained). It is important to ensure that water circulates all around each piece of product, i.e., there is no dead zone in the tank. Spraying will ensure that there is no dead zone as long as the spray covers all exposed area (which can easily be checked visually).

Water thawing requires more specialized equipment and better control than air thawing. If unwrapped meat is thawed in water, there could be a high effluent load, with protein, blood, and solutes being leached out of the meat. Microbial growth and cross-contamination could also be a hazard, because the warm recirculating water is a good growth medium. Because microbial growth takes place only between 5 and 40 °C, fast thawing can be obtained by using water at approximately 45 °C (high temperature will cook the surface of the meat). This temperature is not allowable in air thawing or the water thawing of wrapped products, because, due to the lower surface heat transfer, the meat surface would remain below 45 °C and hence near the optimal temperature for growth. High water temperature should also be avoided in the water thawing of comminuted product, where microbial growth may occur inside the meat.

Owing to the high heat transfer rate, the product's surface temperature will usually be much higher than the center temperature and very close to the water temperature; hence, water cannot be used for tempering. In the home and small commercial premises, water thawing should be avoided because of the microbial risks, except for very small product with short thawing times.

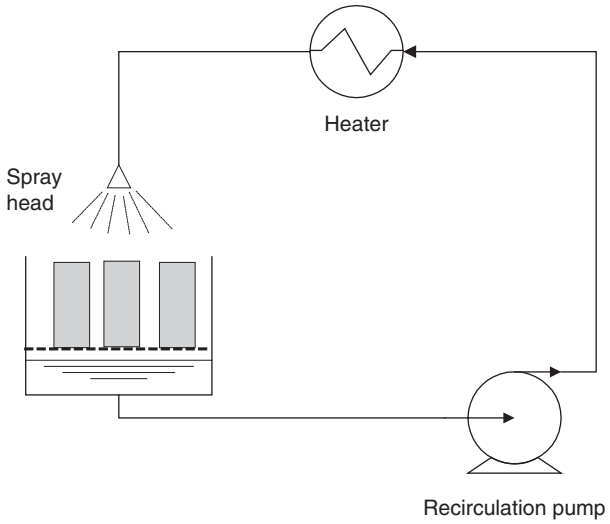

Figure 8 Spray thawing.

Vacuum Thawing

Vacuum thawing involves the introduction of steam into an evacuated chamber where the frozen product has been placed. Steam condenses readily on the surface of the meat giving a very high rate of heat transfer as it surrenders its latent heat. The low pressure depresses the condensation point of steam and ensures that the temperature stays low. Vacuum thawing is even faster than water thawing, especially for small products. Microbial contamination is negligible but growth of existing microorganisms can still occur if the surface temperature is suitable. Weight loss is low, or there may be a weight gain. With large cuts or blocks of meat, the thawing rate is limited

by heat conduction inside the meat and thus the advantage in thawing time will be less noticeable. Equipment for vacuum thawing is expensive, however, and the process must be carried out as a batch operation due to the need for evacuation.

Pressure-Assisted Thawing

High pressure causes a decrease in the freezing point. The maximum freezing point depression that can be obtained is approximately 21 °C at 210 MPa. Because the freezing point is lower, the difference between air and product temperatures can be increased by several times, causing the thawing time to decrease by approximately the same ratio. For example, at a surrounding temperature of 5 °C, the temperature difference between meat undergoing thawing (with a freezing point of −1 °C) and the surroundings is only 6 °C. If the freezing point is decreased to −7 °C by applying pressure, the temperature difference between environment and product and hence the thawing rate will double. Pressure-assisted thawing has been tested on pork and beef. For beef at pressures of up to 210 MPa the color, drip loss, cooking loss, and penetration force are not significantly affected. For pork at pressures of up to 100 MPa, quality factors are improved by pressure-assisted thawing. However, the application of pressure-assisted thawing is unlikely to be widespread for meat due to the cost of the equipment and small batch sizes.

Internal Heating Methods

Internal heating methods comprise electrical resistance heating and dielectric heating, the latter including heating by RF waves and microwave. Resistance heating is not widely used. Both RF (or capacitive) heating and microwave heating work on similar principles, the heat being generated inside the product by the rapid reorientation of dipolar molecules of water or other molecules under the influence of an alternating electromagnetic field. RF covers the frequency range 1–300 MHz, whereas microwave covers the range 300 MHz to 300 GHz. The equipment for generating and transmitting the alternating field differs: in RF the product is placed between two parallel plate electrodes, whereas in microwave it is placed in a chamber or tunnel with microwaves being generated and transmitted into the tunnel via one or more wave guides or antennas. RF can only be used with slab-shaped product that can be placed between the electrodes, whereas microwaves can be used on products of any shape.

Internal heating methods are much faster than external heating methods, because heat does not have to travel from the surface to the center of the product. However, a common problem with all these methods is uneven heat distribution, often leading to runaway heating. All materials are partially opaque to electromagnetic waves (if they were completely transparent, there would be no heating at all) and thus the surface layers will absorb more of the energy and heat up faster. The penetration depth increases with wavelength (decreases with frequency), and hence RF has more penetrating than low-frequency microwave, which is more penetrating than high-frequency microwave. However, the shape and orientation of the meat surface serves to focus the microwave, so that the center of a cylindrical or spherical product may heat up faster than the rest.

Nonuniform product composition will also cause problems. Fat, for example, is particularly effective at absorbing microwaves and may start to fry before the water melts. Ice has much lower absorbance than water, and thus any spot that melts first will preferentially absorb energy and quickly heat up, while the surrounding remains frozen. A mixture of cooked and frozen meat will then be obtained. Various methods are used to improve heat distribution: turntables, microwave stirrers, multiple antennas, and intermittent heating to allow the generated heat to disperse by conduction, but they do not completely solve the runaway heating problem.

For the reasons above, internal heating methods have not been widely used for thawing in industry, except for microwave tempering (not thawing), which is quite successful. In microwave tempering, no part of the product is allowed to rise above melting point, and therefore runaway heating is not likely to occur. The product should not be prewarmed in air before tempering but should be taken straight from cold storage, to avoid the prior thawing of corners and edges, which would lead to runaway heating. Carton-sized meat blocks can be tempered by microwave in approximately 10 min, as opposed to several days by air.

Conclusions

- Air thawing is the most easily applied thawing method, followed by water thawing.
- Microbial growth is the primary factor that determines the suitability of a thawing regime.
- Thawing time depends on the size and shape of the product, the temperature of the surroundings (for external heating), and the HTC, the last being a function of the thawing medium, the circulation velocity, and any wrapping present.
- As soon as thawing is completed, the product should be processed or returned to normal chilled storage conditions, to avoid microbial growth and quality loss.
- The effect of thawing conditions on drip loss and eating quality is not well known and is probably of little importance in the design of thawing processes.
- Two-stage thawing in air, with a short high-temperature period followed by a longer low-temperature period, can be carried out industrially but must be tailored to the product and closely controlled to avoid microbial growth at the surface.
- Computer software is available for calculating thawing time and designing thawing regimes to limit microbial growth.
- When conditions are not well controlled, thawing should be carried out in the refrigerator at 5 °C or below, although an hour or two in a cool room is acceptable.
- Water thawing is faster than air thawing but carries increased risks of cross-contamination and gives the meat a bleached appearance, which is unsuitable for retail display.
- Water thawing should not be used in the home or in small commercial premises.

- Microwave heating is highly effective for tempering to temperatures just below freezing, but not for complete thawing, due to runaway heating when complete melting occurs. It is important to avoid any heating before microwave tempering.
- Vacuum thawing is fast and hygienic but the equipment is very expensive.
- Pressure-assisted thawing is unlikely to become commercially viable.

See also: Modeling in Meat Science: Refrigeration

Further Reading

Cleland, D.J., Cleland, A.C., Earle, R.L., Byrne, S.J., 1986. Prediction of thawing time for foods of simple shape. International Journal of Refrigeration 9, 220–228.
Creed, P.G., Bailey, C., James, S.J., Harding, C.D., 1979. Air thawing of lamb carcasses. Journal of Food Technology 14, 181–191.
Gonzalez-Sanguinetti, S., Anon, M.C., Calvelo, A., 1985. Effect of thawing rate on exudate production of frozen beef. Journal of Food Science 50, 697–700, 706.
Hossain, M.M., Cleland, D.J., Cleland, A.C., 1992. Prediction of freezing and thawing times for foods of two-dimensional irregular shape by using a semi-analytical geometric factor. International Journal of Refrigeration 15, 235–240.
James, S.J., Creed, P.G., 1980. Predicting thawing times of frozen beef fore & hindquarters. International Journal of Refrigeration 3, 237–240.
James, S.J., James, C., 2002. Meat Refrigeration. UK: Woodhead, Abington.
Merts, I., 1998. A review of methods for thawing meat. Technical report MIRINZ 988. Hamilton, New Zealand: MIRINZ Food Technology and Research.
Ohlsson, T., 1984. Low-power microwave thawing of animal foods. In: Zeuthen, P. *et al*., (Eds.), Thermal Processing and the Quality of Foods. London, UK: Elsevier, pp. 579–584.
Park, S.H., Ryu, H.S., Hong, G.P., Min, S.G., 2006. Physical properties of frozen pork thawed by high pressure assisted thawing process. Food Science and Technology International 12, 347–352.
Pham, Q.T., 2001. Prediction of Cooling/Freezing/Thawing Time and Heat Load. In: Sun, Da-Wen (Ed.), Advances in Food Refrigeration. Leatherhead: Leatherhead Food RA, pp. 110–152.
Pham, Q.T., Lowry, P.D., Fleming, A.K., Willix, J., Fitzgerald, C., 1994. Temperature and microbial growth in meat blocks undergoing air thawing. International Journal of Refrigeration 17, 280–287.
Ross, T., 1999. Predictive Microbiology for the Meat Industry. Australia, Sydney, Australia: Meat and Livestock.
Vanichseni, S., Haughey, D.P., Nottingham, P.M., 1972. Water- and air-thawing of frozen lamb shoulders. Journal of Food Technology 7, 259–270.
Wang, J.F., Pham, Q.T., Cleland, D.J., 2010. Freezing, thawing and chilling of foods. In: Farid, M. (Ed.), Mathematical Analysis of Food Processing. Boca Raton, FL: Taylor and Francis/CRC Press, pp. 375–398 (Chapter 16).
Zhao, Y., Flores, R.A., Olson, D.G., 1998. High hydrostatic pressure effects on rapid thawing of frozen beef. Journal of Food Science 63, 272–275.

Relevant Websites

http://www.fda.gov/Food/FoodSafety/RetailFoodProtection/FoodCode/FoodCode2001/ucm089132.htm
Food and Drug Administration.
http://www.foodsafetycentre.com.au/refrigerationindex.php
Food Safety Centre Australia.

RELIGIOUS SLAUGHTER

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Glossary

Bedika A careful examination of the internal organs to ensure that the animal was healthy before slaughter.
Cincinnati pen A restraining device approved by the American Society for the Prevention of Cruelty to Animals for restraint in an upright position before slaughter.
al-Dhabh The Muslim method of ritual slaughter.
Halal Arabic word meaning 'lawful'; indicating a food acceptable for consumption by Muslims.
Kosher Hebrew word meaning 'fit'; indicating food acceptable for consumption by Jews.
Neveila Hebrew word indicating an animal that died of natural causes and so is not kosher.
Shechita Jewish ritual slaughter.
Stick The act of cutting open the throat of a meat animal.
Terefah Unfit for kosher consumption.
Weinberg casting pen An animal holding pen that could be rotated 180° on its long axis to permit an easier and more rapid neck cut.

Introduction

As human societies evolved from nomadic hunting and gathering to settled agriculture, they developed increasingly sophisticated techniques and rituals for harvesting meat from livestock. These grew out of the knowledge, understanding, and beliefs of the day. In some societies the rituals were enshrined in religious law, and the animals that were permitted for consumption were cataloged. In modern society, these systems of ritual slaughter sometimes find themselves in conflict with the standard operating procedures of high-speed, high-efficiency abattoirs; they have also been challenged in some quarters for being inconsistent with scientifically developed standards of animal welfare.

Since biblical times, adherents to the Jewish dietary rules have restricted their meat and fish intake to those classes of creatures conforming to theologically prescribed characteristics. The Mosaic Law of the Torah, Judaism's holiest scripture, further dictates that all birds and mammals (there is no stipulation for fish) destined for the Jewish table be killed according to a divinely revealed method of ritual slaughter – albeit the precise nature of the system is found nowhere in the Bible. Today, for a meat product to be deemed 'kosher' (Hebrew, 'fit') it must come from a biblically prescribed species of animal that has undergone shechita and that has been processed according to Jewish butchering methods. The consumption of meat from hunted animals is strictly prohibited.

The theological origins of Muslim slaughter are found in Islam's holiest book, the Q'uran. Only the meat from (permitted) animals killed according to the customs and methods of al-Dhabh may be deemed 'halal' (Arabic, 'lawful'), although Muslims are allowed to consume meat that has been hunted in accordance with religious guidelines. Whereas the rules of shechita require conscious slaughtering, animals killed in the halal process are, according to some Islamic authorities, permitted to be rendered temporarily unconscious before slaughter. The development of this practice has occurred relatively recently and is by no means universal; however, it has been adopted in a number of prominent meat-exporting nations and continues to grow in popularity.

Kosher Slaughtering (Shechita)

Theological Basis

In Leviticus XI:3–8, and in a slightly modified form in Deuteronomy XIV:3–8, the Torah dictates that mammals fit for consumption by Jews must be ruminant and have completely split hooves. For fish to be suitable, they must have scales and fins (Leviticus XI:9–12; Deuteronomy XIV:9–10). The Torah adjudges all birds to be clean and thus edible, except for those species that it specifically prohibits (Leviticus XI:13–20; Deuteronomy XIV:11–18). One of the chief exegetical conclusions that can be drawn from the proscribed avian species lists is that, in general, common domestic fowl are suitable whereas migratory species and birds of prey are an 'abomination' to eat.

The actual reference to the slaughter of food animals occurs in Deuteronomy XII:21. 'Thou shalt kill of thy herd and of thy flock, which the Lord hath given thee, as I have commanded thee, and thou shalt eat within thy gates, after all the desires of thy soul.' It is generally interpreted from this passage that there existed in Biblical times a canon of oral law (Hebrew, halacha) that was to be used as an exegetical guide for understanding and executing the written Torah.

With the razing of the Second Temple in 70 AD and the Roman persecutions that followed, the leading Jewish thinkers of the era began to commit the oral law to script. The fruit of their labors is the encyclopedic work known as the Mishnah (Hebrew, 'repetition'), the totality of Jewish oral law and tradition compiled during the second century AD. In the three centuries immediately following the creation of the Mishnah, learned halachic scholars in both Palestine and Babylonia began the process of interpreting the codified oral law. In doing so, they created a second body of commentary, often presented as a glossary to the text of the Mishnah, known as the Gemerah (Aramaic, 'tradition'). Contained within these two tracts, collectively known as the Talmud, is the basic philosophy guiding shechita. Subsequent rabbinical interpretations, particularly during the Middle Ages and Renaissance, increasingly refined the rules and defined the methods permissible in shechita, leading, ultimately, to the procedures in place today.

Basic Procedures

At its most fundamental level, kosher slaughter involves cutting the throat (sticking) and bleeding out (exsanguination) of a fully conscious, restrained animal by way of an expertly wielded, razor-sharp blade. The precise cause of death is cerebral hypoxia brought on by the severance of the carotid arteries and jugular veins. In most countries where shechita is practiced, legislation requires that it be performed in approved premises (usually a government-inspected abattoir) and be subject to governmental hygiene and welfare regulations in addition to the religious requirements.

The Talmud identifies five conditions or situations that, should any of them occur during slaughter, would render the entire carcass unfit for kosher consumption (Hebrew, neveila, referring to an animal that dies of natural causes):

1. Shehiya (delay): The throat must be cut with one, rapid continuous motion from start to finish. There cannot be any pause in the blade's motion.
2. Derassa (pressing): No upward or downward pressure may be exerted on the knife beyond that which is absolutely required to create the incision. Hacking cuts are specifically forbidden.
3. Chalada (digging): The incision must not close back on itself and cover the surface of the blade, which must be visible at all times. There can be no burrowing of the blade or stabbing action.
4. Hagrama (slipping): The incision must occur laterally across the throat between the larynx and the top of the inflated upper lung. The cartilaginous cricoid ring located below the larynx must be avoided, as must all other bony structures, in order not to damage the blade (see point 5).
5. Ikkur (tearing): The esophagus and trachea must be cleanly cut and not torn. To achieve this, the blade must be extremely sharp, without even the smallest nick or other imperfection. It must be examined immediately after sticking to determine whether any damage was incurred that would lead to a declaration of neveila on the carcass.

From these five definitive criteria of an acceptable slaughter, many other common characteristics of shechita may be understood. For example, it is recommended that the ritual knife (Hebrew, chalaf) have a broad, rectangular blade, with a length at least twice the width of the animal's neck, thus reducing the likelihood of chalada or shehiya. Typically, knives are 6 inches long for the slaughter of fowl and 18 inches long for cattle. In addition to creating the incision, the slaughterman (Hebrew, shochet) is responsible for ensuring that the blade is sharpened and that the cutting edge smooth and unblemished before and after every animal, or, in the case of fowl, entire lots of birds.

Bedika

If the act of sticking does not violate any of the five prohibited conditions, the shochet or his assistant will then carry out the next level of the kosher process: bedika. This involves an examination of various internal organs, which is made to ensure that the animal's health at the time of death met Talmudic standards of wellness. The Talmud defines eight pathological conditions in food animals, including missing organs, torn organ walls, bone fractures and perforated organs, which would render them unfit for kosher consumption (Hebrew, terefah). It is from this list that shechita's wholesale rejection of stunning is largely derived.

A postmortem examination of the lungs, initially via an incision in the thoracic cavity (on the ventral side) and later once the organs have been removed, is the focal point of bedika. The shochet's pulmonary inspection seeks chiefly to identify evidence of pleural adhesions that might, for example, indicate a punctured lung. If such masses are found during the initial inspection, they are carefully excised on the inspection table. The lungs are then filled with air and submerged in water as a test of pulmonary integrity. Air-tightness, indicating an absence of organ wall damage, is sufficient evidence for kosher certification (assuming the presence of no other prohibited condition). The significance that a bedika places on pulmonary integrity is predicated on the belief that any systemic morbidity in an animal would be evident in the lungs. Regardless of a shochet's verdict, any carcass from an animal processed in a licensed abattoir must ultimately be approved by a government inspector/veterinarian.

Halal Slaughtering (al-Dhabh)

Whilst shechita and al-Dhabh share several philosophical and practical characteristics, there is also much that is dissimilar about the two systems. The annual worldwide halal slaughter is dramatically larger than the yearly kosher kill, with most of the former occurring in the developing world. By contrast, the vast majority of kosher meat is processed under the animal welfare and technological standards of the industrialized west. Further, al-Dhabh is influenced to a great extent by the scriptural interpretation of local Islamic authorities and local customs, whereas the canonical nature of shechita promotes a greater level of homogeneity in its practice. There also exists no Islamic equivalent of the shochet; al-Dhabh retains the tradition of individual Muslims slaughtering their own livestock. As a consequence, millions of sheep, goats, and calves are slaughtered for the halal market outside licensed abattoirs every year. (In most Western jurisdictions, such as the

European Union (see Directive 93/119/EC), it is illegal to market meat from animals killed outside registered establishments.) The most significant difference, however, is that a great many Muslims will now accept as halal meat that has been produced from animals subjected to preslaughter stunning.

Theological Basis

The rules and specific interdictions respecting the Islamic slaughter of food animals are contained in several surah (Arabic, 'chapter') of the Q'uran. They are strictly concerned with the humane killing of God's creatures and not with sacrificial practice per se. The most basic and definitive characteristic of al-Dhabh, the requirement that Allah's name be pronounced at the moment of slaughter, is a product of Surah Al-An'am, 6:118: 'So eat of (meats) on which Allah's name hath been pronounced if ye have faith in His Signs.'

Like observant Jews, Muslims carry a staunch antipathy toward swine flesh, blood, and carrion, which is borne out of Surah Al-Baqara, 2:172–3. This passage, which reinforces the importance of invoking Allah's name during slaughter, states: 'O ye who believe! eat of the good things that We have provided for you and be grateful to Allah if it is Him ye worship/ He hath only forbidden you dead meat and blood and the flesh of swine and that on which any other name hath been invoked besides that of Allah….' In addition to these three foodstuffs, Islamic scholars have identified in the Q'uran several misadventures that can befall an animal that will render its flesh haram (Arabic, 'prohibited'). These include death by strangulation, a fall, goring, and violence from a wild creature.

Basic Procedures

Halal slaughtering may be conducted by any sane, sober, adult Muslim, who must follow the scriptural principles of al-Dhabh. The methodology may vary in detail from location to location, but in general the restrained animal has its carotid arteries and jugular veins severed via an incision in the neck made with a sharp knife. In cases where the knife is of a sufficient width, the stick can be performed with the same continuous slicing motion that is mandated by shechita. More often, however, a short, sometimes curved blade is employed, necessitating one or more stab incisions followed by retrograde severance of the major blood vessels of the neck. Other requirements include the following.

- The slaughterman must face the animal in the direction of Mecca and speak the name of Allah (and no other) either before or during the process. The precise nature of this practice, however, will depend on local customs.
- In the case of animals stunned before slaughter, the cause of death must be as a direct result of the stick and subsequent exsanguination (i.e., prolonged cerebral hypoxia, resulting from profound loss of blood) and not the stun itself. Owing to the requirement that animals must be alive at the time of slaughter, head-only electrical stunning, which has been shown to allow recovery, is the most commonly used method.
- The cut should result in a rapid and thorough exsanguination. It must also minimize pain. Knives must, therefore, be kept sharp.
- Knives must not be sharpened in front of animals before slaughter.
- The animal should be clean and not thirsty at the time of death.
- Animals (but not necessarily fowl) must not be slaughtered in the visual presence of other similarly destined animals.

The greater flexibility of al-Dhabh relative to shechita has facilitated the ever-increasing adoption of preslaughter stunning with the former. In the late 1970s, domestic and international Muslim authorities worked with representatives from government and industry in New Zealand in the development of a method of stunning that would be compatible with the principles set out in the Q'uran. It was agreed that any method that was not inherently painful and which would permit an animal both to regain consciousness and to eat within several minutes was acceptable. As a result, head-only electrical stunning was made mandatory for al-Dhabh in New Zealand. Preslaughter stunning is now a legal requirement for all slaughter of sheep, goats, and cattle in Australia, New Zealand, and a growing number of European and other nations. As a consequence, these jurisdictions have effectively outlawed kosher slaughter and permit the halal process only when it includes preslaughter stunning.

Restraint

Where ritual slaughter is performed without stunning, it becomes necessary to restrain the animal so that its neck can be presented to the knife and held relatively still until the stick is complete. Until the twentieth century, the common method of preparing a large animal for either shechita or al-Dhabh was to first 'cast' it to the ground using ropes and/or chains. The creature – most commonly an adult bovine; sheep and calves are small enough to be held manually or placed in an inverting cradle – was then secured until a slaughterman forcibly extended its neck for sticking.

A variation on this process, eventually mandated by US federal law (in 1906) did away with the casting step entirely. The stated purpose of the regulations was to prevent disease-causing filth from gaining entry to the interior of the carcass through the neck wound. As a result, sticking of animals lying prone on the floor was prohibited; consequently, shochetim were forced to adopt the practice of shackling and hoisting cattle directly to the rail. This procedure called for a single hind leg to be shackled by a chain (or rope) connected to a hoist on an overhead rail system. As the hoist was engaged, the animal's hind quarters were gradually lifted off of the ground. With an unnatural proportion of its body weight now borne by its fore legs, the bovine would inevitably crash, often head-first, onto the floor. Eventually, the creature would hang with its entire mass supported by its shackled leg. Both the 'cast with ropes' and 'shackle and hoist' methods were slow and awkward, and could be perilous to abattoir staff; furthermore, they were surely stressful to the animal and could lead ultimately to carcass damage sufficient for a ruling of terefah.

The first holding pen specially designed to improve both the humaneness and efficiency of shechita and al-Dhabh was developed in the UK in 1927. The Weinberg casting pen consisted of an adjustable enclosure, large enough for adult cattle, which

could be rotated on circular rails about its long axis. With a 180° turn, the animal became inverted, thus allowing for easier neck extension and a more rapid incision. This was hailed by many at the time as a great step forward, and in the United Kingdom became mandatory for religious slaughter in 1958.

However, the Weinberg casting pen was never embraced as a permanent solution to the problems associated with conscious restraint. Inverting any animal, but particularly a large ruminant, inevitably causes distress as well as potential suffocation through pressure of the abdominal contents on the thoracic cavity. These problems were addressed in the design of the first upright slaughter pen in 1963. Known as the ASPCA (American Society for the Prevention of Cruelty to Animals) or 'Cincinnati' pen, it provided for the gentle restraint of both the head and body of a still, standing animal (see Figure 1). With the animal's body supported ventrally and caudally within the enclosure, and its neck extended by a hydraulic chin lift, the physical force applied to the animal was greatly reduced.

Stunned versus Conscious Slaughter

Before preslaughter stunning of cattle became common, there was little rational basis on which to criticize religious slaughter from an animal welfare perspective. To all intents and purposes, all animals harvested for meat before the late nineteenth century were slaughtered in a fully conscious state. Stunning became more common in the twentieth century and was eventually enshrined in animal welfare legislation in many nations. This led to an increasingly spirited debate on the relative humaneness of bleeding conscious and unconscious animals. Until relatively recently, such discussions were more philosophical than scientific; however, the current focus on applied animal ethology research has facilitated a broadening of this debate.

Early devices for the stunning of cattle, such as the poleax and sledgehammer, unless wielded by an extremely skillful 'knocker,' probably did little to enhance the animal's preslaughter welfare. Similarly, before the advent of upright restraining systems it is likely that the struggle to restrain, stick, and bleed a fully conscious bovine for religious slaughter would have severely compromised its welfare. By the 1920s, however, the captive bolt pistol had been developed and refined to such a point that it was widely used to facilitate the secular slaughterman's craft. In those early days, however, it was probably more valued for its ability to reduce struggling and increase processing speeds than for any perceived improvement in welfare.

Modern research indicates that when a penetrating captive bolt stunning device is maintained and used properly, the

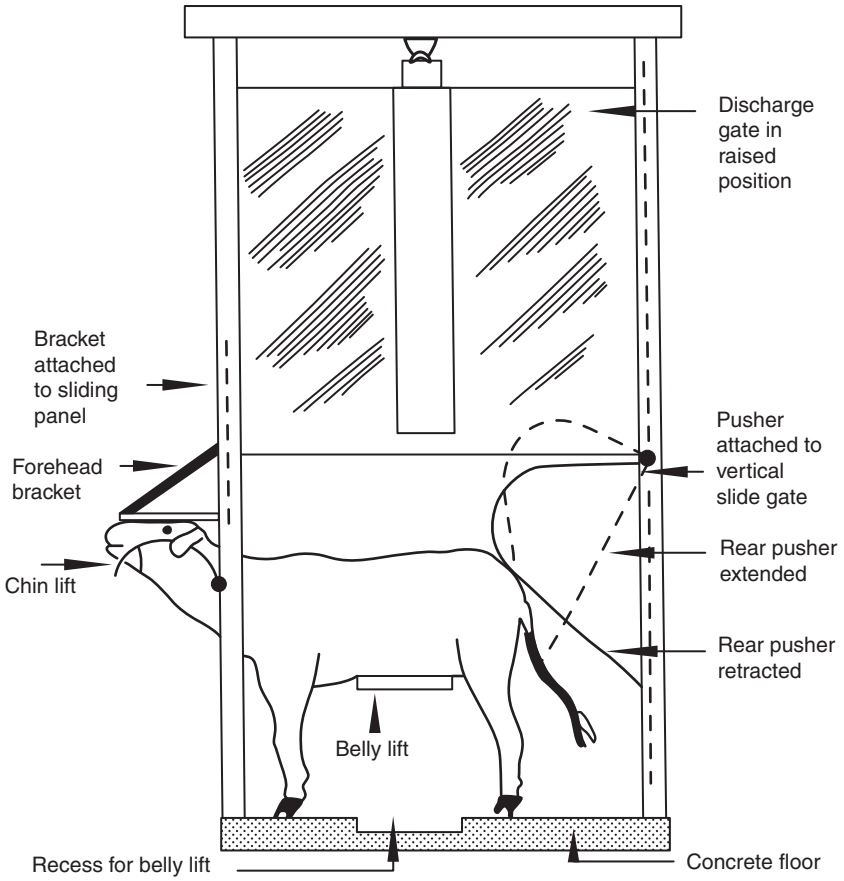

Figure 1 Modified ASPCA pen showing the animal in the correct position for low-stress restraint. Both the forehead and the back should be in level. Excessive pressure must be avoided. Reproduced from Grandin, T., Regenstein, J.M., 1994. Religious slaughter: a discussion for meat scientists. Meat Focus International 3, 115–123.

animal will be painlessly rendered insensible; however, because these devices cause such massive injury to the animal's brain, they effectively kill (or, at the very least, mortally wound) rather than temporarily stun. This point is crucial to any critical evaluation of religious slaughtering methods, which require that the animal be alive at the time of exsanguination. There can be no doubt that both the 'cast with ropes' and 'shackle and hoist' methods of religious preslaughter restraint are, by any reasonable, twenty-first century standard, inhumane. They commonly result in bruising, broken legs, and jaws, dislocated hips and gouged eyes, conditions that, paradoxically, would render a carcass neveila.

Appropriate restraint is a prerequisite to humane religious slaughter, and it is now clear that the ASPCA pen is an improvement on the Weinberg and other inversion-type pens. Research has demonstrated that adult cattle subject to shechita in an ASPCA pen show fewer physiological and behavioral symptoms of distress than those slaughtered while inverted. But the question remains: is it innately cruel to cut a fully conscious animal's throat? Certainly there is strong anecdotal evidence of humans feeling no pain in the face of massive, even fatal wounds; those, for example, received on the battlefield or as a result of a shark attack. The scholarly literature, too, contains numerous reports of cattle and calves displaying virtually no awareness of their throats having been cut during shechita. With their heads freed from restraint, stuck bovines will generally collapse immediately or else stand as normal, despite the gaping neck wound, until the onset of a hypoxic state.

With that said, most of the discussion on this issue has focused on adult cattle in part because their size and strength makes restraint far more difficult than for smaller animals. But there is also evidence showing that the delay in unconsciousness is species dependent because of differences in arterial architecture in relation to the brain. Bovines possess a vertebral arterial structure, which is absent in caprines and ovines, that will continue to supply the brain even following a complete ventral neck cut. As a consequence, cattle may take longer than goats and sheep to lose consciousness and the time taken may be more variable. Indeed, recent research with cattle and calves has shown that even when all procedures are properly conducted, some animals will remain conscious for a period of greater than 60 s after being stuck, during which time they may be expected to experience pain and distress. Other research using an electroencephalogram (EEG) has indicated that following a ventral neck incision nociceptive nerve fibers continued to carry noxious stimuli to the brain where they may be perceived as pain until the animal loses consciousness. It was also found that some animals regained their feet after collapsing, a sure sign of continued awareness.

The Future

Experimental evidence and industrial observations recorded for well over a century strongly indicate that the induction of permanent insensibility in the animal by way of a mechanical, electrical, or gas stunning device is a prerequisite for an ideal (i.e., humane, safe, and rapid) slaughter operation. Further, an increasing sensitivity to animal welfare is gradually leading to mandatory stunning in a number of jurisdictions, effectively rendering all kosher – and some halal – slaughter illegal. In other countries, though, including the United States and Canada, conscious slaughter continues to be tolerated in the name of religious freedom.

In the final analysis, it remains unclear whether the maximization of animal welfare and protection of minority rights are mutually exclusive goals. In fact, these two objectives are likely to become more compatible as religiously justifiable compromises are found to (a) reduce the frequency of conscious slaughter and (b) eliminate inappropriate methods of restraint and sticking. With respect to al-Dhabh, this may be manifest in community acquiescence to the mandatory stunning of all meat animals. In addition, the normalization of training, licensing, and supervision of Muslim slaughtermen by an appropriate authority may serve to improve standards and reduce the inconsistencies associated with halal slaughter. In the case of shechita, one technique that may reduce the potential for suffering – albeit, one that is currently shrouded in some controversy – is the administration of a stun immediately following sticking. A penetrating captive bolt pistol wielded by a skilled slaughterman could render a bovine insensible within 10 s of the shochet's stroke. Although this practice does not address all of the perceived welfare deficiencies of conscious slaughter and is not yet known to have been accepted by Jewish authorities, it certainly has the potential to minimize many of the most acute concerns. Despite the lack of its widespread adoption, postslaughter stunning of cattle does appear to represent a significant improvement in both the humaneness and efficiency of shechita. It is a procedure that deserves further study.

See also: Slaughter, Ethics, and the Law. Stunning: Electrical Stunning

Further Reading

EFSA Scientific Panel on Animal Health and Welfare, 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal 45, 1–29.

Gregory, N.G., Fielding, H.R., von Wenzlawowicz, M., von Holleben, K., 2010. Time to collapse following slaughter without stunning. Meat Science 85, 66–69.

Mellor, D.J., Gibson, T.J., Johnson, C.B., 2009. A re-evaluation of the need to stun calves prior to slaughter by ventral neck incision: An introductory review. New Zealand Veterinary Journal 57 (2), 74–76.

Relevant Website

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775454.htm European Food Safety Authority.

RESIDUES IN MEAT AND MEAT PRODUCTS

Contents

Feed and Drug Residues

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Glossary

Acceptable daily intake (ADI) The amount of an undesirable substance or chemical residue that will not cause adverse health effects, even when consumed over the entire life span.

Exposure assessment Evaluation of the exposure of human consumers to potential harmful substances present in meat. It is based on human consumption patterns, taking into account vulnerable groups of the human population, such as high consumers of meat and meat products, children and the elderly, pregnant women, and diseased people.

Food chain The food chain describes all steps involved in the production of meat or meat products intended for human consumption. It starts with the food-producing animal and its environment and feed supplies, including animal health aspects and the use of therapeutic agents, and addresses meat processing and packing.

Illicit substances Substances that are not allowed to be used in food-producing animals or in their diet (feed), as they may adversely effect animal health and well-being, the quality of animal products, or cause a risk for consumers if residues of such substances enter the food chain.

Maximum permissible level (ML) The amount of an unavoidable food or feed contaminant that can be tolerated, as at this level under the conditions of normal consumption the amount reaching the consumer will not pose a health risk.

Maximum residue level (MRL) The tolerated maximum residue level of any substance that is used intentionally in the meat production chain, including substances that are applied to food producing animals such as veterinary drugs. This maximum residue level is based on the ADI and aims to avoid that consumers are exposed to harmful residues.

National Residue Control Plans (NRCP) Prescribed chemical analyses to identify potentially harmful residues in meat. NRCPs address undesirable residues of contaminants or veterinary medicinal product in meats and are established every year by the competent authorities taking into account the food chain information (risk factors) and the number of slaughter animals per animal species.

Premarketing authorization Any product that is used in food-producing animals or added to their diets requires a pre-marketing authorization. In this scientific evaluation conducted by the competent authorities, the safety of the product for the animal and the safety of animal products under the defined conditions of use is assessed. Products that are not safe are not licensed for use, and even for safe products a withdrawal period might be prescribed to avoid human exposure to residues in meat of meat products.

Risk assessment A complex scientific evaluation of exposure, the likelihood and nature of potential adverse health effects, the relation between such adverse effects and the (ingested) amount of a harmful chemical substance. Risk assessment provides the basis for legal measures (MRL or MLs) and aims to guarantee that the food is safe for all consumers.

Veterinary Medicinal Products (VMP) VMPs are medicines, also denoted as veterinary drugs, that are intended to be used in animals to cure or prevent diseases. Major classes of VMPs used in food-producing animals are antibiotics (to combat bacterial infections), antiparasitics (against parasites), and substances used in the control of pain in the animal.

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Preamble

Animals come into contact with numerous chemical substances in their daily life, being either part of their environment or occurring in feed and fodder (grass, hay), water (offered drinking water or water ingested from rivers and lakes), and the air (industrial and urban air pollution). In addition, animals might be treated for infectious, parasitic, or other diseases with veterinary drugs, and residues of these treatments may form undesirable residues in animal tissue as well. The analysis of this entire food chain aims to prevent consumers from exposure to undesirable residues when consuming meat or meat products (Figure 1).

Terms of Reference and Definitions

As indicated in the title of this article, this short review aims to provide an overview of residues originating from feed and from the use of veterinary drugs (veterinary medicinal products (VMP)) in animals. This excludes a detailed discussion of residues from the environment, the use of pesticides and the occurrence of natural toxins in the diet of the animal. Contamination of meat and meat products with biological hazards, such a pathogenic bacteria, viruses, or parasites and the potential risks to public health are covered in other articles.

How Feed Is Defined

Animal feed includes any material that is offered to animals with the aim to meet the nutritional requirements of the individual animal at the different stages of life. In Europe, an extensive catalog of feed components that are permitted to be used for food producing animals (including slaughter animals) is published in Commission Regulation (European Union (EU) No 575/2011). Other catalogs of feed materials have been established for example by the US Food and Drug Administration or at the global level by the International Feed Industry Federation (IFIF). The main objective of these listings is to ensure animal health and well-being as well as to exclude toxic plants and potentially harmful waste products from animal feeds.

To improve animal health, natural feed materials can be fortified with so-called 'feed additives,' such as vitamins and minerals. Moreover, colorants, appetizers, and technical and zootechnical additives may be added to feeds to improve their quality and digestibility. These substances that are voluntarily added to feed, have to pass an evaluation procedure (marketing authorization) by the competent authorities (for details see Regulation (EC) no. 1831/2003). In this authorization process, the efficacy (benefit for the animal), safety for the animal, and safety (absence of any residues) of animal-derived products is assessed.

How Veterinary Drugs Are Defined

Veterinary drugs (VMPs) are pharmacologically active substances that are administered to animals in the case of disease. Prominent examples are the class of antibiotics that are used in the treatment of bacterial infections, antiparasitics applied in the control of endo- and ectoparasites, drugs that are used in the management of pain, or any other drug with a specific indication for use in a diseased animal. All VMPs used in animals have to undergo a premarketing approval process by the competent (national of EU) authorities, or have to be approved for use human patients (see Council Regulation (EC) No 37/2010 for the procedures applied in Europe). The premarketing approval process control the composition and pharmaceutical quality of a VMP, its efficacy for the claimed indications, its safety profile in the animal species for which it is licensed (this can included one or more animal species) and the safety for the applicant (farmer or veterinarian). In the case of food producing animals, the dossier includes also a special part in which the deposition of residues of the parent compound or its metabolites is studied and the possible impact of residues in edible tissues is assessed (see Section How Risk Assessment is Defined). This implies that only drugs that have passed this premarketing approval can be used in animals for the indications provided in the list of specific product characteristics. If potentially harmful residues of a VMP or its metabolites can be detected in edible tissue of an animal, the

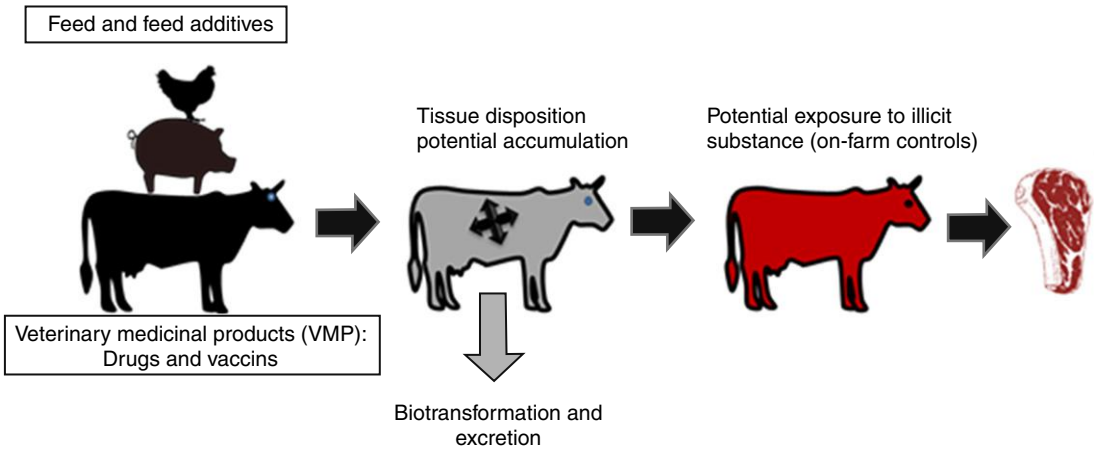

Figure 1 The food chain and the origin of chemical residues in meat.

competent authorities may prescribe a withdrawal period (time between last treatment and slaughter) to avoid that these residues will be present in consumable products (see Section Residues of Feed Additives and Veterinary Drugs).

How Meat Is Defined

In this article the term 'meat' refers to

- Muscle tissue including interstitial and body fat of slaughter animals including pig, poultry, ruminants (bovine species, sheep and goats), and horses (solipeds) and where appropriate rabbit and farmed game meat.
- Offal: edibles organs such as liver and kidney that are commonly collected for human consumption.

Several other parts of the carcass such as intestines, bladders, and stomachs that might be processed for sausage making, as well as local specialties such as tripe (rumen), legs and ears, and bone tissues (spare ribs) are considered as fresh meat and special regulations are in place to ensure that these products meet all criteria set for meat in general. Moreover, other foods of animal origin such as milk and eggs need to comply with the same food safety standards, but details of their evaluation is not within the scope of this review.

How Chemical Residues Are Defined

The term chemical residue refers to any substance originating from the production process of a given food and hence is not a natural constituent of meat. Residues can originate from very different sources or describe residual amounts of production aids (here feed additives or VMPs) that have been used in the life phase of the animal and that may be present in residual amounts in the carcass at the time of slaughter. The term residue covers not only the parent substance actually applied to or being ingested by the animal, but also metabolites such as substances that retain biological activity and hence require an assessment of potential adverse effects for meat consumers, or serve as an indicator of nonapproved use of drugs or other chemicals in/on the animal.

How Feed and Food Safety Is Defined

Considering the definition of feed or feed materials, it is obvious that these should be free of substances that are harmful for the animal. However, in the case of food-producing animals also the safety of animal products (food) needs to assessed. This so-called food chain assessment (from farm to fork) was first formalized in Europe with the enforcement of the General Food Law (Council Regulation (EC) Nr. 178/2002) (Regulation (EC) No 178/2002 of the European Parliament and the Council of 28 January 2002, laying down the general principle and requirements of food safety, and, establishing the European Food Safety Authority (EFSA). This approach is now common practice around the world and implies that for all substances that might be ingested with feed or water by the animal, maximum tolerance levels are established that are indeed based on a careful assessment of the potential risks for animal and human health. If during the life time of an animal chemical substances in the form of feed additives or VMPs (drugs) are used, the prerequisite for use are defined in a premarketing authorization procedure (see Section How Veterinary Drugs are Defined) again taking into account the potential risk for the animal (animal safety assessment) as well as the potential risk for the consumer of animal-derived products that may contain residual amounts (drug residues) of such substances.

How Risk Assessment Is Defined

In the frame of feed and food safety, risk assessment is defined as a standardized procedure taking into account the following criteria.

Hazard identification

The potential of a substance to cause an adverse effect in animals or the human consumer of animal-derived products.

Hazard characterization

The quantitative analysis of the dose–effect curve the quantity of residues in individual tissues and the potential risk for the consumer of animal-derived products.

Exposure assessment

The expected exposure of consumers following the common consumption quantities of an animal product.

Risk characterization and risk assessment

The assessment of the outcome of these previous assessment steps and an overall assessment of the potential risk for animals and the human consumer of animal-derived products as a guidance for risk avoidance or risk management strategies (Figure 2).

Feed Residues

Feed, presented either as natural forage or mixed feed contains a large diversity of chemical substances. The first step in the hazard identification of natural toxins and contaminants in animals feed materials is an analysis of the overall occurrence and the potential adverse effect in animals (toxicodynamics) under the conditions of current farming practice. Although previously descriptions of toxic effects were generally based on case studies, reporting clinical signs of intoxications in a target animal, current approaches aim to identify the mode of action (MoA) to identify potentially hazardous substances, to define intervention strategies and to set priorities for analytical surveys and hazard characterization.

In most cases, a prerequisite for a toxic effect is that the chemical substance under consideration is absorbed from the gastrointestinal tract of the animal and reaches the systemic blood circulation that distributes the substance into the different organs. This fate of a substance in the body of an animal is generally described as the kinetic phase (toxicokinetics) and includes also the biotransformation (metabolite formation) and the excretion with urine of feces. The absorption phase and hence the biological availability (the percentage of the

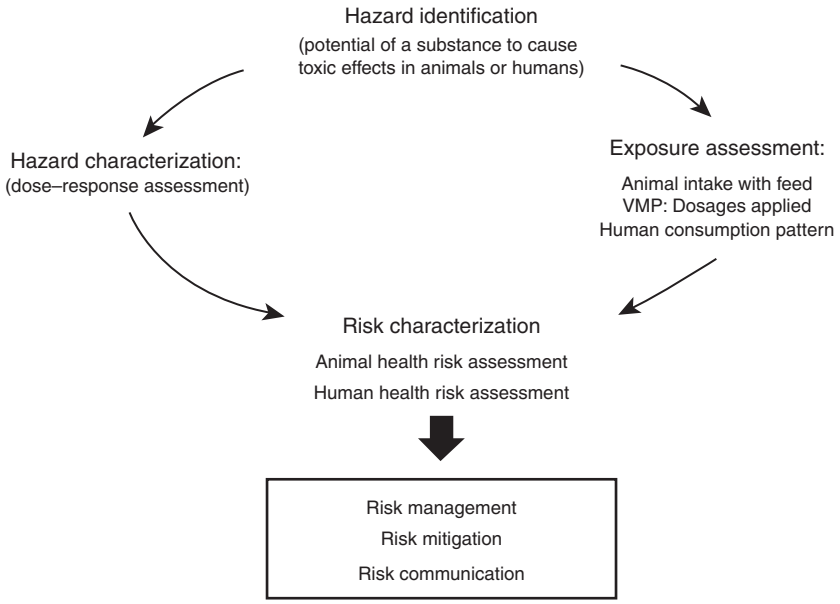

Figure 2 The individual steps leading to risk characterization.

toxin that reaches the systemic circulation) depend not only on the chemical structure of the substance under consideration, but also from the physiological and anatomical differences in the gastrointestinal tract of the animal. Ruminant species (cattle, sheep, goats, and others) share a system of forestomachs, of which particularly the rumen harbors a great diversity of microorganisms, such as bacteria, protozoa, and certain fungal species. The physiological function of this rumen flora is the digestion of fiber (cellulose) that otherwise could not be used as source of nutrients by mammalian species. This forestomach flora is not only able to transform or degrade many potential toxic substances occurring naturally in forage plants, but also as environmental pollutants that contaminate feed materials. The rumen is a very effective first defense making ruminants less sensitive to toxins than horses (solipeds) that are strict herbivorous species as well. The large bowl (cecal) fermentation of monogastric herbivors is less protective as small molecules, including most of the known toxic substances are already absorbed in the upper (proximal) intestines. Nevertheless, all herbivorous species during evolution have developed specific protection systems, such as efflux transporters and biotransformation enzymes expressed in the gut wall, to prevent toxins that are in the daily diet can reach the systemic circulation and exert toxic effects. Omnivores, like pigs and poultry (and of course carnivorous species) are less protected by nature and in turn are more vulnerable to undesirable substances in animal feeds.

A toxicokinetic analysis of undesirable substances in animal feeds addresses also the disposition in edible tissues as a concentration- and time-dependent process and hence identifies the possible risk of residue formation. Many chemical substances, particularly substances that are naturally occurring in (feed) plants, even if they are potentially toxic, are rapidly excreted and do not form residues in meat. In contrast, organic pollutants, of which the dioxins are one of the most prominent examples, have a low immediate toxicity and often no specific adverse effects can be observed in the animal. These substances, however, accumulate in the animal's body (fatty tissues) and are of high concern for the consumers of meat and organs like liver, kidney or other edible products tissues (milk and in particular, eggs). Human exposure is associated with adverse effects on the (neuro)immune system and in other cases with an increased risk to develop cancer. A similar accumulation in the animal's body is also observed for certain heavy metals, particularly for cadmium that accumulates in the kidneys (of animals and humans) during the entire lifespan and may occur in undesirable high concentrations even in horsemeat.

In a recent evaluation by the EFSA (2012–13) (Documents for all individual animal species are available in the free-access *European Food Safety Authority Journal*), it was concluded that feed contamination with dioxin and dioxin-like polychlorinated biphenyls (PCBs) is of high potential concern for consumers due to their specific toxicological profile and their accumulation in the food chain. Other potentially accumulating substances such as cadmium and non-dioxin like PCBs are of medium concern. For many related halogenated substances such as polybrominated diphenylethers and hexabromocyclododecanes and several perfluorinated compounds a decreasing environmental burden is noticed, but comprehensive data on potential residue formation in farm animals are currently lacking. To avoid adverse effects in consumers due to residues of these compounds in meats and other edible tissue, milk, and eggs, maximum permissible levels will be (or have been) set for feed and food and specific control measures of all feed materials (including imports) are enforced in all EU member states. The control of undesirable substances in animal feeds is governed by the Directive 2002/32/EC on Undesirable Substances in Animals Feeds.

Residues of Feed Additives and Veterinary Drugs

In contrast to natural toxins and environmental pollutants that may contaminate feed materials including water supplies of

food producing animals, feed additives, and veterinary drugs may be used only in animals if the individual substance has passed an intensive (scientific) evaluation and a premarketing authorization process by the competent authorities (see Sections How Feed is Defined and How Veterinary Drugs are Defined). This evaluation includes the assessment of the efficacy (claimed beneficial health effect) and animal safety under the conditions of use (including a safety margin for involuntary common mistakes in daily practice, such as poor mixing or incorrect dosing). An important task is the evaluation of potentially harmful residues of the parent compound or its metabolites in animal tissues, as described before. If the elimination of residual amounts of a feed additive or drug is slow, withdrawal times (time between last use and slaughter) will be set to ensure that the carcass is free of residues. In turn, if harmful residues seem to be unavoidable under the conditions of use, a substance is rejected and older substances that have been used in animals before the implementation of the European Food Law have been banned. This ban or prohibition of use applies to all substances that are potentially harmful for the consumer, particularly all substances that may contribute to the development or promotion of cancer.

Drug Residues and Consumer Risks

VMPs (commonly named veterinary drugs) can only be used in food producing animals if they have passed the above mentioned premarketing authorization procedure. This applies also for drugs (e.g., antibiotics or substances used in pain management) that have already achieved an authorization for use in humans. The detailed procedure of premarketing assessment includes an evaluation of the pharmaceutical product quality, the safety for the target animal species, the potential disposition of residues in edible tissues and finally the potential risk for the human consumer of animal-derived foods. The overall objective is risk avoidance, as these substances are used intentionally, and any risk for consumers is considered as unacceptable. To achieve this and to facilitate control measures (see Section National Residue Control Plans) maximum residue levels (MRLs) are established and published by the competent authorities. The 'MRL' is based on a No-adverse effect level (NOAEL) derived from studies in model animal species and is divided by an uncertainty factor (the default factor is 100, accounting for the extrapolation of animal data to humans and the variability within the human population) and defines the 'ADI' (acceptable daily intake by consumers of the food products such as meat, milk, and eggs). On the basis of the ADI (which guarantees the absence of undesirable effects in consumers over the entire lifespan) the MRL (permissible concentration in edible tissue) is calculated taking into account a high consumption of the animal-derived product (for meat the standard consumption of a high consumer is set to 300 g per day to which 100 g of liver and 50 g of fat and kidneys are added). Where necessary, a withdrawal period (time between use and slaughter) is prescribed to ensure that a carcass does not contain any drug residues that exceed the MRL. In the case of antibiotics, potential microbiological effects are evaluated as well.

For a number of compounds this risk assessment resulted in a high level of uncertainty, as genotoxic effects, and hence the possible contribution to the development of cancer could not be excluded entirely. These substances, including for example the group of 5-nitroimidazoles, nitrofurans, and individual substance such as chloramphenicol or aristolochic acid, have been banned for use in food producing animals (EC 37/2010, Annex Table 2).

In the recent reevaluation by the EFSA, noncompliant samples with drug residues of authorized substances were classified as being of negligible concern for consumers due to the precautionary safety margin applied during the establishment of the MRL values, and the nature of the drugs licensed for food producing animals in Europe. This is in contrast to the illicit use of non-authorized substances that may be of medium concern due to potential adverse health effects following long-term ingestions. To avoid such any repetitive exposure of consumers, the national residue control plans prescribe the routine control and targeted sampling of all slaughter animals.

Mandatory Residue Testing: National Residue Control Plans

Within the EU (a similar situation is present in other countries), Council Directive 96/23/EC prescribes the monitoring of residues in slaughter animals, defining the number (percentage) of slaughter animals that needs to be tested for the presence of residues. Each Member State has to present a National Residue Control Programmes (NRCPs) plan based on its specific animal husbandry system. The NRCP is annually updated and is evaluated by the Commission services (DG Health and Consumers) in cooperation with a statistic evaluation and assessment by the EFSA. NRCPs focus on the control of residues from contaminants identified as being of high concern for consumer health (within category B3), the illicit use of nonauthorized substances (within category A) and a general drug residue monitoring to check the compliance with drug use and withdrawal periods (categories B1 and B2). The analytical procedures applied in the framework of the NRCP controls are validated (performance criteria are set) to achieve a high quality standard and to allow a comparison of findings among individual Member States.

In Table 1 an aggregated summary of the results of the NRCPs (all results of the NRCPs are published on the EFSA website) over a period of 5 years is presented. This summary should serve as reliable indication of the results of the European residue control program, although it contains some minor uncertainties: such as for example the presence of two residues in one animal (counted twice), or the fact that from a large batch of slaughter animals (pigs, poultry, and calves) only a limited number of animals have been investigated while it can be assumed that more animals have been treated in the same way when they originated from the same farm and the same age group.

Considering the outcome of this unique and comprehensive survey it can be concluded that in all animal species the percentage of carcasses containing undesirable residues of veterinary drugs (VMPs and pharmacological active substances

Table 1 Residues in meat: Noncompliant samples of the European NRCP 2005–10

Animal species	*Nature of the residue*	*Total number of samples analyzed*	*Number of noncompliant samples*	*Noncompliant samples (%)*
Bovine (all age groups)	Veterinary drugs	282 557	618	0.22
	Illicit and banned substances	476 550	899	0.19
	Chemical elements (including cadmium)	18 798	439	2.3
	Pesticides and organic pollutants	25 216	38	0.15
Small ruminants (sheep and goats)	Veterinary drugs	99 167	235	0.24
	Illicit and banned substances	32 502	208	0.64
	Chemical elements (including cadmium)	6 205	164	2.64
	Pesticides and organic pollutants	13 773	35	0.25
Pigs (all age groups)	Veterinary drugs	502 406	833	0.07
	Illicit and banned substances	294 114	329	0.11
	Chemical elements (including cadmium)	20 500	263	0.78
	Pesticides and organic pollutants	34 019	11	0.05
Poultry (mainly chicken)	Veterinary drugs	174 796	896	0.51
	Illicit and banned substances	188 346	91	0.05
	Chemical elements (including cadmium)	10 689	52	0.49
	Pesticides and organic pollutants	16 709	9	0.05

Source: Data are derived from the reports to the EU Commission of 25 (27) EU Member States and have been aggregated by the European Food Safety Authority.

applied with feed such as coccidiostats) is rather low varying between 0.07% in pigs and 0.51% in poultry. Noncompliant results are expected when veterinary drugs are applied to the animal directly in the period before slaughter. This corresponds to the observation that older, fatting pigs in the last weeks before slaughter are seldom affected by diseases, whereas in poultry, for example, disease outbreaks are possible during the entire (very short) production period.

The categories 'illicit compounds' and banned substances comprise all residues of substances that are either illegal (illicit use) or banned (not licensed for use) in food producing animals (listed in Annex 2 of 37/2010 EC). The percentage of noncomplaint samples varied between 0.05% in poultry and 0.64% in sheep. The latter are apparently treated more frequently with substances not licensed for this animal species, which belongs in many Member States to the group of minor species, with a limited number of drugs licensed for therapeutic purposes.

The category of 'chemical elements' contains all toxic heavy metals including cadmium that accumulates in the kidneys and to a lesser extent in other tissues. The percentage of noncompliant samples ranges from 0.49% in poultry to 2.64% in small ruminants (predominantly sheep). The percentage of positive samples reflect the husbandry system and total lifespan of an animal, with a higher percentage of noncompliant samples in small ruminants and bovines, which are kept on pasture grass and ingest involuntarily earth and sands that contribute to the body burden. In contrast, poultry is kept mainly indoors and has a very short lifespan when used for chicken meat production. As in most cases, these residues of heavy metals are found in kidneys that are generally not consumed in large quantities (or only incidentally used), the overall contribution to human exposure is still considered to be low.

The category of pesticide residues comprises substances that are incidentally used in the control of pests in the direct environment of the animal, but more importantly that enter the food chain due to feed contamination. Although the statistic incidence of these residues is still low (0.05–0.25%), this category encompasses substances such as dioxins that are of high potential concern for the consumer, as they accumulate not only in animal tissues, but also in the body of a human consumer when exposed to residue containing foods.

Risk Mitigation, Risk Management, and Risk Communication

The increasing awareness that food safety is an inevitable right of consumers and the enforcement of strict regulations for has resulted in a continuous decline of the prevalence of undesirable residues in meat (muscle tissue and edible organs) in the past decade. Forthcoming strategies for risk mitigation will be built on further integration of the food chain assessment, aiming at an exchange of information (and mandatory reporting) of all the results of analytical feed (and environmental) controls for the presence of undesirable and potentially harmful substances and a full record of the use of veterinary drugs before the use of foods from animal origin. This could allow corrective measures to further reduce the prevalence of residues in carcasses, and hence meat and meat products. At the global level, the challenge for risk communication remains, to ensure that an equal level of food safety is achieved in all parts of the world. Risk communication should address also directly the consumer's concerns about undesirable residues in meat and other animal-derived products and explain the measures taken to guarantee that food is safe.

See also: Chemical Analysis: Physicochemical Analysis Methods; Sampling and Statistical Requirements. Chemical Analysis for Specific Components: Major Meat Components; Veterinary Drug Residue Analysis. Environmental Contaminants. Residues in Meat and Meat Products: Residues Associated with Meat Production

Further Reading

Andrée, S., Jira, W., Schwind, K.H., Wagner, H., Schwägele, F., 2010. Chemical safety of meat and meat products. Meat Science 86, 38–48.

Dorne, J.L., Fink-Gremmels, J., 2013. Human and animal health risk assessments of chemicals in the food chain: comparative aspects and future perspectives. Toxicology Applied Pharmacology 270, 187–195.

EFSA, 2011. Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). EFSA Journal 9, 2351–2549.

EFSA, 2013. Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals). EFSA Journal 11, 3266–3527.

Fink-Gremmels, J., 2012. Introduction into animal feed contamination. In: Fink-Gremmls, J. (Ed.), Animal Feed Contamination, first ed. Cambridge, UK: Woodhead Publishing, pp. 1–8.

LeDeaux, M., 2011. Analytical methods applied to the determination of pesticide residues in food of animal origin. A review of the past two decades. Journal of Chromatography A 1218, 1021–1036.

MacLachlan, D.J., 2009. Influence of physiological status on residues of lipophilic xenobiotics in livestock. Food Additives Contaminants Part A Chemical Analytical Control Exposure Risk Assessment 26, 692–712.

Mantovani, A., 2012. Chemical risk assessment of animal feed. In: Fink-Gremmels, J. (Ed.), Animal feed Contamination, first ed. Cambridge, UK: Woodhead Publishing, pp. 449–466.

Reeves, P.T., 2010. Drug residues. In: Cunningham, F. (Ed.), Handbook Experimental Pharmacolology, vol. 199. Germany: Springer Verlag Berlin-Heidelberg, pp. 265–290.

Sofos, J.N., 2008. Challenges to meat safety in the 21st century. Meat Scinece 78, 3–13.

Van den Berg, M., Birnbaum, L.S., Denison, M., *et al.*, 2005. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Science 93, 223–241.

WHO, 2012. Evaluation of certain veterinary drug residues in food. World Health organization Technical Report Series 969 Geneva, Switzerland: World Health Organization, pp. 1–101.

Residues Associated with Meat Production

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Glossary

Biogenic amines Low molecular alkaline substances with aliphatic, aromatic, or heterocyclic structure. They are biologically active and occur as metabolic products in animals and plants. In foodstuffs, they are formed mainly by microbial decarboxylation from the correspondent-free amino acids.

Heterocyclic amines The derivatives of quinoline, quinoxaline, and pyridine, all of them containing one amino group. During severe heating, these substances are formed from creatine, present in all animal tissues, other amino acids and monosaccharides.

Nitrosamines Carcinogenic and they are formed during a reaction of nitrite with secondary amines or N-substituted amides. Their formation is also possible with primary amines, diamines, and tertiary amines.

Polyamines Polyamines (spermine, spermidine, putrescine, and cadaverine) are natural components in meat that are biologically active and belong to the so-called natural, which are essential for cell growth and cell proliferation. Hence, besides being natural polyamines. Because polyamines are so important for cell proliferation, cancer growth also requires polyamines, most of them deriving from the diet.

Polycyclic aromatic hydrocarbons The organic compounds that consist of at least two aromatic rings without hetero atoms and substituents formed during incomplete combustion (smoking).

Introduction

Although residues in meat products may result from contamination of the raw material (as is, for instance, the case with pesticides, heavy metals, and antibiotics) they are first and foremost formed during product manufacture, i.e., they are associated with a particular process technology. The latter category of residues may be defined as undesirable substances, which as a rule are only formed in small amounts as by-products of a generally desirable processing procedure such as smoking, curing, and fermentation. In essence, their formation in small quantities is inevitable but when high concentrations are observed this is generally associated with mistakes as regards hygiene and/or process technology during product manufacture. The following procedures have been linked to these procedures (Table 1).

Biogenic Amines

Biogenic amines are low molecular alkaline substances with aliphatic, aromatic, or heterocyclic structure, they are biologically active and occur as metabolical products in animals and plants. In foodstuffs they are formed mainly by microbial decarboxylation from the correspondent-free amino acids as follows:

Histidine	Histamine
Tyrosine	Tyramine
Tryptophane	Tryptamine
Phenylalanine	Phenylethylamine
Lysine	Cadaverine
Ornithine	Putrescine and spermidine (putrescine +aminopropyl group)
	Spermine (spermindine+aminopropyl group)

Spermine and spermidine are actually not products of bacterial decarboxylation but are natural components in meat and are sometimes considered as an indicator of spoilage of meat. They are biologically active and together with putrescine and cadaverine belong to the so-called natural polyamines, which are essential for cell growth and cell proliferation. Hence, besides being natural polyamines putrescine and cadaverine are also biogenical amines as a result of microbial decarboxylation. Because polyamines are so important for cell proliferation, cancer growth also requires polyamines, most of them deriving from the diet. The toxicological effects of biogenical amines and polyamines are described in Table 2.

In heated products, for example, emulsified sausages or cooked ham, the occurrence of biogenical amines reflects the hygiene status of the raw material. If the amounts of biogenical amines in the heated product exceed concentrations of fresh meat usually below 10 mg kg^{-1}, it is likely that spoiled meat was included in its manufacture. Thus, the occurrence of elevated amounts of biogenical amines can be used as an indicator of the hygiene level of the raw material. In meat mixtures prepared from fresh and spoiled meat in different ratios,

Table 1 Processing steps associated with occurrence of residues

Processing step	*Associated residues*
Handling of raw material	Biogenical amines in heated products and fermented sausages
Smoking	Polycyclic hydrocarbons
Heating	Nitrosamines and heterocyclic amines
Fermentation	Biogenical amines
Packaging	Residues from plasticizers and additives
Cleaning and disinfection	Residues from these substances
Use of additives	Nitrosamines and residual additives

the concentration of putrescine, cadaverine, and also tyramine is higher with increasing percentages of spoiled meat.

In raw sausages purchased randomly from retail shops, tyramine, histamine, putrescine, and cadaverine were found in all samples at various concentrations (Table 3). Histamine content was varying from one producer to the other, suggesting a certain manufacturer-dependency. The concentrations of spermine and spermidine showed no remarkable differences (data not shown).

The pathway of amine formation in raw sausages is quite different in comparison with heated meat products. The occurrence of biogenical amines in raw sausages is a consequence of microbiological activity during fermentation. It cannot be excluded that some of the microorganisms that are predominant during fermentation (particularly lactic acid bacteria) have the ability to decarboxylate amino acids. Hence, commercial starter cultures should be controlled regarding their ability to form biogenical amines. Generally, enterobacteriaceae and pseudomonades that also have the ability to form biogenical amines are outcompeted by the fermentation flora. However, the ubiquitous anaerobic and amine-forming microorganisms cannot be suppressed by starters. These microorganisms are generally unable to metabolize proteins. Therefore, the substrates for decarboxylation have to be already available in the raw material. In most cases, excessive concentrations of amines cannot be detected in the raw material, but the occurrence of rather high amounts of the precursor amino acid leads to corresponding amounts of amines in the final product. Tyramine seems to be a very sensitive indicator for the detection of the use of long-stored and hygienically doubtful meat for the production of raw sausages. High amounts of putrescine, and to some extent also cadaverine, indicate the use of spoiled meat. An excessive formation of histamine could only be observed in the presence of specific microorganisms and is strongly dependent on specific flora present on the production site. Tryptamine and phenylethylamine could be found in very different concentrations, but spermine and spermidine do not increase during ripening. Although small amounts of biogenical amines cannot be avoided, an excessive formation of amines indicates that the raw material used for production was not in a hygienically perfect condition.

In sliced raw sausages, biogenical amines are formed only in products where the water/protein ratio is higher than approximately 1.8. This is due to the fact that the moisture content plays an important role in microbial growth.

As mentioned above, the use of hygienically doubtful meat for the production of raw sausages leads to increased formation of biogenical amines during ripening. Tyramine seems to be a very sensitive indicator. A further increase of biogenical amines in sliced raw fermented sausages can only be observed in products in which the moisture content allows bacterial activity.

Recently the European Food Safety Authority (EFSA) conducted a qualitative risk assessment of biogenical amines in fermented foods, using data from the scientific literature including consumption data. The study was focused on histamine and tyramine as these substances were considered as the most toxic and most relevant for food safety assessement of fermented foods. For healthy individuals, no adverse health effects were observed after exposure to 50 mg histamine and 600 mg

Table 2 Toxicological effects of biogenical amines

Amine	*Effect*
Histamine	Nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, bright red rash, oral burning, hypotension, and migraine
Tyramine	Hypertension, respiratory distress, migraine, hot flushes, sweating, and heart palpitation
Tryptamine	Hypertension, respiratory distress, hot flushes, sweating, and heart palpitation
Phenylethylamine	Hypertension and headache
Cadaverine	Hypotension and amplification of histamine effect
Putrescine	Hypotension, amplification of histamine effect, and effect on tumor growth
Spermidine/ spermine	Effect on tumor growth

Source: Data from Beutling, D., 1996. Biogene Amine in der Ernährung. Berlin, Germany: Springer Verlag, pp. 15–29.

Table 3 Biogenical amines in various raw fermented sausages

Product/number of samples	*Amine*	*Minimum (mg kg^{-1})*	*Maximum (mg kg^{-1})*	*Median (mg kg^{-1})*	*Mean (mg kg^{-1})*
	Putrescine	8	808	247	231
Austrian Salami/$n=27$	Cadaverine	1	287	16	37
	Histamine	<1	489	51	104
	Tyramine	34	751	195	209
Austrian Salami-Hungarian style/$n=28$	Histamine	4	449	70	151
Austrian Salami/$n=52$	Histamine	1	654	148	162
Austrian raw sausage/$n=32$	Histamine	4	519	41	127
	Putrescine	1	186	44	56
Italian Salami/$n=10$	Cadaverine	<1	13	5	5
	Histamine	<1	106	<1	13
	Tyramine	<1	143	95	76
	Putrescine	43	521	287	259
Hungarian Salami/$n=8$	Cadaverine	8	931	30	144
	Histamine	<1	356	94	117
	Tyramine	88	291	193	194

Source: Data from Bauer, F., Paulsen, P., 2001. Biogenic amines in meat and meat products. In: Morgan, D.M.L., Milovic, V., Krizek, M., White, A. (Eds.), COST 917 Biogenically Active Amines in Food, vol. 5, EUR 19882. Luxembourg: Office of the Official Publications of the European Community, pp. 88–94. ISBN: 02-894-1630-0.

tyramine per meal. For persons with histamine intolerance or those taking monoamino oxidase inhibitors, the limits are considerably lower. The aforementioned EFSA opinion concluded that further research on toxicity, control measures during production (including process hygiene in fermented foods), and validation of analytical methods is essential.

Polycyclic Aromatic Hydrocarbons

Meat products are often smoked with the intention to improve their sensory and microbiological condition. Smoke is prepared by incomplete burning of wood whereby the components of wood cellulose, hemicellulose, and lignin are degraded through pyrolysis. During this procedure, not only sensorically beneficial and preservative components (phenols, carbonyls, and organic acids) but also the carcinogenic, so-called polycyclic aromatic hydrocarbons (PAH) are formed.

Generally, the formation of PAH cannot be avoided, but taking relevant technological precautions (glowing temperature, cooling of the smoke, and kind of wood) the concentration can be reduced to an acceptable level. The formation of these substances begins at approximately 400 °C, which temperature is also slightly above the ideal temperature for pyrolysis (300–400 °C) and therefore for the building of the sensorically relevant, preservative substances. To reduce the formation of the PAH, the glowing temperature should be as low as possible and only hardwood (e.g., beech) should be used. Furthermore, the site of smoke production and the position of the product to be smoked should be far enough apart to allow the PAH to condense together with tar.

Approximately 40 polycyclic hydrocarbons have been detected in smoke with benz(a)pyrene suggested as a representative indicator. In 2008, EFSA recommended that relying merely on benzo(a)pyrene as a marker is unsuitable for detecting the occurrence of PAH in food. Rather, an approach is recommended relying on an analytical system with either four specific substances (PAH4 – benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) or with eight (PAH8 – PAH4 plus benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenz(a,h)anthracene, and indeno(1,2,3-c,d)pyrene). Consequently, new maximum levels for the sum of four substances (PAH4) have been introduced, although maintaining a separate maximum level for benzo(a)pyrene. The EU regulation prescribes that for smoked meat and smoked meat products, the maximum residual amounts of benzo(a)pyrene should be 5.0 μg kg^{-1} until 31 August 2014 and 2.0 μg kg^{-1} as from 1 September 2014 and of PAH4 with 30 and 12 μg kg^{-1}, respectively.

Incidentally, polycyclic hydrocarbons are also formed during the burning of fat. This fact should especially be taken into consideration if the meat is barbecued using charcoal.

Residual Additives

Generally only such additives are allowed for use in foodstuffs, which are licensed and therefore represent no health risk for the consumer eating normal amounts of the food. An exception is sodium- or potassium-nitrite, which is used for the production of color-stable products. Nitrite is actually a poison and so this substance is only allowed to be sold admixed in sodium chloride (usual concentration of $NaNO_2$ in NaCl: 0.4–0.6% in Austria, 0.4–0.5% in Germany). According to the Food Additive Regulation of the EU not more than 150 mg $NaNO_2$ per kg can be added to meat products during production, but in the case of sterilized products ($F_0 > 3$) only 100 mg $NaNO_2$ per kg is permissible. The fact that ascorbic acid is used for rapid reduction of nitrite to nitric oxide explains additionally why only very small amounts of residual nitrite can be found in the final product. The main reason is the sequestering of oxygen in the batter by nitrite, which is oxidized to nitrate. The second curing agent nitrate per se is not very toxic but is excreted in the saliva and reduced to nitrite in the mouth. When curing meats with nitrate, the reduction of nitrate to nitrite is carried out by microorganisms. Consequently, undesirable amounts not only of nitrate but also of nitrite may be determined in the case of faults during production. The limit for adding nitrate according to the regulation mentioned above is 150 mg $NaNO_3$ per kg. For traditionally cured meat products like bacon, dry cured ham, or salami exceptions are prescribed in the regulations. Often, maximum residual amounts at the end of the production process instead of the maximum added amount are prescribed. Therefore, a toxicological risk from nitrates or nitrites itself is unlikely, however, the formation of nitrosamines remains a possibility.

Nitrosamines

Nitrosamines have been found to be carcinogenic and they are formed during a reaction of nitrite with secondary amines or *N*-substituted amides. Their formation is also possible with primary amines, diamines, and tertiary amines. The most carcinogenic nitrosamine is nitrosodimethylamine. In meat products nitrosopiperidine and nitrosopyrrolidine are important. The amines corresponding to these substances are cadaverine and putrescine, respectively, which can either be formed during fermentation or are originally present as part of the polyamine pool in meat. The formation of nitrosamines is mainly influenced by the amount of nitrite, the pH of the product and not by the concentration of amines. The ratio between nitrite and amine should be approximately 1:10. Nitrosamines can either be formed during commercial product manufacture and during preparation in the household, or in the stomach where acidity favors the reaction. The pH optima for formation of most nitrosamines lies between 1 and 3, for heterocyclic nitrosamines approximately at 3.8. The formation of nitrosamines is accelerated by increasing temperature and is hampered by increasing alkalinity. Halogen ions and the pseudohalogenide thiocyanate catalyze the reaction. The fact that thiocyanate is also a component of smoke from tobacco explains why an evaluated formation of nitrosamines can take place in the stomach of smokers, which may represent a risk as regards the development of stomach cancer. Formaldehyde also catalyzes the formation of nitrosamines by increasing the pH optimum of the reaction. Because formaldehyde is also a component of smoke, it has been suggested that the formation

of nitrosamines is facilitated in meat products, which are cured as well as smoked.

The fact that nitrosamines may be formed in the stomach is the reason why nitrate can also not be rated as toxicologically harmless. Nitrate is excreted in the saliva and is reduced to nitrite in the mouth. The sources of nitrate are mainly vegetables and potable water and not cured meat products.

Whereas in pasteurized sausages and cooked ham nitrosamines are generally not detected, this is often the case in small quantities in raw fermented sausages and raw ham. Recent data show that the concentration in raw sausages and raw ham ranges from 'not detectable' (n.d.) to 5 $\mu g\,kg^{-1}$ for dimethylnitrosamine, from n.d. to $>5\,\mu g\,kg^{-1}$ for nitrosopiperidine, and from n.d. to 0.5 $\mu g\,kg^{-1}$. Although most of the values of dimethylnitrosamine were between 0.5 and 1 $\mu g\,kg^{-1}$, the predominant part of the two other nitrosamines was below 0.5 $\mu g\,kg^{-1}$ for nitrosopyrrolidine. Further sources of nitrosamines in higher concentrations are fried and cured products where nitrosopyrrolidine is developed by thermal cleavage of carbon dioxide from nitrosoproline. Proline can be nitrosated easily; its reaction product nitrosoproline is not carcinogenic. Black pepper may be the reason for the formation of nitrosopiperidine, paprika for nitrosopyrrolidine. Formation of nitrosopyrrolidine is promoted by heating over 170 °C, i.e., during frying of bacon and dry heating of 'Bavarian Leberkäse' (resulting in crust formation), but also when salami is used on pizzas. In the latter case, the possible presence of putrescine and cadaverine increases the risk of nitrosamine formation.

Lowering the added nitrite and nitrate to meat products, the use of ascorbic acid for rapid and complete reduction of nitrite to nitroxide, and the decrease of nitrate in vegetables and in potable water are considered to contribute to the decrease in nitrosamine uptake. First of all, frying of cured products at high temperatures should be avoided, a simple precautionary measure within the responsibility of each consumer.

Heterocyclic Amines

At the end of the 1970s, Japanese researchers found that in the crust of barbecued sardines substances could be detected, which according to the Ames test should be considered as mutagenic. They identified these substances as heterocyclic amines or more precisely as derivatives of quinoline, quinoxaline, and pyridine, all of them containing one amino group. During severe heating, these substances are formed from an amino acid and a monosaccharide provided creatine is present. Creatine is present in all animal tissues. The most important and also the most carcinogenic substances are 2-amino-3-methyl-imidazol[4,5-f]quinoline (IQ), 2-amino-3,4-dimethyl-imidazol[4,5-f]quinoline (MeIQ), 2-amino-3,8-methyl-imidazol[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-dimethyl-imidazol[4,5-f]quinoxaline (4,8-DiMeIQx), and 2-amino-1-methyl-6-phenyl-imidazol[4,5-b]pyridine (PhIP). So far, more than 20 of these substances are known. During heating of meat in boiling water or steam, no heterocyclic amines have been found. Only when temperatures increase the formation starts. The amount formed increases with higher temperature and longer heating times. PhIP is formed most abundantly with observed levels up to 480 $\mu g\,kg^{-1}$. The concentrations of the other amines are generally lower, i.e., ranging from n.d. to 15 $\mu g\,kg^{-1}$ IQ, MeIQx and 4,8-DiMeIQx and up to 50 $\mu g\,kg^{-1}$ for MeIQx.

Residues from Wrapping and Packaging Materials

Substances that can be a health risk or can affect smell and taste deterioration should generally not migrate into the foodstuff from the wrapping or packaging material or from artificial casing. The most common wrapping and packaging materials for meat and meat products are metal (tins) or plastic.

Modern cans are internally coated with an epoxy resin. This coating may release bisphenol-A-diglycerolether (BADGE) or bisphenol-F-diglycerolether (BFDGE), which migrate into the food. BADGE is an intermediate product during the production of these epoxy resins, which is degraded mainly after polymerization. Besides these very flexible coatings, also other added epoxy resins are applied, which are only polymerized partly so that the percentage of BADGE is increased. BADGE was found to be mutagenic in the Ames test, which could not be confirmed in animal experiments. However, as a guideline for the content of the sum of BADGE and BFDGE 1 $mg\,kg^{-1}$ food has been suggested.

Packagings and wrappings of plastic may contain residues of the correspondent monomer, for example, styrole in polystyrole, or residues of additives that are used in their production (catalysts, stabilizers, and emulsifiers). Furthermore, some other additives are able to migrate into the foodstuff, for example, antioxidants, antiblocking and antistatic agents, fillers, and pigment colorants. For packaging of fresh meat, soft and stretchable polyvinyl chloride foils are used, which contain diethylhexyladipiate (DEHA) and epoxidized soy bean oil (ESBO) as plasticizers.

Residues from Cleaning Agents and Disinfectants

Working areas, working surfaces, and equipment of meat processing plants must be cleaned and disinfected to remove residues from meat and fat and to avoid microbial residues. The proper procedure includes rinsing of the cleaned and disinfected surfaces and equipment with potable water to remove the disinfectants and to avoid contamination of meat. Consequently, transition of residues of cleaning agents and disinfectants is only possible when good practices are abused. A health risk is not caused by the direct uptake of these substances but – similar to antibiotics – microorganisms may be resistant against these substances. Unfortunately, prescribed monitoring procedures of the effect of cleaning and disinfection are targeted toward proving the absence of microorganisms, rather than the absence of disinfectants. As long as limits for cleaning agents and disinfectants are not laid down in directions, for example, within the European Union, this situation is unlikely to change.

See also: Chemical Analysis for Specific Components: Curing Agents. Cooking of Meat: Flavor Development; Maillard Reaction

and Browning; Physics and Chemistry; Warmed-Over Flavor. **Fermentation. Packaging:** Technology and Films. **Smoking:** Traditional

Further Reading

Ackermann, P., Jägerstad, M., Ohlsson, T., 1995. Foods and Packaging Materials – Chemical Interactions. Chambridge: The Royal Society of Chemistry.

Alaejos, M.S., Afonso, A.M., 2011. Factors that affect the content of heterocyclic aromatic amines in foods. Comprehensive Reviews in Food Science and Food Safety 10, 52–108.

Bauer, F., Paulsen, P., 2001. Biogenic amines in meat and meat products. In: Morgan, D.M.L., Milovic, V., Krizek, M., White, A. (Eds.), COST 917 Biogenically Active Amines in Food, vol. 5, EUR 19882. Luxembourg: Office of the Official Publications of the European Community, pp. 88–94. ISBN: 02-894-1630-0.

Belitz, H.D., Grosch, W., Schieberle, P., 2009. Food Chemistry. Berlin, Heidelberg, New York: Springer Verlag.

Beutling, D., 1996. Biogene Amine in der Ernährung. Berlin, Germany: Springer Verlag.

EFSA, 2008. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. EFSA Journal 724, 1–114.

EFSA, 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. EFSA Journal 2393, 1–93.

Murkovic, M., 2004. Chemistry, formation and occurrence of genotoxic heterocyclic aromatic amines in fried products. European Journal of Lipid Science and Technology 106, 777–785.

Paulsen, P., Bauer, F., 1997. Biogenic amines in fermented sausages. 2. Factors influencing the formation of amines in fermented sausages. Fleischwirtschaft international 4/1997, 30–32.

Pegg, R.B., Shahidi, F., 2000. The *N*-Nitrosamine Problem and Nitrite Alternatives. Crumbull: Food and Nutrition Press.

Piringer, O.G., Baner, A.L., 2000. Plastic Packaging Materials for Food. Weinheim: Wiley-VCH.

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., Jira, W., 2012. Contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages depending on smoking conditions using glow smoke. Meat Science 90, 176–184.

Rauscher-Gabernig, E., Grossgut, R., Bauer, F., Paulsen, P., 2009. Assessment of alimentary histamine exposure of consumers in Austria and development of tolerable levels in typical foods. Food Control 20, 423–429.

Ruiz-Capillas, C., Jimenez-Colmenero, F., 2004. Biogenic amines in meat and meat products. Critical Reviews in Food Science and Nutrition 44, 489–499.

Skog, K.I., Johansson, M.A.E., Jägerstad, M.I., 1998. Carcinogenic heterocyclic amines in model systems and cooked foods: A review on formation, occurrence and intake. Food and Chemical Toxicology 36, 879–896.

Relevant Websites

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:215:0004:0008:EN:PDF
Commission Regulation (EU) No. 835/2011.

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:295:0001:0177:En:PDF
Commission Regulation (EU) No. 1129/2011.

RISK ANALYSIS AND QUANTITATIVE RISK MANAGEMENT

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Glossary

Appropriate Level of Protection (ALOP) The level of protection deemed appropriate by the World Trade Organization (WTO) member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO). This concept is also referred to as the acceptable level of risk.

Food Safety Objective (FSO) The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the ALOP.

Good Hygienic Practice (GHP) Basic conditions and activities that is necessary to maintain a hygienic environment suitable for the production, handling and provision of safe food for human consumption.

Hazard A biological, chemical or physical agent, or condition in food with the potential to cause an adverse health effect.

Hazard Analysis and Critical Control Points (HACCPs) A system which identifies, evaluates, and controls hazards that are significant for food safety.

Performance Objective (PO) The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

Risk A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Safety factor (SF) A numerical factor, usually 10 or 100, applied as a safety margin to take into account uncertainty associated with quantitative estimates.

Introduction

Risk analysis is a formalized, scientifically based approach that is recognized by the World Trade Organization (WTO) as the tool for addressing food safety issues and on which food safety regulations are founded. When carried out correctly, risk analysis provides a tool for the identification, assessment, management, and communication of risk.

In the area of food safety, risk analysis approaches have been applied for many years to assess and manage chemical food hazards (e.g., food additives, veterinary drugs, and pesticides). Most recently, efforts have been made to develop risk analysis techniques for addressing microbiological food risks.

The application of risk analysis techniques is a discipline that develops constantly. This article attempts to provide an up-to-date overview of the concept and the individual steps of risk analysis, introducing the emerging concept of quantitative risk management based on the target expressions food safety objective (FSO) and Performance Objective (PO).

Purpose and Role

Traditionally, the approach to food safety control, both by the food industry and by public authorities, has been technical, ad hoc, and mainly reactive, based on utilization of experience obtained from many years of exposure to various hazards, taking into account local practices, traditions, and technological possibilities. This approach has proved insufficient to ensure public health and fair international trade in foods.

Analyses of the major food safety problems that have occurred through the last decades teach that these are, most of the time, the consequences of organizational deficiencies – an indication of a lack of effective global organization in approaching food safety.

Risk analysis provides an opportunity to address these organizational difficulties by the systematic integration of scientific understanding of the risks involved and the legitimization of decisions taken. The rationale for using a formal risk analysis approach has multiple components:

1. To assist in the control of multiple foodborne risks in a proactive and cost-effective way. The multiple aspects of food safety include:
   a. Potential microbiological foodborne risks (for instance, from 'classical' salmonellosis to emerging pathologies due to protozoa or viruses).
   b. Chemical/toxicological risks, such as naturally and environmentally occurring toxicants and residues of chemicals and drugs used.
   c. New areas of concern, such as endocrine perturbation, allergenicity, genetic engineering, etc.

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2. To support national food safety regulation by providing a sound, science-based, systematic, and target-focused tool that in addition facilitates fair international trade. The Agreement on Sanitary and Phytosanitary Measures (SPS Agreement) of the WTO has established the tenet that 'members shall assure that their sanitary and phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risk to human, animal, or plant life or health, taking into account risk assessment techniques developed by relevant international organizations.' Codex Alimentarius is in the process of establishing international principles and guidelines for risk analysis.
3. To address the increase in the social unacceptability of food risks. As food becomes objectively safer, the remaining and occasional risks are even less tolerated by the public at large, a trend that is enhanced by the general public feeling increasingly alienated from food safety control activities (decisions are perceived to be mainly the affair of the food industry and/or the public agencies with relevant jurisdiction).

However, the application of the risk analysis concept also has some disadvantages when it is used to support national food legislation. Many legislative measures are multifunctional as they address public health issues as well as other issues such as wholesomeness/suitability of foods, environmental protection, and basic animal welfare. Therefore, the challenge for legislators is to achieve sufficient transparency in the objectives of any such measure to avoid confusion.

Risk analysis is usually described as a process consisting of three elements: risk assessment, risk management, and risk communication. It is a decision-oriented process, and making decisions is a managerial activity.

Hazards and Risks

The technical meaning of the terms 'hazards' and 'risks' is often confused. This is mainly due to translation problems as, in many languages, these terms are directly translated into the same word. Although they are often synonymous in everyday life, they have taken different meanings in the technical language used in risk analysis.

A hazard is a biological, chemical, or physical agent in food, or a condition of food, with the potential to cause an adverse health effect (Codex Alimentarius). Risk is the function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard in food (Codex Alimentarius). In more common words, risk is the likelihood and severity of a failure causing death or illness among consumers. For instance, the probability of humans being affected by the presence (or number) of a pathogenic microorganism in the food is the risk, whereas the microorganism itself is the hazard.

The same confusion exists between the terms 'risk analysis' and 'hazard analysis' (of the hazard analysis and critical control point (HACCP) system). In current practice, hazard analysis is targeted at one particular food produced in one particular food plant and is, in principle, independent of any presence of the same hazard in other foods; risk analysis is a more horizontal tool for use by a government to protect the health of the population.

Risk Assessment

Risk assessment is the scientific part of risk analysis that is initiated and commissioned by risk managers. The purpose is to estimate the severity and likelihood of harm from exposure to a certain hazard by furnishing all scientific data relevant to the evaluation. The output might, for example, be an estimate of annual rate of illness per 100 000 inhabitants or an estimate of the rate of human illness as a function of the incidence of consumption.

The scientific data needed are both qualitative and quantitative. They concern the nature and sources of the hazard, how it affects human health, and how it behaves under various conditions. In addition, scientifically based information on the potential exposure to humans is needed.

The risk assessment process comprises four steps: hazard identification, exposure assessment, hazard characterization, and risk characterization. The information is passed on to the risk managers to assist them in conducting the risk management process. Codex Alimentarius has established general principles for the conduct of risk assessments.

Hazard Identification

Hazard identification is predominantly a qualitative process, the purpose of which is to identify the hazards of concern in the food under study. Hazards can be identified from relevant data sources. Information on hazards can be obtained from the scientific literature, from databases such as those in the food industry and government agencies, and through expert consultation. Relevant information includes data obtained from, for instance, clinical studies, epidemiological studies and surveillance, laboratory animal studies, investigations of the characteristics of the hazards, the interaction between hazards and their environment through the food chain, and studies on analogous hazards and situations.

Exposure Assessment

The purpose of exposure assessment is to obtain a quantitative assessment of the actual or anticipated human exposure of a food hazard. It is normally based on realistic exposure scenarios, including the potential extent of food contamination and on actual dietary information. Susceptible and high-risk population groups with regard to acute, chronic (including long term), cumulative, and/or combined adverse health effects should also be brought into consideration. Typical factors considered include:

1. The frequency of food contamination and its level in the foods over time, which are influenced by
   a. The characteristics of the hazard,
   b. The nature and ecology of the food,
   c. The initial contamination of the raw material,
   d. The level of process controls,

e. The methods of processing, packaging, distribution, and storage of the foods.

2. Patterns of consumption, which relate to socioeconomic and cultural backgrounds, ethnicity, seasonality, age differences (population demographics), regional differences, and consumer preferences and behavior.

In practice, exposure assessment of foods can be qualitatively categorized according to (1) the likelihood that the foodstuff will or will not be contaminated at its source and (2) whether or not the level of the hazards in the food can increase over time, taking into account the potential for abusive handling.

Hazard Characterization

The purpose of hazard characterization is to provide a qualitative or quantitative description of the severity and duration of adverse effects that may result from the ingestion of a hazard in food. The level of the hazard that causes an adverse health effect (dose–response assessment) should be estimated if such data are obtainable.

Several important factors that are considered in hazard characterization relate both to the hazard itself and to the human host.

Factors related to the hazard include:

- Potential for the hazard to replicate,
- Virulence and infectivity of the hazard,
- Impact of interactions between the host and the environment,
- Potential for transfer of genetic material (e.g., antibiotic resistance and virulence factors),
- Potential for spread through secondary and tertiary transmission,
- Incubation period (clinical symptoms can be substantially delayed following exposure),
- Potential for changed pathogenicity due to the attributes of a food, for example, fat content.

Factors related to the host include:

- Genetic factors,
- Increased susceptibility due to breakdowns of physiological barriers,
- Individual host susceptibility characteristics, such as age, health and medication status, concurrent infections, immune status, and previous exposure history,
- Population characteristics, such as population immunity and population behavior, and persistence of the organism in the population.

Risk Characterization

Risk characterization represents the integration of the results of hazard identification, hazard characterization, and exposure assessment, the purpose being to provide qualitative or quantitative estimates of the likelihood and severity of the adverse effects that could occur in a given population. The data may permit only a qualitative estimate of risk. The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions made in all previous steps.

Variability represents heterogeneity within biological systems and populations, whereas uncertainty represents a lack of precise knowledge, associated either with the data themselves or with the choice of model, and both arise at all steps of the risk assessment process:

- Hazard identification, where uncertainty or variability may arise because of (1) misclassification of the agent; (2) the potential unreliability of the screening method used for identifying the hazard; or (3) problems in extrapolating the information provided by the screening test for predicting human hazards.
- Hazard characterization, where uncertainty and variability arise when extrapolating from high to low doses and from one species to another and when considering varying sensitivities within human populations. When models are used, additional uncertainty as to whether they represent actual biological processes is introduced. For instance, the transfer of data from animal studies into estimates relating to humans involves uncertainties. For this reason, a 100-fold safety factor (SF) is often applied to account for likely interspecies differences in susceptibility.
- Exposure assessment, where many uncertainties arise owing to lack of detailed data on, for example, the level of the agent in food, the frequency, duration and magnitude of human intake of food products, changes in concentration of the chemical or microbiological agent during storage, and processing and preparation of the food product. There is also a great deal of variability in dietary habits.
- Risk characterization, where uncertainty and variability arise because of the uncertainties and variability involved in its constituent steps and in the model used for constructing the distribution of individual or population risk.

Risk Assessment of Chemical Hazards

Chemical risk assessment in one form or another has been applied to evaluation of various chemical hazards in foods for many years. Assessments of food additives, of veterinary drug residues, and of contaminants differ fundamentally because food additives and veterinary drugs are deliberately added to food or administered to the animal, whereas contaminants are unavoidable. Further, contaminants generally demonstrate greater potential toxicity, as additives and veterinary drugs are normally subjected to safety evaluation before first use. Food additives and veterinary drugs can be easily controlled, whereas the elimination of contaminants from foods incurs costs, such as reduction in food availability and/or affordability.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Joint FAO/WHO Meeting on Pesticide Residues (JMPR) have traditionally carried out a so-called safety assessments of food additives, food contaminants, veterinary drug residues, and pesticide residues. 'Safety assessments' is a scientifically based process consisting of:

1. Determination of a no observable effect level (NOEL).
2. Subsequent application of SFs to establish an acceptable daily intake (ADI) or tolerable daily intake (TDI).

3. Comparison of the ADI or TDI with probable exposure to the agent.

The approach is somewhat different from risk assessment but it does have the advantage of avoiding problems associated with deciding on an acceptable level of risk.

The acceptable or tolerable intake is an indication of both the magnitude and the duration of acceptable intake. The ADI usually represents an acceptable average daily intake for the lifespan of an individual. TDIs for contaminants should be compared with intake surveys of appropriate duration. In cases where no threshold is thought to exist, such as for aflatoxins, JECFA does not allocate tolerable intake values but recommends that the level of contaminant in food be reduced to as low as reasonably achievable (ALARA). The ALARA level is regarded as the concentration of a substance that cannot be eliminated from a food without having to discard that food or severely compromising the availability of major food supplies.

Codex Alimentarius has initiated a process intended to align the 'safety assessment' with the risk analysis approach.

Microbiological Risk Assessment

The risk assessment process was originally developed for chemicals. Extending the practice to pathogens poses significant difficulties. Accordingly, most microbial risk assessments currently have a qualitative base. However, in recent years, the interest in qualitative approaches to microbial food safety has dramatically increased and quantitative models for specific pathogen–food combinations have been developed.

One difficulty relates to the fact that pathogens can multiply as food moves from the farm to the table, making intake assessment very difficult. In addition, many gaps exist in the data, limiting the precision necessary for quantitative risk assessments. For example, little information is available to estimate accurately the relationship between the quantity of a biological agent and the frequency and magnitude of adverse human health effects, particularly for susceptible populations. Further, risk assessment of raw food (e.g., raw chicken) is further complicated as it has to take into account the probabilities of cross-contamination of other foods in the kitchen during domestic handling and preparation by the consumer.

Pathogens multiply and die, and their biological interactions are complex. The contamination levels of the raw material entering the food chain dictate the character of the initial microflora, but this can be markedly modified by subsequent events. Additionally, there are marked differences in the virulence and pathogenicity of animal and environmental strains, and the individual interactions of host and pathogen are highly variable.

Factors to consider for exposure assessment include the frequency and level of contamination of raw materials and the possibilities of postcontamination and cross-contamination levels in the food during shelf life. The characteristics of the pathogenic agent; the microbiological ecology of the food; the level of basic hygiene; sanitation and process controls; and the methods of processing, packaging, distribution, and storage of the foods all impact on these factors.

Pathogen levels may be kept low, for example, by proper time–temperature controls during food processing, but they can substantially increase under abusive conditions. Therefore, the exposure assessment should include different scenarios describing the pathways from production to consumption and should be constructed to predict the range of possible exposures.

Scenario trees constructed for all steps from production and processing to intended end uses of a food describe the pathway for exposure, and targeted research is often required to accumulate appropriate microbiological data. Predictive modeling is playing an important role in this respect. Unfortunately, dose–response data to allow the hazard characterization component are currently very limited.

Because of the wide variability inherent in much microbial data, Monte Carlo simulation modeling is increasingly being used to generate probabilistic risk estimates that are biologically realistic. The FAO and WHO have recently established an expert body system, Joint Expert Committee for Microbiological Risk Assessments (JEMRA), which operates similarly to JECFA and JMPR and whose tasks are to carry out microbiological risk assessments.

Physical Risk Assessment

Risk assessment for physical hazards can be readily achieved. The characteristics of the hazard do not usually change once they have been introduced to the food, and adverse health effects can usually be subjected to simple ranking systems to generate estimates of risk.

Risk Management

Risk management is a continuing process and constitutes the managerial and political part of risk analysis. It concerns the transfer of the results of risk assessment into actions in accordance with established political priorities. Risk management sets priorities, commissions risk assessments, and implements, monitors, and reviews the chosen strategies and options.

The risk management process comprises four steps: risk evaluation, risk management options assessment, implementation, and monitoring and review.

Risk management is traditionally semiquantitative but, owing to improvements in risk assessment disciplines and statistical models, a development toward fully quantitative risk management has been initiated.

Risk Evaluation

The initial part of the risk management process sets the stage for a risk assessment and evaluates the outcome of the risk assessment process, which should result in a risk estimate.

Risk profiling

A risk profile is developed when a new food safety problem has been identified or if surveillance information shows an unacceptable level or an increase in the level of a disease or a hazard. The food safety problem and its context are briefly

described, including the size and nature of the problem, available data, type of foods involved, main sources, the values expected to be placed at risk (e.g., human health and economic concerns), stakeholders' perceptions, distribution of risks and benefits, and what immediate actions may be necessary, including whether a risk assessment should be carried out. The risk profile assists the risk manager in identifying the questions that need to be answered by the risk assessment.

Target setting and appropriate level of protection

The targets for the risk management activity should be identified as early as possible to guide the rest of the decision-making process. However, the results of a subsequent risk assessment process and subsequent steps of risk management may identify needs to modify or redefine the targets.

Any target should be related to the appropriate level of protection (ALOP) – defined by the WTO (through the SPS Agreement) as the level of protection deemed appropriate by the member state to protect human life within its territory. An ALOP can be implicit or explicit. An implicit ALOP is most often stated in terms of broad public health goals or in relation to legal requirements ('reasonable certainty of no harm'). However, effective implementation of the ALOP may benefit from a more explicit articulation of public health expectations. An explicit description of an ALOP may be in terms of the probability of an adverse public health consequence or the incidence of disease (e.g., the number of cases per 100 000 population per year). Usually, when no significant food-related public health problem exists, the ALOP is the level obtained from the sanitary measures already practiced.

The key point in the SPS Agreement is that any sanitary measure has to be based on science. A government cannot restrict trade or maintain a restriction against scientific evidence. Science can, of course, be misused. Therefore, the scientific agreement also specifies that the scientific approach applicable rests on the scientific assessment principles and evaluation procedures established by international organizations, such as Codex Alimentarius.

The SPS Agreement recognizes governments' rights to decide what they regard as the ALOP, or in other words, the right to decide on the appropriate level of risk that should be valid on their territory. Accordingly, the level of protection may differ between countries, but it shall be determined using harmonized risk analysis procedures. The importance of transparency in the risk assessments carried out is, therefore, obvious. A government must be able to show which factors it has considered and what the results have been of its considerations. This is to ensure that potential differences between the regulations of two countries (e.g., differing maximum limits) are not due to differences in scientific evidence but only to differences in the politically decided acceptance levels.

Decisions on ALOP should be determined primarily by human health considerations, but other factors may legitimately be taken into account, such as technological feasibility and economic, political, or social concerns.

Different approaches to acceptable levels include:

- 'Zero-risk' policies, for example, *de minimis*, ADI.
- Risk-balancing policies, for example, cost–benefit, or ALARA.
- Risk threshold policies, for example, specified levels of risk deemed acceptable.
- Risk comparison policies, for example, comparison between sources and precedence.
- Procedural approaches, for example, negotiation and consensus building.

Risk assessment policy

Risk assessment policy setting serves to protect the essential scientific independence and integrity of the risk assessment. It provides guidelines for value judgments and policy choices that may be needed at specific decision points in the risk assessment process and addresses how to ensure transparency, clarity, and consistency of outcome and how to deal with uncertainties (e.g., application of SFs).

The JECFA and JMPR use the following risk assessment policies at specific decision points in their work.

- Reliance on animal models to establish potential human effects.
- Use of body weight scaling for interspecies comparison.
- Assumption that absorption in animals is approximately the same.
- Use of a 100-fold SF to account for likely interspecies and intraspecies differences in susceptibility, with guidelines for deviations that are permitted in specified situations.
- Decision not to assign ADIs and TDIs to food additives, veterinary drugs, and pesticides that are found to be genotoxic carcinogens.
- Establishment of temporary ADIs and TDIs for additives and residues of veterinary drugs pending submission of requested data.

Commissioning of risk assessments

Commissioning of the risk assessment process is a risk management activity that aims at ensuring that the needs of the risk managers are addressed and that resources are used in the most effective way. Typically, it includes clear statements of purpose and scope of the assessment addressing the risk management targets.

Consideration of the result of risk assessment

When the results of the risk assessment are available, a risk estimate is established. Risk estimates should take into account variability, uncertainties, and assumptions made during the risk assessment process. The need for taking action to reduce the risk is then decided.

Risk Management Options Assessment

Risk management option assessment typically includes four steps:

- Identification of available management options.
- Selection of preferred management option, including consideration of an ALOP.
- Evaluation of impact on other factors of the preferred management options.

Table 1 Risk management options

• Establishing regulatory requirements and/or creating incentives for the introduction of specific food safety management tools (e.g., good agricultural practices, good hygienic practices (GHPs), HACCP or HACCP-like tools, and product tracing systems) or for changes in attitudes in food preparation, handling, and use that will likely contribute to risk reduction in exposure to hazards.
• Establishing public inspection schemes and certification and approval procedures.
• Carrying out educational and/or information programs to the population at large and/or affected subgroups about steps they can take to reduce their exposure to hazards.
• Setting FSOs for ready-to-eat foods in general or for specific groups/types of food.
• Advising on POs at specified stages in the food/feed chain where such levels generically apply and are of critical importance to the overall chain performance.
• Establishing control measures, such as codes of practice or performance criteria specifying 'safe harbor' or 'default' measures for such parties that do not have the means to establish appropriate measures themselves or who elect to adopt such control measures.
• Prohibiting foods/feeds with a substantiated history of contamination or toxicity.
• Requiring import certificates for certain products.
• Promulgating awareness, education, and training programs or, where appropriate, initiating public inspections/audits to stimulate (1) prevention of contamination and/or introduction of pathogens being addressed at all relevant stages in the food/feed chain, including reduction in the level of specific pathogens in primary production; (2) products being labeled properly with consumer information regarding additional guarantees of safety or information being provided that either instructs regarding safe handling practices or warns regarding microbiological hazards that are likely to occur and for which adequate controls are unavailable.

- Final management decision targeted at appropriate stages throughout the food chain.

Identification of available options

Risk management options include consideration of all general and hazard-specific measures. Examples are listed in Table 1. A number of socioeconomic and technological factors may be taken into account and these could, for instance, result in the best management option being:

- Control at the source rather than later in the food chain.
- Regulation through prescribed and detailed good hygienic practice (GHP) rather than, for example, mandating the individual application of HACCP principles (e.g., for small businesses).
- Food safety verification through end-product testing rather than reliance on HACCP systems (for instance, where the origin of the food is unknown).

Selection of options

The SPS Agreement states that sanitary measures must not be more trade restrictive than required to achieve the ALOP, taking into account technical and economic feasibility. A measure would be more trade restrictive than required if another equivalent and reasonably achievable measure were significantly less restrictive.

The outcome of the risk management process for a specific hazard will differ in various societies owing to natural or cultural differences. Such a difference can be scientifically justified, for instance, in relation to the exposure situations in different countries. Differences in established levels of a hazard may, for instance, be justified where the average body weight differs and where relatively little average consumption permits higher threshold levels in a particular food. Also, the prevalence of various foodborne pathogens in the food chain and variation in foodborne disease patterns may justify different risk management outcomes.

In selecting the preferred option, the consequences of impact on other factors should be estimated, such as:

- Impact on consumption patterns (e.g., nutritional consequences of restricting food availability).
- Possible introduction of substitute risks (i.e., increasing another risk by reducing one risk; for instance, increasing microbial risks when not allowing the use of preservatives).
- Impact on public acceptability of measures that interfere with cultural patterns and traditions (e.g., restriction in the availability of tartare (raw minced beef)).

Equivalence

Differences in regulatory food safety systems inevitably exist between countries. Therefore, determination of the equivalence in the sanitary measures applied in importing and exporting countries is becoming a priority issue in international trade. The SPS Agreement requires that sanitary measures of other countries be accepted as equivalent, even if they differ from their own or others, if the exporting country objectively demonstrates that its measures achieve the ALOP established by the importing country.

Codex Alimentarius has developed a framework for the determination of equivalence that requires the development and application of principles and guidelines similar to the risk analysis approach.

Monitoring and Reviewing Implemented Risk Management

An important part of risk management is monitoring whether it is effective, for instance, through ongoing gathering, analysis, and interpretation of data to determine how well risk management activities have been performed and to determine what steps may need to be taken next to better improve public health. Monitoring and surveillance activities provide important information for reviewing whether the ALOPs are being achieved. In most cases, monitoring and reviewing public health outcomes will be a measure of the effectiveness of regulatory food control programs.

Reviews are also necessary when new data become available that might substantively alter the conclusions of a risk assessment or its associated degree of uncertainty. Such data include new information on the virulence, the prevalence and levels of the hazards in foods, the proportion of sensitive populations, changes in dietary intake patterns, changes in food processing patterns, as well as data from epidemiological studies and surveillance and monitoring programs in relation to foodborne diseases.

Quantitative Risk Management

The philosophy behind quantitative risk management is the full recognition of the fact that food safety (i.e., that the food is safe when consumed) is the result of a continuum of control measures (all actions and activities that impact the level of hazards in food) applied along the whole food chain, from the production of feed material to the preparation of the food by the consumer. Quantitative risk management activities address the whole continuum and not individual parts of the food chain in isolation.

Traditionally, the stringency of a food safety system has been articulated using control measures at various points within the food chain; the actual impact on consumers' exposure to a hazard has, at best, been inferred. For instance, risk management in the earlier stages of a food chain is traditionally based on the development and implementation of codes or guidelines on GHP. In many countries these are developed and established by public health authorities and are intended to be applied uniformly, independently of HACCP plans at the manufacturing level. In other words, the quality of raw materials is often defined by public health authorities, and it is left to the HACCP system of the manufacturer to adapt controls to the hazard levels resulting from compliance with these codes. As a result, the continuum is not fully assured (e.g., new hazards or sporadically occurring hazards at primary production levels that require immediate action cannot wait to be addressed by the amended codes of practices in place). Further, the HACCP concept introduced in the 1990s introduced a systematic approach to identifying hazards that need to be controlled by a particular establishment (focusing on the food manufacturing stage) and for the identification and management of key control measures applied directly to the food. However, this system does not address the degree of stringency needed in regard of food safety outcomes.

With the development of risk assessment techniques, it is increasingly possible to derive the maximum tolerable consumers' exposure in relation to the degree of acceptable risk. Quantitative risk management is aimed at achieving a coherent food chain approach that achieves this.

The key objective for the quantitative risk management approach will be to provide guidance on the stringency required to control a specific risk through the identification of targets for acceptable hazard levels at various stages along the food chain and to ensure that these targets are mutually linked to the ALOP.

Food Safety Objective

Recognizing the difficulty of relating control measures directly to an ALOP, the concept of FSO has been introduced to assist in the practical implementation of an ALOP. Conceptually, the FSO can be viewed as the consumers' maximum level of exposure to a hazard that provides or contributes to the ALOP. As such, an FSO articulates the overall performance expected of a food chain in order to reach a stated or implied public health goal. The overall performance results from the level of control achieved by the food safety system deployed from 'farm to fork.'

FSOs express the tolerable level of exposure to consumers in terms of the maximum level of a hazard (or maximum frequency of occurrence of a hazard) in a food at the time of consumption. FSOs can be established for the following purposes:

- To translate the ALOP (whether explicit or implicit) into a more useful parameter for interested parties and to encourage change in operational food safety management, or in the behavior of consumers, in order to enhance the safety of certain products.
- For communication to parties involved in food trade: implemented FSOs may not be universally common and will take into account national and regional situations.
- By the food businesses to design their operational food safety management systems, for example, through selecting appropriate target levels in the end products and to determine the necessary control measures to achieve them.
- To encourage the use in food businesses of quantitative HACCP and other food safety management systems.

Other Quantitative Food Safety Targets

Quantitative risk management requires that additional risk-based milestones be established that articulate how different stages of the overall food safety systems must function to achieve the ultimate food safety outcome required (e.g., the FSO). As a means of addressing this need, two related terms, PO and Performance Criterion (PC), have been introduced.

Performance Objective

The purpose of a PO is to articulate the maximum level of a hazard in a food at a particular stage in the food chain that provides or contributes to an FSO.

The etiology of some foodborne diseases predominantly involves the direct consumption of a contaminated ready-to-eat food (e.g., *Listeria monocytogenes* in soft cheese, *Staphylococcus aureus* in fermented meats, and *Salmonella* in fresh product). In this instance, the consumers' risks are determined by the frequency and extent of contamination in the product at the moment of ingestion. To manage the exposure to consumers of hazards associated with such foods, the establishment of FSOs is appropriate.

With other combinations of disease and foods (particularly raw products that are cooked just before consumption), the etiology of a disease involves cross-contamination. Here a raw food serves as a source of a contamination for other ready-to-eat foods, i.e., ones that are not cooked just before consumption (examples include *Campylobacter jejuni* on raw poultry and *Salmonella* on raw foods of animal origin). The consumers' risks are determined by the frequency and the quantitative level of contamination of the raw product and by the level of the

pathogen after final cooking (except in the case of appropriate cooking to kill pathogens). To manage the exposure to consumers of hazards associated with such foods, the establishment of POs will be appropriate, provided that there is a valid link between it and the FSO that it is trying to address.

Within the HACCP context, a PO is equivalent to 'acceptable level' of a hazard in the end product. A PO intended at a specific step in the food chain for a product that is delivered from one establishment to another for further processing is established taking into account knowledge, such as (1) the probability and extent of growth under specified storage and transport conditions and (2) the requirements of the other establishment to enable it to meet the PO for the further processed product. A PO intended for a ready-to-eat food at the point of delivery from the processing establishment is established taking into account knowledge of (1) the probability and extent of growth under specified storage, transport, and distribution conditions, (2) the expected shelf life, and (3) the expected changes in hazard levels during preparation by the consumer.

Performance Criterion

How the required PO can be achieved can be articulated through Performance Criteria (PCs). A PC expresses the change (effect) in the level of a hazard in a product that must be achieved by the application of one or more control measures to provide or contributes to a PO. The change required can be a minimum reduction, a maximum increase, or no change, depending on the nature and use of the control measures. This can be conceptualized in eqn [1].

$$\text{Performance Criterion} = [\log_{10}(C_0) - \log_{10}(C)] + \log_{10}(\text{SF}) \quad [1]$$

where C_0 and C are the initial and final (i.e., after application of the control measure) concentrations of the hazard, respectively. An SF can be imposed to take into account uncertainties in the effects of subsequent control measures. (For instance, if C_0 is 10 cfu (colony-forming units) of the hazard per gram and if the final concentration C has to be no more than 10^{-6} cfu of the hazard per gram with an SF of 100, then the PC is equal to 12; thus the calculation specifies that the control measure must deliver a performance of a 12 log reduction in the concentration of the pathogen.)

The following conceptual eqn [2] can help express the dynamics in the hazard control applied by a food business:

$$H_0 - \sum R + \sum I \leq \text{PO or FSO} \quad [2]$$

where H_0 is the initial concentration of the hazard, $\sum R$ is the sum of reductions (PCs) in the hazard concentration achieved by control measures and $\sum I$ is the sum of increases (PCs) in hazard concentration as a result of growth and/or contamination.

Risk Communication

Risk communication is the third component of risk analysis and is a central and integral part of effective food safety management.

Every stage of risk management should rely on a wide exchange of information and opinions about risk between risk managers, risk assessors, and all other stakeholders concerned about or affected by the problem and the risk management decision. Risk communication and the involvement of stakeholders are crucial for open, transparent, and effective decision making. Communication of correct and updated risk assessment information to the food manufacturers is also crucial to enable them to conduct correct hazard analyses and designs of HACCP plans.

Risk communication aids in considering the different, and at times conflicting, interpretations of the nature and magnitude of the risk; it offers the opportunity to bridge gaps in understanding, language, values, and perceptions; it ensures that public values are considered; and it generates better-accepted and more readily implemented risk management decisions. In brief, it supports democratic decision making. Poor risk communication will almost always increase conflict and distrust over risk management decisions.

See also: Hazard Analysis Critical Control Point and Self-Regulation

Further Reading

Codex Alimentarius Commission, 1999a. Guidelines for the development of equivalence agreements regarding food import and export inspection and certification systems, CAC/GL 34–1999. Rome: FAO.
Codex Alimentarius Commission, 1999b. Principles and guidelines for the conduct of microbiological risk assessment, CAC/GL 30–1999. Rome: FAO.
Codex Alimentarius Commission, 2003. Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms, CAC/GL 46–2003. Rome: FAO.
Codex Alimentarius Commission, 2007. Principles and guidelines for the conduct of microbiological risk management, CAC/GL 63–2007. Rome: FAO.
Codex Alimentarius Commission, 2011a. Risk analysis principles applied by the Codex Committee on Food Additives and the Codex Committee on Contaminants in Foods. Rome: FAO Codex Alimentarius Commission Procedural Manual, Section IV, pp. 114–119.
Codex Alimentarius Commission, 2011b. Exposure assessment of contaminants and toxins in foods or food groups. Rome: FAO Codex Alimentarius Commission Procedural Manual, Section IV, pp. 114–119.
Codex Alimentarius Commission, 2011c. Working principles for risk analysis for application in the framework of Codex Alimentarius. Rome: FAO Codex Alimentarius Commission Procedural Manual, Section IV, pp. 105–111.
FAO/WHO, 1995. Application of risk analysis to food standards issues Geneva: WHO Report of the joint FAO/WHO expert consultation. Geneva: FAO/WHO, 13–17 Mar 1995.
FAO/WHO, 1997. Risk management and food safety Rome: FAO Report of a joint FAO/WHO expert consultation. Rome: FAO/WHO, 27–31 Jan 1997.
FAO/WHO, 1998. Application of risk communication to food standards and safety matters Rome: FAO Report of a joint FAO/WHO expert consultation. Rome: FAO/WHO, 2–6 Feb 1998.
FAO/WHO, 1999. Risk assessment of microbiological hazards in foods Geneva: WHO Report of a joint FAO/WHO expert consultation. Geneva: FAO/WHO, 15–19 Mar 1999.
FAO/WHO, 2000. The interaction between assessors and managers of microbiological hazards in food Geneva: WHO Report of WHO expert consultation. Kiel: FAO/WHO, 21–23 Mar 2000.
Hathaway, S., 1998. General concepts of risk analysis. In: Heggum, C. (Ed.), Quality and Risk Management. Proceedings of the 25th International Dairy Congress, vol. 5. Aarhus: Danish National Committee of IDF, pp. 30–39.
Heggum, C., 2002. Risk analysis. In: Roginski, H., Fuquay, J.W., Fox, P.F. (Eds.), Encyclopedia of Dairy Sciences. London: Academic Press, pp. 2456–2463.

Herrman, J.L., Nakashima, N., 1999. Assuring science-based decisions: Expert advice and risk analysis – Validity of the process and dealing with uncertainty. FAO/WHO/WTO Conference on International Food Trade Beyond 2000: Science-Based Decisions, Harmonisation and Mutual Recognition. Rome: FAO.

Jouve, J.L., 1998. Development of risk analysis in the international framework. In: Heggum, C. (Ed.), Quality and Risk Management, Proceedings of the 25th International Dairy Congress, vol. 5. Aarhus: Danish National Committee of IDF, pp. 10–29.

Schlundt, J., 1998. Application and acceptance of risk management procedures. In: Heggum, C. (Ed.), Quality and Risk Management, Proceedings of the 25th International Dairy Congress, vol. 5. Aarhus: Danish National Committee of IDF, pp. 41–47.

Vose, D., 2000. Risk Analysis – A Quantitative Guide, second ed. London: Wiley.

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SAUSAGE CASINGS

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Glossary

Bite The resistance of the surface as the consumer initially bites into a sausage.
Collagen A stromal protein that is the major component of animal skin and intestinal tract.
Cooked sausage A sausage that has been fully cooked during thermal processing. May or may not be smoked as well.
Dry sausage Dry sausages have lost approximately 30% of original weight during subsequent processing. Exact definition may be subject to regulatory requirements.
Semidry sausage Semidry sausages have lost approximately 15% of original weight during subsequent processing. Exact definition may be subject to regulatory requirements.

Animal Casings

In any discussion of sausage casings, it is necessary to begin with the oldest of our packaging materials for sausage: the animal casing. For centuries, animal casings have been the traditional containers for sausage materials and are still associated with high-quality sausage products. They offer a unique combination of properties that provide valuable processing characteristics and fit the requirements for natural products, which are increasingly more popular. The material, made from the animals' intestinal tract that is used for the casings, is actually the submucosa, a collagen layer that provides the strength to the particular organ. For the most part, with a few exceptions, the fatty outer layers of muscle are removed along with the inner layer, the mucosa. Because the material used is collagen, the treatment of the casing at the slaughtering plant and during subsequent steps in casing processing has a very definite effect on the utility of the final product.

As in the case of all collagen materials, the collagen in casings is hardened and rendered less soluble by the application of salt. This is essentially the same process used in the curing of hides. Thus, the salting of the casing at the slaughtering plant has a great deal to do with appropriate curing and the final quality of the finished casing.

The collagen material that is the basic structure of animal casings has unique characteristics that are applicable during processing. Initially, as the collagen is exposed to heat and drying, it becomes less permeable to moisture. This drying also affects smoke penetration. For this reason, the initial step in the processing of a sausage in animal casings requires drying to develop the appropriate smoke permeability. Once the smoke is applied, and the desired smoke color and concentration are attained, further drying will render the casing virtually impermeable to moisture. It can then be cooked in a variety of ways, including steam cooking, without injuring the final product. In fact, at one time it was customary to apply the smoke and dry the casing in the smokehouse and then finish, particularly small-diameter products, by water cooking. This variable permeability becomes a very useful tool in further processing and enables the animal casing to accommodate a wide variety of conditions.

Small-diameter animal casings are derived from the small intestines of hogs and sheep (Figures 1 and 2). The sheep casings are the smallest in diameter and are also the most tender. These are the most adaptable to fresh sausages, where there is no further processing to tenderize the casing, as well as to small-diameter cooked and smoked sausages. There are different grades of sheep casing. The B grade has small holes

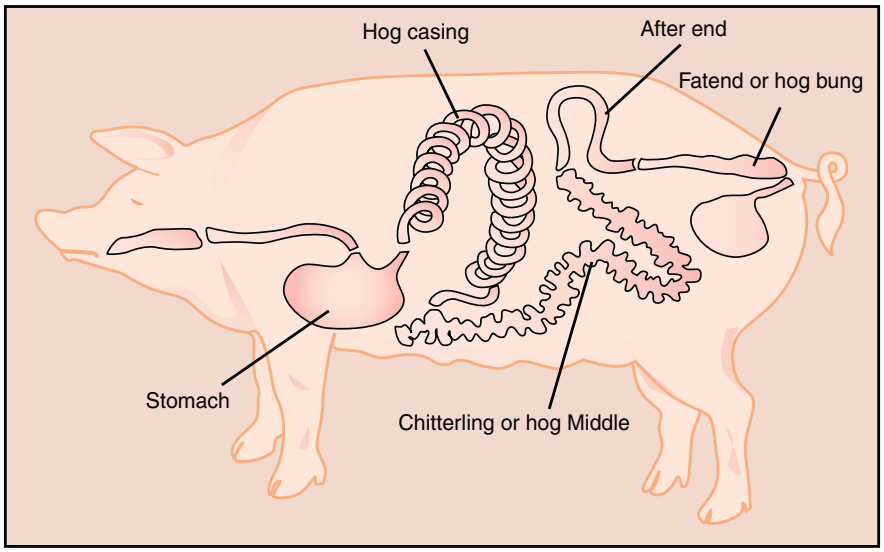

Figure 1 Hog intestines.

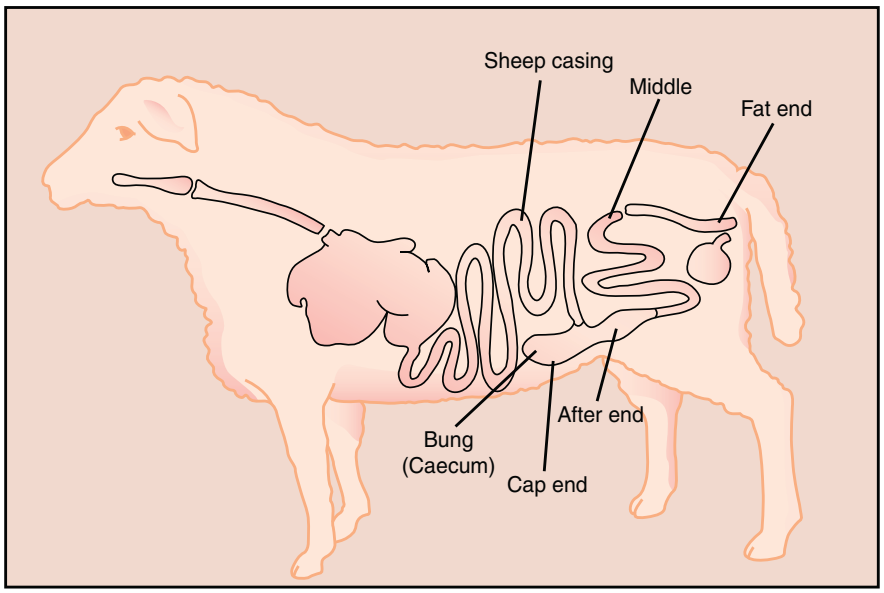

Figure 2 Sheep intestines.

and is applicable primarily to coarse-ground fresh sausage. The A grade is most applicable to an emulsion-type sausage. Hog casings are used for some types of fresh sausage as well as a variety of smoked, dry, and semidry sausages. They are somewhat less tender than sheep casings.

Sheep and hog casings are sold in bundles or hanks comprising of 91 m of casing. Diameters of sheep casings range from 16 mm to approximately 28 mm. Hog casings range from 30 mm to approximately 44 mm. They are normally grouped in 2 mm increments.

Casings of a small diameter are available in a variety of forms. They can be dry salted, and then require flushing before use. During the flushing process, the casing can be examined for strength, punctures, and conformance to size specifications. Preflushed casings are packed in a brine solution. Pretubed casings are shirred onto a plastic sleeve; these can then be transferred directly to the stuffing horn. To improve production efficiency, some processors will flush the casings in a separate location and then shirr them onto a stainless steel mandrel, so that they can be quickly applied to the stuffing horn.

During smoking and cooking, the initial critical steps of drying and smoke application must be watched very carefully. Before smoke is applied, the casing should be dried to the point at which it is tacky. If it is not, the smoke will penetrate through the casing and be deposited on the meat surface, thereby allowing for casing separation and causing a pale, dull appearance. By the same token, if the sausage is overdried, the smoke will essentially be deposited only on the outside surface

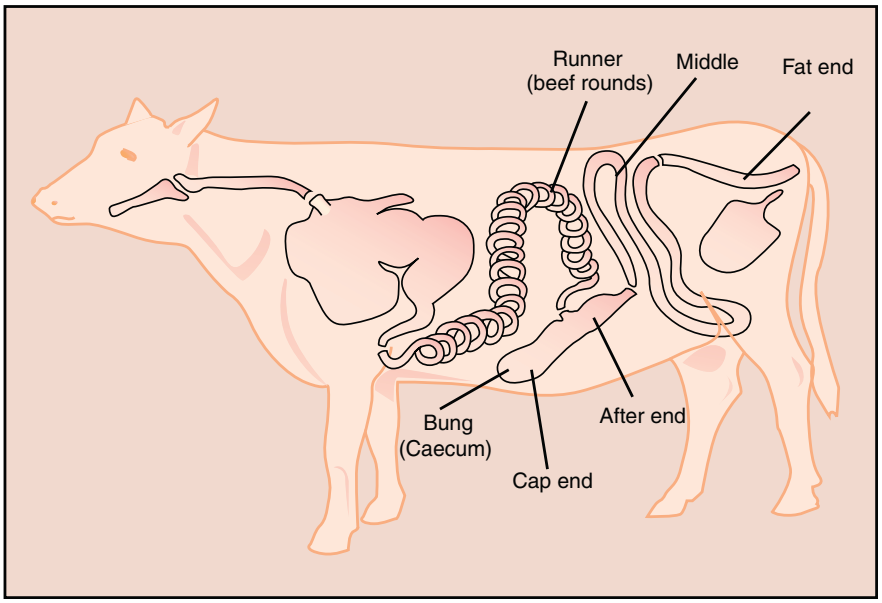

Figure 3 Beef intestines.

with very little flavor effect. Animal casings are ideally suited to liquid smoke application, either by drenching or by atomization.

There is a variety of larger-diameter natural casings available (Figure 3). Beef rounds, from the small intestine, are generally used for ring-type sausages. In terms of handling characteristics, the same type of handling as applied to sheep and hog casings can be used. Beef rounds are probably the largest-diameter casings that one would classify as still being edible from a palatability standpoint. Diameters range from 35 to 46 mm. They are sold in bundles or sets of 9, 18, or 30 m. Other beef casings are beef bung caps, ranging from 75 to 125 mm. These are used for some cooked as well as dry and semidry sausages and are sold by the piece. Beef middles, used for various dry and semidry sausages, range in diameter from 45 to 65 mm and are also sold by the piece. Beef bladders are used for some specialties such as mortadella. Some of these larger-diameter sausages require support when hanging; the support can be either a series of string loops or netting. Most beef casings would typically be removed from the sausage before consumption.

One type of large-diameter casing that is used quite often is the sewn casing, particularly the sewn bung. Sewn hog bungs are used not only for some salamis and cervelats but also for various types of liver products. Sometimes these sewn casings are lined with paper or with another type of animal casing such as hog bungs lined with beef middles. In the case of sewn bungs, the fat layer of the bung is left on the inside of the casing. Particular care should be applied to these casings to check the rancidity level of this fat layer. Salt triggers the development of rancidity and, unless these casings have been carefully and properly handled, rancidity of this fat layer could develop. Subsequently, this rancidity could be transferred to the finished product in the form of an off-flavor. Animal casings should be stored under refrigeration at a temperature of less than 4 °C.

Even when carefully graded, animal casings lack a degree of uniformity and are therefore somewhat limited in their machinability. For this reason, handling animal casings requires somewhat higher labor input than one would experience with the machine-made casings. This is where the processor must make some management decisions depending on the type of the product that is manufactured and the clientele to which it is to be sold.

Laminated Casings

Some large-diameter casings are made by laminating pieces of hog or sheep casings on a mandrel; the casing is subsequently dried. This gives a natural casing with uniform dimensions and also with the possibility of unique shapes. In addition, various netting patterns can be incorporated in the lamination. The primary use of this casing is for dry and semidry sausages.

Manufactured Collagen Casings

A logical development from the animal casing is a manufactured casing made from the same material chemically as the animal casing, that is, collagen. The manufactured collagen casing is commonly made from the regenerated corium layer of beef hides, though actually any source of collagen could be used. The collagen is solubilized and later extruded and hardened in a manner very similar to the production technique used for cellulose casings. Because of its chemical nature, namely collagen, it does have some of the same processing characteristics as the animal casings; however, collagen casings do not require soaking or rinsing steps before stuffing and product diameters of the stuffed sausages are much more uniform compared to animal casings. Once again, the drying step in the manufacture of cooked and smoked sausage is

critical, as is the ability of this casing to dry or to soften during processing of the sausage. Characteristics of these collagen casings vary with different manufacturers. It is therefore a good idea to approach the smokehouse processing of collagen casings on the basis of recommendations from the manufacturer.

As with animal casings, the application of heat together with drying and an acid, such as found in smoke, will toughen the material. The application of heat with moisture will tend to soften it. For this reason, cooking and smoking steps have to be carefully controlled to provide an acceptable 'bite' to the end product, but at the same time, not to soften the casings excessively so that they will drop in the smokehouse. Balancing of drying, smoking, and subsequent cooking steps is extremely critical.

There are a variety of types of collagen casings. Some are specifically designed for fresh sausage, others for cooked sausage, and still others for dry and semidry products like snack sticks. Because of performance differences and differences in tenderness, these specific types are not interchangeable. Collagen casings intended for fresh sausages are designed to be tender, because there will not be a high humidity cooking process applied to this product that would increase tenderness.

With the small-diameter collagen casings, there is another factor to be considered. This is the so-called 'wetting out' immediately after stuffing. When the meat is stuffed into these casings, the casings will absorb moisture from the meat. During this period of moisture absorption, the casing softens and becomes more pliable. If allowed to soften too much, it will not be machinable during the subsequent linking steps. The wetting out of a casing is highly dependent on the material that is stuffed into it, particularly from the standpoints of moisture content and temperature as well as the characteristics of the individual casing itself. Delay times between stuffing and subsequent linking need to be very carefully controlled if casing breakage is to be prevented. Proper wetting out before linking may also affect the performance of the sausage during cooking resulting in a 'dumb bell' effect, that is, protruding ends. This phenomenon can also result when the casings are overstuffed.

Processed meat collagen casings are also available for products that require cooking and smoking. These casings are designed to withstand linking and hanging and are available as colored casings. A type of collagen casing that is applicable to dry and some semidry sausages is made with a layer of collagen reinforced by a cotton or silk mesh or netting. These can also have built-in string loops to resemble the string-supported animal casings.

Collagen casings are available in a variety of sizes. Many of them are designed, in effect, to simulate the animal casings. Some have built-in curves and even built-in irregularities. Collagen casings are sometimes sewn into shapes to simulate a sewn animal casing.

Whereas collagen casing does not require refrigerated storage, the proper storage of collagen casings is critical. Collagen casings can be stored at room temperature but may dry out and become brittle if the original packaging is disturbed. The manufacturer's instructions for proper storage should be followed not only with collagen casings but also with all other casings as well.

For the most part, collagen casings lend themselves well to machine handling because they can be produced with uniform sizes. The manufacturers of both stuffing and linking machines as well as the casing manufacturers should be consulted, however, for appropriate information on how best to handle these particular manufactured casings.

Cellulose Casings

Cellulose casings are made from regenerated cellulose derived from high-grade wood pulp. Cellulose casings are divided into two groups: peelable and fibrous. Peelable cellulose casings are small-diameter casings that are permeable to smoke and moisture but relatively impermeable to fat and some of the larger organic molecules. The permeability can be affected by the moisture conditions surrounding the casings. Higher moisture conditions allow for a greater degree of permeability, whereas drying tends to reduce the permeability somewhat. However, these changes in permeability are not nearly as drastic as one finds in the animal casing. The sausage raw material is stuffed into the casing, the casing is linked, and the product is subsequently cooked (usually with smoke). The proteins at the surface of the sausage are denatured to form a skin, and after chilling, the casing is peeled to produce a skinless sausage. Because of their precise diameters, these casings lend themselves to high-speed linking and stuffing. If the stuffing and linking equipment is precisely controlled along with the further processing steps, precise link weights can be achieved, enabling exact unit weight packaging.

A number of key considerations in the application of peelable cellulose casings affect performance. Adherence to the manufacturer's recommended stuffing diameter (RSD) is one. Overstuffing can cause breakage and poor peelability. Understuffing can cause inexact link weights and contributes to fatting out during thermal processing. These casings are also sensitive to cooking and smoking cycles, and the correct cycle must be used for the particular type of product and the type of casing. There are two types of peelable cellulose, the 'regular' and 'enhanced' peelabilty, each requiring different processing conditions. Peelability is dependent on proper skin formation on the surface of the product. The sausage formulation must have sufficient protein to enable skin formation. Further, there needs to be some source of an acid such as those derived from smoke or liquid smoke to enable the surface coagulation of the protein. Moisture between the casing and sausage surface aids peelability. For this reason, a high humidity cooking step is usually applied at the end of the cooking cycle. Humectants such as dextrose in the formulation also will aid peelability.

Fibrous Casings

Larger-diameter cellulose casings are known as fibrous casings. Fibrous casings are made by impregnating a strong paper-like material with cellulose. The chief advantages of the fibrous casing are its machinability and its uniformity, which adapts it to high-speed operations. Fibrous casings require soaking before use to ensure sufficient pliability during stuffing. When

exact weight control is critical, cellulose casings for small-diameter products or fibrous for large-diameter products come into their own. All of these casings, when properly handled, have good dimensional stability.

There are specific types of fibrous casings for specific uses. For example, on a dry or semidry sausage, it may be necessary to have a casing that will shrink with the product as it dries. For this reason, casings with a special adherence coating are available. These casings adhere very tightly to the surface of the meat and, as the meat begins to shrink during drying, the casing will do likewise. If there were not this adherence at the meat-casing interface, the casing would loosen from the product. Where these casings are used, it is important that the processing steps be observed carefully. If too much moisture is applied before bonding between the protein-coated casing surface and the meat, the casing can be made to loosen from the product. For a product that is designed for slicing, a fibrous casing that is easily peelable is preferable. This casing can be stripped off of the finished product before the slicing operation. Long casing lengths have been employed in slicing operations, in order to minimize the rework from the ends. To maintain dimensional stability during cooking and processing, however, any product over approximately 60 cm long should be heat processed in the horizontal position to preclude dimensional distortion.

When casings are to be applied to a chunked product such as, a sectioned and formed ham, where air needs escape from the surface, a so-called prestuck casing is available. This has minute holes in the surface of the casing, which allow entrapped air to escape. Where large, whole-muscle pieces like hams are stuffed, it is often necessary to drill some large holes at the clipped end of the casing to allow the air to escape as the casing is being stuffed.

Moisture-proof fibrous casings are used for those products that will be water or steam cooked. For the most part, this applies to liver sausage, patés, and delicatessen loaves. A moisture barrier coating, such as polyvinylidene chloride (PVDC), is applied to the fibrous casing to make the casing moisture impervious. Depending on the manufacturer, this coating can be applied either to the inside or outside surface of the casing.

As with all other manufactured casings, it is very important to observe RSD and the manufacturer's recommendations for further processing. The best insurance for maximum performance is to ask the supplier for appropriate directions on proper use. Storage times, temperatures, and humidity should be observed, as all of these can drastically affect performance. The interior of these casings may be coated with a variety of flavorings such as liquid smoke that can ultimately transfer to the surface of the product. In effect, the processor can produce a smoked product without a traditional smoking step. In a similar manner, coloring agents such as caramel coloring can be applied. By applying smoke flavoring along with a dark coloring agent, it is possible to make a product with color and flavor similar to a heavily smoked 'black forest' ham.

Plastic Casings

In some cases, a moisture-impermeable material such as PVDC, mylar, polyethylene, or a polyamide (nylon) is used as a casing. Plastic casings are usually cost-effective but they may have drawbacks such as dimensional instability. These casings generally are impervious to moisture and smoke, although there are now some smoke- and moisture-permeable plastic casings on the market. Where the moisture-proof casings are used, and various flavorings are desired, these must be incorporated directly into the product or coated onto the inside of the casing. Colorings can be similarly applied to the surface of the product by coating them on the inside of the casing.

The impermeable plastic casings have the advantage of protecting the finished product from microbial contamination as long as the plastic casing remains intact.

Fabric Casings

Sewn cotton bags or tubes are used for some types of sausage. Typical was the use of a cotton bag to encase traditional fresh pork sausage made on the farm, particularly in the southern part of the USA. There are now a variety of fabric casings available, some of which are sewn and printed for novelty effects such as animals or holiday symbols. There are also sewn fabric casings that are spice coated on the inside for the purpose of transferring that spice coating to the surface of the finished sausage.

Coextrusion

A relatively recent technology is the coextrusion of collagen dough (the basic material from which collagen casings are formed) on the surface of a sausage raw material as it is extruded through the stuffing horn. The collagen is subsequently hardened to form the casing on the surface of the sausage. This then becomes a continuous process for the manufacture of an encased sausage. The process is applicable to fresh, cooked, and dry or semidry sausages. A more recent introduction is the use of an alginate slurry to encase the sausage as it is extruded. The alginate is hardened and the sausage is cooked, thus forming a sausage with an alginate casing. The process is similar to the coextrusion of collagen, except that the application of the alginate is by coating or enrobing rather than coextrusion as such.

In earlier years, this process required substantial investment in a single-product dedicated equipment. As technology has progressed, capital costs have lowered and production lines have become more flexible. The use of this essentially coextrusion technology is often coupled with packaging of the sausage in a single-layer impervious film where the final cooking steps are accomplished in the sealed package. The result is a product with extended shelf life and freedom from food borne pathogens assuming, of course, that the thermal processing steps are properly designed.

See also: Chemical and Physical Characteristics of Meat: Chemical Composition. Cutting and Boning: Traditional.

Sausages, Types of: Cooked; Dry and Semidry; Emulsion.
Smoking: Liquid Smoke (Smoke Condensate) Application

Further Reading

International Natural Sausage Casing Association, 1997. Facts about Natural Sausage Casings. Kensington, MD: International Natural Sausage Casing Association.

Pearson, A.M., Gillett, T.A., 1996. Processed Meats, third ed. New York: Chapman and Hall.

Relevant Website

http://meatsci.osu.edu
The Ohio State University.

SAUSAGES, TYPES OF

Contents

Cooked

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Glossary

Belly The side and underside of a pork carcass.
Butt The upper portion of the front leg or shoulder of a pork carcass. Often used for making sausage because it contains more connective tissue and is inherently not as tender as a roast or chop compared to other pork cuts.
Chub A short, cylindrical-shaped package typically used for bulk ground products, but may also be used for cooked products.
Die plate A perforated plate through which meat is forced during the grinding process.
Extrusion The process of forcing meat or other materials through a die plate, as occurs during the grinding process.
Fresh sausage 'Fresh' can mean uncooked, uncured, or both, in relation to sausage.
Link The process of twisting, or tying, a filled sausage casing to achieve the desired sausage length. It is also the single unit that sausages are merchandised in, when sold in a casing.
Picnic The lower portion of the front leg or shoulder of a pork carcass. Often used for making sausage because it contains more connective tissue and is inherently not as tender as a roast or chop compared to other pork cuts.
Ready-to-eat Meat products that are fully cooked and ready for consumers to eat directly from the package, without reheating.
Stuff The process of filling a sausage casing.

Introduction

Sausage is typically defined as a mixture of ground meat, combined with spices and seasonings. Sausage is often stuffed into some type of casing and linked, but loose sausage products are also available. Originally, sausage was made as a means to salvage value from trimmings and lower-value cuts of meat. As well as adding value to meat, over time, cooked sausage products have become convenient meat products, as they are 'ready-to-eat' (RTE) and are easy to eat in the form of a sandwich. Cooked sausage products also add great variety to consumers' food choices owing to an endless variety of spices and flavorings that are available when making these products. Traditionally, there were sausage products that were only sold as cooked products, however, processors are now offering many of their traditional fresh or uncooked sausage products in a cooked form. This article focuses on the production and descriptions of the most common types of cooked sausages.

List of Sausages

Andouille, Bangers, Berliner Bologna, Bierwurst, Bologna, Blood sausage, Bockwurst, Bratwurst, Brown and serve sausage, Braunschweiger, Chorizo, Cocktail frankfurters, Cooked or Cotto salami, Corn dogs, Frankfurters, Goetta, Hot dogs, Kielbasa, Knackwurst, Kuemmelwurst, Linguisa, Lyonerwurst, Mortadella, Paté, Pfefferwurst, Pizza toppings, Ready-to-eat products, Schinkenwurst, Scrapple, Smoked sausage, Smokie links, Taco fillings, Thuringer, Vienna Sausages, Water-holding capacity, Weisswurst, and Wieners,

Cooked Sausage Production

Cooked sausage products are made from a variety of cuts or trimmings from various types of meat (beef, pork, lamb, veal, turkey, chicken, etc.). Shoulder cuts, which consist of many

smaller muscles and typically contain more connective tissue, are often used to make sausage products. The 'picnic' portion of the pork shoulder is a good example of a shoulder cut used for making cooked sausage, and the 'butt' portion of the pork shoulder is often selected when a darker product color is desired. Also, the belly portion of a beef carcass, otherwise known as the beef 'plate,' is also used in sausage processing.

Meat from the entire carcass of older animals (e.g., cows and sows) would also be a potential raw material for making cooked sausages. Variety meats, such as livers and hearts, were traditionally used in some meat products, but are used to a lesser extent in sausage products made today.

Meat ingredients are first coarsely ground through a mincer with holes of approximately 12.5 mm (0.5 in.) (Figure 1). The grinding process is essentially an extrusion process and grinding of meat can be compared to the use of the child's toy, the 'Play-Doh Factory.' Meat (like the Play-Doh) is forced through a plate by the action of an auger, and as strands of meat are forced through the plate, revolving knives cut the strands into short particles (similar to how one might cut Play-Doh strands as they are formed after being forced through the die).

Following the coarse grinding process, the meat ingredients are mixed with the nonmeat ingredients, such as salt, sugar, water, nitrite, erythorbate, spices, and flavorings, until a uniform product is achieved.

Salt is added to cooked sausage products for flavor and preservation purposes, as well as for extracting and solubilizing proteins from the meat, which will assist in binding the ground meat particles together to result in the texture associated with cooked sausages.

Sugar (or other forms of sweeteners) is added as a flavoring, as well as to increase the water-holding capacity to prevent moisture loss from these products when they are cooked.

Water is added to cooked sausages to assist in the extraction of the meat proteins and to improve the binding ability of the ground meat particles. Added water also replaces the moisture that is lost from these products during cooking.

Nitrite is the key ingredient in making a 'cured' meat product and is added to give the products the characteristic cured color and flavor. Equally important, nitrite also has antimicrobial properties, preventing the growth of pathogenic bacteria such as *Clostridium botulinum*.

A 'cure accelerator' is typically used in production of cooked sausage products, particularly with modern processing systems, which allow minimal time for the cured color reaction. Cure accelerators include sodium erythorbate, sodium ascorbate, ascorbic acid, and sodium acid pyrophosphate.

Soy proteins bind water and are often added to pizza toppings and taco-filling meat products, as these products are typically served hot and it is critical that moisture and fat loss are minimized during the cooking and hot-holding of these products.

For a ground sausage product, in which protein extraction and binding are important for texture, the mixture is typically given a final grinding through a smaller plate before the final mixing process. The lean portion should be mixed as long as is feasible at the coldest temperature possible, with the salt (and phosphate, if used), to optimize protein extraction. After the fatter ingredients are added, mixing time should be minimized and the product kept as cold as possible to minimize fat smearing during stuffing. Paddle mixers are also preferred for extracting protein, while minimizing fat smearing and uniformly mixing multiple types of meat with nonmeat ingredients. The final grinding step can affect the texture of the product, because the grinder plate holes can have diameters ranging from 12.5 mm (0.5 in.) to 3 mm (0.13 in.). If the product includes nonmeat ingredients such as cheese, peppercorns, encapsulated acids, etc., then the final mixing step needs to follow the second grinding step.

The final grinding step should occur after the mixing step for ground products in which the particle definition is important, but in which the protein extraction for product binding is not so critical, such as cotto salami. Ribbon mixers should be avoided if particle definition is important. This is also true for products in which a more crumbly texture is desired. For a fine-chopped product, the meat mixture would be

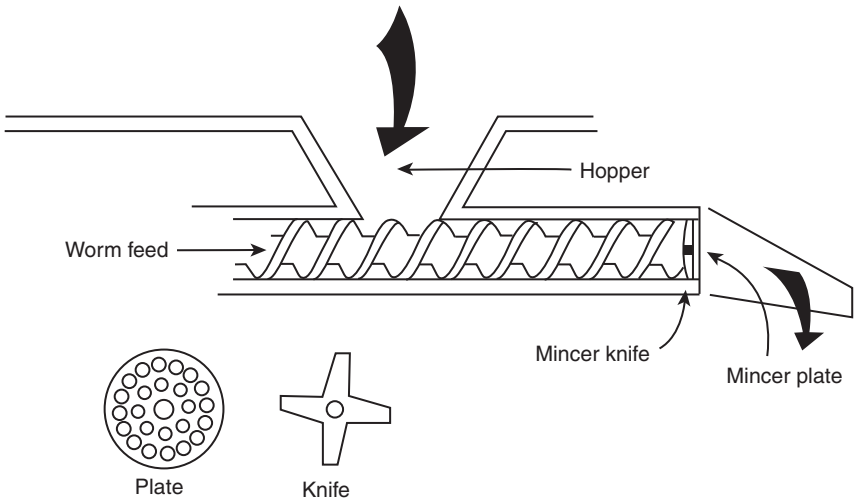

Figure 1 Cross-section of a meat grinder. Reproduced from Kramlich, W.E., Pearson, A.M., Tauber, F.W., 1975. Processed Meats. Westport, CT: AVI Publishing Company Inc., p. 124, with permission of Kluwer Academic Publishers.

chopped or emulsified using either a bowl chopper or an emulsifier.

Vacuum mixing removes air bubbles and pockets, which compacts meat volume and improves appearance of cooked sausage products. It is important for both the quality and the safety of the products to keep the temperature of the raw mixtures below 4 °C (40 °F). For coarse-ground sausages, stuffing the mixture below 4 °C prevents smearing of the fat particles along the inside wall of the casing, resulting in a more desirable particle definition.

After grinding, chopping, or emulsification, the mixture is stuffed into a casing to give it its distinctive shape and appearance, and the resultant sausage is then cooked in an oven, in water, or in oil. Sausages that are cooked in an oven are typically smoked, using either natural or liquid smoke. Figure 2 provides a flow chart of the production process.

Most cooked sausage products are fully cooked and are referred to as RTE products. RTE products can be eaten directly out of the package without further cooking.

The following discussion of cured and uncured cooked sausage products includes general characteristics of a variety of products. The USDA Food Safety Inspection Service has standards of identity for many of these products, as do many other countries. These standards of identity provide requirements that processors must meet in order to use product names on their labels, and these requirements may vary from country to country.

Cured Sausage Products

The majority of cooked sausages are cured, which involves the addition of sodium nitrite to the meat. Examples of cooked, cured sausage products include frankfurters, wieners, hot dogs, bologna, smoked sausage, Italian sausage, and braunschweiger or liver sausage.

Andouille

A spicy coarse-ground, smoked pork sausage, Andouille was introduced to the United States by French immigrants to Louisiana and is often associated with Cajun cuisine.

Berliner Bologna

Berliner is a German-style bologna, made from coarse-ground pork, mixed with pieces of ham and stuffed into large diameter casings. If this mixture is stuffed into a chub, it would be called leona bologna, and if stuffed into a smaller diameter casing it would be called jagdwurst.

Bierwurst

A large diameter, coarse-ground, cooked smoked sausage, of German origin, bierwurst is often stuffed into hog bungs (veined natural casings). It is like beef salami, however, it may contain pork. Garlic is a common flavoring for this product.

Bologna

Although bologna is most commonly made as a finely chopped product, it could be made with a coarse-ground texture, using grinder plate hole diameters ranging from 2.4 mm (0.09 in.) to 3 mm (0.13 in.) for the final grinding. Bologna is most often stuffed into larger-diameter (10–13 cm/4–5 in.) casings, but may be stuffed into beef rounds to make ring bologna. Large-diameter bologna is not typically smoked.

Additional information on finely chopped or emulsified bologna, as well as background and seasoning information, can be found in the article on emulsion sausages.

Braunschweiger

Braunschweiger is a smooth-textured liver sausage, typically made from finely chopped or coarsely ground pork trimmings, livers, and bacon trimmings, and cooked in moisture-impermeable casings in water. If stuffed into a natural casing, it can be smoked as well. Onion and nutmeg are common flavorings for this product. Smoked bacon provides additional flavor, particularly to braunschweiger. Nonfat dry milk is used to improve the harsh flavor of liver. If cooked meat is used instead of raw ingredients, a spreadable product (see section Paté) is produced.

Cocktail Frankfurters

Cocktail frankfurters are short, small-diameter frankfurters, made in a finely chopped form and typically used as appetizers. Currently, cocktail frankfurters are often produced by a co-extrusion process that does not require the use of a casing.

Cooked or Cotto Salami

Cooked or cotto salami are not dry sausages, as is Genoa salami, but are fully cooked products and need to be refrigerated before eating. Cotto salami is made using a large diameter casing, and is typically an all-beef product. It is a mildly flavored sausage, and may be smoked. Black pepper, particularly in the form of whole peppercorns, creates the flavor of cotto salami. Mustard is used heavily in cotto salami, and nutmeg is used in low levels. Other names for cooked salami include: Kosher salami, beef salami and beer salami.

Corn Dogs

Corn dogs are made by dipping frankfurters in a corn meal batter and frying them in oil. In the United States, corn dogs are limited to 65% batter and 35% frankfurter.

Fleischwurst

Fleischwurst is a large diameter, fine chopped German bologna, which typically contains pistachio nuts. Also known as Vienna Bologna, it may also be sold in rings and chubs.

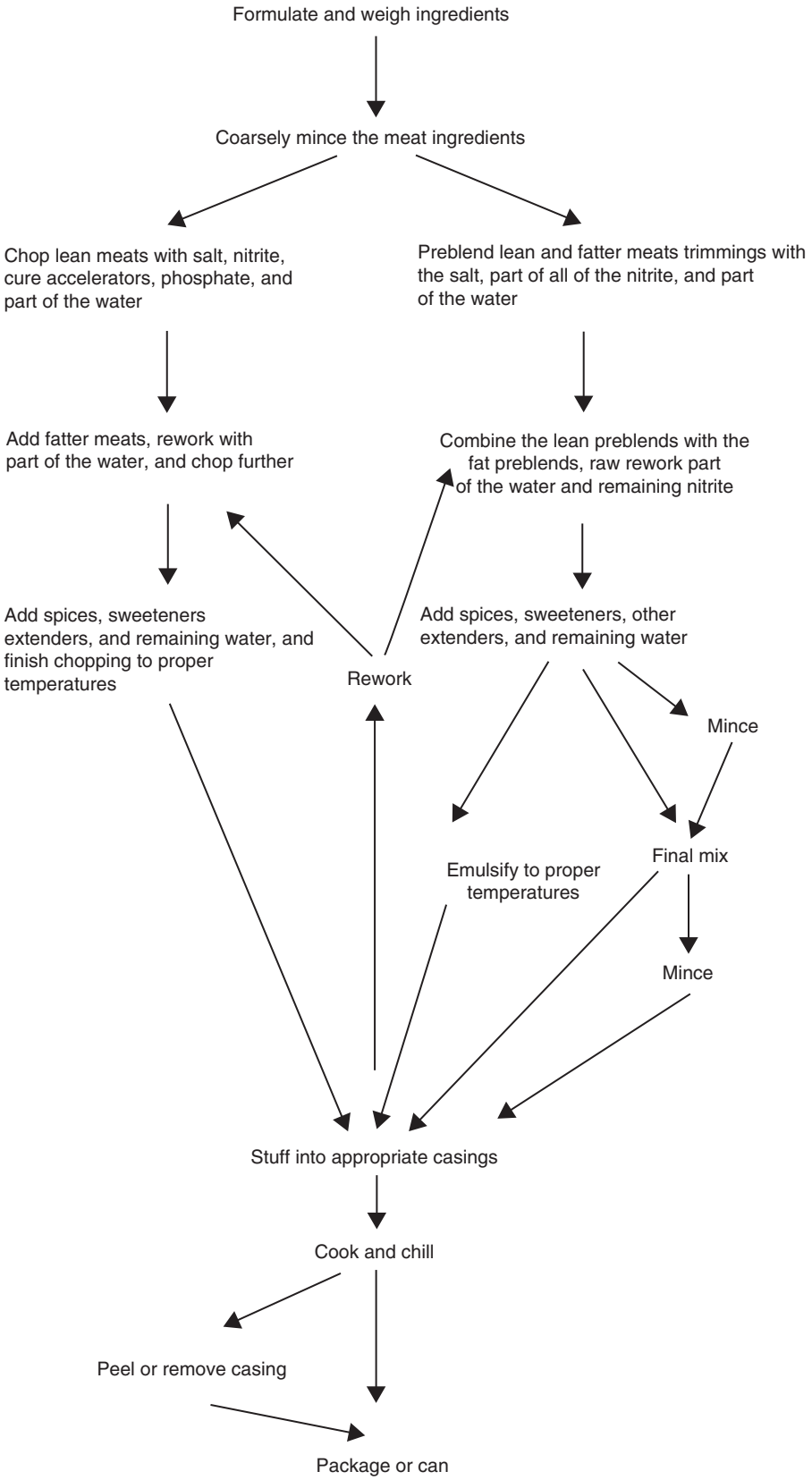

Figure 2 Flow chart for production of cooked sausage products.

Frankfurters, Wieners, and Hot Dogs

These products may be made in either a coarsely ground or finely chopped form. Coarsely ground frankfurters, wieners and hot dogs are made using grinder plates with hole diameters ranging from 2.3 mm (0.09 in.) to 3 mm (0.13 in.) for the final grinding, and are stuffed and cooked in either natural sheep or cellulose casings. These same products can be made in a finely chopped form using a chopper or emulsifier.

Kielbasa

Kielbasa is sold in many forms, but the word kielbasa is technically the Polish word for sausage, so it could be sold in the fresh or cooked and smoked form. Polska Kielbasa, or Polish Sausage, is the most common type of kielbasa in the United States. An uncured (fresh) and uncooked variety is also available. Typical Kielbasa flavors are primarily due to black pepper, dextrose, coriander, marjoram, nutmeg, garlic, and smoke.

Knackwurst

Knackwurst resembles a large-diameter frankfurter and is typically made of pork and beef. It may be made either as a finely chopped or coarse-ground product and is smoked. Garlic flavor is well pronounced in knackwurst.

Kuemmelwurst

Also called Carawaywurst, Kuemmelwurst is a loop- or ring-cooked sausage, containing caraway seeds.

Linguisa

Linguisa is a highly spiced coarse-ground, smoked pork sausage, originating from Portugal.

Lyonerwurst

Lyonerwurst is a fine-ground, beef and pork sausage that contains green peppercorns and is smoked and cooked.

Mettwurst

This product was originally made in a couple of ways in its native Germany. In southern Germany, it was made of pork, smoked and sold as a raw, spreadable product, like teewurst. In northern Germany, it was made similarly, but was dried longer during the smoking process to make a dry product. In the United States, it is made primarily as a linked, cooked, and smoked sausage product.

Mortadella

Mortadella is essentially a fine chopped bologna, with chunks of pork fat added. The base emulsion is typically made from both pork and beef and stuffed into beef bladders. Mortadella may also be sold as a dry or semidry product.

Paté

Paté is made from fresh livers and cooked meats, cooked in a moisture-impermeable casing. This product lacks the binding of liver sausages made from raw meat ingredients and is therefore a spreadable product.

Pfefferwurst

Pfefferwurst is a German-style sausage containing whole peppercorns.

Ring Bologna

Ring bologna is a finely chopped or ground product stuffed into a beef bung, in a loop typically weighing 0.5 kg (1 lb).

Schinkenwurst

Schinkenwurst is a large diameter, German product made by adding ham chunks to a pork and beef emulsion. It is similar to Berliner Bologna, except that Schinkenwurst is made from finely chopped base emulsion and does not contain garlic.

Smoked Sausage

Smoked sausage is typically a coarsely ground product, stuffed into either hog casings or cellulose casings (30–40 mm/1.19–1.5 in. diameter) and smoked. If stuffed into a cellulose casing, which is removed to make a skinless sausage, links are made approximately 12.5 cm (5 in.) long. If stuffed into natural hog casings, smoked sausage is most often not linked, but full strands are looped to make approximately 500 g loops. Typical smoked sausage flavors are primarily due to black pepper, dextrose, and smoke. Red pepper and coriander are occasionally added.

Smokie Links

These are small-diameter, coarsely ground, smoked sausages, which are most commonly co-extruded (so that a casing is not used) and heavily smoked.

Thuringer

Although most thuringer products are semidried (e.g., thuringer cervelot), some are cooked, without drying. It is typically made from pork, sometimes beef, with a pork breakfast link flavor, using marjoram instead of sage. It is sometimes called thuringer bratwurst. This product may also be sold fresh.

Vienna Sausages

Vienna sausages are short small-diameter frankfurters made in a finely chopped form, often cut (open ends) from long lengths of stuffed casings and sold in cans or glass jars. Spices typically used to make Vienna sausages include red pepper, onion powder, cloves, nutmeg, and coriander.

Uncured Cooked Sausage Products

There are a number of uncured products that are sold as cooked products, such as Bockwurst, cooked bratwurst, 'brown and serve' sausage, pizza toppings, etc. Many of these were originally sold as raw products, but more recently have been made available as cooked and RTE products. Some companies have developed cured versions of these products, which have traditionally been uncured.

Bangers

A mildly seasoned, British sausage, which contains rusk or other cereal fillers. The name supposedly comes from the noise it makes when cooked at high temperatures in a frying pay. Typically Bangers are not cured or smoked.

Blood Sausage

A sausage typically made from pork blood and either pork skins, diced back fat or diced pork jowls, and is mildly seasoned. This product is known as blatwurst in Germany, and is very dark in color. A related product, blood and tongue sausage, contains diced, cured, and cooked tongue and may contain ground clove. Blood sausage is typically available in either rings or chubs.

Bockwurst

The traditional bockwurst contains veal and pork, milk, egg, chives, and parsley, with spices similar to those in frankfurters, stuffed into 25 mm (1 in.) diameter casings and cooked in water, but not smoked. It is similar to bratwurst. Originally, bockwurst was sold as a fresh (raw) product, but it is now most often sold as a cooked product, because of its highly perishable nature.

Bratwurst

Bratwurst originates from Germany as an uncooked sausage, but it may also be sold as a cooked or smoked sausage. Traditionally, bratwurst were uncured, however, they are also available as cured sausages. Translated from German, bratwurst means 'sausage for frying,' and it was historically made from pork and veal, and stuffed into casings that were 30 mm (1.25 in.) in diameter and made as 100 mm (4 in.) long links.

Brown and Serve Sausage

These are precooked, uncured sausages, most often targeted as breakfast sausages. They may be stuffed into natural or collagen casings, but are also often extruded into the shape of a sausage link without using a casing. This product was designed for convenience of the preparer and is most commonly used in the food service industry. This product may be sold as links or as patties.

Chorizo

This product is made using a variety of methods, each unique to the various Spanish-speaking countries. Some chorizos are dried, some are a traditional cooked sausage, some are a loose sausage used in other dishes, and some are a fresh sausage. Precooked chorizos are made using pork and are typically hot and heavily spiced.

Goetta

Unique to Cincinnati, OH, this German product is similar to scrapple; however, it is made using pork and rolled oats, rather than corn meal. This product is typically stuffed into either a natural or artificial casing; however, it has a loose texture and is often added to scrambled eggs or other dishes.

Pizza Toppings

These are precooked, often uncured, sausage chunks or crumbles. The product is sold in bulk form, not in a casing, and does not fit the standard identity for sausage products. Pizza toppings are most often cooked in jacketed kettles, continuous thermal screw systems, or continuous belt ovens. In the United States, there is no standard of identity for pizza toppings.

Scrapple

An ethnic product of Pennsylvania Dutch people, this product is believed to be the first pork product to be developed in the United States. It is essentially a mush, consisting of ground pork trimmings (scraps), spices, and corn meal, and is an early example of early settlers' attempts to avoid wasting anything from the pork carcass. This product is typically cooked in a loaf pan, sliced, and fried in butter as a breakfast item. Another Pennsylvania Dutch name for this product is pon haus. An Irish version of this product is called pudding. And, German settlers in Cincinnati, OH, and northern Kentucky made a similar product known as goetta, however, goetta contains oatmeal, rather than corn meal, and is sold in a sliceable form and in a casing as a loose meat for addition to other entrees.

Taco Fillings

These are precooked, often uncured, seasoned meat crumbles that are used in tacos, burritos, and other 'Tex-Mex' type products. This product is sold in bulk form, not in a casing, and does not fit the standard identity for sausage products. Taco fillings are most often cooked in jacketed kettle or continuous thermal screw systems.

Weisswurst

This name means white sausage in German. It is an uncured sausage, similar to bratwurst, typically made of pork and veal. It is more commonly called bockwurst, if it contains milk and eggs.

See also: Chemical Analysis for Specific Components: Curing Agents. Chemistry and Physics of Comminuted Products: Other Ingredients; Spices and Flavorings. Ethnic Meat Products: Biltong: A Major South African Ethnic Meat Product; Brazil and South America; China and Southeast Asia; France; Germany; India and Pakistan; Japan and Korea; Mediterranean; Middle East; North America; Poland. Microbiological Safety of Meat: *Clostridium botulinum* and Botulism. Processing Equipment: Mixing and Cutting Equipment; Smoking and Cooking Equipment. Residues in Meat and Meat Products: Feed and Drug Residues. Sausages, Types of: Emulsion

Further Reading

Anonymous, 1983. A glossary of Old World sausage. Meat Industry Magazine, pp. 46–51.

Faustman, C., Swartz, D., 2007. Basic Meat Chemistry. Meat Processing Technology Series. Savoy, IL: American Meat Science Association.

Pearson, A.M., Gillet, T.A. (Eds.), 1996. Herbs, spices and condiments. In: Processed Meats, third edn. New York, NY: Springer, pp. 311–329 (Chapter 12).

Ranken, M.D., 2000. Handbook of Meat Product Technology. Oxford: Blackwell Scientific.

Rust, R.E., 1971. Sausage and Processed Meats Manufacturing. Arlington, VA: American Meat Institute.

Sierengowski, R., 2004. Ethnic sausages. Food Product Design 13 (11), 76–84.

USDA FSIS (US Department of Agriculture, Food Safety Inspection Service), 2005. Labeling Policy Handbook. Available at: http://www.fsis.usda.gov/wps/wcm/connect/7c48be3e-e516-4ccf-a2d5-b95a128f04ae/Labeling_Policy_Book_082005.pdf?MOD=AJPERES (accessed 15.01.14).

Relevant Websites

http://www.foodsubs.com/MeatcureSausage.html
The Cook's Thesaurus.

http://www.hot-dog.org/ht/d/sp/i/38586/pid/38586
The National Hot Dog and Sausage Council.

http://en.wikipedia.org/wiki/Sausage

http://busycooks.about.com/library/archive/blsausagetype.htm

http://localfoods.about.com/od/meatpoultry/tp/sausagestypes.htm

Dry and Semidry

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Glossary

Decarboxylases Enzymes that transform an amino acid into an amine.

Glycolysis Enzymatic breakdown of carbohydrates with the formation of pyruvic acid and lactic acid and the release of energy in the form of ATP.

Lipase Enzyme that catalyzes the release of fatty acids by hydrolysis of triacylglycerols at positions 1 and 3.

Lipolysis Enzymatic breakdown of lipids with the formation of free fatty acids.

Peptidases Enzymes that catalyze the release of an amino acid from the amino terminus of a peptide (exopeptidases) or that hydrolyze myofibrillar proteins to polypeptides (cathepsins and calpains).

Proteolysis Enzymatic breakdown of proteins with the formation of peptides and free amino acids.

Water activity (a_w) Indicates the availability of water in food and is defined as the ratio of the equilibrium water vapor pressure over the system to that of the vapor pressure of pure water at the same temperature.

Introduction

Dry and semidry sausages constitute products with a long history of consumption. In fact, drying and fermentation may be considered as some of the oldest ways of preservation of meat. The use of drying probably originated in areas with mild winters and relatively low rainfall, like the Mediterranean area, due to its particular climate that allows natural drying and ripening. Dry fermented sausages were already produced and consumed by ancient Romans and Greeks. However, the use of cooking and smoking was applied in northern and colder areas where the climate did not allow natural drying.

The application of pure bacterial cultures as starters constituted a relevant technological advance in the manufacture of fermented sausages. The lack of consistency in quality and safety due to the typical use of back-slopping, a common practice when producing traditional sausages, could be overcome with the advent of such starter cultures.

In general, it is very difficult to classify fermented sausages because of the different processing technologies applied and the large variety of different products available in the market with very different appearance, texture, color, and flavor. One of the most extended definitions, dry and semidry, is mainly based on the extent of drying.

Definitions of Dry and Semidry Fermented Sausages

The main classification of fermented sausages is usually based on the extent of drying and degree of water loss. For a given sausage, this is usually in correlation with the time of ripening. So, fermented sausages may be defined as semidry when the weight loss is less than 20% or dry when the weight loss is greater than 30%. The length of the ripening period will vary depending on the kind of product, the desired final quality and its diameter. Semidry sausages are generally stable at ambient temperature because of a combination of lowered pH (<5.3) and lowered water activity ($a_w<0.95$). This stability is sometimes enhanced by heat treatment and/or smoking to inactivate most undesirable microorganisms except resistant spores. Dry sausages have lower a_w values (usually <0.89) as a consequence of the extensive drying, which make them stable even if the pH is not lowered so much. These sausages may be smoked but never cooked.

There are also large differences depending on the type of processing technology (use of starters, temperature applied, surface molds, smoking, etc.). For instance, traditional sausages are manufactured at low temperatures, using natural casings and without the addition of starter cultures. However, most of the industrial sausages produced worldwide involve the use of starter cultures. In some particular cases, chemical acidulants (e.g., glucono-delta-lactone) may be used; of course, there is no fermentation in this type of product and the sensory quality is rather poor due to the lack of flavor. Also, the presence of surface mold growth is desired by some populations whereas others request mold-free surface. The same can be said about smoking. Culture and gastronomic heritage have a strong influence on the type of products preferred by each particular population.

Technology of Dry and Semidry Sausages

It is important to use raw materials and ingredients of high hygienic standard because there are no alternative ways for efficient reduction of the initial microbial contamination. Once the hygienic requirements are met, care must be taken for the composition of the sausages in order to obtain a good quality. The right amount and type (mono-, di-, oligosaccharides) of carbohydrates must be used to regulate the lactic acid production. A low concentration ($<2\%$) of curing salts (generally a mixture of 0.5% $NaNO_2$ and 99.5% NaCl) favors the growth of undesirable microorganisms more than starter cultures; a high concentration ($>4\%$) inhibits the growth of the starter cultures. A relatively high concentration ($>10^5$–10^6 organisms per gram in sausage) and high

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metabolic activity of the starter culture is a precondition for a good sausage. Nitrite is very reactive and exerts specific protection against *Clostridium botulinum*. When nitrate is used, as for the slow-cured traditional sausages, the enzyme nitrate reductase slowly generates nitrite. It is important to control the amounts of nitrate and/or nitrite added to the initial mixture to those amounts strictly necessary for protection against botulism due to the risk of generation of N-nitroso compounds which have toxic effects.

An acceptable compromise is also necessary for the right fermentation and ripening temperature, considering the temperature requirement of the starter culture. To achieve an optimal rate of weight loss, an essential requirement from both the economic and quality points of view, the drying rate has to be controlled very carefully by adjusting the differences between relative humidity (RH) and equilibrium relative humidity (ERH) and air velocity depending on the type (low- or high-acid) and the diameter of the product. The typical process flow of dry sausage manufacturing is shown in **Figure 1**.

For the manufacture of dry and semidry sausages only casings with water vapor permeability should be used. Natural casings and some types of artificial casings are appropriate. For large-diameter sausages (salamis, 60–70 mm), natural casings from equine and bovine sources have previously been used; but due to an insufficient supply (horse) and because of the risk of bovine spongiform encephalopathy (BSE) (cattle), artificial casings are primarily used now. Artificial casings are usually based on cellulose or collagen because such materials have good water vapor permeability, which makes them suitable for dry sausage production. For smaller diameter sausages (25–38 mm), natural hog casing (small intestine) and collagen casings are mainly used. For intermediate calibers (40–50 mm), artificial casings are the appropriate choice.

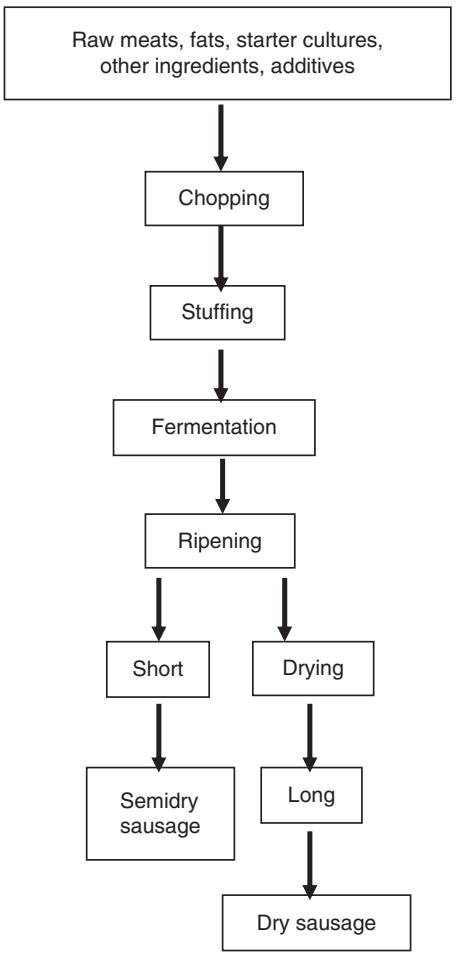

Figure 1 Process flow for dry and semidry sausage manufacture.

Sausages can be classified depending on different criteria; acidity rate, mincing size, starter addition, diameter, and type of casing used. A classification of dry fermented sausages can be done based on the manufacturing process, either industrial or traditional, considering traditional sausages those with no starter culture added. In addition, the industrial sausages can be separated into those submitted to a fermentation step (fast-fermented) and those not submitted to a distinct fermentation process (slow-fermented), resulting in high-acid or low-acid sausages, respectively.

Traditional dry sausages (naturally fermented sausages) are those manufactured in an artisanal way without the use of a starter culture. These sausages present a high diversity in sensory properties and are highly appreciated by consumers. These sausages are based on the natural fermentation of the meat batter through the autochthonous flora. Generally, these sausages reach a final pH greater than 5 and are therefore considered low-acid sausages. However, this manufacturing process is slow and may include safety problems. The low-acid dry sausages tolerate only slow drying rates that can be controlled via the relative humidity and the velocity of the drying air; with closer RH and ERH values, higher air velocity can be applied, and vice versa. Temperature has to be kept low ($<15\,^{\circ}C$) to control the growth of harmful microbes. In addition, traditional sausages dried for a long time use both nitrite and nitrate as curing agents.

Industrial dry sausages are manufactured using starter cultures in order to increase the quality and safety of the meat product. These sausages can be also manufactured with or without a distinct fermentation step. It is generally accepted to name those submitted to a step including fermentation conditions as fast-fermented sausages, and those without a distinct fermentation step as slow-fermented. The fast-fermented sausages can be processed by two different fermentation treatments or conditions; long fermentation done with constant conditions for 1–3 days at 18–24 °C or short fermentation using fermentation conditions and dwell times for up to 24 h. The fast-fermentation conditions favor the growth of lactic acid bacteria and the pH decline to values lower than 5. However, very fast pH decline can negatively affect the color of the product due to the inhibition of coagulase negative staphylococci (CNS) and also, result in an excessive acid taste that may be rejected by consumers. The fast-fermented sausages are dried for a relatively short time and only nitrite is used as the curing agent. These high-acid sausages can also be manufactured using glucono-delta-lactone (GDL) as an acidifying agent.

In the case of slow-fermented sausages that are not submitted to a distinct fermentation step, the process consists of a first stage where low temperatures are applied (10 °C) and then, the sausages are dried at higher temperatures (16–18 °C)

in order to dry and develop the sensory characteristics. These slow-fermented sausages have a mild pH decline (pH > 5), and are also known as low-acid sausages, but are considered safe because the starter added in the process improves the microbial safety of the product.

Semidry sausages with low pH tolerate relatively fast drying rates because the pH of the sausage is close to the isoelectric point of muscle protein, and the loosely bound moisture is easily lost. Starter cultures generally require higher temperatures (20 °C and above in Northern Europe and up to 37 °C in the United States) for their growth and metabolism, but the resulting pH decline controls the growth of harmful microorganisms. The smaller diameter products represent less risk in drying for both types of sausages.

Because elevated temperature also favors undesirable microorganisms, acidification should proceed as soon as possible for safety reasons. According to the voluntary guidelines of the American Meat Institute, the time elapsed until the pH drops below 5.3 can be up to 80 h if the temperature is 24 °C, but only 24 h if the incubation temperature is 38 °C. These guidelines are intended primarily to control coagulase-positive *Staphylococcus aureus*.

Cold smoking is applied in some cases and in some countries, less often in Mediterranean countries. Smoking has several advantages in addition to the special and typical flavoring effect. It helps by combating surface growth of undesirable microorganisms, and through its antioxidative effect it improves the stability of the product. Owing to the NO components in these products, color formation and color stability are also enhanced to some extent.

One of the main inhibitory factors against pathogenic and spoilage microorganisms in dry and semidry sausage is the low water activity. If a_w is low enough (< 0.89), it gives sufficient inhibition *per se* and the product becomes practically stable and safe regardless of the pH value. If it is higher (e.g., 0.95), combination with a low pH is necessary to ensure food safety. If the pH goes below 5.3, a product with an $a_w \leq 0.95$ becomes stable and safe. Reduction of a_w is usually achieved by adding common salt and especially by drying. In China another method is applied: in addition to a low amount of salt, a high amount (20–25%) of sugar is added, and a high drying temperature (50 °C or above) is used. Because small diameter sausages are usually manufactured in China, the required a_w decline is achieved quickly. The taste of these Chinese products is completely different from that of European or American types.

Processing Changes

During fermentation, ripening and drying significant changes take place resulting in a product with characteristic sensory qualities and a high level of safety.

During fermentation, the pH is reduced as a result of breakdown of carbohydrate to lactic acid (Figure 2). Application of homo-fermentative lactic starters is desirable because these only produce lactic acid as a major product with only very minor amounts of other components. Proteins coagulate due to the pH decline as a consequence of lactic acid generation and accumulation, giving better product cohesiveness and good sliceability. The low pH and the reduction in a_w during drying contribute to the selection of the microflora. The pH increases slightly in the later stages of drying as a result of the generation of free amino acids and ammonia.

One of the most important changes during processing is the weight loss as a consequence of loss of moisture by drying (Figure 2). This weight loss tends to be larger for long-ripened products (reaching up to 40%) and this is why they are also named dry sausages (Figure 3). The a_w value decreases in parallel with the weight loss. But for semidry sausages, the loss is lower (less than 20%). The final moisture of long-ripened sausages can be as low as 20%. The moisture:protein ratio (M:P) in this case is most often below 1.5:1. With short-ripened products, the weight is lost more rapidly due to the low pH and because the drying time is shorter. The typical weight loss with these items ranges from 15% to 25%, and the final moisture content of 30–40% gives sufficient stability and safety at a pH below 5.3. The M:P ratio in this case is above 1.5:1. In the case of nitrate (if used) and nitrite, the usual levels by the end of the process are generally very low (Figure 4).

The drying rate also depends on the initial moisture content, which can be adjusted by changing the meat to fat ratio or by lowering the moisture content with dry ingredients (nonmeat protein, oligosaccharides, etc.). If the initial moisture content is low owing to a higher fat content (lower a_w), the drying rate is slower than with higher initial moisture.

Flavor Development

The flavor of dry sausages is based on many chemical and biochemical reactions that occur during the fermentation and ripening/drying stages. Aroma formation is a complex process that generally begins with the enzymatic hydrolysis of protein and fat, generating flavor precursors. These precursors, free amino acids and free fatty acids, act as substrates for further chemical and microbial reactions generating flavor compounds. Also, fermentation of carbohydrates and spices contributes to dry sausage flavor.

Muscle enzymes (e.g., cathepsin D) play an important role only at the beginning of the ripening. In general, the enzymes from microorganisms play an active role in the breakdown of proteins and lipids by proteolysis and lipolysis, respectively (Table 1). Proteolytic enzymes hydrolyze muscle proteins and as a consequence, the amount of myofibrillar and sarcoplasmic proteins decreases whereas the concentrations of nonprotein nitrogen (NPN) compounds, polypeptides, amino-N and ammonia-N, increases, the latter contributing to the pH increase. Some lactobacilli (*Lactobacillus casei*, *Lactobacillus plantarum*) show an intense proteolytic activity whereas others (micrococci, staphylococci) contribute to lipolysis. Enzymes are affected by pH, a_w and temperature; the activity of cathepsin D is enhanced by a lower pH whereas aminopeptidases, active in the breakdown to amino acids, is enhanced by neutral pH. Molds are also active in the proteolysis as well as in other processes that have significance in mold-ripened sausages.

Endo- and exoenzymes take part in the lipolysis process, which play a decisive role in aroma formation. This rather complicated process can be characterized by hydrolytic and oxidative changes. Lipolysis consists of the enzymatic

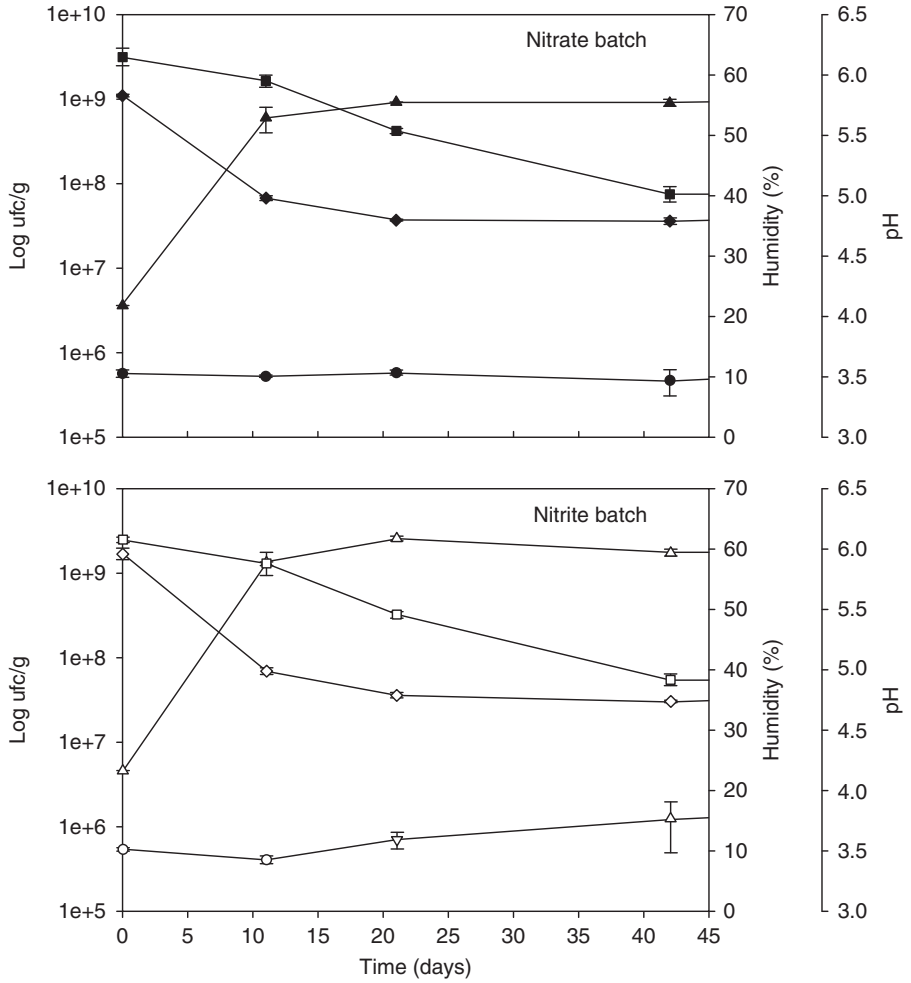

Figure 2 Changes in physico-chemical and microbiological parameters in slow-fermented sausages manufactured with nitrite or nitrate as curing agent. Lactic acid bacteria (LAB, ▲), staphylococci (●), pH (◇), and humidity (■).

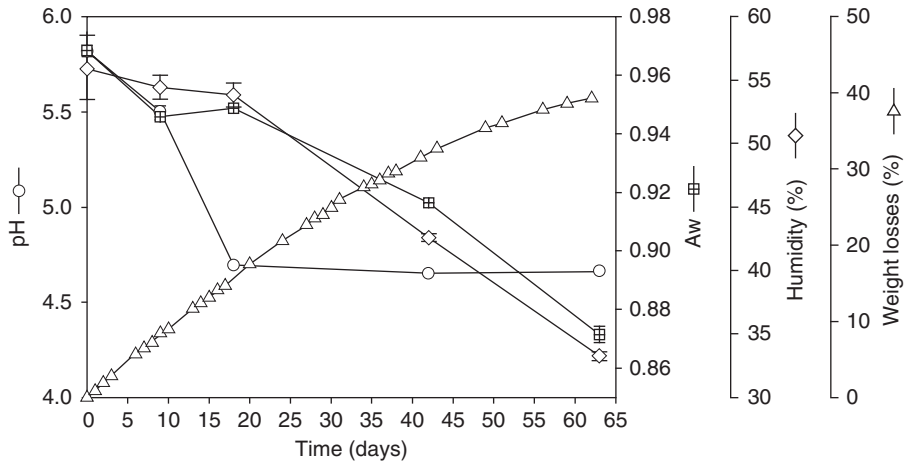

Figure 3 Changes in physico-chemical parameters in slow-fermented sausages manufactured with nitrite and nitrate as curing agent.

hydrolysis of muscle and adipose tissues generating free fatty acids. The initial free fatty acid content in the meat batter is approximately 1–2% of the total fatty acid content and it can increase up to 5% during the process. Many factors affect the lipolysis process such as processing parameters (curing agents, spices, starter cultures, pH) and the raw material used.

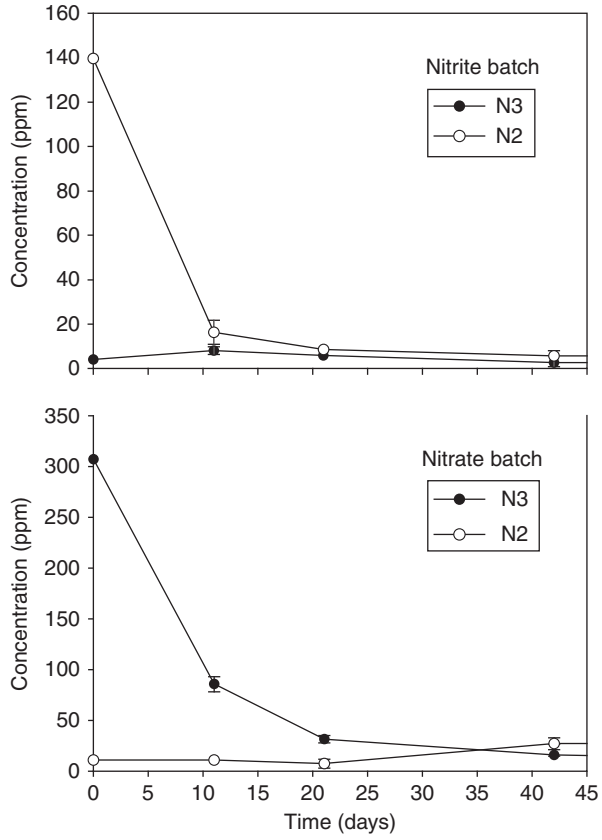

Figure 4 Changes in nitrite (N2) and nitrate (N3) concentrations in slow-fermented sausages manufactured with nitrite or nitrate as curing agent. Reproduced from Olivares, A., Navarro, J.L., Flores, M., 2009. Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. Food Chemistry 115, 1464–1472.

Finally, dry sausage flavor is generated through chemical and microbial reactions. The most important reaction is the chemical fat auto-oxidation that affects primarily the unsaturated free fatty acids. Depending on the metabolic pathway and its extent, lipid oxidation may contribute to develop a desirable flavor, but may also cause off-flavors. Lipid oxidation is autocatalytic and is mostly associated with unsaturated fatty acids; this is also why higher concentrations of unsaturated fatty acids in the sausage have an adverse effect. An excess of unsaturated fatty acids can occur, if the fat of animals on diets high in these fatty acids is used or if a significant amount of fat is replaced by vegetable oil in the recipe. Both of these may occur with an intention of improving the saturated:unsaturated fatty acid ratio with the aim of producing healthier food.

The extent of oxidation can be reduced either by using an antioxidant (not always successful when the drying lasts several months) or by smoking. Mold growth on the surface also helps by protecting lipids against light and oxidation by consuming oxygen, and by physical protection against access of oxygen to the fat.

In addition, other chemical reactions take part during the dry sausage process, such as the Strecker degradation of amino acids. Although the pH and temperature of the process limit this reaction, the low a_w, long ripening times and the increase in free amino acids favors the Strecker degradation. Moreover, other amino acid degradation reactions result from microbial metabolism (Table 1).

The microbial metabolism is responsible for the generation of many flavor compounds through different reactions; fermentation of carbohydrates, microbial amino acids degradation, lipid beta-oxidation, and staphylococci esterase activity. The fermentation of carbohydrates is performed in anaerobiosis through lactic acid bacteria producing lactic acid and other volatile compounds with high aroma properties (diacetyl, acetaldehyde, ethanol, acetic, propionic, butanoic acids, etc.). Microbial amino acid degradation is mainly done by coagulase negative staphylococci and yeasts (*Debaryomyces hansenii*) generating many aroma compounds through the degradation of sulfur, aromatic, and branched chain amino acids. Generally, these reactions are performed through a transamination reaction and a further decarboxylation reaction of the amino acid producing aromatic aldehydes. The lipid beta-oxidation is an oxidation reaction performed by the microbial metabolism (mainly *Staphylococcus* and yeast) generating short chain fatty acids and beta-ketoacids that are further decarboxylated producing methyl ketones that can be reduced to secondary alcohols. Finally, the staphylococci esterase activity produces ester compounds (mainly ethyl esters) through the use of an acid and alcohol by the esterase activity present in *Staphylococcus*. The generation of ester compounds is highly dependent on the substrates present in the sausage and the *Staphylococcus* strain used.

Hundreds of volatile compounds have been identified in dry sausages although not all of them contribute to the aroma. The differences in aroma compounds between naturally dry fermented and industrial dry fermented sausages are shown in Table 2. Many of the identified volatile compounds contribute to different aroma notes in both sausages such as acetic acid (vinegar odor), hexanal (fresh cut grass odor), 1-octen-3-ol (mushroom odor), methyl-3-methyl-butanonate (fruity odor) and 4-methylphenol (stable odor). However, several aroma active compounds were exclusively detected in one of the sausages being the detection frequency (DF) value, an indication of aroma potency (Table 2). Naturally dry fermented sausages were characterized by a highest proportion of ethyl ester compounds contributing to fruity and floral notes whereas industrial dry fermented sausages were characterized by aldehydes, compounds contributing to green, herbal, and tallowy odors.

Color Development

The intensity of color depends on the concentration of myoglobin in the meat used as raw material. Nitrite is depleted during ripening/drying. In fact, nitrite is reduced to nitric oxide and reacts with myoglobin to generate nitrosomyoglobin giving sausages a typical bright-red cured color. When only nitrate is used, which is the case of traditional dry sausages, an initial reduction to nitrite through the action of nitrate reductase is needed. Afterwards, nitrite rapidly reduces into nitric oxide that reacts with myoglobin. In these sausages, it is essential that pH does not drop below 5.0 because nitrate

Table 1 Examples of the main biochemical and sensory effects of purified enzymes isolated from microorganisms used as starter cultures in fermented meats

Group of enzymes	*Microorganism of origin*	*Main biochemical actions*	*Main sensory effects*
Aminopeptidase, tri- and di-peptidase, dipeptidyl-peptidase	Lactic acid bacteria and yeasts	Exopeptidase, generation of free amino acids and/or small peptides	Increase of free amino acids for flavor development
Catalase	Lactic acid bacteria	Dismutation of hydrogen peroxide into water and oxygen	Antioxidant properties. Decrease in the level of volatiles arising from lipid oxidation
Lipase	Lactic acid bacteria and Micrococcaceae	Generation of free fatty acids	Increase of free fatty acids for flavor development. Acceleration of maturation
Superoxide dismutase	Micrococcaceae	Detoxification of superoxide radicals into water and oxygen	Antioxidant properties. Decrease in the level of volatiles arising from lipid oxidation
β-Oxidation and thioesterase activity	Micrococcaceae	β-Oxidation involved in the synthesis of methyl ketones	Contribution to the cured aroma
Decarboxylase	Micrococcaceae	Final step of the β-decarboxylation process leading to CO_2 and methyl ketone	Increase in the levels of methyl ketones contributing to the cured aroma
Nitrate reductase	Micrococcaceae	Reduction of nitrate to nitrite	Nitrite generation. Antioxidant properties
Glutaminase	Yeasts	L-Glutamine amidohydrolase	Neutralisation of acidity by ammonium generation and flavor enhancement through the generation of L-glutamate
Protease	Yeasts and molds	Endopeptidase, able to hydrolyze sarcoplasmic and/or myofibrillar proteins	Protein degradation during meat fermentation and reduction of hardness. Acceleration of proteolysis

reductase is inhibited at lower pH values and thus nitrate would not be reduced to nitrite.

Texture Development

Texture development is the result of chemical and physical processes. Owing to the decline of pH during fermentation, myofibrillar proteins tend to coagulate and thus increase the product consistency which is then accelerated during the drying process. Of course, the consistency of the sausage depends on the extent of drying and subsequent water losses; dry sausages usually have a hard texture. However, the consistency may be soft if there is an excess of fat and water. So, the final texture depends on the degree of drying, pH decline, amount of fat, and extent of proteolysis, especially of myofibrillar proteins. A good consistency is necessary for an adequate sliceability of the product.

Safety of Dry and Semidry Fermented Sausages

Important conditions for assuring safe and stable dry and semidry sausages are the raw materials and ingredients of high hygienic quality and good manufacturing practices (GMP) and good hygienic practices (GHP) observed during manufacturing. The low temperature used for traditional sausages inhibits the growth of harmful microorganisms, and the reduction of the water activity inactivates most of them. In the case of semidry sausages, the low pH and the low a_w together inhibit growth and inactivate most of the harmful microbes.

Some pathogenic bacteria (*Listeria*, *Staphylococcus*, enterohaemorrhagic *Escherichia coli*) can tolerate the a_w reduction, whereas others (*Salmonella*, coliforms, clostridia) are destroyed during drying. As for staphylococci, low temperature and low pH are inhibitory for growth, and low a_w is inhibitory for enterotoxin formation. Enterohaemorrhagic *E. coli* (EHEC) are resistant to low water activity, although they are also inactivated (due to metabolic exhaustion) under the influence of a relatively low a_w for long periods at ambient temperature. Most spoilage bacteria are also inactivated due to the conditions found in the sausage.

In semidry sausages with low pH, staphylococci are also inhibited and inactivated in addition to Gram-negative microorganisms, but *Listeria* and EHEC strains may survive. This makes it even more important to use high-quality raw materials for raw fermented sausages. Highly active starter cultures also contribute to inactivation of harmful microorganisms by their high number (competition for nutrients), the rapid pH decline and eventually by the production of specific inhibitory substances (antibiotics, bacteriocins). Some semidry sausages are heat-treated, either immediately after stuffing or after a short period of fermentation. The heat treatment is sufficient to kill practically all vegetative forms of microorganisms and the less resistant spores. This ensures good hygienic conditions for drying.

Mycotoxic molds such as *Aspergillus* or *Penicillium* may release toxins so that the presence of a mold cover on the surface of sausages may be controlled. Preventive measures consist of inhibiting the growth of mycotoxic mold strains and the production of toxins by using nontoxigenic mold starters, the use of low temperature, control of relative humidity, and/or the application of smoking.

Table 2 Aromatic compounds in (traditional) naturally fermented and industrial fermented sausages

KI[a]	*Compound*	*Gas chromatography-olfactometry (GC-O) descriptor*	*DF*[b]	
			Naturally fermented sausage	*Industrial fermented sausage*
472	Methanethiol	Rotten	7	10
590	2-Methyl propanal	Fresh, cologne	5	6
630	Diacetyl	Butter, caramel	5	10
631	Methyl ethyl sulfide	Rotten onion, unpleasant		4
642	Ethyl acetate	Vegetal	4	
691	3-Methyl butanal	Green, herbal		10
702	Acetic acid	Vinegar	17	20
740	2,3-Pentanedione	Sweet, milky	5	
753	Methyl butanoate	Fruity	4	
784	Ethyl 2-methyl propanoate	Strawberry	12	
802	Methyl 3-methylbutanoate	Fruity	11	14
824	Ethyl butanoate	Strawberry, fruity	17	5
835	Hexanal	Fresh cut grass	12	17
861	Ethyl 2-hydroxy-propanoate	Fresh	4	
870	Ethyl 2-methyl butanoate	Fruity	9	
874	Ethyl 3-methyl butanoate	Fruity, floral	8	
872	Butanoic acid	Cheese	11	18
898	Unknown 1	Meat broth, savory	20	11
924	Ethyl pentanoate	Floral, fresh, fruity	4	
937	Heptanal	Herbal	5	8
963	Unknown 2	Roasted nuts, toasted	15	
966	Methional	Cooked potato	15	15
1007	2-Heptenal	Unpleasant, cabbage	12	
1010	2-Pentyl furan	Meat broth, savory, metallic		18
1020	1-Octen-3-ol	Mushroom	20	20
1025	Ethyl hexanoate	Flowery, sweet	10	
1031	α-Terpinene	Wood, metallic	14	
1035	2-Octanone	Floral, geranium		16
1045	Octanal	Citric	4	13
1109	Benzeneacetaldehyde	Roses	16	10
1147	Methyl benzoate	Fruity, sweet, waxy, metallic	9	
1159	Methyl octanoate	Fruity		10
1162	Heptanoic acid	Fresh, herbal	6	
1178	Unknown 3	Roasted nuts, toasted	14	15
1189	Unknown 4	Mustiness, woody	10	
1190	4-Methylphenol	Stable	16	15
1195	Phenylethyl alcohol	Roses	9	
1201	Unknown 5	Mustiness	9	9
1220	2-Nonenal	Medicinal		19
1222	Unknown 6	Wood, toasted, herbal	15	
1236	Methyl benzeneacetate	Caramel, sweet, toffee	7	
1288	2,4-Nonadienal (E, E)	Tallowy		7
1424	Ethyl decanoate	Fruity	4	
1443	Caryophyllene	Spicy, cloves	10	

[a]Kovats index calculated in a capillary column (DB-624; J&W Scientific 60 m×0.32 mm×1.8 μm).
[b]Detection frequency value.

Biogenic amines (tyramine, phenylethylamine, histamine, tryptamine, putrescine, cadaverine, agmatine) may be formed during fermentation by microorganisms that have decarboxylase activity. Amines may cause potential toxic effects in consumers due to the vasoactive and psychoactive properties of these compounds. These effects are more severe in those consumers treated with inhibitors of monoamine oxidase (IMAO) drugs. In most cases, biogenic amines are formed as a consequence of the activity of contaminant bacteria. Preventive measures consist of controlling the hygiene quality of raw materials as well as verifying the absence of decarboxylase activity in the microflora used as starter cultures.

Finally, the shelf-life of dry and semidry sausages depends on the combination of several factors, namely, pH, a_w, temperature of storage and type of packaging. Other factors that limit the shelf-life of these products are the extent of physical changes (i.e., fat melting or hardness due to excessive drying) and chemical changes (i.e., rancidity, discoloration) that occur in these products during the drying process.

See also: Chemical Analysis: Raw Material Composition Analysis; Standard Methods. Chemical and Physical Characteristics of Meat: Water-Holding Capacity. Drying. Ethnic Meat Products: Mediterranean. Fermentation. Sensory Assessment of Meat

Further Reading

Demeyer, D., Stahnke, L., 2002. Quality control of fermented meat products. In: Kerry, J., Ledward, D. (Eds.), Meat Processing: Improving Quality. Cambridge, UK: Woodhead Pub. Co., pp. 359–393.

Flores, J., 1997. Mediterranean vs northern European meat products. Processing technologies and main differences. Food Chemistry 59, 505–510.

Flores, M., 2011. Flavour of meat products. In: Nollet, L.M.L., Toldrá, F. (Eds.), Sensory Analysis of Foods of Animal Origin. Boca Raton, Florida: CRC Press, pp. 131–145.

Flores, M., Toldrá, F., 2011. Microbial enzymatic activities for improved fermented meats. Trends in Food Science & Technology 22, 81–90.

Marco, A., Navarro, J.L., Flores, M., 2007. Quantitation of selected odor-active volatile constituents in dry fermented sausages prepared with different curing salts. Journal of Agricultural and Food Chemistry 55, 3058–3065.

Olesen, P.T., Meyer, A.S., Stahnke, L.H., 2004. Generation of flavour compounds in fermented sausages – the influence of curing ingredients, *Staphylococcus* starter culture and ripening time. Meat Science 66, 675–687.

Olivares, A., Navarro, J.L., Flores, M., 2009. Establishment of the contribution of volatile compounds to the aroma of fermented sausages at different stages of processing and storage. Food Chemistry 115, 1464–1472.

Olivares, A., Navarro, J.L., Flores, M., 2011. Effect of fat content on aroma generation throughout the processing of fermented sausages. Meat Science 87, 264–273.

Stahnke, L.H., 2002. Flavour formation in fermented sausage. In: Toldrá, F. (Ed.), Research Advances in the Quality of Meat and Meat Products. Kerala, India: Research Signpost, pp. 193–223.

Talon, R., Leroy, S., Lebert, I., 2007. Microbial ecosystems of traditional meat products: The importance of indigenous starters. Meat Science 77, 55–62.

Talon, R., Leroy, S., Lebert, I., *et al.*, 2008. Safety improvement and preservation of typical sensory qualities of traditional dry fermented sausages using autochthonous starter cultures. International Journal of Food Microbiology 126, 227–234.

Toldrá, F., 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Science 49, 101–110.

Toldrá, F., 2002. Dry-cured meat products. Trumbull, CT: Food & Nutrition Press.

Toldrá, F., 2006. The role of muscle enzymes in dry-cured meat products with different drying conditions. Trends in Food Science & Technology 17, 164–168.

Toldrá, F., 2007. Fermented meat production. In: Hui, Y.H., Chandan, R., Clark, S., *et al.* (Eds.), Handbook of Food Product Manufacturing, vol. 2. Hoboken, NJ: John Wiley Interscience, pp. 263–277.

Relevant Websites

http://www1.clermont.inra.fr/tradisausage/
Assessment and Improvement of Safety of Traditional Dry Sausages from Producers to Consumers. European Commission Research Project QLK1 CT-2002–02240, 2003–2006.

http://www.iata.csic.es/IATA/dcie/carn/
Research of the Author's Group on Cured Meats.

Emulsion

CL Knipe, Ohio State University, Columbus, OH, USA

Glossary

Cellulose casings Sausage casings made from cotton linters, which need to be removed before the sausage is eaten.

Chub A short, cylindrically shaped package typically used for bulk ground products but may also be used for cooked products.

Coextrusion The process of forming sausage into a cylindrical shape while simultaneously coating the sausage with a layer of collagen. The collagen sets up on the surface of the sausage to serve as a casing.

Link The process of twisting, or tying, a filled sausage casing to achieve the desired sausage length. Moreover, it is the single unit that sausages are merchandised in when sold in a casing.

Protein extraction The process of dissolving proteins from meat by mixing a salt solution with the meat.

Salt-soluble proteins Proteins that can be dissolved or extracted from the fibrous structure of muscle by using a salt solution and some form of size reduction and mixing.

Stuff The process of filling a sausage casing.

Introduction

Emulsion-type sausage products are made by finely chopping meat to form a stable mixture that binds water and traps and holds fat to form the characteristic texture of an emulsified product when cooked. The stable meat mixture is formed by extracting salt-soluble proteins from the meat ingredients by using either a bowl chopper or a combination of a mixer and an emulsifier. The mixing action of both the mixer and the bowl chopper creates a more even distribution of the functional nonmeat ingredients, such as salt (sodium chloride) and alkaline phosphates, with the meat and added water. Salt and phosphates are considered functional ingredients, as they alter the salt-soluble proteins in the meat in such a way that the proteins are more easily extracted and solubilized from the fibrous meat tissue. Salt increases the ability of meat to hold water during cooking, i.e., its water-holding capacity. Alkaline phosphates adjust the pH of the meat mixture to a point of higher water-holding capacity and protein extraction.

There is a synergistic effect of the combination of salt and phosphates on protein extraction and water-holding capacity of the meat mixture. The pyrophosphate form of the alkaline phosphates commonly used in producing emulsions has been shown to partially reverse the formation of actomyosin, returning some of the actin and myosin to their separate prerigor forms. Myosin is the most functional of the muscle proteins in terms of ease of extraction in a salt solution and the binding ability of the mixture when cooked.

The meat ingredients are commonly preblended with a portion of the meat block to maximize the functional properties of the added salt and water. Preblending is done by mixing coarse-minced pieces of 0.5 in. (1.5 cm or larger) meat trimmings with salt and water and held at refrigerated temperature for up to 72 h. This holding period allows time for the salt and water to migrate into the meat trimmings to enhance the protein extraction and the water-holding capacity of the meat. This process is most effective when applied to the lean portion of the mixture, because the protein content is much higher in the lean portion. However, it is known that salt stabilizes the fat cells against melting during the cooking process. For emulsified or finely chopped emulsions or batters, it has been shown that phosphates are not needed if the preblending procedure is used.

Air incorporated into the finely chopped batter is undesirable, both from the standpoint of binding ability of the protein and the visual appearance of the final product. Vacuum chopping, mixing, and emulsification are important factors in removing this air.

The extracted protein, also known as a protein exudate, serves as the protein glue to bind the protein fibers together into a viscous matrix, which traps or holds the fat particles and water in the product when the product is cooked. Ice is often added during the chopping process to keep the meat mixture cold during the time the chopping and mixing would normally cause a temperature increase in the mixture. By keeping the mixture cold longer, the chopping and mixing times are maximized, thus maximizing the extraction of the salt-soluble proteins. The water that results from the melting ice further dissolves the added salt to form a salt solution that aids the extraction of the salt-soluble proteins. If the mixture becomes too warm, the fat in the mixture may become soft and melt, forming unsightly fat pockets on the surface of the products that become apparent during the cooking process.

Comminution Methods

The two basic processes for forming a finely chopped mixture are mixing–emulsifying and chopping. Both processes share the initial coarse grinding process that reduces meat chunks to manageable-sized meat particles.

The more traditional process of chopping involves the use of a bowl chopper or cutter (Figures 1 and 3), in which the protein extraction process can be maximized by first adding lean meat ingredients, salt, and ice or water first, to maximize protein extraction from the lean meat components, before the less functional or desirable ingredients are added to the chopper. With a bowl chopper, the variable speeds of the

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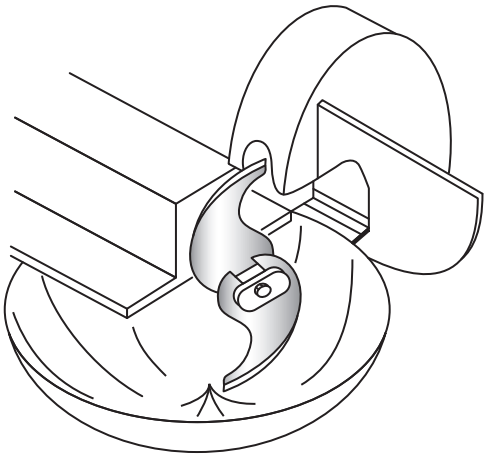

Figure 1 Bowl chopper. Reproduced with permission from Aberle, E. D., Forrest, J.C., Gerrard, D.E., Mills, E.W., 2001. Principles of Meat Science, fourth ed. Dubuque, IA, USA: Kendall/Hunt Publishing Company, p. 127.

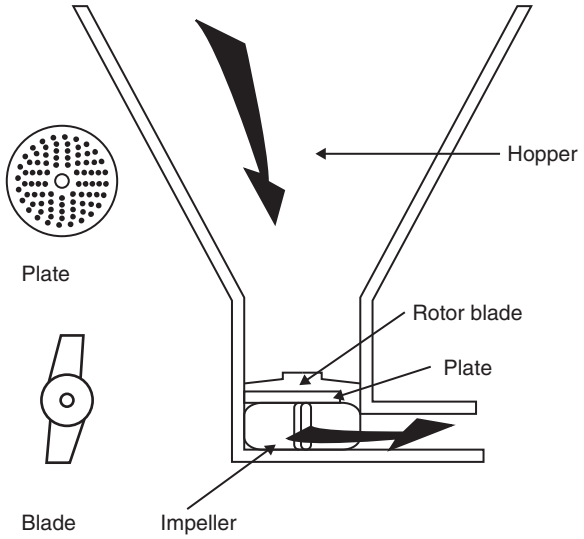

Figure 2 Cross-section of an emulsifier. Reproduced from Processed Meats, p. 127, 1995. AVI Publishing Company Inc., Westport, CT, USA, with permission of Kluwer Academic Publishers.

knives and the bowl allow extremes in action from simple mixing to high-speed emulsification. This process also allows the chopper operator to have control of the temperature of the mixture, which is important for the protein extraction process as well as for the stability of the fat component, particularly for products containing traditional fat levels. Proper use of the mixing speeds of a chopper also allows a processor to add nonmeat ingredients, such as cheese, pickles, pimentos, olives, etc., without damaging or more finely cutting these inclusions. The major disadvantage to the chopping process is that the chopper has a fixed limit to its capacity, making the process a batch process. This results in lower production volumes, compared with the mixing–emulsifying processes.

The mixing–emulsifying process involves two steps – a mixing step and an emulsifying step (Figures 2 and 3). Although some protein extraction occurs during the mixing and emulsifying steps, it is normally not as efficient as with the chopper process. Once all the ingredients are sufficiently mixed, the mixture is added to an emulsifier, which is a high-speed mincer or grinder. The emulsifying process is a high-volume, continuous process in which a finer texture is achieved, compared with that produced using a bowl chopper. Traditionally, the operator had less control of the mixture temperature, and the meat mixture temperature rise was proportional to the number of times the mixture was passed through the emulsifier. New technology now allows more precise control of the meat batter temperature as it exits the emulsifier.

Combining the chopping process with the emulsifying process takes advantage of optimizing both protein extraction with chopping and the finer texture of the emulsifying process.

In the true sense of the word 'emulsion,' a meat emulsion is considered to be a misnomer. True food emulsions involve an emulsifying agent, a protein, which binds to both fat and water to prevent the fat separating from the water (effectively allowing the fat to be dissolved and distributed in the water). In its role as an emulsifying agent, the hydrophobic end of the protein binds to the fat and the hydrophilic end of the protein binds to the water. In the case of meat emulsion, there are so many other factors affecting the stability of the mixture – such as muscle fibers, connective tissue, etc. – that the true emulsion theory probably does not apply. The final, finely chopped or emulsified, mixture is better described as a batter, mixture, or matrix than as an emulsion; however, the word emulsion continues to be most commonly used in the meat industry.

Emulsification Procedures

Whether mixing or chopping, the lean meat should be combined first with the salt and part of the water, usually approximately one-third of the total water to be added (Figure 3). If sodium nitrite and a 'cure accelerator' (sodium erythorbate, sodium ascorbate, ascorbic acid, or sodium acid pyrophosphate) are used, they should be added in the early stages with the lean meat ingredients. For chopping of emulsions, the fatter meat and another third of the water should be added, once sufficient protein extraction from the leaner meat has occurred. Finally, the spices, binders, cooked rework, etc. are added, along with the final third of the water, and chopping continues until the emulsion reaches the proper temperature.

In the case of a chopped emulsion, the final chopping temperature, before stuffing, would be determined by the dominant type of fat in the meat ingredients used (Table 1). These final chopping temperature guidelines are due to the inherent crystallization and melting temperatures of fatty acids of different species. Beef fat is unusual in that it has two final endpoint temperature ranges. Companies that traditionally make all-beef emulsified products typically use the lower temperature of 4 °C (40 °F). When using a mixer–emulsifier combination, preblended lean and fat meat mixtures are combined in a mixer, to which the spices, binders, cooked rework, etc. are added (Figure 3). The mixing process should continue only until the meat mixture has reached a

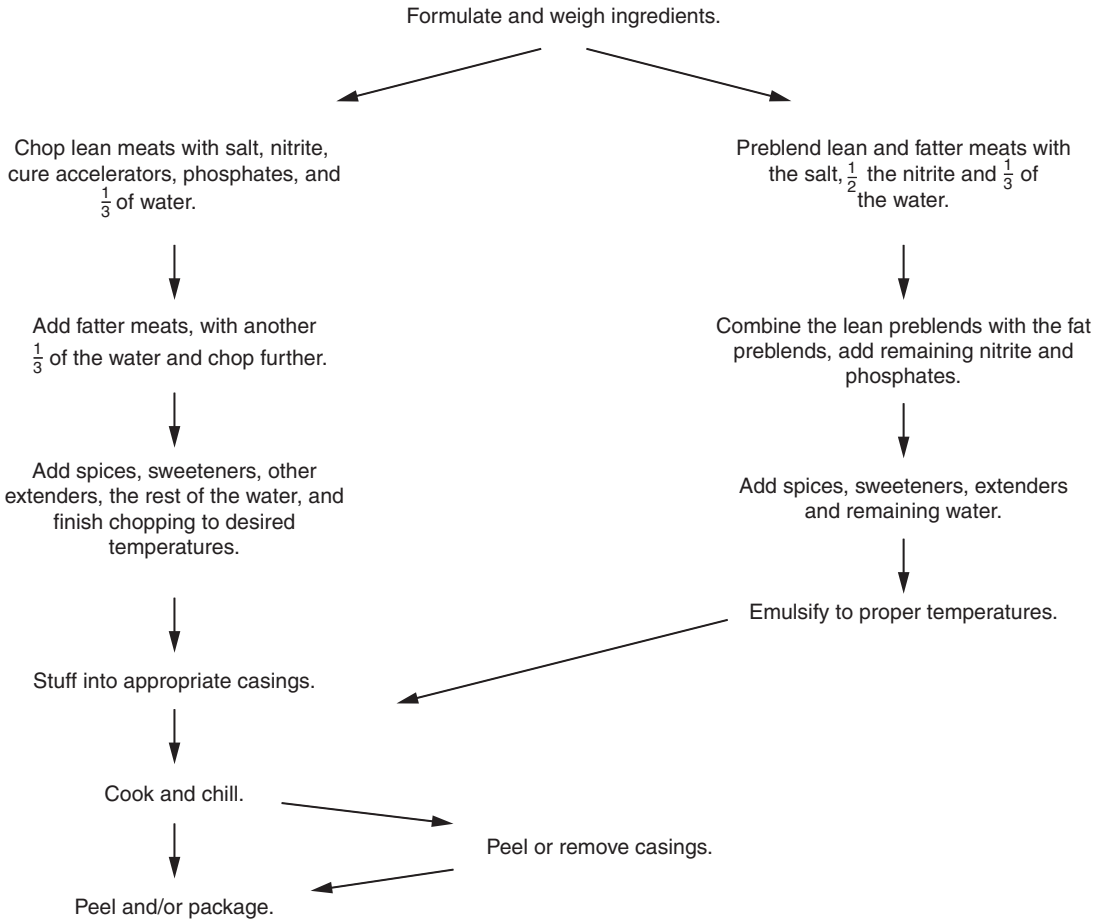

Figure 3 Flow chart for producing emulsified meat products.

Table 1 Final emulsion temperatures based on the formula's predominant fat source

Predominant source of fat	*Final emulsion temperatures in °C (°F)*
Chicken or turkey	7 °C (45 °F)
Pork	12–15 °C (55–60 °F)
Beef	4 °C (40 °F) or 18–24 °C (65–75 °F)

temperature 5–7 °C below the proper final emulsion temperature (Table 1). More precisely, the temperature difference between the final mixing and emulsifying should equal the temperature rise that is typical for the number of passes that the product is sent through the emulsifier. This mixture is then passed through an emulsifier one or more times, depending on the equipment and the temperature rise.

After chopping or emulsification, the mixture is stuffed into a casing to give it its distinctive shape and appearance and is then cooked either in an oven or in water. Cooked sausages that are cooked in an oven are typically smoked, using either natural or liquid smoke. Most cooked sausage products are fully cooked and referred to as ready-to-eat products.

The emulsion production system just described is based on the use of meat proteins as a base material. Some cooked sausage products, such as spreadable liver sausage or paté, are made without using the binding ability of raw meat proteins. The nonbinding materials in these products are essentially trapped in the cooked meat mixture. This lack of bind allows the product to be spreadable. There is also a sliceable version of liver sausage, which does depend on the bind of meat proteins discussed previously.

Rind Emulsions

Emulsions can also be made using pork rinds or skins. Rind emulsions are made by first cooking the pork rinds in water. The cooked rinds are then finely chopped, and sodium caseinate or soy isolate is added to stabilize the collagen and fat in the chopped rinds. This product is then chilled and added as a fat replacement ingredient in emulsified products.

Fat Preemulsions

Fat preemulsions are made to stabilize fat against the stress of cooking the sausage, thereby preventing the fat from rendering out of the product during cooking. Either sodium caseinate or soy isolate can be added to fat during a chopping process and after this mixture has set up, it can be added as a stable fat source to lean meat in order to produce a fine-chopped or

emulsified meat product. As a pre-emulsion, the fat is less likely to melt during cooking process than the fat that has not been preemulsified.

Causes of Emulsion Failure

Emulsion formation is based on the successful use of meat and nonmeat ingredients, stuffing these into casings and cooking the resultant sausages. Emulsion failures, often referred to as 'fatting out,' most often result in fat separation from the meat mixture and coalescence of the fat on the surface of the product, just under the casing. Emulsion stability is typically as good as the weakest link in the whole process. Most commonly, emulsion failures are due to the inability of the lean meat ingredients to emulsify or trap the fat sufficiently to prevent fat separation. This could be due to insufficient levels of salt or phosphates, too much fat or water being added to the formula, or chopping or mixing to a too high or too low temperature before stuffing and cooking. Also, emulsion failure may be due to damaged proteins, resulting from using improperly frozen trimmings or trimmings that are too old and partially spoiled. Abusing an emulsion by pumping it too far or holding an emulsion too long between emulsification and cooking may also cause an emulsion breakdown. Emulsion failures may also occur with properly made emulsions that are either improperly stuffed into the casings or cooked too rapidly or at too high relative humidity in the oven.

Emulsion failure may also result from the formation of gel pockets under the casing, which is normally due to the use of high-collage meat ingredients in combination with high-humidity cooking processes.

Types of Emulsified Sausages

Examples of emulsified sausages include: frankfurters, wieners, hot dogs, bologna, white hots (cooked bratwurst), bockwurst, knockwurst, Braunschweiger, or liver sausage. The descriptions of these products, and more, follow.

Bockwurst

The traditional bockwurst contains veal and pork, milk, eggs, chives, and parsley and is seasoned similarly to frankfurters; however, bockwurst does not contain nitrite and is not smoked. Bockwurst is stuffed into 1 in. (25 mm) diameter casings and cooked in water. Originally, bockwurst was sold as a fresh (raw) product but is now most often sold as a cooked product, because of its highly perishable nature.

Bologna

Bologna, which originated from Bologna, Italy, is made much like frankfurters and wieners. Bologna is most often stuffed in larger diameter (4–5 in./10–13 cm diameter) casings, sold in chubs or sliced for use as cold sandwich meat. It is also available as ring bologna, in which beef rounds are used for the casing and stuffed as 1 lb (454 g) loops. Bologna could be made with a coarse-ground texture but most commonly is a finely chopped product.

Bolognas often have a similar flavor to hot dogs, but they often contain more garlic to enhance the flavor, as they are most often eaten cold. Mustard is heavily used in most bolognas. Large-diameter bologna is not typically smoked.

Braunschweiger

Braunschweiger, or liver sausage, is typically made from pork trimmings, livers, and bacon trimmings and cooked in moisture-impermeable casings in water. This product may be cured or uncured, spreadable or sliceable, or fine chopped or coarse minced. The spreadable liver sausages are not considered emulsified, because to retain spreadability protein-to-protein bind needs to be minimized. Onion, nutmeg, and smoked bacon are typical flavors found in liver sausages. Nonfat dry milk is used to soften the harsh flavor of the liver. If cooked meats are used instead of raw ingredients, a spreadable product (see paté) is produced.

Cocktail Frankfurters

These are short, small diameter, finely chopped frankfurters, typically used as appetizers. Currently, cocktail franks are often produced by a coextrusion process, which does not require the use of a casing.

Frankfurters

This product is believed to originate in Frankfurt, Germany in 1487. The name frankfurter is often used interchangeably with frank, wiener, and hot dog. Frankfurters are typically 3/4 to 1 in. (20–25 mm) in diameter and are linked in 5 in. (12.5 cm) lengths. Frankfurters are prepared using either a natural casing or a cellulose casing and are typically smoked. The cellulose casing is removed before packaging of this product, which results in what is called a skinless product. Skinless frankfurters make up more than 95% of this product category. Frankfurters are most commonly sold in the fine-chopped, emulsified form but may be made as a coarse-ground product. Frankfurters are most often sold in packages of 10 per pound (454 g).

The flavor of frankfurters is typically due to black pepper, nutmeg, and possibly coriander. Garlic and onion may also be used in frankfurters. Mustard is heavily used in most hot dogs. Other spices may be used to produce a particular regional flavor. Because they are normally eaten hot, frankfurters would not be as heavily spiced as bologna, which is most often eaten cold.

Hot Dogs

Officially, hot dogs are wieners or frankfurters, which are sold and eaten in a bun. Hot dogs were first seen in the late 1800s, at baseball games in the United States, at which vendors decided to use bread, instead of the 'gloves' that had been used previously, to hold the hot sausages while eating. Frankfurters were often called 'dachshund' or 'little dog' sausages by German vendors. Although there is not total agreement on the origin of the name 'hot dog,' one version is that a *New York Journal* sports cartoonist, Tad Dorgan, was not sure how to

spell 'dachshund' in a cartoon he was drawing, so he simply wrote 'hot dog!'

Knackwurst

Knackwurst sausages are very similar to frankfurters. They are made with a fine-chopped texture, except that they are made using a larger diameter casing (1½ in./4 cm) than most frankfurters and linked in 3–4 in. (7.5–10 cm) lengths. Knockwurst sausages are known for their garlic flavor.

Mettwurst

This product was originally made in a couple of ways in its native Germany. In Southern Germany, mettwurst is made of pork smoked and sold as a raw, spreadable product. In Northern Germany, it is made similarly, but it was dried longer during the smoking process to make a dry product. In the United States, it is made primarily as a linked, cooked, and smoked sausage product.

Paté

Paté is a finely comminuted product like other emulsified products, but in order to make it a spreadable product, the emulsification process (discussed earlier) is not used for this product. Paté is made from fresh livers and precooked pork and cooked in a moisture-impermeable casing. This product lacks the bind of liver sausages made from raw meat ingredients and is, therefore, a spreadable product.

Vienna Sausages

Vienna sausages are short, small-diameter frankfurters often cut (open ends) from long lengths of stuffed casings and sold in cans or glass jars.

White Hots

Also known as a cooked bratwurst, this product is made much like frankfurters except that its product contains no nitrite and is typically not smoked.

Wieners

Wieners originated in Vienna, Austria. The wiener is essentially the same as a frankfurter, with the exception that originally it was made primarily from veal, rather than from pork and beef. Currently, there is very little difference between a wiener and a frankfurter, and the names are often used interchangeably, even though wieners have been defined as being smaller than 20 mm in diameter and frankfurters as being 20 mm or larger.

See also: Additives: Functional. Sausages, Types of: Cooked

Further Reading

Faustman, C., Swartz, D., 2007. Basic Meat Chemistry. Meat Processing Technology Series. Savoy, IL: American Meat Science Association.

Frye, C.B., Myers, K.L., Knipe, C.L., Sebranek, J.G., 1991. Effects of method of addition of sodium tripolyphosphate on the processing and storage parameters of preblended, coarse ground and emulsified sausage. Journal of Muscle Foods 2 (1), 37–48.

Pearson, A.M., Gillet, T.A., 1996. Herbs, spices and condiments. In: Processed Meats, third ed. Weimar, Texas: Hospitality Industry Publication Services, pp. 311–332 (Chapter 12).

Price, J.F., Schweigert, B.S., 1971. The Science of Meat and Meat Products. New York: W. H. Freeman and Company.

Ranken, M.D., 2000. Handbook of Meat Product Technology. Oxford: Blackwell Scientific.

United States Department of Agriculture Food Safety and Inspection Service, 2003. Labeling and Consumer Protection: Food Standards and Labeling Policy Book (May 2003). Available at: www.fsis.usda.gov/OPPDE/larc/policies.htm (accessed 04.02.04).

Relevant Website

www.hot-dog.org
National Hot Dog and Sausage Council, Chicago, IL (2014).

Fresh

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Glossary

Bockwurst German sausage made from ground pork, and/or lamb, turkey, chicken.
Hot boning Boning before chilling (before rigor).
Meat emulsion A two phase system, with the dispersed phase containing solid or liquid fat particles and the continuous containing water.
Mechanically deboned tissue (mechanically separated meat (MSM), mechanically recovered/reclaimed meat (MRM)) Obtained by forcing meat and bone, under high pressure through a sieve or similar device to separate the bone from the edible meat.
Mettwurst German strongly flavored sausage made from raw ground pork, which is preserved by curing and smoking.
Thuringer German mildly seasoned fresh or smoked sausage.

Introduction

Fresh sausage under the US regulations is defined as fresh, uncured (however, some are cured). Its taste, texture, tenderness, and color are related to the ratio of fat to lean content, and trimmings from primal cuts are often used as raw material. Ice or water is limited to 3% of the total formula. Fresh sausage must be stored under refrigeration and thoroughly cooked before consumption. Binders and extenders are permitted except when prohibited by regulation. There are essentially seven decisions when making fresh sausage: selection of raw materials (species, location of tissue in a carcass, fresh or frozen, and fat content); comminution of the tissue (emulsion to coarse grind); selection of seasonings (flavor) and additives (chemical and physical properties); mixing (uniformity); alteration of the protein (physical propersties and yield); shaping of product (selection of desired form and casing type, if used); and type of storage (normally refrigerated or frozen). Consumers are responsible for cooking (effecting flavor and safety of the sausage). The definition of fresh sausage is not always clear and covers a variety of items manufactured from meat tissue. Many nationalities often have their own specialities of fresh sausage that are tailored to their own organoleptic preferences. The most popular US fresh sausage is 'fresh pork sausage' made from individual pork cuts or from the total muscle mass of carcasses (e.g., whole hog carcasses), which is minced and seasoned. However, even the US fresh pork sausages can differ in texture, tissues used, seasonings, type of casings (or without casings), and meat/fat content. The most popular formats of pork sausages are links or patties that are usually consumed at breakfast with gravy (often pork sausage gravy) and eggs in a variety of cooking procedures. Fresh pork sausage is usually not smoked, but smoked variations are also available. All fresh sausages should be cooked before eating, even those that have been smoked.

Definitions

A definition (according to the US meat inspection) of fresh sausage can be found in the Code of Federal Regulations, 9 CFR 319 Subpart E.

'Fresh pork sausage' can be prepared from fresh or frozen pork, (not including pork by-products), can contain some mechanically separated (species) tissues, can be seasoned, cannot contain more than 50% fat in the finished product, and can contain a maximum of 3% water or ice.

'Fresh beef sausage' can be prepared from fresh or frozen beef (not including beef by-products), can contain some mechanically separated (species) tissues, can be seasoned, cannot contain more than 30% fat in the finished product, and can contain a maximum of 3% water or ice.

'Breakfast sausage' can be prepared from fresh or frozen meat (including meat by-products), can contain some mechanically separated (species) tissues, can be seasoned, cannot contain more than 50% fat in the finished product, and can contain a maximum of 3% water or ice.

'Whole hog sausage' is similar to 'fresh pork sausage' except that the pork must be in the same proportion as found in a single carcass and mechanically separated (species) tissue must likewise be in proportion of the species used.

'Italian sausage products' are more difficult to define because there are several subcategories, such as cured (must be in the product name) or uncured, cooked (must be in the product name) or uncooked, smoked (must be in the product name) or unsmoked, and seasoned (can include paprika, vegetables, and monosodium glutamate). They can include a maximum of 3% ice or water.

'Uncooked smoked sausage,' is smoked with hardwood or other nonresinous material, seasoned, and shall not contain more than 3% added moisture and no more than 50% fat.

'Pork sausage' or 'sausage meat' in the UK has 65% meat with 60% lean meat, of which 80% is pork; for beef and other sausages, the meat content is reduced to 50%, of which 50% must be lean meat and 50% must be named meat.

"Fresh Sausage" or "Sausage Meat" is usually pork but can contain some beef, chicken, and tripe. All fresh sausage should be cooked before serving.

"Smoked Sausage" is made from meat that is often cured (with some exceptions that take it out of some of the definitions of the fresh sausage category) and can be smoked and uncooked or smoked and cooked (excluded from fresh sausage category). Smoked-only products should be cooked before consuming.

Raw Materials

Tissues used for manufacturing fresh sausages should have a low bacterial count (because no heat is used to reduce their numbers) and the products should be handled under strict sanitation and the temperature should be maintained at or below 2 °C (35 °F) during mincing and mixing. The temperature should be lowered to 0 °C (32 °F) during holding and transit. For extended shelf life, the temperature could be lowered to −18 °C (0 °F) until shipped. Meats utilized for manufacturing include pork trimmings (e.g., 85–95% lean), regular pork trimmings, and cheek meat.

Color of the trimmings will influence the color of the sausage. Pale, soft, and exudative pork tissue will not only result in a lighter color but also result in more shrinkage when cooked. Dark, firm, and dry pork tissue will result in darker sausages with a pH more favorable for bacterial growth; however, this tissue will have increased water-holding capacity. The percentage of red (darker) as opposed to white (lighter) fibers in muscles is influenced by the animal's genetics and the location in the carcass, with the more active muscles (e.g., the heart, shank, neck, and many muscles in the fore quarter) being darker. Even in the hind leg area, different muscles have different amounts of pigmentation and different muscle fiber types. Species, age of the animal, and exercise will also influence the color. Beef and lamb tissues are usually darker than pork tissue, which is usually darker than chicken tissue. As animals age, muscles usually become darker, and as tissue dries out, it darkens. Mechanically deboned tissue contains more red pigment due to an increase in bone marrow and a decrease in connective tissue.

The quantity of connective tissue can have influence on tenderness and chewiness as well as on emulsifying stability in emulsified products. Again, the active shank muscles, cheek muscles, and a portion of the fore quarter are higher in connective tissues. However, fresh sausage is minced and is usually not an emulsion; therefore, tenderness and emulsifying stability are usually not a major problem. Fresh pork sausage in the US must use pork trimmings that do not exceed an average maximum of 50% trimmable fat. An entire pork carcass will yield approximately 50–70% of sausage tissue, if the whole carcass is utilized.

Seasoning varies according to the individuals manufacturing the products. Some of the more popular seasoning mixtures can be found in Table 1. In most cases, the proper blend must be selected to suit the required taste. There is a wide range of ethnic and regional preferences in seasoning. Areas that are more tropical and/or produce spices (e.g., India) generally prefer more spice intensity. Even in regions of the country that are in close proximity, flavor desirability can vary a great deal (e.g., fresh pork sausage with or without sage).

Binders and extenders are permitted (under the US regulations 9 CFR 319 Subpart E) in fresh sausage except where prohibited by regulations; for example, 'pork sausage' (9 CFR 319.140), 'beef sausage' (9 CFR 319.142), 'whole hog sausage' (9 CFR 319.144), and 'Italian sausage' (9 CFR 319.145).

Sex and age can influence boar taint, which can impart an undesirable flavor and odor to sausage products. Older, intact boars are the major (but not the only) contributors of this undesirable problem.

Casings

Sausage might be stuffed into casings, linked or not linked, or sold as sausage meat (loose or unstuffed). Natural casings are the small and large intestines of hogs and come in sizes of extra narrow (29 mm or less), narrow (28–32 mm), narrow medium (32–35 mm), English medium (35–38 mm), wide (38–43 mm), and extra wide (43 mm and above). Sheep casings can also be used; popular sizes are 20–22 and 24–26 mm. Animal casings are highly contaminated and fragile when removed from animal carcasses and must be cleaned immediately after harvest. Cleaning involves removal of mesentery, passage through a manure stripper, water soaking, passage through a crusher, further water soaking, passage through a mucosa stripper, passage through a finishing machine, and soaking in salt water. Factors that influence casing quality include age of animals, breed, fodder consumed, other factors related to animals (e.g., parasites), or conditions under which they were raised. After cleaning, the casings are sorted, salted, packed in salt, and shipped. Before the casings are used, they are often soaked in water to remove the excess salt. Reconstituted collagen (corium layer extracted from animal hides, usually beef) and extruded fibrous cellulose, polyethylene, and cloth casings or bags are also sometimes used.

Production and Types of Sausage

Prerigor sausage production (muscle removal from carcasses by hot boning before chilling) is gaining in popularity. This technique is particularly utilized when whole hog sausages are manufactured because tenderness is not a major problem when utilizing minced tissue. One major advantage of prerigor sausage manufacturing is extended shelf life (can almost be doubled) of the sausage as a result of the salt microbiologically stabilizing the fairly clean tissue before extensive microbial growth and increased chilling efficiency compared with chilling and then boning. Hot boning can also increase yield. Modern whole hog processors today often utilize 240–350 lbs (114–200 kg) butcher hogs to make this sausage. To facilitate mincing and mixing, ice or water may be used, provided it does not exceed 3% of the total ingredients used.

In principle, 'fresh sausage' is one of the simplest types of sausage to make, as it is usually neither cured and cooked nor smoked before being sold. It is simply minced tissue that is seasoned, mixed, and stuffed into a casing or sold in bulk.

The typical 'fresh pork sausage' is processed by mincing through a 12.5 mm (0.5 in.) plate, mixing, remincing through a 5 mm (0.18 in.) plate, stuffing, and then selling as fresh or frozen.

However, some products are cooked (meat precooked before manufacture). Some products after manufacturing are fried. Some are cold or hot smoked and then, in some cases, cooked (62 °C/142 °F; excluding it from the fresh sausage category). In some cases, the casing is peeled or the sausage may be molded rather than being in a casing. Precooked and peeled sausage is often called 'brown and serve' sausage.

Other types of fresh sausage are subsequently described.

1. *Pork sausage*: Owing to the large surface area exposed and the lack of heat treatment, maintaining an attractive color

Table 1 Additives and seasoning for some common fresh sausage products

The following values are per 45 kg (100 lbs) of the meat block[b]	*Pork sausage*[a]	*Pork sausage, highly seasoned*[b]	*Bratwurst*[b]	*Fresh Thuringer*[b]	*Bockwurst (white sausage)*[b]	*Mettwurst*[b]	*Polish (Kielbasa)*[b]
Salt, lbs[b]	1.1–2	2–2.5	2–2.5	2–3.5	1–3.2	2.5–3	2.3–3
Nitrite, oz[b]				0–0.15		0.25	0.25
Nitrate, oz[b]				0–0.15		0–3	0–3
Sodium erythorbate, oz[b]				0–0.87		0–0.87	0–0.87
Dextrose, lbs[b]	1/3–1/2	1/3–1/2 Corn sugar	0–1	0–1.5	0–2	0–0.7	0–1
Corn sirup, lbs[b]					3–4		0–2
Pepper, black, oz[b]	2–6 (or coarser butcher's)	3/8–2 Red pepper		0–6		0–4.8	0–6
Pepper, white, oz[b]	2–6	3–8	4–7	0–6	1–7	0–8	0–6
	2–4 oz Sage	3/8–1 oz Ginger	1/2 oz Ground celery seed	0–0.5 oz Mustard	0–1 oz Mace	0–1 oz Caraway	0–03 lbs Garlic
	0.25–2 oz Ginger	3/8–4 oz Mace	1/2 oz Mace	0–0.05 oz Nutmeg	0–4 oz Onion powder	0–4 oz Coriander	0–3.5 lbs Nonfat dried milk
	0.75 oz Nutmeg	3/8–1 oz Thyme	0–1 oz Rubbed sage	0–2 oz Coriander	1 oz Sage	0–4.8 oz Mustard seed	0–2 oz Coriander
	1 oz Thyme	2–5 oz Sage	2–8 oz Onion powder	0–4 oz Paprika	3 lbs Eggs		0–4 oz Garlic powder
				Sometimes starter culture	4.75 lbs Nonfat dry milk		0–3 oz Nutmeg

[a]Fresh country-style pork sausage' is ground a little coarser and is usually stuffed in casings 1–3/8 in. diameter, often unlinked or can be linked 8–10 in. long, or sold in bulk. It should be refrigerated before preparation and cooked before serving.
[b]Per 45 kg (100 lbs) of the meat block.

and shelf life in this sausage is difficult. Therefore, proper sanitation and product temperature at or below 0 °C (32 °F) should be maintained throughout the distribution and retail merchandising system as well as during home storage before cooking.

2. *Breakfast style*: This is a finely minced, all pork sausage that is seasoned with salt, pepper, and sage and stuffed into medium and large sheep casings, small hog casings, or regenerated collagen casings. It is linked in a variety of lengths to make different sizes of sausage. It is also merchandised in bulk and in patties. This product is rarely smoked, but it will not wrinkle if the smoking temperature is between 90 and 100 °F. This 'fresh' sausage is now also made precooked, often in links, but without casings or in patties (sometimes sold with biscuits). It is often sold at retail in the unfrozen state. The precooked product has a longer shelf life, and color is not a major merchandising problem.
3. *European pork and beef sausages*: These sausages can contain additional ingredients such as 10–15% binders (often rusks, stale ground bread), 11–26% water, phosphates, and a variety of seasonings, eggs, and tomatoes.
4. *American pork and beef fresh sausages*: Sausages that contain beef, in addition to pork, are sometimes smoked but are not cooked.
5. *'Smoked country-style pork sausage' and 'Smoked country-style sausage'*: These are smoked, uncooked sausages that should be cooked before consumption.
6. *Country style*: This type of sausage usually contains 10–20% beef and 80–90% pork. It is coarsely minced (0.18 in. or 5 mm plate) and does not contain sage. It is sold in bulk or stuffed into different sizes of hog casings or regenerated collagen casings and is not linked. This sausage is sometimes also smoked (2–3 h), and the added leaner beef will reduce wrinkling if the temperature does not get too high.
7. *Bratwurst*: This is a sausage manufactured from lean pork plus seasonings and sometimes with the addition of beef trimmings. Also, nonfat dried milk and eggs are sometimes incorporated. This mixture is minced through a 0.18 in. (5 mm) plate, stuffed into narrow hog casings, and usually not smoked, although the sausages can be smoked for 3–4 h at 120–130 °F. Sometimes, the product is also cooked (excludes it from the fresh sausage category) before it is sold at retail.
8. *Fresh Thuringer*: This is a beef sausage, sometimes with added pork, that is minced through a 6 mm (0.25 in.) plate and stuffed into medium casings. It may be cured and/or fermented, usually with a starter culture, and/or cooked before sale. If these treatments are used, it is excluded from the fresh sausage category.
9. *Bockwurst (white sausage)*: This is a veal or beef and/or pork sausage that often contains eggs and nonfat dried milk. It is minced through a 0.125 in. (3 mm) plate and stuffed into casings. It is very perishable and must be cooked before consumption.
10. *Mettwurst*: This is a pork or pork (30–80%) and beef (20–70%) sausage that is sometimes cured and stuffed into casings (1.5–1.75 in.), usually smoked and uncooked but should be cooked before consuming.
    a. Polish (Kielbasa): This is a pork or pork (70%) and beef (30%) sausage that is minced through a 6 mm (0.25 in.) plate, seasoned with garlic, stuffed into 1.5 in. casings, and cold or hot smoked; it should be cooked before consuming. It is sometimes cooked before retail sale.
    b. Italian pork sausage: Some varieties are uncooked, smoked sausage and should be cooked before consuming. 'Hamburger or burger' has several meanings to different people, depending on where it is purchased. Retail hamburger is simply fresh (uncooked) ground beef without any additives that is sold refrigerated, packaged with oxygen-permeable film or vacuum packed (which extends shelf life but the product is darker in color when opened), or frozen, thus fitting much of the definition of fresh sausage. Burger at retail is fresh ground beef often containing seasoning, additives, and/or cheese and vegetables, which is closer to the definition of fresh sausage. Hamburger and burger

Figure 1 Some of the forms of fresh Sausage. Top left: fresh pattey, top right: smoked, and bottom: fresh links.

if purchased at a fast-food restaurant is cooked (outside of fresh sausage category) and served with a bun (Figure 1).

'European fresh sausage-type products' include 'meat loaves,' 'meat pies,' 'puddings,' flans,' 'pates,' and fermented products such as 'salamis'.

Many other 'International products' also fall into the fresh category. For example, those containing soy can include 'Boerewors' (South Africa), 'Longaniza' (Philippines), 'Native Longaniza' (Philippines), 'Formulated Beef Patties' (USA), 'Formulated Gyro Patties' (Philippines), 'Mutton Patty or Maharaja' (India), 'Grilled Chicken Patty' (India), 'Beef Patty' (Indonesia), 'Pork Breakfast Patty' (USA), 'Turkey Sausage Patty' (USA), 'Turkey Breakfast Patty' (USA), 'Chicken Patty' (USA), 'Beef Kekaba' (Pakistan), 'Beef Burger' (Italy), 'Chicken Sausage' (Indonesia), 'Food Service Chicken Burger' (UK), 'Veal Pojarski Steak' (UK), 'Chicken Mignon' (UK), 'British Breakfast Banger' (UK), and 'Pizza Topping' (USA) from Hoogenkamp (2001).

Many more international fresh sausages containing other additives and extenders could be added to the list if space permitted.

Summary

Spices sometimes utilized in fresh sausages can be seen in Table 1, processing procedures are listed in Table 2, and finished forms for these fresh sausages are listed in Table 3. Because there

Table 2 Some examples of processing of fresh sausage

Pork sausage	*Bratwurst*	*Fresh Thuringer*	*Bockwurst (white sausage)*	*Mettwurst*	*Polish (Kielbasa)*
Chill 32 °F	Mince 0.18 in. plate or chop	Mince 0.125–0.5 in. plate	Chill to 34 °F	Chill	Chill
Mince 0.5 in. plate	Mix	Mix	Mince 0.125 in. plate or chop	Mince	Mince 0.25 in. plate
Mix	Stuff	Stuff	Mix	Mix	Mix
Mince 0.18 in.	Very perishable	Cold smoke	Stuff	Remince 0.18 in. plate	Stuff
Stuff	Sometimes water or steam cooked	Cure	Very perishable, can be frozen	Cure	Cold smoke
Chill 28 °F	Chill	Sometimes water or air cooked	Store at 28.5 °F	Mix	Sometimes water cooked
Sell fresh or frozen (1 month maximum storage)		Shower Chill If fermented, add starter culture, chop and allow 2–3 days for flavor to develop	Sometimes steam or water cooked	Stuff Cold smoke Shower Sometimes cooked Chill	Shower Chill

Conversions: °C = 5/9 (°F − 32); 1 in. = 25.4 mm; in plate indicates the diameter of holes in the plate.

Table 3 Finished form of different sausage types

Pork sausage	*Bratwurst*	*Fresh Thuringer*	*Bockwurst (white sausage)*	*Mettwurst*	*Polish (kielbasa)*
24–26 mm Hog casing	30–32 mm Hog casing	Beef middles	24–26 mm Hog or sheep casing	Beef rounds	30–32 mm Casing
28–30 mm Hog casing	35–38 mm Hog casing	2.75–3 in. sewn single-wall beef middles	4–6 in. Link	Wide hog casing	32–35 mm Hog casing
3.5–4 in. Links	4 in. Links	Sewn, defatted hog bungs	Wide beef casing		40–42 mm Hog casing
20–22 mm Sheep casing	21–23 mm Collagen casing	2.5 in. Casings	1 lbs Chub cellulose casing		Export wide beef round
24–26 mm Sheep casing		Fibrous casings			Beef middles
12 mm Cellulose casing		30–32 mm Hog casing			Cellulose casing
24–26 mm Cellulose casing					
Polyethylene bags					
3 in. cloth casing					
Bulk					

are many local and individual specialties in each category, selection as to which procedure to follow for a particular result needs to be made. Experiments should be carried out with various combinations before a large batch is manufactured.

See also: Sausage Casings. Sausages, Types of: Cooked; Emulsion

Further Reading

A.M.I.F. Center for Continuing Education, 1960. The Science of Meat and Meat Products. Chicago: AMIF.
Baley, J.W., 1970. Encyclopaedia of Labelling Meat and Poultry Products. St. Louis: Freezing Provisioning.
Brown, M.H., 1983. Meat Microbiology. London and New York: Applied Science Publishers.
Clemen, R.A., 1927. By-Products in the Packing Industry. Chicago: University of Chicago press.
Gerrard, F., Maliton, F.J., 1980. The Complete Book of Meat. London and Coulsdon: Virtue.
Hedrick, H.B., Aberle, E.D., Forrest, J.C., Judge, M.D., Merkel, R.A., 1994. Principles of Meat Science. Dubuque, IA: Kendall/Hunt.
Hoogenkamp, H.W., 2001. Soy Protein and Meat Formulations. St. Louis: Protein Technologies International.
Lawrie, R.A., 1985. Meat Science. Oxford, New York, Toronto, Sydney, Braunschweig: Pergamon Press.
Long, L., Komarik, S.L., Tressler, D.K., 1982. AVI Food Products Formulary Series, vol. I. Meat, Poultry, Fish, Shellfish. Westport, CT: AVI Publishing.
Mountney, G.J., 1976. Poultry Products Technology. Westport, CT: The AVI Publishing Company.
Ockerman, H.W., 1989. Sausage and Processed Meat Formulations. New York: Reinhold.
Ockerman, H.W., Hansen, C.L., 2000. Animal By-product Processing and Utilization. Lancaster: Technomis.
Romans, J.R., Ziegler, P.T., 1977. The Meat We Eat, eleventh ed. Danville, IL: The Interstate Printers and Publisher.

Relevant Websites

http://www.gpo.gov/fdsys/pkg/CFR-2011-title9-vol2/pdf/CFR-2011-title9-vol2-sec319-140.pdf
Government Printing Office.
http://www.gpo.gov/fdsys/pkg/CFR-2011.title9vol2/pdf/CFR-2011-title9-vol2-sec319-142.pdf
Government Printing Office.
http://www.gpo.gov/fdsys/pkg/CFR-2011-title9-vol2/pdf/CFR-2011-title9-vol2-sec319-145.pdf
Government Printing Office.
www.fsis.usda.gov/OPPDE/larc/Policies/Labeling_Policy.082005.Pdf
Labeling Policy Hand Book.

SENSORY AND MEAT QUALITY, OPTIMIZATION OF

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Glossary

Aging The process of meat tenderization and flavor intensification that occurs over time – it commences after rigor mortis.
Calpains Components of the enzyme system that acts on cytoskeletal proteins during meat tenderization.
Conditioning One of the terms used to describe the process when meat enters rigor mortis; it is sometimes used interchangeably with aging.
Connective tissue The noncontractile fibrous proteins (epimysium, endomysium, and perimysium) that are contained within or surround muscle fibers, bundles, and whole muscles – the amounts dictate the basal or 'background' tenderness.
Cooking Application of heat to meat that denatures the various proteins at different rates, depending on the temperature, resulting in various textures, colors, and flavors of cooked meat.
Harvest Used in preference to 'slaughter' in some countries and is the period from stunning to exsanguinations to processing of carcasses.
Myofibrillar proteins The muscle contractile proteins: actin and myosin.
Postmortem The period after harvest when the pH falls until rigor mortis and subsequent aging.
Preslaughter The period before harvest where factors such as stress can affect meat quality.
Rigor mortis A term where muscles stiffen after all muscle fibers enter rigor.
Shear force The force (N) applied to a standardized core, slice, or cube of meat to shear it.
Shortening A process that occurs when prerigor muscle is cooled below 10 °C – additionally, it also occurs as muscles enter rigor at high temperatures (rigor shortening).
Tenderization An enzymatic process that takes place after rigor mortis and makes meat tender.
Toughening Procedures that inadvertently cause meat to become difficult to bite or chew.

Introduction

Success of any product in the marketplace depends on its appeal and acceptability to consumers. Perceived quality, both at point of purchase and at point of consumption, usually depends on a combination of variables that are still screened and evaluated by human panels, even though many of the individual parameters can be measured or estimated by objective means. However, superimposed on the combinations of variables are national or cultural preferences for products. Brand names of products in many countries are important for the profitability of meat marketing and sales, but, in reality, brands are not an automatic guarantee of sensory aspects of quality. A shift to value-based marketing from the traditional commodity marketing system will require optimization of production and processing in order to guarantee quality.

This article briefly covers aspects of meat production, preslaughter stress, and processing that affect the sensory aspects of meat. Rather than merely providing satisfactory quality, these variables can interact to produce an optimum product by addressing the way meat animals are farmed and meat is processed. Other situations are more widely considered under texture variations. Endeavors to produce meat of optimum quality involve numerous technologies that are covered in articles throughout this Encyclopedia, impinging on aspects such as microbiological safety, processing, packaging, and transport. This article covers only those that have a direct effect on sensory aspects of eating quality of fresh meat. Processed meat products are not covered.

The sensory aspect of meat quality needs to be distinguished from food safety or the microbiological aspects of quality. In fact, the processing conditions for maximum food safety considerations often are contradictory to optimum sensory aspects of meat quality. It is informative to consider the way various aspects of refrigeration, microbiology, and meat quality interact by examining modeling processes.

The main requirement of consumers is palatability, which comprises tenderness, texture, juiciness, mouthfeel, and flavor with an overriding assurance of food safety. Tenderness is very important to consumers in most countries (high 'scores' indicate increasing desirability) and it is most conveniently determined objectively using a tenderometer (e.g., Warner–Bratzler shear instrument, Lee–Kramer instrument, or MIRINZ tenderometer), where low values indicate increasing desirability. Acceptability can occur over a range of values, so there is no

optimal value. A high degree of tenderness by itself does not guarantee a high degree of acceptance, for example, a high degree of tenderness is not necessarily acceptable if the meat is dry or mushy or contains off-flavors. In addition, other factors such as texture (affected by connective tissue content) and mouthfeel (conveyed by level of lipid components) are required for optimal palatability. Certainly, there should be a very desirable flavor and no off-flavors.

Visual Impact at Point of Sale

At the point of purchase, consumers depend almost exclusively on visual properties in order to assess perceived quality or on labels such as 'Prime' or 'Certified Angus Beef,' whereas at the point of consumption, they depend on olfactory, taste, and mouthfeel sensations to assess eating satisfaction. Hardly ever is there direct feedback from consumers to determine which visual attributes influence quality, but color generally is the most important trait affecting consumers' purchase decisions. Overlying the purchase aspects are obvious health-related concerns, such as levels of saturated fats or total fat content. An example is the level of marbling in meat, which is generally associated with desirable meat quality, particularly in beef. However, it has been shown that marbling accounts for only 12–14% of the variation in tenderness or even of other of palatability traits. Nevertheless, a certain degree of marbling is essential for optimum palatability, but excess marbling beyond a certain level does not increase palatability significantly. Also, marbling is not always seen as a positive attribute by some consumers, particularly those who are diet/health conscious.

Species, Gender, and Genetics

The factors influencing the sensory aspects of meat quality include intrinsic animal properties, such as gender, species, and genetics. To some extent, gender aspects, such as toughness in bulls, are related to a higher proportion of more insoluble collagen or stress effects, but gender-related effects, such as boar taint, are more predominant in males. Meat from cattle is generally less tender than meat from lambs and pigs. In general, tenderness and other palatability traits are often less desirable for intact males than for castrate males and females.

Genetics

Genetic properties of animals are important for obtaining the right characteristics for various production situations. Angus, Red Angus, Shorthorn and South Devon cattle, and Duroc and Berkshire swine breeds are best for high marbling. Animals with a *Bos indicus* content (≥50%) have generally less tender meat in most major muscles. However, with good electrical stimulation, these differences are less significant. Hindsaddle muscles of callipyge sheep are much less tender than those of normal sheep. Hampshire swine have tender meat but a lower water-holding capacity because of the Napole gene. Charolais or Simmental × Angus or Red Angus crosses of cattle optimize composition and meat quality. Heritability of tenderness and of marbling in cattle is approximately 0.40 and 0.50, respectively. Expected progeny differences for tenderness (measured by Warner–Bratzler shear force) have been published by some breed associations in the US. There are now commercially available genetic markers for beef and swine for quality traits. Within the beef industry, genetic markers for marbling and tenderness can be used. For swine, genetic markers for the Napole gene, the Halothane gene, and marbling are available.

Production

Production aspects, such as extensive pastoral management situations or intensive feedlot management situations, influence marbling and/or meat palatability traits. Feeding of supranutritional levels of vitamin E to cattle (e.g., 2000 IU α-tocopherol acetate kg^{-1} feed per day for at least 50 days) or pigs (e.g., up to 700 mg kg^{-1} α-tocopherol acetate from weaning to harvest) is a practical and effective way to improve meat color and can extend shelf life by 2 to nearly 5 days, with no reports of negative effects on feed intake or performance. It is suggested that this effect arises from accumulation of α-tocopherol in muscle tissue and this antioxidant delays both lipid and myoglobin oxidation and decreases drip loss. Such supplementation is less important for pasture-fed animals, for which there is usually sufficient vitamin E in the diet over most of the year.

Aggressive anabolic implants and some β-agonists can decrease tenderness. Growth rate and animal age influence connective tissue cross-linking that, in turn, affects tenderness. These characteristics mean that younger animals are preferred. Cattle and sheep finished on pasture produce meat that is different in color, flavor, and sometimes in tenderness than those finished on high grain diets. Consequently, there are two different consumer perceptions, and often preferences, of the subsequent quality from the two systems. Regardless of the production system, the rate of growth of muscle should be as near maximum as possible in order to minimize the negative role that collagen maturation can have on tenderness in cattle or lambs. It is probably fair to say that the best of both pasture and grain finishing systems is equally appreciated and depends on the quality characteristics to which consumers are accustomed.

Preslaughter

Even if genetic and production aspects are optimal, preslaughter factors can still modify the final product. Ideally, stress effects should be reduced by appropriate genetics, but some animals, such as Pietrain pigs (other pig breeds can also have similar effects), have an exceptionally fast glycolytic rate that can be triggered by immediate preslaughter events, leading to an aberrant meat quality defect known as pale, soft, and exudative (PSE) condition. In addition, stressing of normal pigs just before slaughter without allowing 2–4 h of rest can result in PSE pork. Long-term stress over 24 h can reduce muscle glycogen, leading to elevated ultimate pH values.

When the ultimate pH is in the range of 5.75–6.1, the meat is generally less tender, particularly for cattle, than the meat of lower ultimate pH. When the pH is too high, a condition such as dark, firm, and dry (DFD) is produced with meat that has undesirable color, flavor, and textural defects. Beef with high pH is acceptable for tenderness but not desirable for visual appearance or flavor. The DFD defect cannot be significantly modified by any aspects of processing once animals are harvested. To some extent, rapid chilling of pork carcasses can minimize PSE, but the rapid chilling can introduce other serious effects in non-PSE meat, such as cold toughening.

Processing

Assuming that intrinsic animal properties are optimal and preslaughter stress is minimal, the major extrinsic component of meat quality is processing. The effect can be so dramatic that it can override the benefits of any optimal production and preslaughter handling.

Cold Shortening and/or Toughening

When rapid chilling causes the meat temperature to fall below 8–10 °C while the pH is above 6, the muscles shorten to cause cold toughening. However, this situation does not occur in poultry until the muscle falls below a critical temperature of approximately 2 °C. The temperature for minimum shortening of muscle is 15 °C, although environmental temperatures may be well below this, and this is an optimum temperature for rigor mortis to occur. Cold shortening/toughening is more likely to occur in carcasses with thin fat covering. Therefore, rapid chilling to achieve microbiological safety has to be balanced with optimum temperature for tenderization.

Temperature and Reduced Aging

Mere avoidance of cold shortening by keeping temperatures elevated during processing can have another effect called 'hot toughening,' which really is a reduced aging capability rather than toughening per se. Hot toughening/reduced aging was previously labeled 'heat shortening,' but it now appears that the shortening, which does indeed occur, is not the main reason for toughening but rather the enzymes ordinarily responsible for tenderization are denatured, possibly through pH/temperature effects, with the result that the meat cannot tenderize to the same extent as in more conventional chilling. The protein denaturation that can occur also reduces juiciness and hence palatability. This so-called 'hot window,' where pH 6 is reached when muscle temperatures are above 30 °C, reduces palatability for nonelectrically stimulated beef and lamb. Heat toughening and reduced aging with rigor mortis occurring above 20 °C are far more significant in poultry than in sheep and cattle.

Electrical Stimulation

With a more rapid and controlled glycolysis induced by electrical stimulation, muscle pH values fall more rapidly, thus entering rigor mortis early and avoiding temperatures at which cold shortening/toughness would otherwise occur. For this reason, electrical stimulation has become a major processing technology. The situation in which a rapid fall in pH causes a PSE-like condition in pork is rare in beef and lamb, and once muscles are in rigor mortis, myosin denaturation is minimal. Thus, for electrically stimulated beef and lamb carcasses, not only does more rapid rigor mortis reduce myosin denaturation and drip, but also increasing stimulation effectiveness reduces the time to rigor and exposure to potential denaturation conditions. This occurs because some muscle fibers enter rigor almost immediately on stimulation and the rest of the muscle fibers rapidly follow. Some drip does eventually occur because tenderization occurring through cytoskeletal denaturation produces drip. The early appearance of drip following stimulation occurs as muscles tenderize rapidly, but there is no more drip for equivalent tenderization. Conditions that reduce drip generally reduce tenderization.

Tenderstretch

When carcasses are suspended by the pelvic bone (called 'tenderstretch'), some muscles are stretched, which minimizes or prevents shortening, although it cannot prevent it with extremely fast chilling. The tenderstretch method results in longer sarcomere lengths for some important muscles, consistent with tender meat. However, the hind legs fall in such a way that some leg and loin muscles may be shortened, but not to the degree that causes significant toughness. There are practical physical problems created in large plants with close rails with the hind legs extending laterally and cause movements of carcasses on rails difficult. Consequently, the 'tenderstretch' technique is not used in the larger North American plants. There are various modifications of this technology, such as 'tendercut,' in which the vertebrae are sawed at one or more places to allow some stretching of the longissimus muscle.

Hot and Cold Boning

The normal meat-processing situation in which the carcass is suspended by the Achilles tendon until rigor mortis, with subsequent boning the next day, is termed cold boning. Hot boning occurs when meat is excised before rigor mortis while carcass temperature is relatively warm, and as early as 45 min postslaughter. Rapid chilling of hot-boned meat can result in cold shortening, which is exacerbated by the absence of skeletal restraint. This is avoided to some extent by electrical stimulation because the accelerated rigor mortis occurs before the muscles have reached temperatures conducive to cold shortening. If the muscle temperatures are approximately 15 °C at rigor mortis, hot- and cold-boned meats are similar in tenderness.

If excised muscles are wrapped tightly during chilling, shortening can be minimized and the meat will be tender. A modification of this system has been developed in which the meat is fed into a tube containing a film that is stretched over the tube in such a way that allows the open tube to have meat placed in it. The stretched film then contracts to its prestretch dimensions over the meat, forcing out air and bringing the film into close contact with the meat product. The package

is then heat sealed and the meat is conditioned. The very tight packaging prevents muscle shortening.

Aging

Meat is actually least tender at the time rigor mortis is completed because of strong actin–myosin binding and minimal enzymatic proteolysis of the cytoskeletal proteins at that point but becomes tenderer over time by a process known as aging. Aging allows proteolysis (tenderization) to occur and has to be substantial in order for the full tenderness to be realized. It is fastest at warm temperatures, but clearly this has implications in terms of accelerated microbiological deterioration, so quality aspects still have to be considered in terms of food safety. At 15 °C, for example, it can safely be completed in 3–4 days, but other commercial aspects relating to food safety become an issue.

Aging of beef for a minimum of 10 days under commercial situations at a temperature of approximately 4 °C is often practiced. After electrical stimulation, aging commences earlier and at higher temperatures, so overall acceptable tenderness occurs earlier. Lower aging temperatures can be used; for chilled lamb held at −1.5 °C, the aging will take up to 6 weeks, allowing transport to markets distant from production and processing plants. However, it cannot overcome genetic contributions to toughness, toughness from low marbling scores, or toughness arising when the meat has been severely cold shortened. Aging can occur on the hanging carcass or wholesale cuts, where surface dryness prevents significant microbiological growth, or alternatively, the meat can be excised and placed in a vacuum package where the environmental conditions minimize spoilage situations. Tenderization is the same whether resulting from hanging of carcasses ('dry' aging) or storing of cuts in a vacuum bag ('wet' aging), but the flavor profile is reported to be different between dry and vacuum aging. 'Dry aging' results in a browner, roasted flavor, whereas 'wet aging' results in a more metallic, serumy flavor. Although the mechanism of aging is more likely to be the same for all species, there will be differences because of different glycolytic rates preceding aging and differences in the rate of aging itself, even at the same temperature. Poultry ages to an acceptable level in a few hours, pork takes longer, and beef and lamb take considerably longer again. The rapid growth of poultry and pigs to market weights mean that contributions from toughness associated with connective tissue are also lower.

Packaging and Storage

Packaging is covered in detail in other articles. Essentially, packaging is designed both for protection of the meat and for food safety. The packaging environment, of which there are many types, allows aging to develop to the stage at which meat has optimum quality. However, packaging is also important in that meat has to be shipped, not only within countries but also around the world. The storage conditions at low temperatures allow a high degree of aging to be achieved with minimal deterioration. Such meat is exceedingly well received by consumers because of the combination of microbiologically safe packaging and adequate aging.

Freezing of meat (either sufficiently slowly or at a rapid rate to avoid cold shortening) is used to preserve it. Frozen meat, if thawed before cooking and held for a few hours for water reuptake, is similar in palatability as fresh meat. Rapid thawing, such as at room temperature, will result in greater moisture loss and less juicy meat when cooked. However, there can be a significant problem if the meat is aged for long periods before freezing, in which case there can be some off-flavor development, such as rancidity.

Evaluation of Meat Quality Sensory Properties

Evaluation of meat objectively goes a long way to establishing protocols for producing high-quality meat acceptable to a wide range of consumers. However, in reality, sensory properties can only be measured by consumers or sensory evaluation panels. Both of these latter methodologies are expensive, primarily because they are very time consuming. Trained sensory panels consist of highly trained individuals who objectively evaluate the products. There are no accurate objectives or instrumental procedures for measuring flavor differences. Therefore, highly trained descriptive attribute or flavor profile panels are the most accurate methods for measuring them. However, in special markets, only consumers in those markets can be used for sensory evaluation. This makes it difficult for those countries that export meat products throughout the world and it adds to the costs of processing.

Cooking

The method, rate, and endpoint temperature of cooking can have highly significant effects on tenderness and palatability of meat and therefore constitute part of the major factors affecting the optimization of meat quality. Meat is cooked to kill microorganisms, to develop cooked flavors and aromas, and to improve tenderness of muscles with moderate to high levels of connective tissue. For muscles (cuts) with low levels of connective tissue, use of moderate to moderately high cooking temperatures in a dry environment (broiling and oven roasting) to endpoint temperatures of 60–70 °C optimizes tenderness. With moderately high, dry heat cooking on a belt grill, optimum tenderness of beef longissimus, biceps femoris, and deep pectoralis occurs at approximately 55, 60, and 60 °C, respectively. The primary advantage of higher levels of marbling is when meat is cooked to higher degrees of doneness. Cooking at a moderately high temperature and dry environment allows browning and development of cooked aromas and flavors and avoids toughening that results from excess moisture loss at higher endpoint temperatures. However, personal preferences for degree of doneness to attain desired flavor do not always allow optimum tenderness. With proper and controlled cooking, sufficient browning can result to optimize flavor and yet obtain optimum tenderness.

For cuts high in connective tissue, use of moist heat cooking (pot roasting and braising) at moderately low cooking temperatures optimizes tenderness by allowing conversion of connective tissue to gelatin without the toughening of muscle tissue itself that results from collagen shrinkage and associated

excess moisture loss. Maximum conversion of connective tissue to gelatin occurs when meat is in the temperature range 60–64 °C for a considerable time. An alternative to moist heat cooking is dry roasting at low temperatures for a relatively long time. This solubilizes the connective tissue without extensive collagen shrinkage and excessive moisture loss. It also allows browning and optimum aroma and flavor development.

Cooking meat to high endpoint temperatures (75–80 °C) causes myofibrillar toughening because of excessive moisture loss and shrinkage of the muscle fibers, but eventual tenderization occurs to some extent with solubilization of connective tissue. Conversely, the use of thin slices, as in a 'stir fry,' ensures that meat can be cooked to less than 70 °C rapidly but not be overcooked to the stage where connective tissue shrinkage causes toughness.

Guaranteed Tenderness

There is increasing interest in the US for guaranteeing acceptable tenderness of beef. The US Department of Agriculture's Agriculture Marketing Service has developed 'Standard Practice for Verifying Tenderness Marketing Claims Associated with Meat Cuts Derived from Beef.' The marketing claims that requirement can be used by all parties interested in highlighting production and marketing practices of tender beef. Because a significant proportion of beef is marketed before near maximum tenderization occurs, retail stores, restaurants, and food service operations might need to further age beef in order to meet the marketing claims criteria before it is sold to consumers. Because meat tenderizes throughout the distribution chain, measurements at packaging really need to be backed up by time and temperature monitoring. Marination and mechanical tenderization are considered 'noninherent' processes and are precluded from the use to meet tenderness requirements. Beef can be 'United States Department of Agriculture (USDA)-Certified Tender,' when Warner–Bratzler shear force (WBSF) is ≤4.4 kg (43.2 N) or slice shear force (SSF) is ≤20.0 kg (196.1 N), or 'USDA-Certified Very Tender,' when WBSF is ≤3.9 kg (38.2 N) or SSF is ≤15.3 kg (150 N). It is anticipated that this system will be used extensively. One major US beef processing company began using this program in 2013.

Conclusions

The optimization of sensory aspects of meat quality should not be considered as a single, unchangeable process. Factors to be considered depend on the end use of the meat and even the way meat is cooked. However, the main consumer requirement is palatability. Tenderness alone is not enough and other aspects of palatability, such as juiciness, texture, and flavor development, are also necessary.

See also: Boar Taint: Biological Causes and Practical Means to Alleviate It. Chemical and Physical Characteristics of Meat: Palatability. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Cooking of Meat: Cooking of Meat; Flavor Development; Heat Processing Methods. Cutting and Boning: Hot Boning of Meat. Electrical Stimulation. Human Nutrition: Cardiovascular and Obesity Health Concerns. Meat, Animal, Poultry and Fish Production and Management: Beta-Agonists. Meat Marketing: Market Requirements and Specifications. Microbial Contamination: Microbial Contamination of Fresh Meat. Modeling in Meat Science: Meat Quality; Microbiology; Refrigeration. Packaging: Equipment; Modified and Controlled Atmosphere; Overwrapping; Technology and Films; Vacuum. Preslaughter Handling: Preslaughter Handling. Refrigeration and Freezing Technology: Freezing and Product Quality. Sensory Assessment of Meat. Tenderizing Mechanisms: Mechanical. Tenderness Measurement

Further Reading

De Geer, S.L., Hunt, M.C., Bratcher, C.L., *et al*., 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Science 83 (4), 768–774.

Devine, C.E., Bell, R.G., Lovatt, S., Chrystall, B.B., Jeremiah, L.E., 1996. Red meats. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York: Marcel Dekker, pp. 51–84.

Dikeman, M.E., 1996. The relationship of animal leanness to meat tenderness. Reciprocal Meat Conference Proceedings 49, 87–101.

Ferguson, D.M., Jiang, S.-T., Hearnshaw, H.R., Rymill, S.R., Thompson, J.M., 2000. Effect of electrical stimulation on protease activity and tenderness of M. longissimus from cattle with different proportions of *Bos indicus* content. Meat Science 55, 265–272.

Gruber, S.L., Tatum, J.D., Scanga, J.A., *et al*., 2006. Effects of postmortem aging and USDA quality grade on Warner-Bratzler shear force values of seventeen individual beef muscles. Journal of Animal Science 84, 3387–3396.

Gursansky, B., O'Halloran, J.M., Egan, A., Devine, C.E., 2010. Tenderness enhancement of beef from *Bos indicus* and *Bos taurus* cattle following electrical stimulation. Meat Science 86, 635–641.

Jeremiah, L.E., Tong, A.K.W., Gibson, L.L., 1991. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. Meat Science 30, 97–114.

Obuz, E., Dikeman, M.E., Grobbel, J.P., Stephens, J.W., Loughlin, T.M., 2004. Beef longissimus lumborum, biceps femoris, and deep pectoralis Warner-Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. Meat Science 68, 243–248.

Rosenvold, K., North, M., Devine, C.E., *et al*., 2007. The protective effect of electrical stimulation and wrapping on beef tenderness at high *pre-rigor* temperatures. Meat Science 79, 299–306.

Thompson, J., 2002. Managing meat tenderness. Meat Science 62 (3), 295–308.

Watanabe, A., Daly, C.C., Devine, C.E., 1995. The effects of ultimate pH of meat on the tenderness changes during ageing. Meat Science 42, 67–78.

Relevant Websites

http://www.themainmeal.com.au/index.htm
Beef and Lamb Australia.

http://busycooks.about.com/od/howtocook/a/howtocookbeef.htm
Busy Cooks.

http://www.mla.com.au/Publications-tools-and-events/eNewsletters/fridayfeedback/General/Beef-essentials-iPhone-app
Meat and Livestock Australia.

http://en.wikipedia.org/wiki/Temperature_(meat)

SENSORY ASSESSMENT OF MEAT

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Glossary

Check all that apply (CATA) A sensory technique suitable for consumer studies. The consumers are presented with a list of words and are asked to check the words that describe the product.

Core temperature The temperature measured in the center of the meat.

Holistic by DMRI™ A sensory technique used in consumer studies. The consumers are presented with a list of abstract words such as appetizing, traditional, etc and are asked to rate the words depending on how descriptive they find them for the product.

International Standard Organization (ISO) An organization, which produces standards for various processes including that for sensory training and sensory laboratories.

Napping® A sensory technique which can be used both with a trained panel and consumers. The panelists are asked to place the samples on a piece of paper depending on how similar or different the products are.

Quantitative descriptive analysis (QDA) Describes the sensory profile of a product using trained assessors and standardized conditions.

Introduction

Sensory science has been defined as 'a method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing,' and this applies to all food categories. The assessment of meat can be conducted for both hot and cold samples depending on the purpose of the given assessment and the product at hand. Sensory assessment can be conducted using trained panels, where the panelists are used to give an objective description of the product, or untrained consumer panels.

The assessment of meat faces several challenges including the inevitable animal-to-animal variation that always exists despite attempts to remove this factor. Variation within the same meat cut is also an important factor to consider while planning a study. Even small variations in cooking procedures can introduce variation in the samples. Planning and performing a sensory assessment of meat, including subsampling within muscles, therefore needs careful consideration.

This article describes the conduct of sensory panels using both trained and consumer panelists of fresh meat and processed meat products including visual, oral, and odor assessments. The terminology as well as the standardization of procedures including cooking and presentation are described. Specific description for the sensory assessment of pork, beef, and lamb is also described.

Assessment Performed by a Trained Panel

A trained panel consists of assessors who have, as a minimum, passed an initial screening and have received a general training procedure. This can be a systematic training such as that described in the ISO standard no. 8586-1. The assessment is most commonly performed in sensory booths located in a room designed for the purpose in which light and air can be controlled according to the ISO standard no. 8589. If these facilities are not available, it is still important to perform the assessment in a well-ventilated and quiet room (Figure 1).

Classical Sensory Methods for the Evaluation of Meat

There are several methods available for the assessment of food in general. Roughly, the methods are divided into descriptive

Figure 1 Sensory assessment in a booth with controlled light and air flow.

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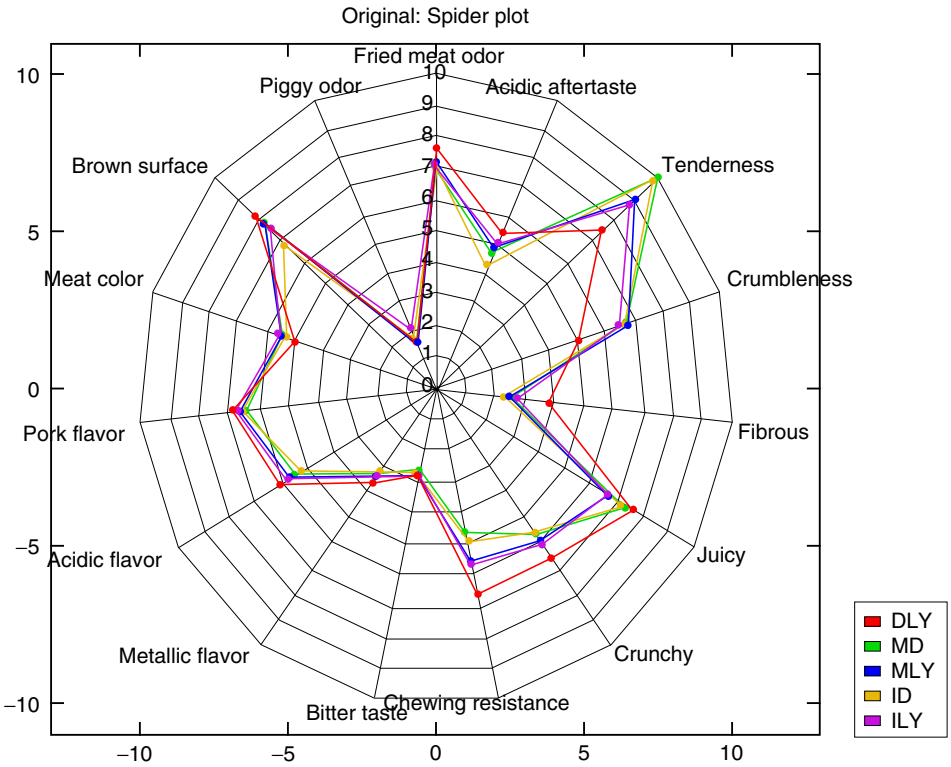

Figure 2 Example of a spider web plot of a sensory profile data of pork loin chops from five different crossbreeds D: Duroc, I: Iberian black foot, L: Landrace, M: Mangalitza, and Y: Yorkshire. It can readily be seen that the difference between loin chops from these pig breeds was especially related to texture attributes.

and discriminating methods. Most descriptive methods are various modified versions of the original QDA® (Quantitative Descriptive Analysis), also known as sensory profiling test. This type of analysis requires a trained panel and standardized settings as described above. The common features of these methods are (1) the initial training sessions, in which the panel agrees on a common vocabulary and (2) the following intensity measures of the selected attributes. The assessment is usually performed by 8–12 trained assessors with replicate assessments of each sample. The panels might be product-specific and thereby perform hundreds of hours of training to create consensus both related to the description of attributes and to the use of the scale (profile panels). The American Meat Science Association Meat Cookery and Sensory Evaluation guidelines involve initial screening of potential panelists by triangle tests, training, and then testing of trained panelists. Only those panelists who have statistically acceptable test scores are used on panels. However, some panels are oriented or trained for only 2–5 sessions before product assessment (descriptive attribute panels). The method is widely used to obtain detailed information about the products as shown in Figure 2. However, as this method is also time consuming and expensive, discrimination tests or rapid sensory methods might be a good alternative depending on the amount of information needed.

The group of discriminating methods is built upon various comparisons, often with a reference, for example, paired comparison test (two samples are compared), triangle test (two similar and one odd sample), duo-trio test (five samples are compared of which two vs. three are similar) and A-not-A test (two samples – are they similar or not). These tests can be used to gain information about differences in one attribute, for example, flavor, or if one needs a general difference between the products to be assessed. There is no training before the assessment.

Rapid Sensory Methods for Comparison with Untrained Assessors

In recent years, the development of rapid sensory methodology has provided several new methods. All these methods use untrained assessors and no replicate samples. With methods like Napping® and Projective mapping, it is possible to compare several samples at the same time. The samples are placed on a piece of paper according to their similarities and differences. The positions of the samples are then measured. This method is applicable in, for example, product development; as it is fast, there is no need for replicates or training, and it visualizes the product space.

Assessment Performed by Consumers

If the consumers opinion is wanted, it is necessary to do consumer studies instead of assessments with trained panels. Most frequently, consumer studies concern liking and preference, as these are subjective. To obtain significant results, it is recommended to include a minimum of 40–50 consumers per

consumer group (segment). If several segments of consumers are targeted such as two genders, different age groups, etc. then 40–50 consumers are needed in each group.

Alternatively, if 'linked' products can be used between consumer panel sessions, it is possible to use as little as 10 consumers, as is used in the Meat Standards Australia (MSA) grading scheme. 'Linked' means that the same product is used across consumer panels, to ensure standardization. The use of 10 consumers in the MSA program was determined after investigating the test accuracy and variation between muscles and balancing this with the physical restriction of the number of samples that can be derived from any one muscle as well as the cost restrictions. In addition, the same descriptions and consumer assessment sheets are used each time a consumer panel is conducted and products are 'linked' between consumer panels. The MSA scheme for assuring beef meat quality has used 10 consumers per product (muscle), linked products, and more than 67 900 consumers to determine the attributes mostly critical to consumer satisfaction of beef. Using the consumer data, a model has been developed to predict the quality of the muscles in beef carcasses and this model has been successfully tested and verified in more than nine countries around the world. This model and scheme is used to assure the quality of meat from 2 million beef carcasses per year and has resulted in increased returns to producers, meat processors, and retailers in Australia.

Questions asked in consumer surveys are often "how much do you like this meat sample?" or "which meat sample do you prefer?" and these two approaches can give slightly different results. As an example, Danish Meat Research Institute (DMRI) developed a healthy Frankfurter sausage with dietary fibers added and with a low fat content. Consumers tasted this sausage with a regular Frankfurter sausage and scored liking, followed by indicating which of the two sausages they preferred. Interestingly, the healthy sausage was equally liked by 66% of the consumers. However, in the preference test, most consumers preferred the regular sausage.

Moreover in the field of consumer science, rapid methods have been developed. One such method is CATA (Check All That Apply), in which the consumers check off all the words presented that they can apply to the meat product at hand. In principle, these words can be any words, but often abstract words are used such as traditional, appetizing, and boring.

Holistic by DMRI™ is a new method using abstract words for the investigation of consumer perception. In this method, a 15 cm unstructured line scale, with anchor points 'very little' and 'very much,' is used to measure the degree of association between the individual abstract words and the given product. In this way, it is possible to gain more insight by knowing how traditional or how appetizing consumers find the product. A close correlation between liking and a word like appetizing has been shown, so liking is also investigated using this method, though not directly asked. This method was used in a consumer survey regarding marinated pork chops. It was evidently shown that the consumers prefer marinates with a clear color and visual herbs and spices (**Figure 3** PCA-bi plot).

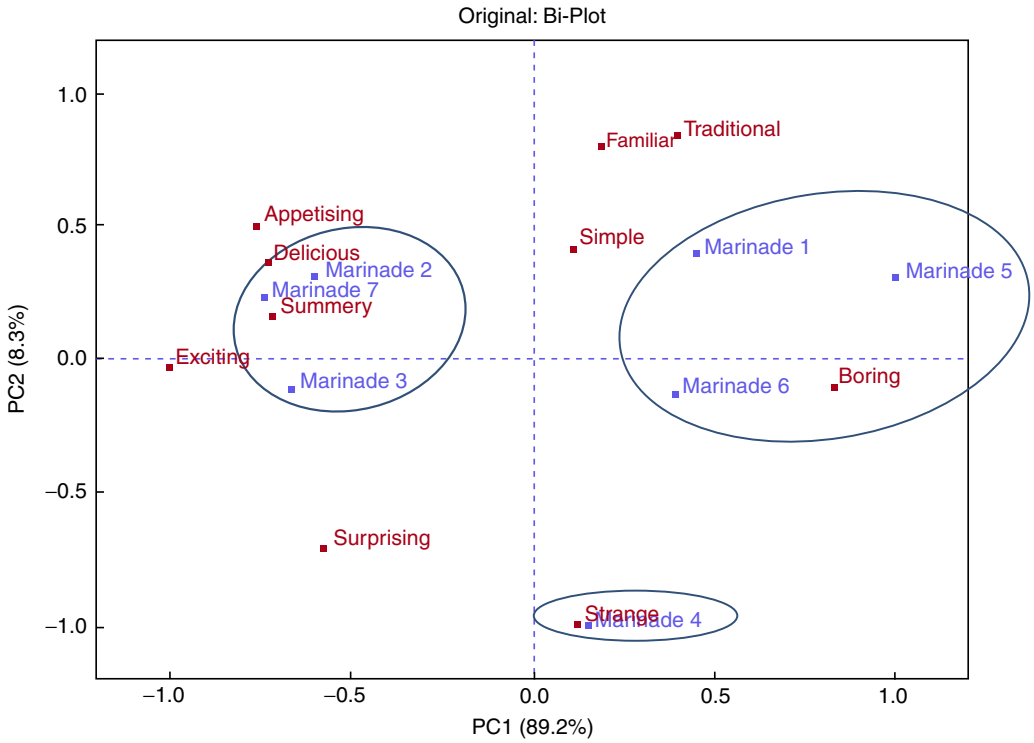

Figure 3 PCA plot of a holistic by DMRI analysis of marinated pork chops Marinades no. 2, 3, and 7 had a clear color and visual herbs and spices, and these marinades were associated with the words: appetizing, delicious, summery, and exciting. Marinades no. 1, 5, and 6 were more common BBQ-like marinades, and these were associated with the words boring, traditional, and familiar. Finally marinade no. 4 was associated with the word strange, probably due to a very pale color.

Factors to Be Considered before Performing a Sensory Analysis of Meat

Several factors have to be considered before starting a sensory analysis of meat. First of all, the cooking procedure, including cooking method, heating rate, and core temperature, is very important. The possibility of detecting differences in raw meat qualities depends on the cooking method used. As an example, the differences in crumbliness of longissimus dorsi and biceps femoris of pork are larger when the meat is cooked in an oven compared with pan frying. Furthermore, the two muscles cooked to a core temperature of 65 °C differ in 'piggy flavor' only when fried on a pan and not when cooked in an oven.

Choosing the Core Temperature

Several structural changes take place in the meat at approximately 65–68 °C. Random variations between samples are therefore relatively large in this temperature range. A slightly higher core temperature such as 70 °C can, therefore, be recommended unless special focus is on juiciness for which a lower core temperature of 60–65 °C will be more suitable. If the aim is to replicate the cooking used by consumers, the core temperature will depend on the cultural habits in the country for the given meat species.

Choosing the Cooking Method

As mentioned in the beginning of this section the cooking method needs to be chosen with care depending on the purpose of the study. For example, if the sensory analysis is going to show differences in fried flavor that might be dependent on the raw meat quality such as the glycogen content, a cooking method like frying or grilling is necessary to increase the amount of Maillard reactions generating fried meat flavors.

Sample Preparation and the Sensory Setup

When the cooking procedure has been decided upon, the assessors need to be trained. This is to ensure a consensus on the understanding of the attributes. During the training, references can be used, especially for the odor, flavor, and taste attributes.

It is very important to cook and present all of the meat in exactly the same way because it is differences in the meat that are being investigated, and not random variations in cooking. The frying pan or oven has to be checked regularly for the uniformity and stability of the temperature. In addition, the core temperature of each piece of meat, at the completion of cooking, needs to be measured to ensure that the desired core temperature is reached (Figure 4). Typically, the core temperature determines when the cooking process is completed.

Before presenting to the panelists, the meat is cut into a reasonable serving size. In most assessments, the meat is cut into a standard cube to mask differences in size of the cuts, but depending on the aim of the study, a whole or half a steak or roast slice is served, and this leads to the opportunity to assess the meat edge, as well as the fat cover.

Figure 4 A handheld thermometer can be used for assessing the core temperature of each pork chop.

To keep the meat warm until assessment, the meat should be placed on preheated plates and covered with a lid. The temperature of the meat should be measured by random sampling to ensure a uniform serving temperature.

The number of replicates within muscles and the number of animals depends on the aims of the study. If the aim is to investigate the effect of, for example, cooking procedure or aging time, a block design can be used in which animal is the block and all treatments are represented within each animal. This can minimize the number of animals required. However, if the study aims to investigate a treatment in the primary production such as breed or feed, more animal replications are needed, taking into account all possible sources of variation such as gender, growth rate, live weight, fat depth, etc. As a rule of thumb, 15–20 animals per treatment are needed to take the animal-to-animal variation into account whereas 5–10 replicates are necessary if the animal can be a block effect. For uniform meat products, as low as three replicates can be performed because the random variation between samples from the same experimental group is low. Furthermore, if consumers are used instead of trained sensory panelists, the variation between consumers in their response can mean that a higher animal replication is required. Thus a replication of >30 would be required.

Sensory Assessment of Pork, Beef, and Lamb

The attributes that the sensory panel is asked to rate for assessments of meat depend on the aim of the study and the cooking method. Fried flavor is only relevant if the meat is fried, whereas assessment of warmed-over flavor is only relevant if the experiment includes handling of the meat that is expected to result in these flavors.

Appearance can be assessed both on the meat surface and in the cutting edge. The surface reflects the meat's ability of obtaining the fried color and is often correlated with fried flavor. Furthermore, it is very sensitive toward small changes in the pan temperature, and the attribute is, therefore, seldom included. Several attributes can be assessed in the cutting edge

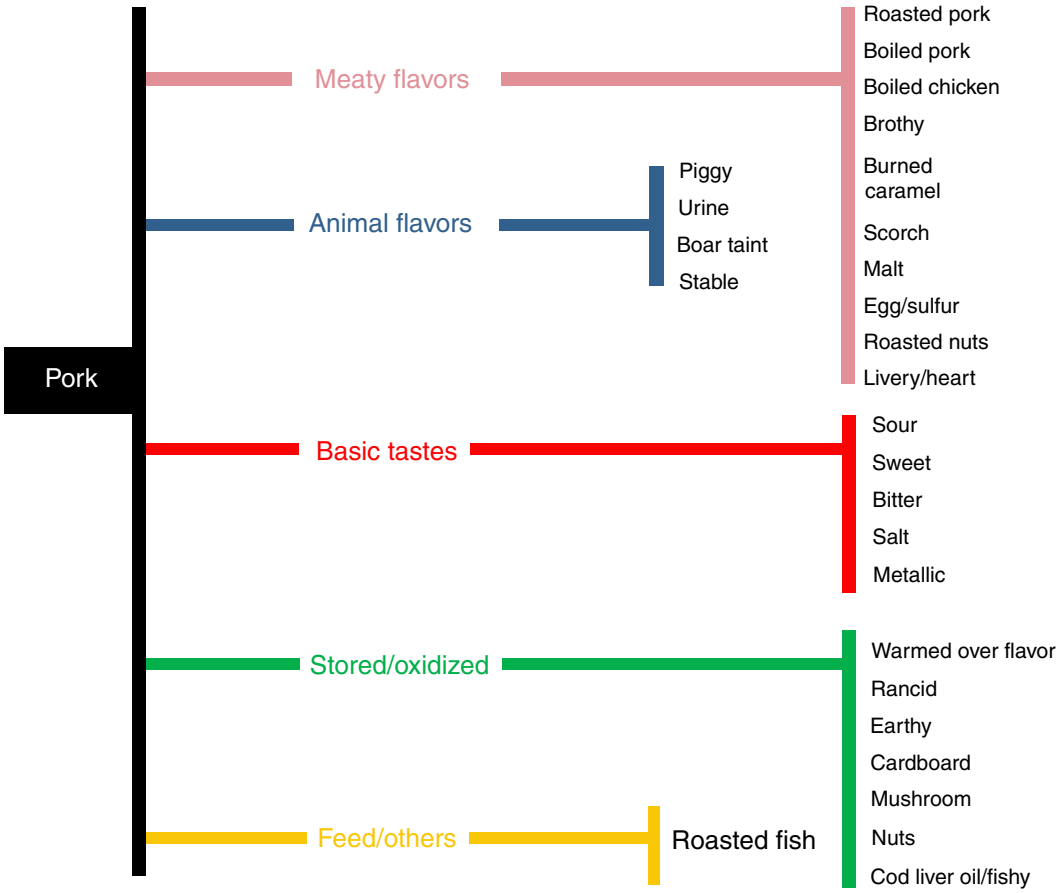

Figure 5 A flavor-tree of pork.

of the meat: degree of cooking (from red to gray) and meat color (from light to dark) represent the color attributes, whereas fibrous (small invisible fibers to large rough fibers) represent the meat structure.

Texture attributes include the group of attributes for which it is easiest to gain consensus among panelists. The attributes vary depending on the meat structure and include among others: bite resistance (hardness at the first bite with the molars), tenderness (easiness of breaking down the meat), springiness (the degree of springiness after a bite), and chewing rest (the amount of product in the mouth when ready to swallow). A comprehensive list of texture attributes is present in the ISO standard 11036. Juiciness is a special texture attribute. Early in the chewing process, the juiciness depends on the amount of loosely bound water in the meat, but later it is more dependent on the intramuscular fat content and on the panelists' ability for saliva production, as the meat at this time has grown up to 40% by absorbing saliva.

Flavor, taste, and odor attributes are the most complex and most difficult to gain consensus among the panelists. Flavors can be divided into several groups as demonstrated in the flavor tree in Figure 5, using pork as an example. Depending on the purpose, the overall descriptor such as stored/oxidized flavor can be used, or the more detailed descriptors such as rancid or earthy can be used.

Although the taste attributes (sweet, sour, acidic, bitter, salt, and umami) often relate to few compounds present in meat, the more complex flavor attributes relates to a cascade of different chemical reactions during cooking such as Maillard reactions, lipid degradation, etc. Using references is, therefore, very important while training the assessors, to ensure the same understanding of the attributes. Table 1 shows a suggestion of reference samples for flavor attributes of meat.

Sensory Assessment of Flavor of Lamb and Beef

Many of the same considerations from sensory assessment of pork are also true for beef and lamb. Differences between species in sensory assessment predominantly derive from the differences in flavor, as the texture and juiciness tend to be similar between species. Flavor is a very complex attribute of meat palatability and refers to the components of food responsible for chemosensory stimulation – volatile aroma and nonvolatile taste compounds. There are literally hundreds of compounds in meat that contribute to flavor and aroma. Many of them are altered through storage and cooking, making meat flavor an incredibly complex topic. Many compounds that contribute to meat smell and flavor are thermal lipid breakdown products.

Owing to the differences in digestive systems between ruminants and nonruminants, deposition of fatty acids is different. Poultry and pork muscle typically have higher levels of polyunsaturated fatty acids than lamb or beef. Furthermore,

Table 1 Examples of references for flavor and odor attributes of pork that can be used for training of panelists

Attribute	*Production of reference*	*Comments*
Meat flavors		
Roasted pork	Loin, pH 5.55–5.65, a boneless chop is dry roasted at 150–160 °C pan temperature, until a core temperature of 65–68 °C	Only the crust is tasted
Boiled pork	Loin, pH 5.55–5.65, a boneless chop simmered for 20 min	
Boiled chicken	Chicken breast simmered for 20 min	
Brothy	MSG powder or the cooking loss from a boiled pork chop	Is a little acidic as well
Burnt caramel	Sucrose melted on a pan for 5 min. Served chilled	
Burnt	Loin, pH 5.55–5.65, a boneless chop dry roasted at 250–280 °C pan temperature, core temperature of 80 °C	
Malt	Malt powder from SFK 5670/550973	
Egg/sulfur	Chopped boiled egg	
Roasted nuts	Fresh hazelnuts, chopped and roasted	
Livery	Pork heart boiled for 30 min or cooked beef liver	Pork liver is sometimes too strong to be used as a reference (therefore the heart is used)
Grassy	Hexanal	Accepted odor standard for 'grassy'
Hay	Sugarcane mulch	
Animal odors		
Piggy	Melted pork fat or a piece of cloth that has been into contact with live pigs	
Urine	Androstenone	
Boar taint	Skatole	
Stable	A piece of cloth from a stable	
Sheep meat	Not defined	
Mutton-like	Not defined	
Barnyard/farmyard	*p*-Cresol	
Basic tastes		
Sour	0.3 g Citric acid in 1 l water or sodium lactate 60 % added to minced meat yoghurt natural	
Sweet	1 g Sucrose in 1 l water or 1 g sucrose in 100 g minced pork	
Bitter	0.09 g l^{-1} Quinine chloride or 0.25 g l^{-1} caffeine	
Salt	0.5 g NaCl in 100 g minced meat	
Metallic	Copper coins or a silver spoon	
Stored/oxidized flavors or odors		
Warmed over flavor	Minced meat cooked as a pattie. Kept refrigerated for 1–3 days at 2 °C. Reheated in an oven	
Rancid	Linseed oil simmered for 10 min	Only odor
Earthy	Earth from a garden or 2, 3, 5-trimethyl pyrazine	Only odor
Cardboard	8 g Cardboard in 250 ml water for 24 h, filtered	Only odor
Mushroom	1-Octen-3-ol	Only odor
Nuts	Nuts at least ½ year old kept in an open bag	
Cod liver oil/fishy	Cod liver oil	
Feed/others		
Roasted fish	Roasted salmon fillet smelled at the skin side	

the feeding systems influence the fatty acid composition in both ruminants and nonruminants. In particular for ruminants, pasture, in comparison to grain, introduces a different flavor to the final product, which is perceptible by both trained and consumer sensory panels. The flavor resulting from pasture feeding is sometimes associated with the species-specific flavor, particularly in sheep meat. Sheep meat from pasture-fed animals is often described as having a 'pastoral' flavor, which is unacceptable to some consumers. The compounds 3-methylindole (skatole) and *p*-cresol have been implicated as the main volatile contributors to the 'pastoral' aroma evident in the cooked meat of pasture-fed animals. Interestingly, skatole is also one of the two compounds associated with 'boar taint' as described in Table 1. Another unacceptable flavor associated with cooked sheep meat is 'mutton' flavor, which is traditionally associated with meat from older sheep. Mutton flavor is derived from branched chain fatty acids. More recently, diet has also been implicated in the formation of 'mutton' flavor in sheep meat.

In beef, the main unacceptable flavors that might arise are 'liver' flavor, which appears to have unknown causes, and metallic flavor.

Sensory Assessment of Meat Products

Meat products differ from fresh meat as they are often served cold or at room temperature. This makes it simpler to standardize the protocol for handling the samples. Before performing a sensory assessment, it is necessary to decide at which temperature the samples should be assessed. As meat products can be spicy and salty, compared with fresh meat, it has to be taken into account while designing the study.

The selected attributes must discriminate between samples, and be relevant to the specific product and the aim of the investigation. It is optimal to have reference samples especially for color, odor, and flavor attributes in order to achieve agreement among panelists.

The texture of meat products is related to mechanical, geometrical, and surface attributes. Mechanical attributes are characterized as hardness, cohesiveness, viscosity, springiness and adhesiveness, whereas the geometrical attributes describe size, shape, and particles within the product during chewing. The surface attributes are related to the sensations produced by moisture or fat content. Similar to sensory assessment of flavor, the terminology for texture can be established using samples representing the full range of textural variation.

Panelists performing color assessment must have normal color vision. A test for color blindness can be performed using 'ISHIHARA's Test for Color Deficiency.' When evaluating color in meat products, a color chart can be used as a reference. Several color chart systems are available on the market like Natural Color System (NCS) and Pantone Color Guide. In some countries, persons must pass the 'Munsell Color Test' before being considered qualified to score color.

Air can influence the color of a product markedly, as observed on sliced products lying in open air before the evaluation, and a standardized time and temperature sequence before the evaluation is, therefore, important to ensure identical evaluation conditions. Moreover, the lighting during assessment must be standardized as well.

The color chart can be used on a number of different products. If products have two colors, for example, a 'meat color' and a 'fat color,' an interval of colors can be defined instead of a single color code (Figure 6).

Figure 6 Example of color chart for an emulsified meat product.

Physical and Chemical Measurements of Sensory Related Properties

Physical Texture Analyses

The texture analyses methods most often used are the Warner Bratzler shear force (WBS) and the texture profile analysis (TPA). The texture is measured as the strength to cut a defined piece of meat; the more force needed, the tougher the meat is. During the years, many researchers have tried to correlate the physical texture measures with the tenderness assessed by a trained panel, and with varying success. It is possible to get indications of tenderness by the physical measures and this is relevant especially if many samples need to be analyzed. However, it has not been possible to translate, for example, a shear force value to a specific score on the sensory scale.

Color Measurements

Color is an important characteristic of meat, and the red color of beef is, by many consumers, related to freshness. Meat color can be measured by various reflectance colorimeters. One widely known and used color scale for meat is the L*, a*, b* color scale. Generally, the instrumental color measures are based on average values. However, measuring meat color is a challenge due to the uneven surface including marbling, and so the new generation of methods is measuring color with a vision camera. By this method, it is possible to measure the color of, for example, a fermented sausage and, subsequently, extract the color of both the fat and the meat parts. With this vision technology, it is possible to generate far more information than before.

Odor Measurements

For the investigation of meat aroma and the presence of specific aroma compounds, gas chromatography (GC) methods are widely used. There are several ways of sampling the aroma compounds (volatiles), though all include initial trapping of the headspace that contains the volatiles. The volatile extracts are subjected to chromatographic separation and instrumental detection by either flame ionization detection (FID) or mass spectrometry (MS), and the gas effluent is simultaneously sniffed by human assessors. The characteristics of each odor is described and the intensity can by quantified by 'present or not' (summing up over the panelists) or the length of time, the given odor can be smelled by the panelist.

In this way, aroma compounds related to a given food can be identified and also quantified. In addition, the trained human assessor evaluates the odors coming into the funnels and adds both a description of the perception of each detected odor as well as intensity. Using GC-O-MS, it is possible to gain knowledge on the individual volatiles constituting the

overall aroma of a given food. For instance, the odor of fried pork is characterized by aldehydes, pyrazines, and sulfur-containing compounds. However, the overall odor attributes of meat or any food are still assessed by the more classical sensory methods.

See also: Cooking of Meat: Cooking of Meat; Flavor Development; Maillard Reaction and Browning; Physics and Chemistry; Warmed-Over Flavor

Further Reading

Aaslyng, M.D., Bejerholm, C., Ertbjerg, P., Bertram, H.C., Andersen, H., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Quality and Preferences 14, 277–288.

AMSA, 2014. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat.

Bejerholm, C., Aaslyng, M.D., 2003. The influence of cooking technique and core temperature on eating quality of pork – depending on the raw meat quality. Food Quality and Preferences 15, 19–30.

Berry, B., Dikeman, M.E., 1994. AMSA cookery and sensory guidelines. Proceedings of the 47th Reciprocal Meat Conference of the American Meat Science Association, pp. 51–52. Chicago, IL: National Live Stock and Meat Board.

Calkins, C.R., Hodgen, J.M., 2007. A fresh look at meat flavor. Meat Science 77, 63–80.

Lawless, H.T., Heymann, H., 2010. Sensory Evaluation of Food, Principles and Practices, second ed. New York: Kluwer Academic/Plenum Publishers.

Meinert, L., Schäfer, A., Bjergegaard, C., Aaslyng, M.D., Bredie, W.L.P., 2009. Meat Science 81, 419–425.

Moskowitch, H., 1996. Experts versus consumers: A comparison. Journal of Sensory Studies 11, 19–37.

Polkinghorne, R., Thompson, J.M., Watson, R., Gee, A., Porter, M., 2008. Evolution of the Meat Standards Australia (MSA) beef grading system. Australian Journal of Experimental Agriculture 16 (48), 1351–1359.

Stone, H., Sidel, J.L., 1993. Sensory Evaluation Practices, second ed. San Diego, CA: Academic.

Watson, R., Polkinghorne, R., Thompson, J.M., 2008. Development of the Meat Standards Australia (MSA) prediction model for beef palatability. Australian Journal of Experimental Agriculture 16 (48), 1368–1379.

Young, O.A., Braggins, T.J., 1998. Sheep meat odour and flavor. In: Shahidi, F. (Ed.), Flavor of Meat, Meat Products and Seafoods. London: Blackie Academic & Professional, pp. 101–130.

Relevant Websites

http://www.csiro.au/
CSIRO.

http://www.dti.dk/services/sensory-analyses/31883
Danish Technological Institute.

SLAUGHTER, ETHICS, AND THE LAW

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Glossary

Bleeding/exsanguinations A procedure where the blood vessels in the neck or chest are severed.
Halal slaughter A slaughter procedure that adheres to Islamic teachings where the animal is dispatched by severing the blood vessels of the neck following a prayer.
Kosher The meat produced following Shechita.
Shechita The Jewish method of slaughter carried out by a trained Jewish slaughterman, shochet.
Slaughter The process of exsanguination that ensures the animal cannot recover sensibility.
Soclap Society, Oneself, Customer/client, Legal, Animal, and Profession.
Stunning The procedure where an animal is rendered unconscious before exsanguination or another killing method, pelt removal, and evisceration.

Introduction

Killing animals for food can be a troubling experience or concept. Most of us probably recall our first realization that meat comes from muscle, which in turn is part of a living animal that has feelings. For some, this is a disturbing experience that is difficult to reconcile. The discomfort usually has two facets. First, there is abhorrence at the thought of the physical act of slaughter. Second, there is remorse about the fact that an animal had to be killed for the consumer's enjoyment or benefit. In a survey conducted in the United Kingdom in the early 1990s, it was found that when meat eaters were confronted with the hypothetical prospect of having to kill animals themselves to eat them, the majority said that they would cease eating meat altogether. The connection between slaughter and eating meat can be unnerving.

These concerns depend to some extent on the species being considered and the connection that is conjured up. There is a hierarchy of meats that ranks a meat according to how soon a semivegetarian or reduced meat eater strikes that meat off from her or his food list (Table 1). The reduced meat eater would typically progress toward abstention by first giving up beef, then lamb, pork, poultry, and finally fish. The species of origin, the appearance of blood, and the redness of the meat are thought to be key features that create this hierarchy, but other issues may be involved such as perceived 'lightness' of eating, convenience of preparation, and views about body image. The way a meat is presented may also be pertinent. Some meat eaters dislike confronting a fish with the head, and in particular the animal's eyes, on the plate in front of them. These points demonstrate how ethical concerns can be intertwined with other beliefs and attitudes, and this can make it difficult to rationalize the ethical component when it needs to be considered on its own. A current example is religious slaughter that has received much attention and continuing to be controversial in relation to religious rights, rules, animal welfare, and legislation.

Table 1 Hierarchy of meats among semivegetarian women

Type of meat	*Number of semivegetarians who eat the respective meat for every meat-eater who consumes the same meat*
Beef sausages	0.41
Pork	0.43
Crumbed veal	0.46
Lamb	0.46
Steak	0.47
Bacon	0.47
Roast beef/veal	0.51
Casserole (not chicken)	0.55
Mince meat	0.56
Cold meats	0.65
Processed meats[a]	0.67
Chicken	0.82
Fish	1.05

[a]Sausage rolls, pies, and hamburgers.
Source: Reproduced from Gregory, N.G., 1997. Meat, meat eating and vegetarianism. In: Kerry, J., Kerry, J., Ledward, D. (Eds.), Proceedings of the 43rd International Congress of Meat Science and Technology, pp. 68–85. Auckland, New Zealand: International Congress of Meat Science and Technology.

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Applied Ethics and Decision-Making

For some, but by no means all, people, there is an overlap between ethical concerns and squeamishness. Most people manage squeamishness by avoiding a particular thought, by confronting it and overcoming it through familiarization, or, if they are in a position to do so, by modifying the procedure so that it is more aesthetically acceptable. Ethical concerns can be managed or resolved by weighing up responsibilities, and forming a judgment or decision based on careful assessment of all the responsibilities and potential outcomes of a course of action. A useful way of going about this is to use a procedure that has been developed for veterinary practitioners when they are confronted with particularly thorny ethical conflict. It is known as 'soclap:' Society, Oneself, Customer/client, Legal, Animal, and Profession. These elements represent the facets or parties usually involved in any ethical conundrum that involves animals. In the present context it could be used, for example, when trying to decide whether a particular slaughter method is ethically acceptable. In the case of society's expectations, one needs to ask 'What would society expect in this situation?' There will be widely differing views on this, according to race, religion, and tradition, and so limits or a context have to be set. The ethical decision-maker puts him or her in society's position and should adopt a reasonably broad perspective. 'Oneself' covers one's own expectations and sensitivities, and 'customer/client' encompasses, for example, sensitivities of the retail company buying the meat, whether the meat is destined for retail or the catering trade, and any other feature that impacts on the image or reputation of a meat or company in the marketplace. Legal requirements often try to reflect society's expectations, while allowing for fair and honest production or procurement practices. This is included in soclap to ensure that the practice that is being considered is operating within existing law. Responsibilities to the animal can fall into two categories: animal rights and animal welfare. The animal rights outlook considers whether it is fair to the animal to impose on it in a particular way. This could include the right to life, when the ethical issue being considered is whether slaughter is justified. Animal welfare is concerned with how the slaughter is conducted and how to avoid or control animal suffering. Lastly, there is responsibility to the profession; in this case the focus could be on the abattoir industry. How does the industry as a whole view itself in this context, and does it have any position statements?

In regard to killing animals the inevitable question of whether death is a welfare issue or not comes to the fore. Epicurus in ancient past claimed that death should not be a worry but sense-experiences would be more important. This view is still not seriously challenged. However, assumption that death is not a welfare issue for animals creates conflicts for those who deal with animal ethics that an animal's death is ethically significant. One could argue that a meat animal's life has positive attributes, and in this case death becomes a welfare issue. Conversely, if positive states cannot be provided, for example, by changing husbandry methods, then killing an animal may not be a welfare issue provided that it is carried out in a quick and painless way.

This decision-making process may be a personal exercise, or it may be conducted by a company considering the ethical or moral issues of a particular practice, or by an industry as a whole. It does not instruct the parties about what to conclude, but it does direct them in how to arrive at a conclusion.

Stereotypes in Ethical Discussions on Animal Welfare and Slaughter

When a controversial ethical or animal welfare issue is being discussed, particular approaches can usually be identified within each faction. Both sides have a characteristic *modus operandi* in their arguments; these are simplified in Table 2. Assessment of the debate by the public probably works in several ways. People who have strongly developed internal value orientation will consider the arguments in terms of benefits to themselves and the parties concerned. Externally oriented people are more likely to look to state-appointed experts or legislation as controllers of the outcome. These points are relevant, as no single approach to a debate on the ethics of animal practices will satisfy all individuals.

Ethics of Stunning

Preslaughter stunning was probably the first used way of immobilizing animals that were about to be slaughtered. An early wood carving, dating back to mediaeval times, shows an unrestrained pig about to be struck on the head with an axe. This was a practical approach to controlling the animal so that it could be killed, in a convenient manner, by bleeding it out. An added advantage with stunning before slaughter was that it helped satisfy concerns about the pain and distress the animal could experience during the sticking procedure. Cutting the skin in the neck is potentially painful, and many people foster a sense of ethical or moral responsibility for minimizing that pain, as well as subsequent suffering, when animals are being killed for human benefit. Knowing that an animal was stunned when it was slaughtered also helps ease the conscience about taking the animal's life. If one is assured that it was a humane death involving little or no suffering, there is less concern about eating the animal as meat.

When stunning was introduced into law, in some countries there was the added requirement that animals must not

Table 2 Stereotypes in debates over livestock ethical and welfare issues

Approaches used by animal advocates	*Approaches used by livestock trade*
Appeal to public opinion	Consultation with experts
Emphasis on intuition	Emphasis on knowledge
Risks portrayed in broad terms incorporating political and social factors	Narrow framing of the risk analysis, considering only measurable uncertainties
Broad symbolic and political goals	Narrow business goals
Emotion	Rationality
Openness	Secrecy
Suspicion of standards	Trust in standards

be slaughtered within sight of another conscious animal. The reason was to reduce the likelihood of an animal recognizing that it was about to be killed. The basis for this has been questioned, as research has shown that when sheep and pigs have been in a position to watch one of their penmates being stunned and slaughtered, they did not demonstrate any stress response such as a rise in heart rate or plasma cortisol.

Not everyone chooses to eat meat, and part of the reason for this can be ethical (Table 3). Some people become vegetarian, while others are highly selective in the types of meat they eat. Concern is particularly strong among women, and especially among young women. A common view is that they would feel partly responsible for the death of an animal if they ate its meat, and also that knowledge does not rest comfortably with their conscience. Instead, they would prefer to be guilt-free in their eating style. It is not necessarily their purpose to convert the rest of the world into vegetarianism, but they feel the need to dissociate their involvement with animal slaughter. This viewpoint is a personal ethic.

Religious ethics provide another reason for not accepting animal slaughter or meat eating. In the case of Buddhism, there is a belief in reincarnation and that it is inappropriate to take any form of animal life let alone eat it. In other religions, the doctrine requires the believer to avoid eating certain parts of an animal, either because they are defiling or because they hold or represent the soul of the animal. Most religious faiths have one or more sects that ascribe to vegetarianism (e.g., Brahmin, Seventh Day Adventist and Rastafarian sects), and in many instances the reasons for vegetarianism are founded on fundamental precepts connected with metaphysical well-being of the consumer.

In contrast to refraining from eating animals, other religious faiths allow meat consumption and have prescribed methods of slaughtering and processing. Muslim (Halal) and Shechita (Jewish method) are the major religious techniques subject to much debate. There is also the Sikh religious belief that requires slaughter of animals by decapitation. An EC funded project, DIALREL, has recently disseminated information relating to religious slaughter and a recent comprehensive review on religious slaughter has been commissioned by the English Beef and Lamb Executive (EBLEX).

In religious communities that accept the slaughter of animals to eat meat, there are usually rules that govern what species are allowed and how the animals can be killed. Provision and consumption of meat for Muslim and Jewish communities is an essential part of the religious life and certain conditions must be met, so that the meat is lawful. Muslim method, Halal, is based on interpretation of the Koran and the Hadith (the sayings of the prophet Mohammed). The act of slaughter (Al-Dhabh) is carried out by pronouncing the name of God (Tasmiya) and slaughter is carried out by severing the neck to achieve maximum exsanguination as consumption of blood is forbidden.

Jews require their acceptable Kosher meat from animals slaughtered by their prescribed method called Shechita. Slaughter has to be carried out by a trained Shocet who uses a special knife (chalaf). Preslaughter stunning and damage to tissues are not accepted. In addition, a Jewish Inspector examines the carcass and rejects certain parts (treifa) that includes both hind legs.

Although meat production for religious groups is on the rise because of high consumer demand, there are some

Table 3 Summary of reasons given for reduced meat consumption during the 1980s and 1990s

Animal welfare	Moral reasons associated with the view that modern animal production is ethically unacceptable. By reducing consumption of specific meats or all meats, or by eating trusted, welfare-friendly products only, the individual is divorcing himself/herself from those production systems. It is usually a personal expression of rejection rather than a way of trying to change farming systems or society.
Environment	Moral concern that certain features of animal production harm the environment and have undesirable ecological consequences. The individual's conscience is quelled by abstaining from a particular meat or from all meat, and this has an element of long-term self-interest.
Health	Concern about one's own health. This has three features: 1. Avoiding the consumption of products that are normal ingredients in meat but are viewed as harmful (such as cholesterol and saturated fats). 2. Avoiding the consumption of unnatural ingredients that could be hazardous (such as hormones, antibiotics, coccidiostats, and pathogens). 3. Cutting out meat with a view to avoiding specific health problems where the causal agent may not be clearly understood (cancer, hypertension). The decision to reduce meat consumption for health reasons is made out of self-interest or concern for the health of one's family.
Social priorities	Some people reduce their meat consumption to conform or adapt to the lifestyle or standards of friends, relations, or other influential people. One's own body image is an important example.
Displeasure with meat	This takes several forms. Total abstention from meat can be because of revulsion at the sight of meat and in particular any associated blood or blood-like drip. Some people find the sticky texture of meat abhorrent, whereas others dislike the taste and elastic mouth-feel of meat when they eat it.
Metaphysical	The individual abstains from certain meats for spiritual, religious, doctrinal, or ethnic reasons. Eating meat is believed by some to impart negative effects, for example it arouses animal instincts in humans, including greater aggression.
Expense	Poorer communities and households abstain from certain meats because of their cost.
Inconvenience or inappropriate presentation	Some meats may not be presented in a way that fits into 'light,' informal meals. Some meats may be avoided because they are presented in a form that is inappropriate for the take-away trade or are difficult to cook quickly at home.

concerns about religious slaughter. These relate to the following questions:

1. Is there preslaughter stress;
2. Is the neck incision painful; and
3. Is sensibility and consciousness lost quickly enough following exsanguination 'sticking.'

Of all the above questions, the crucial issue is that whether stunning is used or not, while Shechita, in the Jewish method, stunning is completely prohibited; Halal may accept it under certain circumstances. With regard to whether stunning should be acceptable before Halal slaughter there are 3 views: (1) acceptance under certain conditions (e.g., reversible stunning); (2) rejections on grounds that it is against religious rules, painful induction, and causes insufficient blood loss; and (3) some either not sure or require assurances. Certain types of reversible stunning methods have been regularly used for decades in some countries such as New Zealand that exports Halal meat. Poultry slaughter in large numbers also often employs electrical stunning.

Although legislation usually covers conventional methods and requires preslaughter stunning, exemptions for religious slaughter are in place in most countries. However, slaughter without stunning is illegal in several countries (e.g., Sweden, Denmark, Norway, and Switzerland, New Zealand). The religious dispensation is allowed on the basis of human rights, which, with the exception of one or two countries in Europe, takes precedence over animal welfare. In other countries, cultural rights have been embodied in the human rights legislation, and, in the present context, this could complicate interpretation of the scope of the dispensations. One of the reasons for the increase in Halal meat demand is the desire to maintain traditional as well as religious ways of life.

Stunning is considered impractical in most marine fisheries, and so it is not a legal or ethical requirement. Nevertheless, some fish are dispatched by concussion or brain spiking once they are landed on board the boat. This helps control body movement and the risk of surface damage to the fishes' bodies, and it is considered to be a humane death when carried out proficiently.

In general, it is considered impractical to stun an animal that is being hunted. However, some authorities state that it is best to shoot an animal in the head, as this should cause immediate unconsciousness, and death. Some would go so far as to say that if a head shot cannot be achieved, then the animal should not be shot until there is a clearer view or it is close enough to allow a head shot. The concern is that a body shot may not cause concussion of the brain and so the animal may not be rendered unconscious immediately. Some hunters prefer using a neck shot to the less predictable head shot, as this disables the animal immediately and it is likely to experience a quick death. Similarly, some deer hunters use only a chest shot because they consider this leads to fewer cases of maimed animals escaping. There is always the concern that hunting can result in maiming, and that the maimed animal may escape and experience suffering. The risk of this happening will vary considerably according to the type of gun that is used, the accuracy of the marksman, the species, the terrain, and the method of stalking or chasing.

To summarize, in those countries where stunning is a legal requirement, the main reasons for insisting on stunning are to minimize the risk of the animal (1) experiencing pain during slaughter and (2) becoming distressed while it is dying. There are also practical advantages in stunning an animal before cutting its neck, as it helps to immobilize or control its movements. Advocates of preslaughter stunning usually hold that stunning is more humane than not stunning. In contrast, advocates of religious slaughter methods that disallow preslaughter stunning hold that their particular method is humane. In particular, Halal and Kosher meat producers and their consumers believe that their prescribed methods are not only required as ordered, but also in the meantime provide the most humane and painless techniques that protect animal welfare. Their objections to conventional stunning and slaughter methods are based on assumptions that stunning is painful and animals suffer.

See also: Exsanguination. Human Nutrition: Vegetarianism. Preslaughter Handling: Welfare of Animals. Religious Slaughter. Slaughter-Line Operation: Sheep and Goats. Species of Meat Animals: Cattle; Finfish; Game and Exotic Animals; Pigs; Poultry; Shellfish. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning; Slaughter: Immobilization

Further Reading

Anil, H., 2012. Effects of Slaughter Method on Carcass and Meat Characteristics in the Meat of Cattle and Sheep. UK: English Beef and Lamb Executive (EBLEX).

DIALREL, 2009. Religious slaughter, improving knowledge and expertise through dialogue and debate on issues of welfare, legislation and socio-economic as-pects. Available at: http://www.dialrel.eu/dialrel-results (accessed 02.05.12).

Gregory, N.G., 1997. Meat, meat eating and vegetarianism. In: Kerry, J., Kerry, J., Ledward, D. (Eds.), Proceedings of the 43rd International Congress of Meat Science and Technology, pp. 68–85. Auckland, New Zealand: International Congress of Meat Science and Technology.

Gregory, N.G., 2000. Consumer concerns about food. Outlook on Agriculture 29, 251–257.

Richardson, N.J., Shepherd, R., Elliman, N., 1993. Current attitudes and future influences on meat consumption in the United Kingdom. Appetite 21, 41–51.

Suits, D., 2001. Why death is not bad for the one who died. American Philosophical Quarterly 38 (1), 69–84.

Yeates, J.W., 2010. Death is a welfare issue. Journal of Agricultural and Environmental Ethics 23, 229–241. Available at: http://link.springer.com/journal/10806 (accessed 14.10.13). doi:10.1007/s10806-009-9199-9.

SLAUGHTER-LINE OPERATION

Contents

Cattle

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Introduction

The commercial slaughter process for live cattle generally is uniform across the industry. However, many customizations, innovations, techniques, and practices exist that might not be uniform to all plants. The objective of the slaughter process is to efficiently convert live animals into carcasses, and to harvest dress-off items and offals. Many production processes that precede slaughter have a tremendous impact on the safety and quality of the resulting carcasses and are discussed in detail in other sections of this encyclopedia. Discussion here will focus on those processes that occur between receipt of live cattle and carcass evaluation and grading. The sequence in which these processes are encountered by animals and carcasses as the harvesting proceeds can vary slightly between facilities due to space limitations, slaughter line configuration, and dressing procedures. There also can be numerous modifications and variations to each process as Standard Operating Procedures are tailored within each facility and at each processing step to result in sanitary conversion of live cattle to carcasses.

Cattle Receiving and Animal Handling

Although not technically one of the steps in the conversion of live cattle to carcasses, cattle receiving is often the first step listed on slaughter process flow diagrams. The objective of this step is to take control of incoming live cattle, obtain live weights, and stage animals for entry into the slaughter process. Facility design, maintenance, and employee training are essential elements of this process. Animal handling and animal well-being is paramount to the sustainability of the beef industry, and as a result, beef slaughtering facilities have well-designed animal holding and animal moving facilities to be more efficient and animal friendly (e.g., holding pens with nonslip floors and curved alleyways that eliminate bright lights and shadows). Additionally, slaughtering facilities have implemented strict policies for transportation companies and employees for the transportation and unloading of cattle that are received by the facility. Poor animal handling can result in value reducing defects and impair meat quality.

Antemortem Inspection

Each animal is visually appraised by an inspector (in the United States these are either US Department of Agriculture federal inspectors or state level inspectors) to identify apparent clinical signs of disease or distress. If none are noticed, animals are cleared for slaughter. If the inspector notices any signs of disease or abnormality, an animal can either be identified as 'suspect' and subjected to further evaluation as the slaughter process proceeds, or identified as 'condemned' in which case the animal is not accepted and passed for human consumption. Animals that are nonambulatory at the time of slaughter are 'condemned.' Condemned animals must be appropriately disposed of so that no parts of the animal may enter commerce.

Stunning

Animals cleared for slaughter are presented for stunning, sometimes referred to as 'knocking.' Stunning can be accomplished using several mechanisms, such as captive bolt, electricity, or gas asphyxiation. Regardless of the method utilized, all animals must be stunned and rendered unconscious before starting the dressing process.

To minimize animal stress, proper restraint is required. The most common methods of restraint in large commercial facilities are either 'center-track' restrainers or 'V-Track' restrainers. In smaller plants, a simple 'knocking box' or chute

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can be utilized. In these situations, the animal is prevented from moving forward or backward and is positioned for subsequent stunning. Automated restraint devices are utilized to immobilize and transport cattle from the lead-up alley to the stunning area so that appropriate stunning can occur without causing undue stress to the animal.

The penetrating captive-bolt device is the most common method for stunning cattle. Captive-bolt stunning devices can be powered with powder charges (similar to firearm blanks) or air pressure (pneumatic, nonair injecting) and are composed of a firing mechanism (trigger) and a 'captive bolt' (a steel rod approximately 1.27 cm in diameter and 10 cm in length). The device should be placed in the center of the animal's forehead and fired. An effective stun results in an animal that is insensible; five key observations necessary to ensure animal insensibility are (1) no blinking of the eyes, (2) no movement of the eyes in response to touch, (3) no rhythmic breathing, (4) no 'righting reflex' (i.e., animals try to stand up), and (5) no response to ear/nose pinching (painful stimuli). If these conditions are met, slaughtering and dressing may then continue. In situations where an animal is not rendered insensible, they must be restunned until the five conditions are met.

Shackling and Hoisting

Animals are shackled as the stunning process proceeds. The shackle is applied to the left hind foot of animals during the brief period when animals are in the restrainer. In some smaller plants, shackles may not be applied until following stunning and removal of the animal from the knocking area. The shackle comprises a heavy chain that is attached to a roller trolley on one end that can move freely on metal rails, and an expanded hook on the other end, which allows for constriction of the chain around the hind foot as animals are hoisted.

Following the application of an effective stun, animals are passed out of the restraint device or rolled out of the knocking box, and hoisted to an overhead rail using the attached shackle. Once animals are suspended, they can be moved forward through the dressing process until they are transferred to rolling hooks (trolleys).

Exsanguination (Sticking/Bleeding)

A common misconception is that stunning 'kills' the animal. The actual point at which death occurs is not finite; brain death and metabolic death do not necessarily coincide with respect to time. Normally, animals maintain cardiac rhythms for several minutes following stunning. Blood loss (exsanguination) usually is the ultimate cause of death in the slaughter process and is commonly referred to as 'sticking.'

Insensible animals are bled by opening the hide from the base of the jaw to the point of the brisket. A clean, sterilized knife is inserted ($\sim$15–20 cm) at an approximate 45° angle at the anterior point of the sternum and a cut is made in the direction of the backbone. When performed correctly, the ascending branch of the aorta and the vena cava are severed, resulting in rapid blood drainage. As both the aorta and vena cava are severed, escaping blood should be a mix of bright red, oxygenated blood (from the aorta) and dark red, deoxygenated blood (from the vena cava). Animals should be allowed to bleed for several minutes, and blood should be collected for either edible or inedible use.

Blood Collection

At the time of blood removal, approximately half of the animal's blood is removed equating to approximately 3.0–3.6% of the animal's live weight in blood (e.g., 13–16 kg of blood from a 450 kg steer). Much of the blood removed from the live animal is captured, dried, and sold as-is for miscellaneous uses including, but not limited to, pet food, nonruminant livestock feed, and fertilizer manufacturing. If a processing facility has the ability to collect blood in an aseptic fashion, then blood can be collected for human consumption and pharmaceutical use. Aseptic blood collection is a much more involved process including the use of specialized devices to capture blood directly from major blood vessels including the carotid artery or the jugular vein during exsanguination. Such devices employ a blade or knife to sever the blood vessel and simultaneously vacuum blood into sanitary collection vessels containing anticoagulants. Some facilities are choosing to aseptically collect edible blood postexsanguination at a point in the dressing procedures when the hide has already been removed. An additional 1–2 kg of blood can be collected immediately following mechanical hide removal, particularly when side pullers and low-voltage electrical stimulation is utilized in the process.

Hide Washing/Dehairing

In an effort to reduce the presence of pathogenic organisms on cattle, many packing plants now have initiated processes to 'wash' the exterior of the hide either immediately before exsanguination or immediately following exsanguination. In some cases, chemical pathogen spray-washing intervention technologies are used to accomplish this objective. Cattle should not proceed to subsequent processing steps until water or chemical solutions no longer drip from the exterior of the hide.

Hide Removal

The process of removing hides from carcasss is the most variable, and most labor- and time-intensive process involved in cattle dressing. The objective of this process is to remove the hide at the intersection of the skin and fat/subcutaneous muscles, leaving all fat and subcutaneous muscles intact and attached to the carcass. All hide removal efforts, along with many corresponding modifications/additions to the slaughtering and dressing process, have focused on keeping dirt, dust, mud, manure, hair, and other foreign materials from the surface of dressed carcasses. Hide removal techniques, in conjunction with the equipment utilized to accomplish this task,

are probably the most variable of the slaughter processes from plant to plant.

First Leg Skinning

Leg skinning is the initial dehiding process; it results in the separation of the hide from the 'free' (right hind) leg. This accomplishes separation of the hide from carcasses from just above the hoof to above the hock joint.

Rump/Butt Skinning

Following hide removal from the free foot, the process is continued by removing the hide from the hock joint over the rump of the free side of carcasses. This involves separation of the hide from the rectum, and it is imperative that the hide is removed so that it can be folded back to prevent contamination of the rump area of the carcass.

Hock Removal

Once the first leg and rump (right) are completely dehided, the free foot is then removed. This is commonly accomplished with a large pneumatic or hydraulic cutter ('hock cutters'). The hind foot and shank are removed at a point distal to the hock joint, at the junction of the tarsal and metatarsal bones, ensuring that the anatomical structure of the gambrel (*Achilles*) tendon is intact and undisturbed.

Leg Steam Vacuuming/Blow-Off

To quickly remove potential microbiological contamination that is transferred from hides to the recently exposed hock, many plants now employ use of a washing/vacuuming system to remove contamination. It is important that such technologies do not result in water that is free to run down the hide or carcass surface.

Leg Hang-Off (Transfer to Trolley)

At this point, the free (right) leg of the carcass is completely dehided and the hind foot and shank are removed. A trolley hook is inserted from the medial side of the shank through the natural gap that exists between the gambrel tendon and the tibia/fibula. The trolley is then placed on the open face of the rail and the carcass is transferred to the trolley, which releases the shackled leg. The shackle is removed and sent back to the knocking area for cleaning and reuse.

Left Leg, Rump/Butt, and Hock Skinning; and Steam Vacuuming/Blow-Off

These processes are identical to first (right) leg skinning.

Left Leg Hang-Off

This process is identical to first leg hang-off, but applied to the opposite leg of carcasses. Following this step, the hind legs and rump are completely dehided and carcasses are suspended by two independent trolleys from the rail. The hind legs are spread apart by chain cogs or rail blocks to aid in further dressing.

Midline Split

Focus now turns to the abdominal region of the carcass. At this step, the hide is opened along the midline axis of the carcass from the stick wound to a point that reaches the already dehided hindquarters. This process, along with the process of splitting the hide on the fore and hind limbs, traditionally is referred to as 'patterning' of the carcasses (creating the 'pattern mark').

Fore-Foot Removal

Equipment (hydraulic or pneumatic cutters) similar to that used for the removal of hind feet also are utilized to separate the front feet from carcasses. The location of removal can vary between plants depending on further utilization of flexor tendons and shank bones. If flexor tendons are to be harvested from carcasses, forefeet are removed just above the hooves at the junction of the metacarpal bones and the proximal phalanx. If tendons are not to be harvested, entire foreshanks may be removed at the junction of the proximal and distal carpal bones.

Belly Skinning

Starting at the midline, the hide is separated from carcasses along the midline outward, from the ventral side to the dorsal side, covering the area from brisket to flank. This process prepares carcasses for subsequent mechanized hide removal, and can be achieved with either a fixed blade skinning knife or a pneumatic skinning/dehiding knife. At this point, loose edges of hides on adjacent carcasses are often hooked together using chains or 'bungee' cords to prevent and minimize carcass contamination from the exterior of the hide.

Side Pullers

Side pullers often are the first mechanical aid used in dehiding. These machines are typically hydraulic and are synchronized with the automated slaughter line to travel with a carcass as it moves. This operation involves inserting the loose midline edges of hide into puller arms that are retracted to pull the hide toward the dorsal edge of the carcass while the back of the carcass is held stationary (commonly using low-voltage electrical stimulation to 'stiffen' the carcass). This accomplishes removal of the hide over the carcass sides, an area typified by attachment to the 'cutaneous trunchi' muscle ('fly shaker' muscle).

Back Skinner

This process is generally completed manually by workers with pneumatic skinning/dehiding knives. The objective is to remove the hide from the back of the animal; from a point posterior to the shoulders, to a point anterior to the lumbosacral junction. This not only removes the hide from an area of the carcass that is prone to fat pulls, but also facilitates removal from the hindquarter.

Up Puller

The 'up puller', also referred to as a 'banana bar', is a smooth cylindrical bar that is inserted between the hide and the dorsal edge of the carcass through the hide/carcass separation created during the previous step. Once the bar is passed through the opening, the mechanism is activated and the bar makes an upward sweeping motion that completes separation of the hide from the hindquarter of the carcass. At this point, the hide is draped downward and remains attached only to the forequarter of the carcass.

Down Puller

The 'down puller' is the final step in the removal of the hide from the carcass. This can be accomplished with several types of equipments, all of which apply downward force on the hide to complete separation from the carcass. The two most popular models are 'clothes pin' and 'track' pullers. Clothes pin down pullers are a steel bar that is shaped like a clothes pin (cylindrical with a channel through the center). The posterior portion of the hide, previously separated from the carcass, is inserted into the channel and the bar is rotated so that the hide is 'wound' around the bar. Simultaneously, the bar will be retracted away from the carcass exerting additional force until the hide is removed from the entire body and head of the carcass. 'Track' pullers work similarly, except that the hide is fed between two sets of rotating rubber tracks that grip the hide and exert a downward force until the hide is freed from the carcass. The removed hide is then conveyed or dropped to an adjacent processing area where fleshing, grading, and further value-adding processes may occur.

Steam Vacuuming Stations

Hand-held steam vacuums (for spots of visible contamination <2.5 cm in diameter) are used to remove visible contamination from beef carcasses. Such systems use steam or hot water to loosen soil and kill bacteria, followed by vacuuming to remove contaminants. When applied correctly (e.g., passing the steam-vacuum system slowly enough over the surface of carcasses so that the temperature of the steam is able to kill bacteria), visible contaminants and bacterial counts are reduced.

Pre-evisceration Washing

Before evisceration, carcasses often are subjected to pre-evisceration washing to decontaminate the surface before bacteria have time to 'attach' adequately. This step generally involves washing of carcasses with large volumes of water at ambient temperatures, often followed by application of organic acid (e.g., 2.5% lactic acid).

Tie and Separation of Weasand

The first step in the removal of the viscera is separating the weasand (esophagus) from the trachea. Care must be taken here to prevent leakage from the rumen and to prevent carcass contamination with ingesta. This step is completed with an instrument referred to as a 'weasand rod.' The rod acts to separate the esophagus from the trachea, to ease the subsequent removal of the viscera. Subsequently, an elastic band or clamp is applied to the weasand (esophagus) to prevent leakage. In some instances, the weasand is tied with a butcher's string at the cranial end before separation from the trachea.

Dentition Check

Following exsanguination and prior to head removal or even prior to the removal of the hide, the age of each animal is routinely checked via dentition. An animal exhibiting a loss of three or more baby (deciduous) incisors and the eruption of three or more permanent incisors above the gum line are designated as 30 months of age or older. Owing to concerns relating to encephalopathic diseases, cattle designated as more than 30 months of age or older are processed separately from 'young' cattle and additional specified risk materials (SRMs) must be removed.

Head Removal

The head is removed at the juncture between the atlas vertebra and the occipital condyles (occipitoatlantal joint) of the skull. Following removal, the oral and sinus cavities of the head are flushed with water to remove debris. Each head must be tagged and identified so that the carcass and the head can be reunited for further inspection if necessary; usually using a four-piece 'gang tag' or 'head tag,' which maintains the sequence and identity of each carcass, head, tongue, and organ.

Tongue Removal

Following head removal, the tongue must be removed from the head for inspection purposes and further processing. This procedure is accomplished by severing the cartilaginous joints of the hyoid bones on either side of the tongue and removing it from the jaw bone. Following removal of the tongue from the head, the tonsils (pharyngeal, tonsil of the soft palate, palatine, tubal, and lingual) are removed from the tongue. Tonsils are an SRM that must be removed from all age classifications of cattle. Each tongue is identified with a portion of the 'gang' tag.

Head and Tongue Washing

Following their removal, heads and tongues must be washed. In large commercial facilities, this generally is accomplished using spray-washing cabinets designed for this purpose. In many cases, microbial intervention cabinets also can be used to apply chemical decontaminates to heads and tongues (e.g., a 2.5% solution of lactic acid). In smaller plants, heads and tongues might be washed manually as they hang on racks by workers. In all cases, care should be taken to insure that the

possible presence of pathogenic bacteria has been adequately addressed.

Head and Tongue Inspection

During head/tongue inspection, mandibular and parotid lymph nodes are excised and examined for abnormalities, and cheeks, lips, and tongues are examined for evidence of sores, abscesses, wounds, or splinters. Signs of abnormalities result in condemnation of either the head or tongue, or both, and in some instances can warrant further investigation of the carcass and viscera to determine carcass wholesomeness.

Brisket Saw

This process results in separation of the sternum along its midline to ease the process of evisceration and to facilitate carcass splitting. A specialized saw, equipped with a short ($\sim$10 cm) blade fitted with a blunt end (typically a steel ball), is used. This prevents the blade from damaging or rupturing the rumen as sawing occurs. Sawing is initiated posterior to the xiphoid cartilage, and extends downward causing complete separation of all sternebrae.

Bung Bagging, Tying, and Separation

This step results in separation and covering/sealing of the 'bung' (anus) so that it cannot contaminate the carcass. Complete separation of the rectum from the exterior carcass surface and pelvic cavity is necessary so that it can be removed during evisceration. This process is commonly referred to as 'bunging' or 'bung dropping.' It is imperative that fecal contamination be prevented; therefore, the bung always should be placed in a plastic bag and sealed with twine to prevent further defecation.

Oxtail Removal

The oxtail is removed by severing the cartilaginous joint between the last sacral and first coccygeal vertebra. Tails are then further processed as an edible by-product.

Evisceration

Evisceration is completed by: (a) removal of viscera (rumen, intestines, liver, esophagus, and spleen) and (b) removal of the 'pluck' (heart, lungs, and trachea). Viscera is removed by opening the peritoneal cavity (abdomen) along the ventral midline of the carcass, locating the bagged and tied bung, and gently pushing the viscera downward, pulling the separated and tied weasand through the thoracic cavity and removing the digestive tract. The liver is removed and presented for inspection. All internal organs are removed, except the kidneys, exposing the diaphragm. The diaphragm is severed where the muscular portion meets the connective tissue portion, and the contents of the thoracic cavity (the 'pluck') can be removed by severing the pericardial sac, pushing the heart and lungs downward, and pulling the trachea upward to complete removal. Both viscera and pluck are identified with a portion of the gang tag and presented for inspection. During inspection, the heart is opened and evaluated for abnormalities, the bile duct of the liver is split and evaluated, the liver is palpated for signs of abscess or sclerosis, and the weasand and intestines are evaluated. Abnormalities result in part or all of the internal organs being condemned and, in some cases, the carcass may be retained for further inspection or condemnation.

Carcass Splitting

Beef carcasses are split laterally down the center of the vertebral column using a hanging band saw. It is critical that the cut be made in the center of the vertebral column to prevent damage to loin and rib cuts of carcasses.

Spinal Cord Removal

Today, due to concerns relating to encephalopathic diseases, spinal cords generally are removed from the exposed vertebral foramen of carcasses and rendered. This process might be performed manually, or via use of an automated vacuum system.

Final Trimming

Carcasses are not considered wholesome if visible contamination is found to be present on the exterior. Knives are routinely used to remove visible contamination as is required by 'zero tolerance' performance standards. A designated area for the 'final trimming' of carcasses, just before final inspection, allows an opportunity to remove any remaining visible contamination on carcass surfaces.

Final Postmortem Inspection

Carcasses are evaluated by inspectors under the guidance of veterinarians to detect conditions resulting in products as unwholesome, or not appropriate for human consumption. Today, such conditions include presence of visible fecal, ingesta, or milk contamination, as well as a long list of pathological conditions. Carcasses can be 'retained' for additional processing or inspection after suitable corrective actions, some carcasses then might be approved (released) for continued processing. Other carcasses that are not salvageable are condemned, and deemed inappropriate for use as a food product; they must be disposed of in a manner ensuring that no carcass parts can enter commerce.

Measurement of Hot Carcass Weight

Carcasses generally are weighed before final washing or additional processing. Hot carcass weights are often subsequently utilized in classification and grading systems.

Hot Fat Trimming

Because it is inefficient to chill fat tissue, many plants now incorporate trimming stations (following the first hot carcass weight scale) designed to allow removal of excessive, trimmable fat from the exterior (i.e., subcutaneous) and interior (i.e., kidney, pelvic, and heart) of carcasses. Following removal of fat, plants generally collect a second hot weight from each carcass; these weights are then used to determine carcass value.

Final Carcass Decontamination Interventions

Just before initiation of chilling procedures, carcasses generally are subjected to a sequence of several 'final intervention' decontamination technologies designed to address the possible presence of pathogens on carcass surfaces ('multiple hurdles' systems). Synergistic or additive effects are obtained when combinations of two or more decontamination systems are used in sequence. As a rule, all carcasses are washed with large volumes of ambient-temperature water. Most plants also incorporate a thermal or steam pasteurization system designed to apply water at temperatures in excess of 82 °C, as well as spray cabinets that apply organic acids (e.g., lactic acid and acetic acid) or other chemical decontaminates to the surfaces of carcasses. Lactic acid and acetic acid are the most commonly applied organic acids in practice, both of which are approved by FDA and the Codex Alimentarius Commission as direct food additives. The Food and Agriculture Organization of the United Nations and the World Health Organization has specifically approved lactic acid and acetic acid as a food additive in all meat products (Codex Alimentarius Commission in 2006). Among the many available chemical decontaminates used as interventions, lactic acid sprays (concentrations ranging from 2.5 to 5% of solution) are one of the most consistently effective interventions against pathogenic microorganisms on the surface of beef carcasses. Until recently, the European Commission has refused to allow the use of chemical applications (e.g., lactic acid) onto carcass surfaces as interventions because of a philosophy that required prevention of contamination of live animals. However, that philosophy has now changed, and beginning in 2013, voluntary use of some interventions will become possible.

Carcass Chilling

Carcasses are generally chilled for 36–48 h in 'hot boxes' that are comp of 'bays' of chilling rails cooled via overhead units and high-velocity forced air. The use of chilled water in 'spray chilling' systems facilitates heat dissipation; usually spray chilling time intervals and the quantity of water applied are reduced as chilling progresses. Hot boxes often contain automated 'walking beams' that minimize human contact with carcasses. Carcass chilling should be initiated within 1 h of exsanguination so that a temperature of 4 °C or less is reached within 24 h. Carcass temperature is usually measured at five randomly spaced locations, 1 mm under the fascia of the inside round (rear leg). Sides of beef should be spaced in hot boxes to minimize carcass-to-carcass contact and to allow efficient air circulation.

See also: By-Products: Edible, for Human Consumption; Hides and Skins; Inedible. Carcass Chilling and Boning. Conversion of Muscle to Meat: Slaughter-Line Operation and Pig Meat Quality. Exsanguination. Preslaughter Handling: Preslaughter Handling; Welfare Including Housing Conditions. Slaughter, Ethics, and the Law. Slaughter-Line Operation: Pigs; Sheep and Goats. Species of Meat Animals: Cattle. Stunning: Mechanical Stunning

Further Reading

Frondson, P.D., Spurgeon, T.L., 1992. Anatomy and Physiology of Farm Animals, fifth ed. Philadelphia, PA: Lea and Febiger.
Grandin, T. (Ed.), 2000. Livestock Handling and Transport, second ed. New York, NY: CABI Publishing.
Judge, M., Aberle, E., Forrest, J., Hedrick, H., Merkel, R., 1989. Principles of Meat Science, second ed. Dubuque, IA: Kendall/Hunt Publishing Co.
Lawrie, R.A., 1985. Meat Science, fourth ed. Oxford: Pergamon Press Ltd.
Lawrie, R.A., 1998. Lawrie's Meat Science. Cambridge: Woodhead Publishing Ltd.
Savell, J.W., Smith, G.C., 1998. Meat Science Laboratory Manual, sixth ed. Boston, MA: American Press.

Other Species

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Glossary

Abattoir Slaughter plant.
Carcass The dead body of an animal, especially one slaughtered for food.
Carcass contamination Bacterial contamination of the carcass.
Carcass dressing Removal of the hide, feet, head, tail, and internal organs from the carcass.
Electrical stimulation A current is run through the carcass soon after slaughter which makes the muscles contact and therefore speeds up the normal postmortem glycolysis (pH decline). The carcass can be chilled fast without risk of toughening ('cold shortening') and the tenderizing process (proteolysis, and aging) becomes more efficient.

Introduction

The principles for slaughtering and processing nontraditional species are the same as for the main livestock species. The animals have to be handled and slaughtered in a humane manner, and their carcasses dressed, stored, and transported hygienically. The main differences between the species lie in the way they are handled while still alive, in the approach used in skin removal and in the butchery lines used for the dressed carcass. Evisceration before jointing is the usual procedure in abattoirs if the meat is to be sold for meat consumption, but notable exceptions are the camel and the crocodile, where meat is often taken off the uneviscerated skinned body.

When animals are shot in the wild to be sold as game, the carcasses can be trucked to a central processing station. They may or may not be bled where they were shot. In hot climates, if the processing point is at some distance, the carcasses may be fully or partly processed, stored in a refrigerated larder, or chilled in a refrigerated truck before arrival at the processing station. There are many variations on this theme. From a hygiene perspective, there are two important features. First, the carcass or body should be chilled as soon as possible to control bacterial proliferation. Second, opening or dressing the carcass in unhygienic conditions greatly increases the risk of bacterial contamination and growth. In some situations it is advisable to leave the dead animal undressed until it can be taken to a centralized processing area where dressing can be done hygienically. This raises some issues about how bacteria multiply in uneviscerated carcasses, and the effects of delayed evisceration on the quality of the meat. Research studies have shown that when animals have been slaughtered and refrigerated without evisceration, it has not been possible to recover, from the edible carcass, marker bacteria that had previously been introduced into the gut before slaughter. However, the presence or absence of migration of bacteria through the gut wall postmortem may depend on the types of bacteria that are being considered. It is thought that in some circumstances *Clostridia* may be able to pass through the gut wall. This is a particular concern with fish meat, and it is thought that they can occur in terrestrial meat species, especially when the animals are severely stressed or if they have lesions in the gut wall, for example, from enteroparasites. There is no doubt that gases pass from the gut into the edible parts of the carcass. When a carcass is stored uneviscerated, internal tissues are prone to turn green through chemical combination with hydrogen sulfide that is released from the digesta.

Fine-fibred or fur-bearing animals such as kangaroos, rabbits, hares (jack rabbits), and deer can present problems with the transfer of fine hairs to the carcass during removal of the hide or pelt. This issue can be reduced by allowing the skin to cool before starting skin removal, as this encourages the fibers to set more firmly within the skin. If hairs do transfer to the carcass, it is not always possible to remove them with a washdown spray gun. The pressure from the water can cause the hairs to embed into the surface of the carcass, hence making the situation worse. Instead, the hairs should be removed with a knife along with some underlying tissue. In rabbits, the risk of hair transfer can be reduced by pulling the skin as a sock instead of a jacket.

During field slaughter it can be difficult to hoist a carcass to remove its skin, particularly in long-legged or heavy animals. In those situations, the skin may be removed while the carcass is lying horizontally on a cradle, cratch, or pram. An alternative, but less common, arrangement would be to suspend the carcass by four legs for the purpose of skin removal, and then re-suspend it from an Achilles hook or spreader when the legs have been reduced, to drop the guts. Alternatively, an animal killed in a remote area may be dressed while it is resting on its back and side with the partly attached hide serving as a clean apron between the ground and the carcass. The legs, backstrap, and other joints are removed before evisceration, followed by the red offal and fillet. In some birds, three-point suspension (by the neck and both legs) is used to assist vent opening and removal of viscera.

Deer

Both wild and farmed deer are processed in deer abattoirs. Wild deer are shot in the field and their carcasses are sent to

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the abattoir, sometimes with a holding period in a refrigerated larder. In some countries there is a legal requirement that the carcass must be eviscerated within 3 h of slaughter. To meet this standard, carcasses are gralloched (green offal is removed and discarded) where they are shot, whereas the red offal is usually retained with the carcass, which still carries its hide. Farmed deer and wapiti/elk are processed either in dedicated deer-processing plants or in plants that are registered game premises. It is very important to have pens and stunning facilities that are purpose-built for deer. Deer are versatile jumpers, and high-walled partitions are needed, and the lighting must be adjusted to help control their activity. Deer are usually handled individually between the holding and stunning pens, and some operators use boards to drive the animals in the required direction and to protect themselves from an advancing animal. When the deer is confined in the stunning pen, it is usually shot with a captive bolt gun, and then stuck after shackling and hoisting.

Where inverted dressing is used, consideration needs to be given to health hazards (leptospirosis) for operators, created by hinds that release urine. In some plants the pizzle, as well as the weasand and bung, is bagged and secured with a rubber ring before removal.

New Zealand has pioneered the development of farm-based production systems for deer meat (venison). Consequently, an important component of the research and development for this deer industry has been to define production systems that render distinctive and high-value attributes to venison, together with post mortem processing systems to complement these goals. As a specialist culinary product, venison is processed in specialized facilities licenced by the New Zealand (NZ) Ministry of Agriculture and Forestry. An animal welfare Code of Practice governs the humane treatment of deer before processing to ensure minimum stress and to enhance product quality. Electrical stimulation is standard protocol for the NZ deer carcasses. Venison is processed according to customer requirements. The trend is toward added-value (or further processed) cuts, where the bones and 'silverskin' (i.e., the connective tissue surrounding the muscles) are removed. The most important markets for New Zealand venison are Europe (Germany, Switzerland, Austria, and Scandinavia) and the US. Venison is attractive to the gourmet and to the health-conscious consumer for its unique flavor, low fat content, favorable fat composition, and high mineral content.

Camel

Camels are slaughtered for meat consumption in abattoirs in a number of countries in the Middle East and in Australia. Australia exports live feral and farmed camels for slaughter in Brunei; they kill out at approximately 50%. Most camels are familiar with being handled and this makes the slaughtering procedure a simple business, but feral animals are more difficult as they are prone to sitting down in the yards and refusing to move. When animals are slaughtered individually for local consumption, the tame animal is couched (made to sit in sternal recumbency) in the normal manner or with its hind legs extended backwards, and then shot in the head or slaughtered according to halal regulations. The dressing procedure is unusual in comparison with other species. The hide is opened from the dorsal instead of ventral aspect, and dorsal cuts of the carcass are removed before evisceration. This is done in the following way. While the body is maintained in sternal recumbency, the opening incision is made along the midline of the back, and the hide is freed down the sides. The hide serves as an apron on the ground, which helps reduce contamination of the carcass. The hump is removed at this stage if it is large, or left attached to the carcass if small. The shoulder is separated from the thorax working dorsoventrally, and the ribs are removed, followed by the flank. The carcass is then eviscerated. The camel is not a true ruminant even though it has a rumen. It has three instead of four stomach compartments, the omasum being continuous with the abomasum. In camel abattoirs in Australia, the camels are stunned, slaughtered, and dressed in a similar manner to beef, except that the neck and forelegs are removed before suspension to ensure clearance between the carcass and the floor.

The dromedary carcass is usually less fat than the bactrian. The subcutaneous fat is localized in the hump, and the absence of a continuous subcutaneous fat layer assists body cooling. The fat is usually white and soft, and bactrian fat is valued as a cooking oil in Chinese cuisine. The meat is similar to beef, but it has a lower fat content. Camel hides can flay and flesh easily depending on the level of hydration. They also dehair relatively quickly and are easier to split than cattle hides.

Buffalo

When water buffalo are processed in abattoirs, they are handled and dressed in much the same way as cattle. Difficulties are sometimes encountered in stunning them with a captive bolt gun because of the protection provided by their heavy skulls. Water buffalo in the Indian subcontinent and East Asia have a low dressing percentage in comparison with beef, the normal range being between 38% and 45%. The carcasses have low levels of fat, but the fat is usually white. They have relatively large fore shank, sirloin, and short plate cuts due to well-developed bone, and poorly muscled chuck and ribs, in comparison with beef. The meat is eaten as for beef, but in West Asia and Eastern Europe there are specialist buffalo meat products in the pastrami range of salted dried meats.

North American Bison

North American bison (often called buffalo) production is being tried in a number of countries for curiosity and tourist value. The main centers of production are in the northern parts of North America. Farmed animals are amenable to handling and can be put through processing plants that are designed for beef. There is very little export of the meat.

Horse

Horse and pony meat is exported from a number of countries for human consumption in France, Belgium, and Japan. It is

also sold for human consumption among aboriginal communities in Australia. Horses are slaughtered for the fresh and frozen petfood markets in many countries, but little is used in the canned petfood industry. The horses may be domesticated or mustered from wild populations of brumbies or mustangs. Wild horses and ponies are considered a pest in some regions, and slaughter for meat is a by-product of the culling operation. They are usually killed at dedicated horse export abattoirs, but in Australia there is also some field slaughter in the Northern Territory, and West Australia. Approximately 30% of the horse meat exported from Australia comes from brumbies. In the former USSR, the favored types for meat consumption are the blocky steppe breeds (e.g., Kazakh) and the forest breeds (e.g., Byelorussian Harness and Zhemaichu). Some attention has been given to selecting these types for body and carcass conformation.

On arrival at the abattoir, domesticated horses will sometimes have their shoes removed the day before slaughter to prevent injuries to each other and to staff. They are stunned with either a captive bolt gun or a rifle. Processing and dressing are relatively straightforward in horses, the only peculiarities being that in some countries the head has to be split to allow inspection for the bacterial disease glanders and, as they are hindgut fermenters, they have a large cecum. The dressing percentage varies between breeds, and for a 180 kg live weight harness breed would be approximately 51%. The meat is dark in color, with a strong grain and a sweet odor. Boneless meat yield varies considerably, according to the condition of the animal, and for feral stock it is usually between 125 and 175 kg. The fat can be soft and yellow. In Japan, the meat is used as a manufacturing meat, for example in smoked meats, sausages, and meatballs. In some countries horse meat from feral animals is becoming recognized for being free from veterinary residues. Saleable by-products include horse hide, hair plus, meat, and bone meal. Sound horse hides can command good prices, for example for making the inner soles of fashion shoes. Tail hair is used for making the bows of stringed musical instruments. Hearts and spleens have a market in the pharmaceutical industry.

Various Other Species

Reindeer and Moose

Fennoscandia (Sweden, Norway, and Finland) lead the world in commercial production of reindeer meat. In these countries the traditional Sami reindeer husbandry culture is performed with the reindeer free ranging (i.e., not enclosed in fenced areas) in forests and on the mountain tundra. Traditionally, reindeer were slaughtered at the selection site, i.e., at various locations surrounding the reindeer herding districts of the Sami people. In Sweden, new directives regarding reindeer meat inspection were instituted by the National Food Administration in 1993. The consequences of these new directives were that many of the former outdoor slaughter sites were closed, the numbers of reindeer transported to slaughter increased, and new mobile slaughter facilities were developed and introduced. Reindeer slaughter is seasonal, and almost all slaughter take place in the autumn and winter months, between September and March. The animals are herded on foot, using motorcycles, snowmobiles, or helicopters to gathering corrals where reindeer destined for slaughter are selected. The rules applied for animal transport, veterinary inspection of living animals and carcasses, stunning methods, slaughter hygiene, carcass grading, and chilling conditions for reindeer are similar to those applied for other domestic species, i.e., the specialized reindeer slaughter plants are all EU-approved facilities for slaughter of domestic animals. Reindeer meat is similar to deer venison; however, it is extremely tender and does not require aging before consumption and it has a characteristic wild/gamey flavor reflecting the natural pasture consumed by the reindeer in the forests and on the mountain tundra. Reindeer meat is a very exclusive gourmet product, which is in high demand and always on the menu in the more luxurious Fennoscandian restaurants. The meat is consumed fresh but is also marketed as cold- or hot-smoked and dried meat products.

Several of the dedicated reindeer slaughter plants in Fennoscandia are also approved game handling facilities, which means that they can also process various game carcasses. The processing of game is performed on a separate line from domestic reindeer carcasses and normally kept in a special area within the processing plant. Moose is the most common species processed in the game handling facilities; however, carcasses from various deer species (red deer, fallow deer, and roe deer), wild boar, and brown bear could also be delivered directly by hunters to these processing plants.

Small quantities of reindeer meat are also produced in Alaska, USA, but currently only for domestic/local consumption. Reindeer were introduced to Alaska from Siberia in 1892 to establish a predictable meat source and economic development for Alaskan natives. The extent and management of reindeer in Alaska has changed considerably over time but has recently evolved into a modern animal production system. The current reindeer industry is concentrated in the Seward Peninsula, Alaska, where reindeer are managed over large, rugged, and remote areas with little or no transportation infrastructure. Reindeer meat and velvet antler production generates significant employment and revenue important to the economies of rural Alaskan communities, but lack of slaughtering and transportation infrastructure constrains the development of the industry.

Reindeer and caribou are different. The semidomestic reindeer that inhabit the northern parts of Scandinavia, Russia, Siberia, China, and Mongolia and that have been introduced to Alaska belong to the subspecies of Eurasian tundra reindeer (*Rangifer tarandus tarandus*) and have been domesticated for thousands of years. Alaska caribou (*Rangifer tarandus granti*) and other North American caribou subspecies have never been domesticated, but have been hunted for meat.

Ostrich, Emu, and Rhea

Ostrich, emu, and rhea are often slaughtered in dedicated ratite plants. The birds are led individually to the slaughter point, and either electrically stunned or shot with a captive bolt. Typically, a pair of scissor-type tongs is used for electrical stunning, but a specialized stunner within a box is also used

for ostriches, where the bird's head is shut inside the box and current is then applied. Often, the bird's legs are secured before stunning. This is usually done either with a chain shackle or with a bar applied across the shank while forcing the bird to either sit or stand still within a wedge-shaped restrainer. Leg restraint is used to help control skin damage and to make transfer to the sticking point less hazardous because of flailing legs. Sticking is done with an ear-to-ear ventral throat slit, and this is sometimes followed by an additional thoracic stick once the carcass has stopped convulsing. Ratites do not have a crop, and therefore a chest stick does not usually lead to contamination of the carcass with upper gastrointestinal tract contents. At some plants, ostriches and emus are shorn, using a set of electric sheep shears, before the skin is removed. In the past this has been done on the live animal, but more often it is now done after stunning and sticking. Shearing should be done in a separate dedicated area to help control the spread of dust from the feathers. The skin is removed using a midline ventral incision that deviates around the chest callus over the sternum. The skin is very tight over the back and has to be freed with a knife, whereas at the front and sides it can be punched and pulled by hand. The meat is dark red in color and most is in the thigh and drumstick. There is very little muscle over the keel. The drumstick meat has a lower value than thigh meat because of its higher sinew content. The sinew can be removed with a silver skinner. South Africa exports both frozen and vacuum-packed fresh ostrich meat.

Emus have a well-developed preen gland, which is valued for its oil in medical and health products. It is removed by pulling with one hand while cutting it free from the carcass with the other. It is processed by cutting into small pieces, or grinding with water, then extracting the fat by boiling and pouring it into collecting jars. It may also be skimmed or filtered before despatch to the pharmaceutical company. The skin has a strongly bound layer of subcutaneous fat in well-finished birds, which can be difficult to remove. In addition, the feathers protrude through the skin into the subcutaneous tissues over the back, and it is necessary to remove all these feathers before taking the skin off, otherwise there is a risk of tearing the skin. Manual feather removal can slow the processing considerably. As with ostriches, the shank skin can be valuable in the fancy leather trade. It is removed by pulling after making opening cuts in the sole of the foot and the rear margin of the leg. Rheas have similar feather follicles to emus, and their skins are smaller in overall size. Emus have small caeca in comparison with ostriches and rheas.

Crocodile

Crocodile processing is invariably done at the farms of origin, which also often serve as tourist stops. During slaughter, the animal is snubbed and tied, and carried to the processing area, where it is then shot in the head. The value of the animal is in its skin, and therefore great care is taken to minimize damage during handling. The tail has been the main part of the crocodile used for meat consumption in Africa. It is a pale meat with a characteristic structure, and is usually served as thin slices cut across the length of the tail. In Australia, a standardized jointing procedure has been developed that makes use of the backstrap, leg meat, jowls, neck, flank, and rib covering.

Animals that are killed in the wild are sometimes butchered and eaten where they were shot. The meat can be cooked and eaten immediately, without being unduly tough. In this situation the muscle is cooked and eaten without going into rigor. However, if the meat is cooked slowly, rigor will be accelerated and heat shortening will occur and hence there will be some toughening. The rise in temperature during cooking needs to be rapid to avoid the contraction and thus help ensure acceptable tenderness.

Kangaroo

Kangaroo, wallaby, and euro are usually shot at night time using cross-country vehicles and spot lights. After a shooting run, the carcasses are gathered together and dressed on the spot before loading into refrigerated trucks. African wild ungulates may be treated in the same way, or the uneviscerated carcasses may be trucked to a standing processing depot.

Rabbits

Rabbits are stunned electrically in the larger processing plants. They are handled individually using gloves and the head is pressed into a set of V-shaped wall-mounted electrodes. The stunned animal is then suspended and stuck. In other situations concussion may be used, or the animal may have its neck dislocated manually. There is nothing unusual about the dressing of rabbit carcasses, except that the skin may be removed as a sock, and in some parts of the world the brain is saved for speciality dishes.

Geese and Ducks

Geese and ducks are often processed in automated chicken or turkey plants that can handle birds of larger size than conventional broiler chickens. The plants are often equipped with semiautomated primary feather pullers and baths of molten wax for removing down feathers. Feather removal can be particularly difficult in waterfowl, and it often determines the optimum killing age of the bird; the feathers become more difficult to pull as a bird gets older. Dry salted fermented duck is a traditional product that is popular in China because of its special flavor. The traditional technology of producing dry salted duck is characterized by handcrafted salting and natural drying, which limited the production scale. New processing techniques have been introduced to improve the traditional technology and quality of dry salted duck.

Quail

Quail are sometimes stunned and processed while suspended in shackles from an overhead line. The shackling procedure can be stressful for the birds, and sometimes they attempt to fly out of the shackles. If one leg is removed, the suspended leg may break at the shank. In one system the birds are stunned electrically along the length of the body to simultaneously induce cardiac arrest. They are not bled with a neck cut, but

instead blood is removed at evisceration. At other plants the birds are decapitated either with a cleaver and block, or by a knife or pruning shears, before they are suspended. Both wet and dry plucking methods are used. Dry plucking is done by hand or mechanically, and at some plants the birds are wax dipped in a similar manner to ducks. Gutting can be more difficult in quail because of their small size. Vacuum drawing of the viscera is used in larger plants. Quail products include boneless meat, oven-ready fresh or frozen carcasses, and smoked quail for the delicatessen trade.

See also: Meat, Animal, Poultry and Fish Production and Management: Exotic and other Species. Species of Meat Animals: Game and Exotic Animals

Further Reading

Edwards, D., Hector, D.A., Norman, G.A., Silverside, D., 1979. Slaughter Facilities for Tropical Conditions: A Guide to the Selection and Costing of Appropriate Systems. London: Tropical Products Institute (G123).

Hoffman, L.C., Wiklund, E., 2006. Game and venison – meat for the modern consumer. Meat Science 74, 197–208.

Queensland Department of Primary Industries, 1996. Handbook of Australian Crocodile Meat. Brisbane: Queensland Department of Primary Industries.

Stern, R.O., Arobio, E.L., Naylor, L.L., Thomas, W.C., 1980. Eskimos, Reindeer and Land. USA: School of Agriculture and Land Resources, University of Alaska Fairbanks.

Tetangco, M.H., 1981. Recent Advances in Buffalo Research and Development. Taiwan: Food and Fertilizer Technology Center.

Wiklund, E., Malmfors, G., 2004. The effects of pre-slaughter handling on reindeer meat quality – a review. Animal Breeding Abstracts 72, 1N–6N.

Wiklund, E., Smulders, F.J.M., 2011. Muscle biological and biochemical ramifications of farmed game husbandry with focus on deer and reindeer. In: Paulsen, P., Bauer, A., Vodansky, M., Winkelmayer, R., Smulders, F.J.M. (Eds.), Game Meat Hygiene in Focus. Microbiology, Epidemiology, Risk Analysis and Quality. The Netherlands: Wageningen Academic Publishers, pp. 297–311.

Relevant Websites

http://www.deernz.org/n3.html
Deer Industry New Zealand.

http://icr.arcticportal.org/
International Centre for Reindeer Husbandry.

http://www.slv.se/sv/grupp1/Livsmedelskontroll/Livsmedelsanlaggningar/Eu-godkanda-anlaggningar1/
National Food Administration.

http://reindeer.salrm.uaf.edu/
Reindeer Research Program.

http://www.eng.samer.se/
Sami Parliament.

Pigs

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Glossary

Audit A systematic examination of a system to determine whether documented procedures are being complied with and that the system achieves its objectives.

Carcass The dead body of a slaughtered animal after removal of the offal.

Codex Alimentarius Commission A joint international committee of the Food and Agriculture Organization and the World Health Organization of the United Nations. Its work includes the development of international food standards, guidelines, and codes of practice to protect consumer health and ensure fair practices in the food trade. In many cases, Codex Alimentarius standards are used as a basis for national legislation.

Critical control point A point, step, or procedure at which control can be applied to a hazard so that it is prevented, eliminated, or reduced to an acceptable level and where loss of control may lead to a food safety problem that could harm the consumer.

Evisceration To remove the viscera from the carcass.

Hazard analysis Identification and assessment of all potential and real hazards that may occur at each step of the carcass dressing process. It includes an assessment of the likelihood that each hazard will occur and the severity of the hazards identified.

Hazard analysis and critical control point (HACCP) A systematic approach to the identification and assessment of hazards and risks associated with the production, processing, distribution, and use of a particular foodstuff and implementing measures which either prevent the hazards occurring or correct the process to maintain control.

Pathogens Bacteria capable of causing disease. Meat items infected with pathogens usually look no different to an uninfected item.

Process A set of activities, usually expressed as a sequence of steps that must be performed to prepare a final product.

Processor A commercial entity responsible for the humane slaughter, dressing, and chilling of pigs and their carcasses.

Risk A measure of the likelihood of a hazard occurring and the severity of a hazard.

Introduction

The slaughter-line operations for pigs can be divided into two major sections. Following stunning and bleeding of pigs, activities on the slaughter floor are concerned with processes to remove hair, including scalding, dehairing, singeing, and polishing of carcasses. Evisceration then occurs, which involves the removal of the intestines and pluck sets (larynx, trachea, lungs, esophagus, heart, liver, and spleen) from carcasses. Carcasses are then washed to primarily remove bone dust and blood. The head, kidneys, kidney fat, flare fat, and foretrotters may also be removed from the carcass before entry into the chiller, depending on customer requirements.

The Codex Alimentarius Commission, the United Nations International Standards Organization for food safety, advocates the implementation of hazard analysis and critical control points (HACCP) systems as effective process controls for managing food safety risks associated with the production of meat. The implementation of quality control systems by pork processors, based on good manufacturing practice and HACCP, is legally mandated in many countries, including the US, Australia, Canada, and throughout the European Union. The requirement to limit microbial contamination to as low as what can be practically achieved may result in additional operations being introduced into a plant. Specific interventions, such as chemical sanitizing, hot-water washing, or steam pasteurization, may therefore be needed to reduce microbial contamination, enhance shelf life, and minimize risks to human health associated with microbial pathogens.

A shortage of labor, the need to reduce costs, customer demands for improved carcass hygiene, and the need to provide good working conditions for employees have resulted in the automation of many pig slaughter operations. Semiautomated, welfare friendly lairage systems are available to divide pigs into groups of approximately 15 and move them toward the stunning point. These groups are then further subdivided into groups of approximately five for automated carbon dioxide group stunning, without requiring the use of electrical prodding devices. Only the shackling, singeing, and gambrelling operations in modern, high-throughput plants need to be manually performed; preevisceration, scalding, singeing, scraping, and polishing can be automated. Automated carcass evisceration systems are available as systems for bung dropping, back finning, and carcass splitting on the slaughter line, with those components that are in contact with the edible product sanitized between each carcass to minimize cross-contamination. The principal slaughter-line operations can be fully automated to form a computer-integrated manufacturing system.

A Description of Current Pig Slaughter Practices

Although current pig slaughter practices may vary slightly from country to country, the core activities remain the same. These are shown in Figure 1.

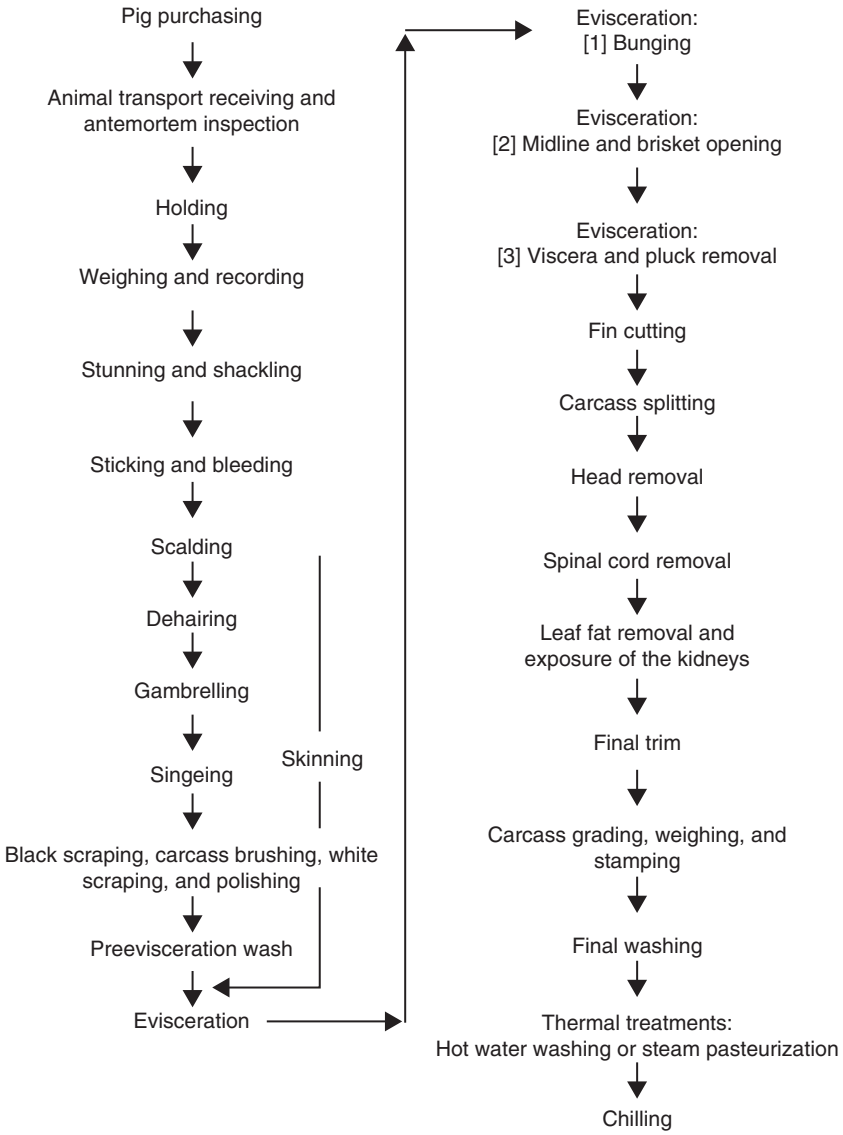

Figure 1 A summary of flow diagram of pig slaughter processes.

On-Farm Pig Quality Assurance Systems

Pigs should be purchased from producers whose production systems meet the standards described in a recognized quality assurance scheme and audited by an independent third party.

Pig quality assurance schemes may include an on-farm *Salmonella* control program (e.g., in Canada and the European Union) based on bacteriological testing. Competent authorities may maintain a central database and each pig producer be issued with a certificate stating the category of the herd. In Denmark, this program also includes serological testing using enzyme-linked immunosorbent assay of meat juice samples at the abattoir to identify previous exposure. A finisher pig *Salmonella* index is calculated based on sample results, and pig herds are assigned on a monthly basis into one of three levels of *Salmonella* infection. The objective of the program is for as many farms as possible to achieve level one status, with fines imposed on producers whose herds fall into levels two and three. The status of each herd is published on a website that details the health status of Denmark's pig breeding herds. Pigs arriving at the abattoir without an up-to-date certificate are usually treated as category three animals. Category three pigs are transported separately, segregated in a lairage, and slaughtered at the end of the day. Unless heat-treated, the head or head meat, gastrointestinal tract, lungs, and liver of these animals are excluded from human consumption. In the US, the Food Safety Inspection Service (FSIS)/United States Department of Agriculture Pathogen Reduction: HACCP Final Rule (61 FR 68806), introduced in 1997, includes *Salmonella* performance standards for slaughter plants to provide incentives for meat processors to reduce *Salmonella* prevalence on their products.

Feed Withdrawal

A feed withdrawal program should be implemented on farm if the time from the farm to slaughter is less than 12 h to reduce the risk of burst viscera during evisceration. Pigs subjected to preslaughter feed withdrawal are less likely to vomit, experience motion sickness, or die during transport compared with pigs transported with a full stomach. Pigs should be slaughtered between 12 and 24 h after they have been removed from feed to minimize *Salmonella* shedding and possible contamination of the carcass.

Animal Transport, Receiving, and Antemortem Inspection

Most countries have guidelines or legislation covering animal transport. Processors may also stipulate that transport companies must be accredited for transporting their livestock. The Australian Standards & Guidelines for the Welfare of Animals Land Transport of Livestock was introduced nationally in July 2012, replacing the Land Transport Standards endorsed in 2009. These standards cover the period before loading when animals are first deprived of feed and water to the period when livestock are unloaded and provided access to water at their destination. Within the EU, regulations on the protection of animals during transport and related operations were amended in December 2004 and are detailed in EU Council Regulation (EC) No 1/2005. In Denmark, requirements for mechanical ventilation, global positioning systems, drinking water, and sprinkler systems on transport vehicles have been introduced by processors.

Antemortem inspection is a requirement of public health legislation as an essential prerequisite to the slaughter of animals intended for human consumption. The antemortem inspection and the judgment of fitness for slaughter are normally restricted to the official veterinarian or other authorized person to whom the inspection is delegated. There is growing evidence that antemortem inspection may be more effective in improving animal welfare and processing efficiency if carried out on farm as part of a quality assurance system by trained personnel familiar with the animals than abattoir antemortem inspection. Adequate facilities should be provided, including appropriate lighting, and the animals should be observed at rest and from both sides while moving.

Holding

Pigs should be rested in lairage pens, with access to clean water, at the abattoir to allow them to settle and overcome any stresses encountered during transport for at least 2 h before slaughter. Pigs should not be mixed with unfamiliar animals during transport and in lairages to minimize fighting and bruising. Apart from the direct loss associated with death during transport, bruising can result in increased carcass trimming or carcass condemnation. Acute stress immediately before slaughter can also result in the development of pale, soft, and exudative pork, negatively affecting its quality, shelf life, and saleability. Lairage facilities should be constructed and maintained so as to prevent physical injuries to the animals and allow for adequate ventilation, light, and shelter from adverse weather conditions. Space allowance in lairages should allow all animals to stand or sit or lie down at the same time. Pigs should be handled calmly and quietly. Pigs have an acute sense of smell, good hearing, poor vision and are curious, but are particularly sensitive to stress. With a poor following instinct, they require firm handling, which can be best achieved using handling aids, such as pig boards, rattles, or flags, to encourage movement through sight or sound. Pigs prefer being handled in small groups of 5–6 pigs and do not like being moved individually or being left alone. The use of electric goads should be avoided as much as possible.

Each batch of pigs should be segregated in a lairage to minimize fighting and the spread of pathogens such as *Salmonella*. Clean drinking water should be available at all times and the pigs should be fed if the delay before slaughter exceeds 24 h. In hot weather, a fine mist or spray of cold water may reduce stress during lairage.

Weighing and Recording

In some plants, live pigs may be weighed using a balanced scale before stunning to allow the calculation of dressing percentage ((carcass weight per live weight)×100). The breed, sex, and ID number may also be recorded.

Stunning and Shackling

To present the pigs for stunning, they must be moved from the lairage. This may be achieved using a series of races and chutes to encourage pigs to move forward. Races should have solid sides and be wide enough for two pigs to walk together. Pig races should never have right-angled bends. Lighting should gradually become brighter toward the stunning point. Chutes may be single or double and include a series of gates to prevent the animals from reversing. In most countries, stunning and exsanguination, or sticking, may only be carried out by accredited persons.

It is a welfare requirement in many countries that pigs are rendered insensible to pain by stunning before slaughter (e.g., EC No 1099/2009 that has repealed Council Directive 93/119/EC and applied from 1 Jan 2013; Australia – Model Code of Practice for the Welfare of Animals: Livestock at Slaughtering Establishments; USA – Humane Methods of Livestock Slaughter Act). All stunning equipment should be maintained and inspected by trained, conscientious employees to ensure that a consistent and effective stun is delivered to each animal. There should always be a backup stunning system within reach of abattoir personnel. Pigs are typically stunned in abattoirs using either a carbon dioxide chamber or by the application of electrical current to the head. Penetrative captive bolt stunning of adult pigs requires accurate placement on the pig's forehead to render the animal insensible to pain in a single shot, as its brain is relatively small and protected by sinuses. This method is not routinely used in abattoirs for pigs as it has a lower efficacy (it is acceptable for 95–98% of animals to be instantly rendered insensible with one shot) compared with electrical or

carbon dioxide stunning. Tonic and clonic movements can also be intense following captive bolt stunning, impacting on worker safety during shackling and hoisting for exsanguination.

Carbon dioxide stunning of pigs is now used in many countries. Pigs should be smoothly moved into gondolas, typically set out in a Ferris wheel-type arrangement. Once appropriately filled, the gondola is lowered into a pit containing a high concentration of carbon dioxide. It is a requirement of the World Organization for Animal Health that the CO_2 concentration used must not be less than 80%. Pigs should be exposed to a maximum concentration of 90% carbon dioxide in air at the bottom of the pit within 30 s, and the total exposure time should be more than 90 s to minimize any chance of recovery. Research has shown that blood oxygen levels fall slightly during stunning, whilst carbon dioxide levels in the blood rise significantly. Carbon dioxide stunning lowers the pH of the blood and brain tissue, leading to analgesia and loss of consciousness. After stunning, the now unconscious, flaccid pigs are tipped onto a shackling table and shackled with a chain by one leg, usually between the dewclaws and the hock, before being hoisted onto an overhead rail and exsanguinated. A semiautomated group carbon dioxide stunning system, developed in Denmark to optimize both animal welfare and meat quality, is now being used in many countries. This system relies on the pigs' natural curious behavior to move forward in small groups toward the stunning unit, with assistance from gates and slowly moving walls. This has removed the need for single file races and use of electric goads. The system allows for stunning of small groups of five pigs at a time and can meet plant line speeds of 830 pigs per hour.

For effective electrical stunning of pigs, a minimum current of 1.3 A at a voltage of at least 240 V must pass through the pig's brain for at least 1 s to reliably induce an epileptic fit. Electrical stunning should only be applied once. Electrical current may be applied by either manual or automatic application of stunning tongs positioned on both sides of the brain between the eye and the base of the ear. Cardiac arrest stunning may also be used either by using head-to-back electrodes (applied either between the ears, on the sides, or top of the head or on the forehead, and on the backbone of the animal behind the heart) or head-to-brisket tongs that are positioned with electrodes placed on the top of the head between the ears and the other electrodes placed between the forelegs on the brisket. Electrical stunning at a frequency of 50 Hz is most commonly used. It is imperative that the electrodes are correctly positioned and the current is applied for a sufficient time so the duration of insensibility persists to ensure that the pig does not recover before it dies from bleeding. A maximum stunning-to-stick interval of 15 s is recommended. For pigs, a minimum time of 37 s to return of rhythmic breathing and recovery from effective electrical stunning has been shown. If the stun is not effective, animals will be merely paralyzed and recover from the stun before brain death following sticking. Electrical stunning of free-standing pigs may result in broken bones, particularly the shoulder blades, as a result of the forelegs hitting the floor when electrical current is applied. Hence, V-shaped restrainers and monorail or double-rail conveyers are often used to hold in position while efficiently moving them toward the point for electrical stunning. High-voltage stunning (up to 1000 V for 1.5–2 s) may also be used, particularly in higher volume plants, and applied in association with the use of a restrainer–conveyer to form an automated system.

Sticking and Bleeding

Sticking should take place within 15 s after stunning to ensure the animal does not regain consciousness and to minimize ecchymosis (blood splashes) in the muscle. Ecchymosis may occur when capillaries restricted by muscle spasm rupture as a result of the build-up in blood pressure when the heart continues beating and is more common in electrically stunned pigs. A knife is used to cut the main blood vessels (anterior vena cava and bicarotid trunk) in the upper chest. This is usually achieved by inserting the knife midline at the base of the neck. Then, with the point of the knife between the sternum and the backbone and directed toward the tail, the operator thrusts the knife toward the anus until it strikes the backbone, cutting the carotid arteries and jugular veins. Blood should flow freely and death should occur within a few minutes. The knife should be sterilized in hot water at a temperature of at least 82 °C between each animal to prevent cross-contamination. Abattoir personnel should ensure that the animal has been effectively stuck by checking for the absence of brain stem reflexes (blinking when cornea is touched and reflexive gasping breaths).

Sticking may push contamination from the skin surface of the pig to the inside of the carcass. Scalding may also contaminate the sticking wound and this should be removed at a later stage and condemned. Bleeding usually takes less than 5 min and death occurs as a result of oxygen deprivation to the brain. Blood may be collected at this stage for later use in the preparation of processed pork products. This can be done using a stainless steel hollow knife, connected to a vacuum pump and storage vessels, which is then sterilized between each carcass in 82 °C hot water. When collected for edible purposes, blood will be batched and held until carcasses have passed inspection. Vision systems for automatic monitoring to ensure that sticking has been performed correctly have been installed in Europe and US abattoirs.

Dehiding

In most countries, skin remains on the carcass. However, in some countries, most notably Japan, China, and Taiwan, skins may be removed from the unscalded pig or immediately after scalding. Leather produced from unscalded pigskins is thicker and has greater tensile strength than that produced from skins that have undergone the scalding process. Skin removal may be performed manually or by machine. Machines capable of skinning the whole carcass are now available.

Scalding

When the pigs are not skinned, the hair must be removed from the carcass. Scalding is the first of the dehairing operations. Scalding facilitates hair removal by denaturing the proteins in

the hair follicles. This can be achieved by immersing the carcasses in a scald tank of hot water. Steam condensation systems are available and are used in countries including Poland, Spain, France, Switzerland, Japan, Australia, and China. The time–temperature combination applied is important, as underscalding does not facilitate hair removal and overscalding causes the skin at the base of the bristles to contract, holding them tighter. Although a typical time–temperature regime may vary from 5–10 min at 60–70 °C, the exact combination applied will depend on the amount of hair and its ease of removal. Factors such as pig breed and seasonal influences should also be taken into account. In the US, for example, there is an easy hair season (February–March) when a scalding combination of 58 °C for 4.5 min is typically used, and a hard-hair season (September–November) when 60 °C for 4.5 min is applied. Throughout the rest of the year, a typical scalding temperature–time combination of 59 °C for 4.5 min is the norm. In Australia, the typical scalding temperature–time combination for immersion systems is 62 °C for 4.5–5 min.

Food safety is also a consideration during scalding as each carcass brings a quantity of dirt, feces, and ingesta to the scald tank and as a result the water quickly becomes polluted and may act as a vehicle for carcass cross-contamination. However, enteric bacteria such as *Salmonella*, *Escherichia coli*, and *Campylobacter* will be destroyed if the temperature is sufficiently high. Studies have found that 1.0×10^6–1.9×10^6 *Campylobacter* per milliliter may be reduced to less than 10 organisms per milliliter after 1–1.25 min in a scald tank water at 56 °C. Similar reductions have been observed for *Salmonella* and *Yersinia* strains at 60 °C after 1.7–2.2 min and 2.5 min, respectively.

Dehairing

Dehairing is usually performed using a dehairing machine. This removes the hairs mechanically by rotating the carcasses using the action of rotating flails while cold or hot water is sprayed over the carcasses. A perforated tray under the machine screens hair and other detritus from the water. The dehairing machine may be an important contributor of *Salmonella*, *E. coli*, *Campylobacter*, *Yersinia*, coagulase-positive *Staphylococcus aureus*, and *Listeria* contamination of pork carcasses during processing. The prevention or reduction of contamination is dependent on effective cleaning of the equipment.

Gambrelling

Pigs are ejected from the dehairing machine onto a stainless steel conveyor belt or gambrelling table. Two parallel incisions are made through the skin on the back of each hind leg to expose the gambrel tendons and the gambrel hook is inserted hooking the hind legs of the animal, which is then hoisted onto an overhead conveyor rail. The toenail may be removed at this stage or after polishing. This is most effectively achieved using a toenail puller. Pruning shears may be used if the toenails are particularly difficult to remove. Carcasses generally remain hung from the Achilles tendon on entry to the chiller, but if split, sides may be transferred from gambrels to individual slides.

Singeing

In the abattoir, singeing is performed to facilitate the removal of any remaining hairs. Intense heat is applied that burns the remaining hairs and tightens the skin, facilitating subsequent polishing or shaving. Singeing may be as simple as using a hand-held gas torch. Larger plants utilize singeing units containing gas jets that are housed in a stainless steel cabinet that is positioned on the line and carcasses pass through it at chain speed. Gas and air are blown into the cabinet, creating a flame that envelops the carcass. The temperature in the chamber may reach up to 1200 °C and is applied for 5–10 s. The surface temperature of the pork carcass may increase to 100 °C during this process. This operation does not eliminate all the bacteria on the carcass, but it significantly reduces contamination. Water is usually sprayed onto the carcasses before and after singeing to remove loose hairs and blood.

Black Scraping, Carcass Brushing, White Scraping, and Polishing

After singeing, a variety of polishing or shaving devices may be applied to remove singed and remaining hairs. The black scraper consists of slowly rotating steel scrapers applied with a cold water spray to remove black singed hairs. Carcass brushers consist of a series of stiff nylon rotating brushes, which contact most parts of the carcass and are usually applied with a spray of cold water to remove loose hairs and other particles. They are particularly useful for reaching areas such as the head, forelegs, and hind legs, which may be unaffected by other dehairing devices. The white scraper is a machine that operates on the same principles as the black scraper. It consists of revolving stainless steel claws, which move up and down the carcass, and is applied dry to give the carcass a polished finish. The polisher consists of horizontal and vertical flails that rotate against the carcass to remove any remaining bristles. The rotating flails are often attached to two or more upright shafts, which typically run at different speeds. For example, the lower shafts may run at 55–60 rpm while the upper shaft rotates at 100 rpm. The whipping action of the flails removes loose hairs, and these machines are particularly effective for carcass cleaning.

Inadequately polished areas may be shaved or scraped by abattoir personnel using a shaver or knife. This includes the removal of eyebrows and hair on the lips and inner ear. If particularly cumbersome, these may be trimmed using a knife. All tools should be immersed in water at temperatures of at least 82 °C between animals to prevent cross-contamination.

Although effective singeing can result in almost complete removal of skin surface contamination, not all singeing is completely effective and bacterial contamination may remain on carcasses postsingeing particularly in skin folds, ears, and hair follicles. Polishing can therefore redistribute those few bacteria that survive the flame treatment across the surface of the carcass, negatively affecting the carcass microbial status.

A second singeing unit after polishing may be used to improve the hygiene status of pork carcasses.

Lean Meat Yield Estimation

Fully automated 3D ultrasound scanning equipment for estimation of lean meat yield of carcasses at a capacity of 1200 carcasses per hour may be installed after dehairing and before evisceration. Alternatively, other systems may be used post-evisceration and be used as a basis of carcass payment to producers. These include automated vision and image-processing systems for lean meat yield estimation. Manual reflectance or ultrasound probes for measurement of carcass fat and muscle depth at a single site are also used. Single-point fat and muscle depth measurements of pig carcasses are generally made over the longissimus muscle at approximately 65 mm from the midline of the carcass between either the third and fourth last thoracic ribs or at the last thoracic rib (referred to as the P2 site). This fat and muscle depth information may then be used in algorithms for the prediction of carcass lean meat yield.

Preevisceration Wash

The polished carcasses then pass through a cold water spray before entering the dressing procedure. This removes any loose hairs from the carcass but it is not a decontamination procedure. Washing may spread localized microorganisms on the skin to other areas of the carcass.

Evisceration

The first stage in evisceration is the removal of the scrotum, testicles, and penis. A cut is made between the hind legs downwards toward the penis and around the preputial pouch. This should expose the penis cord, which is removed along with the penis. Evisceration is then divided into bunging, midline and brisket opening, and viscera and pluck removal. Robots that automate the evisceration process are now available and have been installed in many countries to minimize contamination, improve yields, and optimize labor use. Automated evisceration, using a laser-guided aitchbone saw and belly opener, can reduce contamination and wastage, resulting from the accidental cutting of the stomach and contamination from spilled visceral contents.

Bunging

Bunging or cutting around the rectum and reproductive tract where it enters the pelvic cavity may be done using a knife or bung cutter (consisting of a rectal probe and a sharp rotating cylinder). Some rectal probes use a vacuum to remove fecal material from the rectum during this procedure. All the connective tissue to the bung is then severed, allowing the operator to pull the bung up and out. To prevent cross-contamination between carcasses, the cutting instrument must be immersed in hot water (82 °C or higher) between each carcass. Although the sphincter muscle should prevent leakage of fecal material onto the carcass, this is best prevented by tying off the bung with string or by using a plastic bag to seal the rectum. The operator puts the inverted plastic bag over their left arm (assuming they are right-handed), grasps the rectum with the covered hand and pulls the bag, inside out, over the rectum before sealing it with a plastic band or tying it with a piece of string.

Midline and Brisket Opening

Using a knife, a short incision is made in the inguinal region into the abdomen. The knife is then reversed and the abdomen is opened to the xiphoid cartilage. A cut is then made through the tissue over the sternum to the sticking wound using a brisket saw. Viscera are then lifted out and a cut is made through the esophagus. The pluck is lifted out separately, and this may be done by a separate operator depending on the chain speed.

Viscera and Pluck Removal

The viscera are comprised primarily of the stomach, the small intestine, and the large intestine. In pigs, the pluck set includes the larynx, trachea, lungs, esophagus, heart, liver, and spleen. The liver is released by severing the blood vessel near the top of the organ. The esophagus is cut to free the viscera. Operators must be well trained to ensure that during removal of the intestines the esophagus is cut at the correct distance from the stomach and that accidental cutting into the viscera is minimized. After the pluck set is removed, these organs are usually placed with the viscera on a stainless steel tray to allow for inspection or hung on a moving hook. Either way, the viscera remains linked to the carcass until the completion of inspection. The kidneys may be left in place. The removal of the viscera and pluck is increasingly being performed by robots in larger abattoirs, resulting in reduced levels of microbial contamination on the carcasses. In many countries, including the European Union, USA, Canada, and Australia, evisceration is considered to be a critical control point in HACCP systems. Problems associated with monitoring, critical limits, and corrective actions may be overcome by using an on-line monitoring system.

Fin Cutting

In many countries, long incisions are made exposing both sides of the spinal processes from the tail to the head and the loin is separated from the spine before carcass splitting in a process called fin cutting. This process has also been automated and systems have been commercially installed around the world.

Carcass Splitting

The carcass splitting saw is positioned along the midline between both legs. The carcass is split through the center of the

vertebral column along the midline from the hind to the fore. The glands and any blood clots in the neck and jowl regions may then be removed. The saw should be immersed in water at 82 °C or higher between each carcass to prevent cross-contamination.

Head Removal

Depending on customer requirements, the head may remain on the carcass until after chilling. However, high levels of microbial contamination are associated with the head, particularly tonsils and tongues. Early head removal, including excision of the tonsils and tongue, may be done immediately before evisceration. If not previously done, the eyelids and the inside portion of the ears will be removed immediately before head removal. The head is removed using a knife and trimmed of any residual hair. Head removal is best achieved by cutting directly above each ear before cutting into the seam outlining the jaw on both sides of the head. The atlas joint joining the head to the body may then be cut, followed by the esophagus and trachea. It is important that the esophagus is cut directly above the epiglottis to avoid cutting through the lymph nodes, which will be subsequently inspected. The head may be left attached to the carcass by the remaining skin and muscle or completely severed and washed.

Spinal Cord Removal

The spinal cord is removed using a knife or hook or by suction using a vacuum system.

Leaf Fat Removal and Exposure of the Kidneys

The leaf fat is a heavy layer of fat lining the inside surfaces of the porcine abdominal cavity. This is usually manually pulled away from the carcass to expose the kidneys.

Final Trim

Trimming is performed to remove any excess fat and any visible fecal stains. The knife should be immersed in water at 82 °C or higher to prevent cross-contamination.

Carcass Grading, Weighing, and Stamping

Pork carcass grading is generally based on carcass weight and a measurement of the fat and lean content of the carcass on the slaughter floor before chilling. In Australia, producer payments for pork carcasses are based on hot carcass weight and fat depth at the P2 site, located 65 mm from the midline of the carcass at the last rib. In Canada, the national grading system classifies pork carcasses into indexes based on measurement of fat and muscle depth 7 cm from the midline of the carcass between the third and fourth last thoracic ribs and carcass weight. Within the European Union, carcasses are divided into six classes and assigned a letter (S, E, U, R, O, or P), which indicates estimated lean meat content (S > 60%, E = 55–60%, U = 50–55%, R = 45–50%, O = 40–45%, and P < 40%). In the US, fat depth at the last rib may be measured and the expected yield of four cuts (ham, loin, picnic shoulder, and Boston butt) included in the carcass grading process. Information regarding grade, carcass weight, gambrel identification, and producer's tattoo number may be registered electronically and used in reporting back to producers. In Japan, fat thickness is measured at the narrowest point between the 9th and 13th thoracic vertebrae, carcass weight is obtained, and assessments of carcass appearance, meat, and fat color are then used by the grader to determine the grade of each carcass.

Final Wash

Carcasses are washed with cold (10–15 °C) or warm (15–40 °C) potable water to remove bone dust and blood clots. Washing with cold or warm water should not be considered a decontamination step during slaughter. Its effects are related solely to improving carcass appearance and not to food safety. This wash may also increase subsequent chilling rates (by evaporative cooling) and reduce the moisture loss of the carcasses. Cold water does not reduce the bacterial levels on pork carcasses.

Thermal Treatments

Hot water may be used as a sanitizing procedure to reduce the microbial load on the surface of pork carcasses. The main bactericidal effect of such systems is thermal, although there may also be a physical effect involving the removal of some bacteria as a result of washing. It can be applied either by spraying at high or low pressures, immersion of carcasses, or deluging carcasses with cascading sheets of hot water. In the EU, only the use of potable water is currently allowed in hot-water decontamination units. Recycling of hot water for carcass decontamination may be permitted (e.g., in Canada) to manage environmental considerations and water use and has been shown to be equally effective in reducing bacterial numbers compared with potable water. In Denmark, almost all carcasses from *Salmonella* level three herds and *Salmonella typhimurium* definitive type 104 herds are hot water treated. The FSIS in the US permits the use of specifically designed thermal interventions such as hot-water washing or steam pasteurization for the control of bacterial foodborne pathogens in pork. It may be more cost-effective for *Salmonella* prevalence to be reduced during processing by using thermal carcass decontamination systems rather than through on-farm control strategies, due to the considerable impact of this process on the *Salmonella* status of carcasses.

Steam pasteurization may be applied instead of hot-water washing and kills bacteria by opening pores in the skin. A process of applying pressurized steam to carcasses was approved for use in the USA (Frigoscandia Steam Pasteurization System). In this system, pressurized steam is applied to carcasses inside a chamber to achieve a surface temperature of at least 90 °C for at least 10 s followed by spray cooling of the carcass before chilling. Systems are also available for pork.

Steam treatment at the end of slaughter has been shown to effectively reduce the number of gram-negative bacteria (including *Salmonella*, *Yersinia*, *E. coli*, and *Campylobacter*) by 1.0–2.1 orders of magnitude. The automation of steam vacuuming of pork carcasses is being developed in Denmark. Other interventions, including the use of chemicals (e.g., lactic acid, acetic acid) may require prior approval by regulatory agencies before use.

Chilling

Chilling is the final slaughter-line operation. The duration for processing a pig carcass from stunning point to chiller is approximately 30–45 min in most modern abattoirs. Pork carcasses are usually kept in chillers for approximately 24 h before being sent to the boning room or transported to customers for carcass processing. The rate of chilling of muscles postslaughter is influenced by the size, shape, and fatness of the carcass as well as the chiller temperature, relative humidity, carcass spacing, velocity, and flow pattern of the chiller air. The chilling rate can influence pork quality as it influences the rate of muscle pH and temperature decline of muscles.

Although chilling conditions vary between plants, pork carcasses in Europe are usually chilled rapidly by blast chilling with air in the range of −10 to −30 °C at a fan speed of 0.5–2 ms^{-1} for approximately 2 h (which can result in freezing of the outer surface of the carcass) followed by chilling at 3–5 °C at 0.2–0.5 ms^{-1}. Relative humidity during chilling should be kept high (90–95%) to prevent excessive weight loss; however, relative humidity can be difficult to control. In North America, systems that spray carcasses with water during the first phase of cooling are also used. In other countries, including Australia, batch-chilling systems are used where carcasses are placed into chillers set at approximately 2–4 °C for approximately 24 h postslaughter. Rapid chilling can result in shrinkage losses of less than 1% of hot carcass weight due to a reduction in evaporative weight loss compared to 2–3% when carcasses are conventionally chilled. Chilling carcasses may prevent the proliferation of bacteria on warm carcass surfaces, but it does not always result in a decrease in total bacterial counts.

See also: Automation in the Meat Industry: Cutting and Boning. By-Products: Hides and Skins. Carcass Chilling and Boning. Classification of Carcasses: Pig Carcass Classification. Conversion of Muscle to Meat: Color and Texture Deviations; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening; Slaughter-Line Operation and Pig Meat Quality. Exsanguination. Hazard Analysis Critical Control Point and Self-Regulation. Microbial Contamination: Decontamination of Fresh Meat. Preslaughter Handling: Preslaughter Handling. Spoilage, Factors Affecting: Microbiological. Stunning: CO_2 and Other Gases; Electrical Stunning. Tenderizing Mechanisms: Mechanical

Further Reading

Alban, L., Stärk, K.D., 2005. Where should the effort be put to reduce the *Salmonella* prevalence in the slaughtered swine carcass effectively? Preventive Veterinary Medicine 68, 63–79.

Baptista, F.M., Halasa, T., Alban, L., Nielsen, L.R., 2010. Modelling food safety and economic consequences of surveillance and control strategies for *Salmonella* in pigs and pork. Epidemiology and Infection 139, 754–764.

Beier, R.C., Pillai, S.D., Phillips, T.D., 2004. Preharvest and Postharvest Food Safety. Contemporary Issues and Future Directions. Oxford, UK: Blackwell Publishing and the Institute of Food Technologists.

Berends, B.R., Knapen, F.V., Snijders, J.M.A., Mossel, D.A.A., 1998. Impact on human health of *Salmonella* spp. on pork in The Netherlands and the anticipated effects of some currently proposed control strategies. International Journal of Food Microbiology 44, 219–229.

Codex Alimentarius Commission, 2005. Code of Hygienic Practice for Meat. CAC/RCP 58-2005. Available at: www.codexalimentarius.net/download/standards/10196/CXP_058e.pdf (accessed 07.05.12).

Gill, C.O., McGinnis, D.S., Bryant, J., Chabot, B., 1995. Decontamination of commercial, polished pig carcasses with hot water. Food Microbiology 12, 143–149.

Hamilton, D., Holds, G., Lorimer, M., *et al.*, 2010. Slaughterfloor decontamination of pork carcases with hot water or acidified sodium chlorite – a comparison in two Australian abattoirs. Zoonoses and Public Health 57, 16–22.

Hinrickson, L., 2010. Manufacturing technology in the Danish pig slaughter industry. Meat Science 84, 271–275.

Hui, Y.H., Nip, W.K., Rogers, R.W., Young, O.A., 2005. Meat Science and Applications. New York, USA: Marcel Dekker.

Jackowiak, J., Kiermeier, A., Kolega, V., *et al.*, 2006. Assessment of producer conducted antemortem inspection of market pigs in Australia. Australian Veterinary Journal 84, 351–357.

Loretz, M., Stephan, R., Zweifel, C., 2011. Antibacterial activity of decontamination treatments for pig carcasses. Food Control 22, 1121–1125.

Madsen, K.B., Nielsen, J.U., 2002. Automated meat processing. In: Kerry, J., Ledward, D. (Eds.), Meat Processing – Improving Quality. Abington: Woodhead Publishing, pp. 283–296.

Miller, G.Y., Liu, X., McNamara, P.E., Barber, D.A., 2005. Influence of *Salmonella* in pigs preharvest and during pork processing on human health costs and risks from pork. Journal of Food Protection 68, 1788–1798.

Toldra, F., 2009. Safety of Meat and Processed Meat. New York, USA: Springer.

Relevant Websites

www.codexalimentarius.net/download/standards/10196/CXP_058e.pdf
Codex Alimentarius Commission.

http://www.fao.org/docrep/003/x6909e/x6909e09.htm
Food and Agriculture Organization for the United Nations.

www.grandin.com
Temple Grandin.

Poultry

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Glossary

Broiler stunning The process of using either electrical shock or exposure to controlled atmospheres, such as carbon dioxide, to render the broiler unconscious immediately prior to slaughter.

Carcass chilling The process of lowering the broiler carcass temperature to 4 °C within 1–2 h after slaughter. This is commonly done in a counterflow water immersion chiller that is 4 °C at the entrance and 1 °C at the exit.

Carcass prechilling A common practice after the evisceration and washing of broiler carcasses in which the broilers are exposed to temperatures of 7–12 °C in a water chiller for 10–15 min to lower the temperature with the purpose of moisture loss before the carcass is put in the chiller.

Evisceration The process of removing the viscera from the carcass. Viscera generally includes the gastrointestinal tract, reproductive tract, heart, and lungs.

Picking The process of removing feathers immediately after scalding in which flexible ribbed, rubber fingers rotate rapidly and use abrasion to remove feathers from the carcass.

Scalding The process of immersing broiler carcasses in hot water (commonly at 120 or 145 °F) for 30 s to 2 min to loosen feathers from the cuticles that they are attached prior to the picking process.

Poultry

Large-scale poultry processing plants are specifically designed to process poultry (chicken, turkey, and duck) at a rate that meets the growing consumer demand for poultry. The process not only needs to be efficient but also needs to be optimized at every step of the process to maximize yields and quality and insure product safety. The slaughter-line operation includes stunning, bleeding, defeathering, evisceration, inspection, chilling, and packaging operations. In commercial processing, bird uniformity is crucial for efficient processing so that the machines do not need adjustment during processing and lines can operate anywhere between 70 and 180 birds per minute. These production rates allow processors to minimize costs because the fixed costs of facilities and personnel must be paid irrespective of the efficiency of the processing line. The steps involved in a typical poultry plant are shown in Figure 1; modifications of this arrangement might exist, but the basic steps are fairly similar in all plants. The operation can be automated to varying degrees, depending on required output, capital investment, labor cost, etc. Some of the modern plants include automated evisceration, cut up lines, packaging, and labeling that can handle between 4000 and 12 000 birds per hour on a single line.

Receiving and Weighing

Birds are commonly transported to the processing plant either in individual crates stacked on a truck or in cages permanently mounted on a truck. Trucks typically arrive every 10–15 min, so there is more than one truck in the holding area at all times to minimize plant delays and allow the birds to rest for a minimum of 30 min before unloading. This rest results in lower corticosterone levels in the birds, indicating a recovery from any distress of prior handling and transportation. The weight of the birds received, on the truck, is determined and is used as the basis for calculating the payment to the farmer.

Unloading

Unloading the birds from the crates and placing them on the shackle line so that they are hung by their legs can be done manually, which is a common practice for turkeys. Automated unloading systems, also called dumpers, are usually part of a modular crate system, which can be lifted and tilted slowly so that the birds can pass onto a conveyor belt. As carcass damage, such as broken bones and bruising, can result from unloading, proper training is necessary and the distance between the dumper and the conveyer belt should be minimized to prevent such damage. In plants where gas stunning or low atmospheric pressure stunning (LAPS) is employed, the birds can be left in the crates, where they are stunned by carbon dioxide, argon gas, a mixture of gases, or low atmospheric pressure and later removed from the crates (this can assist in reducing bruising of excited birds taken out of a cage); unloading should then be done immediately after stunning so that no time is allowed for the birds to regain consciousness. Excited birds are more active; they flap their wings and are more likely to be hurt during the unloading process.

Stunning

Electrical stunning (ES), controlled atmosphere stunning (CAS), and LAPS are all effective at insuring that broilers are

Process step	Auxiliary step
Receiving at plant	
Remove containers from trailer	
Weigh empty truck/trailer	
Remove birds from container	
Live hand	
Stun	
Kill/bleed	
Scalding	
Primary picker	
Head removal	
Finishing picker	
Bird wash	
Count	
Hock cutter	
	Foot/paw remover
Transfer/rehang	
Pin feather removal	
Oil sac cutter	
Ventor	
Opener	
Eviscerator	
Fat separator/presenter	
USDA inspection	
Giblet harvest	
Neck removal	
Cropper	
Iobw	
Microbial intervention	
Finisher	
Bird unloader	
Water chiller	
	Wash shackles
Rehang	Grading
Drip line	
Sorting	
Packaging	
Storage	Cooler Blast freezer Holding freezer
Shipping/distribution	

Figure 1 Flow diagram for commercial broiler processing in the United States.

desensitized before slaughter. Stunning should produce a rapid onset of stress-free insensibility of sufficient duration to allow the animal to remain unconscious until the time of death. ES is the most common method used for poultry; the equipment is relatively inexpensive, simple to use (a fiberglass brine/water bath fitted under the overhead shackle line from which the suspended birds are moved forward), does not require much space, and is compatible with current kill-line speeds. The birds pass through the stunner in a continuous procession (e.g., 140–180 birds per min in a modern processing line).

To insure an irreversible stun, high amperage current (120–150 mA per bird) is utilized in the European Union (EU) so that the birds are stunned to death. No clear relationship exists between stunning amperage and whole-carcass quality attributes, but high stunning amperages have been associated with hemorrhaging in deep breast muscles. Hemorrhaging is due to a sharp increase in intravascular pressure, as a result of which blood capillaries may rupture and cause bleeding. Low-voltage ES is used in the US and is an effective method for immobilizing broilers. Low-voltage (10–25 V) and high-frequency (500 Hz) systems are used in 77% of the US poultry plants. Low-voltage ES can decrease the incidence of carcass quality damage and hemorrhaging that is associated with high-voltage ES. However, a bird will regain consciousness if not bled within approximately 2 min of stunning. In 2004, the European Food Safety Authority published a regulation with minimum currents to be used for ES (Figure 2). This requirement was published with the statement: "Since welfare is poor when the shackling line and water bath electrical stunning method is used, and birds are occasionally not stunned before slaughter, the method should be replaced as soon as possible. At present, the inert gas stun/killing method is the best alternative." This regulation went into effect in 2013.

CAS involves controlled changes in the atmosphere surrounding broilers such that broilers lose consciousness due to lack of oxygen (hypoxia); excess CO_2 (hypercapnic hypoxia); a combination of these two methods (hypercapnic anoxia); use of oxygen with inert gases, such as nitrogen or argon (hypercapnic hyper oxygenation); or atmospheric depressurization. The stunning gas or gas mixture should not be aversive to the broilers, and the induction of unconsciousness should be rapid. Several researchers have indicated that there are advantages to stunning with gases. One particular advantage includes the acceleration of rigor mortis. Stunning and bleeding by gas is effective at improving carcass quality by reducing bloodspots, especially those on the thighs and breasts, when compared with ES (EU electrocution).

Inhalation of elevated concentrations of the inert gases (argon and nitrogen) causes hypoxia, which eventually leads to anoxia (complete absence of oxygen). The commonly used gases are nitrogen and argon with or without CO_2 for broilers and nitrogen with CO_2 for turkeys, which results in anoxic loss of sensibility. In commercial situations, gas stunning minimizes the handling of birds (uncrating/shackling) before stunning, thereby reducing preharvest stress. However, gas stunning can also lead to some distress in birds as exhibited by wing flapping, gasping, and head shakes.

LAPS or atmospheric depressurization reduces atmospheric partial pressure of oxygen by evacuating air from an airtight chamber. Broilers are placed in an airtight decompression chamber and pressures of 0.20–0.29 atm have previously been utilized to stun the broilers. LAPS has been in the development and implementation stages in commercial broiler abattoirs since 2005. This technology controls the atmosphere through anoxia by using a vacuum pump to reduce oxygen tension in the atmosphere. Most tissues of the body can survive without oxygen for several minutes and some for as long as 30 min, during which the cells obtain energy through anaerobic metabolism. The brain is minimally capable of anaerobic metabolism because neurons have only a

minimal reserve of glycogen. Thus, a sudden cessation of blood flow to the brain or a sudden drastic decrease in oxygen in the blood will result in unconsciousness in 5–10 s. As of 2013, a few broiler plants in the US have adopted LAPS as their method of stunning.

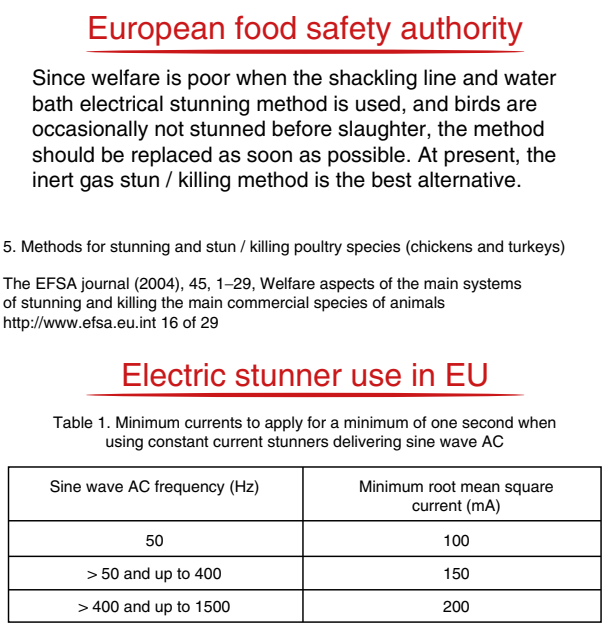
Bleeding

The most common method of bleeding is to cut both carotids just below the jowls to insure fast and efficient bleedout. This method also results in leaving the windpipe and esophagus intact, which is important when automated equipment is later used to pull out the windpipe. When this method is conducted, a cutting machine is used that guides the head so that a circular blade cuts the jugular vein and carotid arteries. Automated high-speed bleeding equipment employs a railing system that positions the neck of the suspended birds in such a way that precise cutting of the blood vessels can be performed. For true kosher slaughter, birds are not stunned and only manual cutting of the blood vessels is permitted. For Halal slaughter, it is dependent on the certifying body on whether or not birds may be stunned before the slaughter process. The bleedout phase takes anywhere between 2 and 5 min, depending on bird size, etc. During the process, approximately 35–50% of the total blood is lost. Other factors affecting blood loss include the stunning method used and the time interval between stunning and bleeding. It is important to note that a poor bleedout can increase the prevalence of carcass downgrading conditions due to bloodspots and, in particular, engorged or hemorrhagic wing veins, which leads to red wing tips and decreased shelf life.

Scalding

In commercial abattoirs, scalding is done in a continuous manner whereby the birds are dipped in a single or multistage scalding bath while suspended from a moving shackle line. There are three commonly employed scalding schemes (Table 1): selection of one over another depends on the degree of difficulty in removing the feathers, the chilling method that is to follow (water and air), and the age of the birds. Soft scalding (50–55 °C) is commonly used for broilers and turkeys. The temperature needs to be closely monitored because a temperature that is less than 50 °C can lead to bacterial contamination or inefficient feather removal. Higher scalding temperatures (60–64 °C) not only are better for loosening feathers from their follicles but are also harshest on the skin (the outer layer of the skin, epidermis, becomes loose and is later removed during the plucking operation). The removal of the epidermis can result in discoloration of the skin if it is dehydrated during subsequent air chilling. In addition, hard scalding can cook the breast meat where the skin is very thin and leads to stripes on the breast meat that has been referred to as tiger striping. However, hard scalding is the only satisfactory way to release the feathers of waterfowl. Generally speaking, hard scalding does not cause as much discoloration in the thick skin of waterfowl as it does in young poultry.

Soft scalding is commonly used for young broilers and turkeys because it does not damage much of the epidermis and allows relatively easy feather removal. Adequate agitation of the scald water and uniform temperature are essential to insure good feather removal. Careful equipment design is required for meat hygiene. Maintaining and controlling the temperature is one of the key features to keep the bacterial load under control. Another means is the use of a counterflow design (clean water introduced at the exit end of the tank, and water flow toward the entrance where the more contaminated birds are introduced). Installing a multistage scalding tank system can further reduce contamination problems; this consists of two to four


European food safety authority

Since welfare is poor when the shackling line and water bath electrical stunning method is used, and birds are occasionally not stunned before slaughter, the method should be replaced as soon as possible. At present, the inert gas stun / killing method is the best alternative.

5. Methods for stunning and stun / killing poultry species (chickens and turkeys)

The EFSA journal (2004), 45, 1–29, Welfare aspects of the main systems of stunning and killing the main commercial species of animals
http://www.efsa.eu.int 16 of 29

Electric stunner use in EU

Table 1. Minimum currents to apply for a minimum of one second when using constant current stunners delivering sine wave AC

Sine wave AC frequency (Hz)	Minimum root mean square current (mA)
50	100
> 50 and up to 400	150
> 400 and up to 1500	200

Figure 2 Welfare aspects of the main systems of stunning and killing the main commercial species of animals. Reproduced with permission from European Food Safety Authority Journal (EFSA), 2005. Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal 45, 1–29.

Table 1 Recommended scalding schedules for defeathering

Technique	*Water temperature (°C)*	*Time (s)*	*Used for*
Hard scalding	>60	45–90	Waterfowl
Medium/subscalding	54–58	60–120	Mature birds
Soft/semiscalding	50–53	60–180	Broilers, roasters, and young turkeys

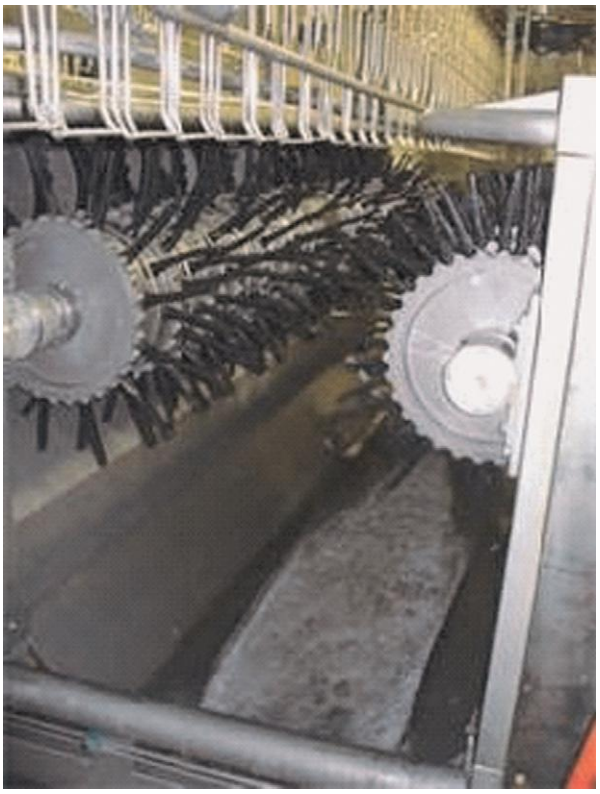


Figure 3 Side view of rubber fingers mounted on rotating disks and used for picking/plucking poultry feathers.

water tanks, where the carcasses are moved from the initial, more contaminated bath, to the cleanest bath at the end.

Feather Removal

In large processing plants, feather removal is done by mechanical pickers/pluckers equipped with rubber fingers that rub the feathers off the carcass. In a continuous operation, this is done while the carcass is hanging upside down on the shackle line between two and three sets of rotating disks equipped with rubber fingers (Figure 3). The fingers are made out of rubber and contain a certain degree of lubricating agent that controls their hardness and elasticity. The elasticity and length of fingers varies, depending on the task required, the machine speed, etc. The fingers can also be mounted on drums that rotate toward the center. The distance between the two sides is adjusted to accommodate size variations.

Electrical Stimulation

Many commercial plants have adopted electrical stimulation after either the bleeding or the picking steps. Either high- or low-voltage electrical stimulation can be used to speed up rigor mortis so that breast meat can be deboned more quickly than the 4 h postmortem standard deboning time in the industry. Although deboning time can often be decreased, it is still necessary to wait until rigor mortis is complete to insure that the breast meat is tender. Breast meat deboning times for electrically stimulated carcasses are highly variable (30 min–6 h) and depend on the bird size, parameters of the electrical stimulation unit, and many additional factors, such as customer, product application, and company standards.

Feet Removal

During feet removal, the knee joint is positioned by guiding bars on an angle along the shackle line, and the feet are cut off by a circular rotating blade at the hock joint. Cutting through the bone can result in dark/red color in the chilled bird and almost black in the cooked product. Some of the new automated leg cutters first bend the leg and then perform a small incision, which allows further bending before cutting through the joint with a rotating circular blade. Feet are separated into two quality classifications that consist of those with defects including footpad lesions and those with dark color.

Rehanging

After removal of the legs, the carcasses are usually transferred to another line because the broilers need to be hung by the knee joints, and this reduces contamination because the dirty shackles that are used for the live birds are replaced. Broilers are generally hung by their hocks so that they are 6–8 in. apart on shackle lines. An automated rehanging device can consist of a large wheel (carrier) with slots for holding the birds from underneath the knee joints and a device to push them into the evisceration line. The advantages of using automated rehanging equipment include labor savings and better hygiene.

Evisceration

This process refers to opening the body cavity and withdrawing the viscera (the intestines, gizzard, gallbladder, and crop). In semiautomated or fully automated evisceration processes, the first step is to cut around the cloaca, using a circular rotating blade. Some of the new devices are equipped with a vacuum device so that potential fecal contamination is reduced, and the cutting device is usually rinsed after each insertion. The viscera are then scooped out from the body cavity and remain attached to the body for inspection purposes. Some of the new automated equipment allows total viscera separation immediately after withdrawal and placement on a parallel line. This can further improve the hygiene of eviscerated carcasses. Once the viscera pack is exposed or removed, the birds are inspected.

Inspection

Inspection is done at this point because the inspector can see all parts at the same time. The attached or detached viscera can reveal diseases or problems within the internal organs. Inspection requirements differ among countries, but inspection is usually carried out by a government official. This process is essential in insuring that only wholesome birds, free of

disease, reach the market place. In some countries, it is required that each individual bird be inspected by a qualified veterinarian; in other countries, inspection is done on a whole-flock basis and only certain carcasses are inspected by a trained inspector. The suspected birds receive a more thorough inspection and all or parts can be salvaged. In large-scale plants, the viscera are pulled out of the bird using a pack puller that places a clamping device into the abdominal cavity to pull the viscera and esophagus out of the abdominal cavity. Multiple inspectors (two to four) are often on the line to make sure that every carcass is inspected.

Giblet Salvage

The viscera are removed after inspection and giblets (the liver, heart, and gizzard) are salvaged and washed on a separate line. The gizzard (stomach used to grind the food, as birds have no teeth) is first cut open, the contents are removed, and the lining is peeled off. Mechanical equipment used for peeling consists of two rollers that 'catch' the lining and pull it away; this is followed by washing and chilling. The hearts and livers are collected, inspected, washed, and chilled. The chilled giblets can then be collected and sold separately to reduce the risk of *Salmonella* and *Campylobacter* cross contamination.

Lung, Head, and Crop Removal

The crop is removed by placing a spinning probe with barbs through the abdominal opening to push the crop through the opening where the head used to be. The probe is then cleaned and the crop is removed before retracting through the carcass. The lungs can be removed through the use of a vacuum gun that is inserted into the thoracic cavity and suctions the lungs from the dorsal surface of the rib cage. A neck breaker and neck puller is then used to cut the neck at the weakest point between the atlas and the axis vertebrae and separate it from the carcass. The neck is often included as part of the giblets, with the heart, gizzard, and liver.

Inside/Outside Bird Wash

Before entering the chiller, carcasses are washed to remove any material that is present inside and outside of the carcasses. The device usually consists of multiple spray nozzles positioned to cover critical areas in removing debris or blood clots both inside and outside of carcasses. Tilting the carcasses can assist in thorough draining of the water through both the abdominal opening (created during evisceration) and neck opening (formed after pulling the windpipe and the crop). Some designs have spray heads positioned along the moving shackle line, washing first the upper part of the carcasses and subsequently the lower part. Bactericidal rinses, such as chlorine and organic acids, can be used. Chlorine is one of the most commonly used chemicals (where permitted) and is typically used at up to 50 ppm in the rinse. Citric, lactic, and acetic acids have all been used at concentrations of 1.0% or less. Propionic acid, peroxyacetic acid (PAA), chlorine dioxide, and cetylpyridinium chloride (CPC) can also be used in spray washes, as dips or in the chiller to inhibit microbial growth. The washing operation is critical in reducing the number of microorganisms on the carcass and decreasing the incidence of pathogenic bacteria, specifically *Salmonella* and *Campylobacter*.

Chilling

After the washing step, which occurs approximately 15 min after bleeding, the meat is placed in a chiller. The most common methods include water-immersion chilling, air chilling, and spray chilling. For immersion chilling, the carcasses are dumped into a trough-like structure, which usually contains either a large-diameter auger or paddles that move the birds forward (Figure 4). The most common design used today is the counterflow design, in which the product moves counter to the flow of the cold, clean water. This is a more efficient way of cooling the carcasses (the coldest temperature is at the end of the tank), which also assists in improving the hygienic conditions. The microbial quality of birds coming out of a water chiller is often better than before chilling because the water helps to wash off bacteria.

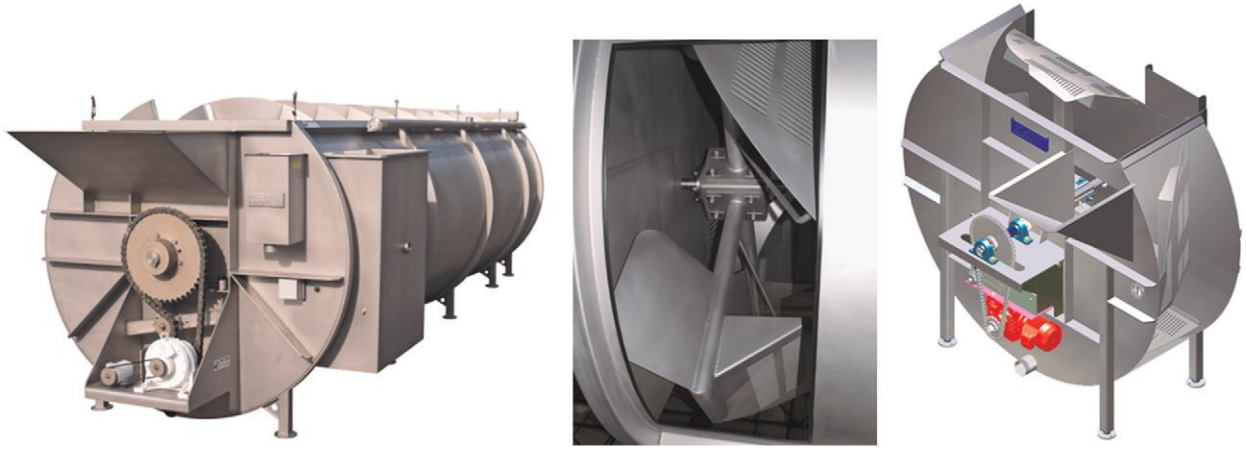


Figure 4 Auger carcass chiller (Left); side view of auger carcass chiller (Center); cross-sectional top view of auger carcass chiller.

A prechiller (7–12 °C) is commonly used in a water-immersion chiller for 10–15 min so that the carcasses have a gradual temperature transition. This is important because the lipids in the skin are liquid when the carcass enters the prechiller. The carcass temperature is generally approximately 30 °C after prechilling, when it enters the main chiller that has a water temperature of approximately 4 °C at the entrance and 1 °C at the exit. This counterflow design is designed to lower carcass temperature to 4 °C within approximately 2 h as well as maximize the heat transfer in the chiller. One problem that can occur in the chiller is thermal layering at the product surface that prevents carcass cooling. Injecting air or using water jets to pump water into chillers at high velocity prevents thermal layering. Chlorine, citric acid, lactic acid, acetic acid, propionic acid, PAA, chlorine dioxide, and CPC all either have been used or have potential for use in chiller systems through either direct addition or use in the pump water. Antimicrobials used in pump water are able to improve contact between the carcasses and the antimicrobials. In addition, a finishing chiller is used, where the carcasses receive a final rinse and antimicrobials to reduce the incidence of *Campylobacter* and *Salmonella*.

Air chillers are commonly used in Europe and are starting to appear in North America and elsewhere. Cold air is used as chilling medium, so care should be taken not to dry the product surface and lose weight. This can be achieved by raising the air humidity or wetting the product at some point (s) along the line; this can reduce weight losses to approximately 1%. A typical setup includes an overhead rail that goes back and forth along a chilling tunnel or room. Depending on the tunnel capacity and the volume of product, chilling can be achieved within 60–150 min. Air chilling generally takes longer than water-immersion chilling because it is less efficient at heat exchange. The air-chilled carcasses are generally exposed to colder temperatures at the beginning of the chiller (−6 to −8 °C) and approximately 1 °C at the end of the chiller. Spray chilling is a hybrid between water and air chilling. Cold water is constantly sprayed over the carcasses while they are moved along the line. Moisture pickup is less during water chilling but higher during air chilling.

Grading, Weighing, and Packaging

Grading is usually not mandatory, but it is done in most large markets to facilitate sales. The grade is based on the relative muscling, bone conformation, presence/absence of tears/bruises/pinfeathers, and missing parts. Each country has its own specifications, but a bird (chicken, turkey, and duck) with adequate muscle deposition and no esthetic defects will generally be classified as Grade A; minor defects will result in Grade B; and more serious defects will result in Grade C. The last two categories will usually not be sold as whole birds but rather as parts (no grade labeling required) or as further-processed products. Grading can be done by a qualified person or with the assistance of a computerized machine-vision system. The final overall grade can be affected by different processing parameters (e.g., stunning and chilling) as well as feeding, growing, and transporting parameters described in other articles.

Weighing of the ready-to-sell birds is usually done by automated weighing equipment connected to a computer network. Alternately, birds/parts can be packaged in bulk and sold to consumers interested in buying only certain parts (wings/drum sticks) or institutions/fast food restaurants interested in large quantities, depending on market demands.

See also: Hazard Analysis Critical Control Point and Self-Regulation. Microbiological Safety of Meat: *Salmonella* spp.; Thermotolerant *Campylobacter*. Spoilage, Factors Affecting: Microbiological

Further Reading

Barbut, S., 2002. Poultry Products Processing – An Industry Guide. New York: CRC Press.
Bilgili, S.F., 1992. Electrical stunning of broilers – Basic concepts and carcass quality implications: A Review. Journal of Applied Poultry Research 1, 135–146.
Bilgili, S.F., 1999. Recent advances in electrical stunning. Poultry Science 78, 282–286.
European Food Safety Authority Journal (EFSA), 2005. Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related to welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal 45, 1–29.
Fletcher, D.L., 1999. Slaughter technology. Poultry Science 78, 277–281.
Gregory, N.G., Wilkins, L.J., 1989. Effect of stunning current on carcass quality in chickens. Veterinary Record 124, 530–532.
Guyton, A.C., Hall, J.E., 2010. Textbook of Medical Physiology, twelfth ed. Philadelphia, PA: Saunders Elsevier. (Chapter 43), pp. 527–533.
Hoen, T., Lankhaar, J., 1999. Controlled atmosphere stunning of poultry. Poultry Science 78, 287–289.
Kranen, R.W., Lambooj, E., Veerkamp, C.H., Van Kuppevelt, T.H., Veerkamp, J.H., 2000. Hemorrhages in muscles of broiler chickens. World's Poultry Science Journal 56, 93–126.
Raj, A.B.M., Grey, T.C., Audsely, A.R., Gregory, N.G., 1990. Effect of electrical and gaseous stunning on the carcass and meat quality of broilers. British Poultry Science 31, 725–733.
Raj, A.B.M., Johnson, S.P., 1997. Effect of the method of killing, interval between killing and neck cutting, and blood vessels cut on blood loss in broilers. British Poultry Science 38, 190–194.
Raj, A.B.M., Wilkins, L.J., Richardson, R.I., Johnson, S.P., Wotton, S.B., 1997. Carcass and meat quality in broilers either killed with a gas mixture or stunned with an electric current under commercial processing conditions. British Poultry Science 38, 169–174.
Raj, A.B.M., Wotton, S.B., McKinstry, J.L., Hillenbrand, S.J.W., Pierterse, C., 1998. Changes in the somatosensory evoked potentials and spontaneous electroencephalogram of broiler chickens during exposure to gas mixtures. British Poultry Science 39, 686–695.
Sams, A.R., McKee, S.R., 2010. First processing: Slaughter through chilling. In: Owens, C.M., Alvarado, C.Z., Sams, A.R. (Eds.), Poultry Processing, second ed. Boca Raton, FL: CRC Press, pp. 25–49.
Sams, A.R., Owens, C.M., 2010. Second processing: Parts, deboning, and portion control. In: Owens, C.M., Alvarado, C.Z., Sams, A.R. (Eds.), Poultry Processing, second ed. Boca Raton, FL: CRC Press, pp. 51–65.

Relevant Website

http://www.efsa.europa.eu/en/efsajournal/pub/45.htm
European Food Safety Authority.

Sheep and Goats

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Glossary

Bleeding/exsanguinations The procedure where the carotid arteries, jugular veins, and esophagus are severed – sometimes a knife is inserted to the heart, cutting additional vessels.
Cardiac arrest Cessation of normal circulation. In this case, the passage of an electrical current through the heart causes fibrillation.
Electrical stimulation Application of an electric current through a carcass postmortem that accelerates rigor mortis and enhances tenderization.
Electrical stunning The procedure where a current is passed through the head of the animal, causing a seizure that ensures that the animal is insensible. If only the head is in the electrical pathway, the heart continues to beat and the animal can recover (head-only electrical stunning). If the body of the animal is in the pathway, the heart stops (cardiac arrest) and the animal cannot recover (head-to-body stunning).
Evisceration Removal of the components of the body cavity, such as the heart, lungs, rumen, and the intestines.
GR Thickness of the fat based on measurement of total tissue depth over the 12th rib at a point 11 cm from the midline.
Halal slaughter A slaughter procedure that adheres to Islamic teachings where the animal is dispatched by severing the blood vessels of the neck while the animal is facing Mecca. The knife cut can be preceded by a head-only electrical stun, provided it does not kill the animal and the animal is able to recover if not slaughtered.
Harvest The whole process of production and processing animals.
Inverted dressing A system where the carcass is suspended by the front legs in contrast to systems where the carcass is suspended by the hind legs.
Pelt removal Removing the skin/hide from the carcass – also called depelting.
Slaughter The process of bleeding/exsanguination where the carotid arteries, jugular veins, and esophagus are severed – sometimes a knife is inserted to the heart, cutting additional vessels. The blood loss ensures that the animal cannot recover sensibility.
Stunning The procedure where an animal is rendered insensible before exsanguination, pelt removal, and evisceration.

Introduction

There were approximately 861 million goats and 1078 million sheep in the world in 2008 with most (805 million goats and 739 million sheep) being in Asia and Africa and, additionally, 247 million sheep and 19 million goats in Europe and Oceania, the remainder being mainly in North and Central America and the Caribbean. This means that traditional non-mechanized methods of harvest predominate over mechanized systems. For sheep and goats, the small size means that they have been harvested and eaten by societies ranging from nomadic tribesmen to most western cultures for thousands of years. Not only do they provide meat but also hides and wool (sheep) are important by-products. The small size of sheep and goats are clearly advantageous, when they are slaughtered by individuals away from plants on farms or the steppes of Eurasia, but in commercial processes, the labor and mechanization required per carcass is disproportionately high compared with larger cattle and pigs. The labor required to produce a given weight of dressed carcass is generally less when the carcasses are large. Sheep are relatively small and traditional dressing methods with low levels of mechanization require approximately 80 man-hours per 10 000 kg of carcass. Beef and poultry in contrast require only approximately 22 man-hours to produce the same weight, due to the large carcasses of the former and highly mechanized processing procedures for the latter. Procedures for sheep, the main species in commercial abattoirs, have changed rapidly and procedures for goats are adapted from those used for sheep. The main differences between sheep and goat harvest methods relate to the need to recover wool in sheep – the leanness and the generally lighter weight of goats mean that with rapid chilling, cold shortening and toughening are more likely to occur unless electrical stimulation is used. This article covers the mechanized processes that dominate in Oceania, North and South America, and Europe.

Over the past 20 years, because of the large numbers exported, the New Zealand meat industry in particular has invested heavily in developing slaughter and dressing technology for sheep and lambs to reduce labor costs, to maintain stringent hygiene, and to improve the quality of the pelt and meat quality through all aspects of processing, including boning, cutting, packaging, and retail distribution. The industry in this country is one of the leaders in the field, and the situation is reflected in many other countries with minor changes in details.

Traditional slaughter methods involve a severing of the throat, effectively cutting the carotid arteries, jugular veins, trachea, esophagus (weasand), and various nerves. The animals are usually hung up by their hind legs and then allowed to go through postmortem movements and to bleed out over a period of 5 min or more before depelting and viscera removal.

Such slaughter procedures without stunning have been shown to be humane for sheep, where unconsciousness will occur 6–10 s post the knife cut. In many parts of the world where sheep are slaughtered in this manner, the procedures are also in line with those required for halal and kosher slaughter. A throat cut has been the main sheep and goat slaughter method throughout human history.

When commercial processing operations with high throughputs are required, the sheep need to be transported to the facilities and lairaged before slaughter. Such procedures potentially involve varying degrees of preslaughter stress and consequent meat quality deterioration, so handling procedures to reduce stress should be implemented (including familiarization of animals to some stressors). In most countries of the world, sheep dressing takes place with dry wool surfaces, and the potential risk of contamination by dust, dirt, and fecal contamination does not appear to be a problem. In New Zealand, sheep have been vigorously washed by swimming through a race or have high-velocity water jets spraying the animals to remove visible dirt and feces, but it is unclear whether bacterial loads are reduced overall, because the moist conditions on the warm skin surface during drying would encourage bacterial growth. The stresses imposed can affect meat quality, and the process is being phased out. Goats do not need to be washed.

Stunning

In commercial operations, preslaughter stunning is generally used, and the most common commercial method is electrical stunning. This is achieved by placing tongs spanning the head and passing an electrical current through the brain. With goats some care is needed in placing the electrodes behind the horns. This type of stun, termed a 'head-only' stun, does not stop the heart and the animal can potentially recover. As the slaughterman cuts the throat before the animal recovers, they are effectively taking the life of the animal and this procedure is, therefore, consistent with halal slaughter. Other stunning and slaughter procedures use a modification of the tong system with a pistol grip-like handpiece, which sometimes may be used for head-to-back (or body) stun and cause cardiac arrest (Figure 1). Cardiac arrest arises when a current passes through the heart but because the spinal cord is in the pathway, it additionally reduces the animal's reflexes with significant movement reduction, making it a good option when halal slaughter is not required.

Ideally, the animals are presented to the stunner in a V-restrainer (Figure 1). Additional advantages of the V-restrainer are that it allows precise location of the head of the animal and therefore facilitates automation of application of stunning. Such automated procedures not only reduce costs but also improve worker safety. One system has a series of grids that contact each side of the head. Nozzle electrodes, arranged throughout each grid, then apply an electrical current to the head, concurrently with water to facilitate current flow.

Figure 1 A slaughterman applying a head-to-back stun to a sheep in a restraining conveyor. Inset: A stun gun showing the pointed front electrodes that are applied to the head and the flat rear electrode through which water is also applied to the wool to lower electrical resistance.

Most countries require preslaughter stunning before slaughter. Head-only electrical stunning is consistent with halal, but it is not acceptable for kosher slaughter.

Bleeding

Once the animals are stunned, the throat is cut, and bleeding takes place either by a traditional gash cut as mentioned above or by a thoracic stick that severs the atria and vessels leading to the heart. In some plants, the animals are suspended by hooks on the rail system before they are bled (Figure 2). The blood leaving the carcass is not affected by the stunning procedures. If the heart is still beating, the animal initially bleeds by blood being pumped via the heart from the carotid arteries. At the same time, blood drains into the heart from the jugular–vena cava system. As the heart refills from the venous system draining into it, the amount of blood being lost by cardiac action reduces when the heart cannot fill due to the cut vessels. Eventually, a stage is reached when all the remaining blood must drain by passive means from the severed vessels. With electrical stunning, and in particular head-to-body stunning, there are other types of sticking, for example, a thoracic stick draining blood rapidly via the venous system so that this becomes the main mechanism of bleeding rather than via the carotid artery. It is important to note that from the moment the atrium/vena cava or heart is pierced, bleeding takes place passively (helped by gravity) – this is true for animals shot in the head with a captive bolt as well as electrically stunned animals. Cardiac arrest, therefore, has little or no effect on overall blood loss (providing vessels are severed before clotting occurs). To prevent contamination by ingesta, the weasand (throat or esophagus) is tied or clipped.

Support of the hind legs facilitates good bleeding. However, with increased efficiency on the dressing line, time delays

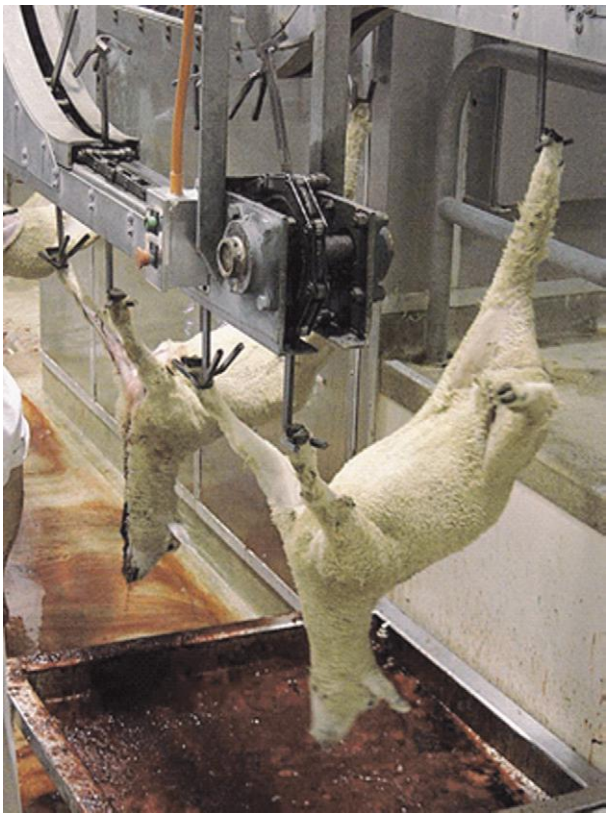

Figure 2 Two sheep carcasses on a processing line. One rear leg is tightly held and the front legs are on a spreader. The nearest animal has just been stunned and not yet bled. The second has just finished bleeding, and knife workup on the neck and fore legs has just commenced. This allows the commencement of head workup and separation of the skin from the brisket area and precedes pelt removal.

are not desired and some procedures are telescoped. For example, carcasses are suspended by both the two rear legs and front legs soon after slaughter commences. This can result in blood pooling in the thoracic area before it bleeds out, if the thorax is lower than fore and hind legs (the correct angle is shown in Figure 2). Clotting occurs because blood that remains in the large vessels, such as the jugular and the vena cava, retains all clotting factors – it, therefore, needs to be rapidly removed. An interesting situation occurs in small vessels (such as those supplying and draining the pelt) where blood leaks out from cut surfaces. This occurs because the biochemical factors that facilitate clotting rapidly diffuse away through the thin vessel walls, so clotting is reduced.

Sometimes there are cosmetic deficiencies with poor bleeding. One type, termed blood splash, results when blood from vessels, torn through muscle spasms (usually, but not always, from an electrical stun), are retained in the muscle. Another cosmetic deficiency, termed speckle bruising, appears as myriads of small red spots lying in the fat over the loin to give a 'salt and pepper effect,' which may coalesce in extreme cases producing a fiery red effect. The defect is particularly visible in sheep, but it can be found in goats, cattle, pigs, and even deer – it is only when it is severe that it is a problem. This defect, essentially the beginning of a bruise, is generally caused by a violent movement just before or during slaughter, for example, a head-to-back electrical stun where there is effectively a shearing action between the fat and muscle fascia initiated by muscle contraction.

Pelt Removal

Traditionally, sheep were depelted while hanging from their hind legs, and this provided the basis for early mechanized systems with various gambrels or hooks placed through the Achilles tendon. By careful knife work, the areas around the rump are cleared, then the legs and the thorax are cleared, and finally the pelt is pulled over the body and head. At all stages, it is desirable to keep the carcass clean; paper is often placed at judicious places to prevent the pelt rolling inward and contaminating the carcass – this is more important for sheep than goats. In the late 1970s, the benefit of depelting the sheep and goats from the shoulder to the hind leg was recognized, and this process was most easily achieved by suspending the animal by front legs. This process, known as the 'inverted' system, formed the basis of many subsequent developments to date with considerable manpower reductions over previous systems. Because the hind legs are at the lowest point, it has the added benefit of reducing fecal contamination and maximizing the return from every part of the carcass, including offal. One of its major attributes is its simplicity and ease of installation as many of the tasks and skills used in the inverted system were used in previous systems.

The inverted manual system requires a mechanical puller to remove the pelt from the rear and then over the front legs. Many variations have been tried in New Zealand and at present at least 10 different designs are being used in meat plants for both sheep and goats. Most of the designs can process at least eight carcasses per min. In one system, the pelt is manually removed around the brisket, cleared from the back area, and the hanging pelt is placed between prongs of the puller shown in Figure 3. The prongs rotate (Figure 4) and trap the pelt, and then the device moves down the carcass, removing the pelt.

Automatic front and rear hock removal machines have also been produced, and machines to assist the transfer of the fore legs from the wide spreader to the narrow hock holder have been developed. Potential contamination from the front trotters onto the carcass is eliminated during the major part of the pelting operation.

Evisceration

After depelting, evisceration and offal handling, such as removal of the intestines, liver, lungs, and heart, are the next biggest users of manpower. Several developments have been undertaken to partly mechanize this area. Brisket cutting and belly opening was the first area studied. A machine not only cuts the brisket but also opens up the belly area. Further developments facilitate viscera removal.

Head Processing

The focus of developments in head skinning and brain and tongue removal has changed, as the European Union (EU)

Figure 3 The four prongs are part of a system to remove the pelt from a sheep carcass. The pelt is freed from much of the carcass, and a hanging portion is placed between the prongs, which can rotate.

Figure 4 The rotating prongs grip the pelt and pull it down over the rear legs in this inverted dressing system.

regulations regarding head inspection required that a totally skinned head had to be presented with the carcass for carcass inspection. A number of head-skinning machines were developed. In one type, a small shaft gripped a flap of skin near the nose and removed the remainder of the skin by a rolling action. The regulation requiring heads to be presented with the carcass was partially relaxed in 1987, so that only those heads from which edible brains and tongues were to be used for human consumption needed to be inspected. Edible brains can still be obtained from sheep in countries without bovine spongiform encephalopathy or scrapie. Small incremental developments include automatic atlas joint severing, automatic head splitting, and automatic brain extraction.

Mechanization

Traditionally, mechanization has focused on direct task replacement. Inverted dressing offered significant advantages in this respect and has generated significant benefits over the past 20 years. With reducing numbers and increased focus on adding value through further processing, the current focus has moved toward more advanced automation and robotics. With the incremental development of mechanized devices, the output from a mechanized sheep dressing system, processing 8 lambs per min, requires 25 butchers plus 11 assistants, the actual labor requirements highly depending on throughput, in contrast with the older systems that needed 44 plus 15 assistants. The recently developed robotic brisket cutting and evisceration robots, combined with a new evisceration inspection protocol, have the potential to remove five evisceration inspection and sorting labor units on a typical eight lambs per min chain. The best-mechanized system is likely to produce carcasses and pelts of a quality equal to or better than any economically viable manual system of a similar throughput. The cost benefit of mechanization has to consider processing quality. Poor hide pulling that impacted on fat over the ribs would have zero impact on value because the fat cap is removed when cutting into French racks; however, an evisceration robot that damaged 1% of runners could be significant.

Modern pelt-pulling equipment senses the force required in removing the pelt. Additional labor units are often deployed to do the necessary workup to ensure product quality as these extra labor units are often doing other part tasks as well; therefore, the combination of mechanization and labor deployment must stack up financially. However, the alternative of a fully manual operation is not viable these days due to throughput requirements and health and safety issues.

Sheep and Lamb Carcass Grading

There is no international carcass grading system for sheep, lambs, and goats, but some generalizations can be made, and

in particular, sex and age are important. Mutton is a female (ewe) or an adult noncastrated male (ram) or a castrated male (wether) with more than two permanent incisors in wear. A hogget is a young male sheep or a maiden ewe having no more than two permanent incisors in wear. Lambs are young sheep less than 12 months of age and without any permanent incisors in wear. New Zealand, Australia, and the EU account for almost 90% of lamb exports, but each country is serving specific markets and therefore the systems are not the same. The greatest sheep meat production is in China, followed by the EU, Australia, New Zealand, and the Middle Eastern countries (where goats are processed as well), all with different grading systems. Links to the various systems can be obtained from web links cited in further reading. VIASCAN systems (video analysis) based on carcass conformation are now being used.

In many systems, grading is based on the overall size and conformation and the fat cover. In New Zealand, the thickness of the fat based on measurement of total tissue depth over the 12th rib at a point 11 cm from the midline, called GR, is used (the fat cover on the longissimus muscle is not such a useful guide as in pigs). The export grades are based on three grades of leanness (A = devoid of external fat, Y = low fat, and P = medium fat). Excessive fat is trimmed and gives rise to another series of grades. There are then, superimposed on this, four weight grades (L = 9–12.5 kg, M = 13–16 kg, X = 16.5–20 kg, and H = 20.5 kg and more). Australia follows a similar, but not identical, system.

Electrical Stimulation, Chilling, and Freezing

Once the pelt and the viscera have been removed from the carcasses, they can be graded and weighed, and they move down the chain to an area where they may be electrically stimulated. There can be either a low- or a mid-voltage system applied early postmortem (Figure 5) or a high-voltage system at the end of processing (Figure 6).

It is ideal for meat to reach temperatures approximately 15 °C at rigor mortis (>12-h postmortem without stimulation) to avoid cold shortening and ensure optimum aging. Compared with beef, the small carcass size of sheep and goats means that excessive rates of chilling or freezing can easily occur with the risk of cold shortening and toughening when temperatures are significantly lower than this. As electrical simulation considerably reduces the time to rigor mortis (3–5-h postmortem, depending on type of stimulation), cold shortening is unlikely to occur. In New Zealand, the accelerated conditioning and aging (AC&A) process was developed and resulted in sheep with a known tenderness specification. The AC&A process consists of high-voltage electrical stimulation using 1130 V peak, 2 A peak, 15.5 sine wave pulses per s for 90 s, which passes from the middle of the back through the legs of many carcasses as they move slowly along the electrode system (Figure 6). Alternative systems utilize a low-voltage system current (80 V peak, 120–300 mA peak, 15 pulses per second) before depelting or a mid-voltage system after depelting (300 V peak 5 ms duration square wave pulses at 15 pulses per s). Chilling or freezing processes together with prior procedures, such as electrical stimulation, have an interdependent effect on optimal processing and on product quality, so a single set of process cannot be put in place.

Figure 5 A low-voltage stimulation unit for sheep that can be used in processing plants with a small throughput. The current (80 V peak, 120–300 mA peak, 15 pulses per s) passes from the front legs to the earthed rail. The duration of stimulation ranges from 30 to 60 s. In this system, the stimulation is applied at the end of dressing but earlier stimulation at the end of stunning may be used and this has the advantage that carcass movement poststun is reduced. Three sheep are connected at a time in this system. The carcasses with the current flowing have the front legs outstretched.

In a typical procedure, the carcasses are aged (tenderized) at air temperatures above 6 °C for 8–12 h before being frozen or packaged for chilled distribution. Sheep carcasses can be further processed into cuts and packaged for distribution. With the best processing hygiene and the use of either vacuum packaging or controlled atmosphere packaging systems, chilled rather than frozen meat can be distributed worldwide for retail distribution for as long as 10–12 weeks postmortem at −1.5 °C.

Future Trends

To date, sheep dressing is based fairly heavily on mechanical principles. In the future, however, rapid developments in the areas of electronic sensing, vision, and robotics are expected to affect carcass dressing. Initially, these new developments will be used to control existing machinery for greater processing accuracy. For significant further manpower reductions in sheep

Figure 6 A typical high-voltage electrical stimulation system for lambs in a meat processing plant. The current (1100 V peak, 2 A peak, 15 pulses per s) passes from the middle of the back through the legs of many carcasses as they move slowly along the electrode system, taking 90 s to complete the process. Reproduced with permission of AgResearch Limited, New Zealand.

and lamb, and even goat, processing, tasks such as opening cuts and clearing cuts, would have to use robotic technologies. To date, the best set of improvements has reduced the labor required for a traditional system by more than 40%, so instead of earlier requiring more than 2.6 times as many man-hours to produce the same weight of carcass as a chicken processing system, it now requires only 1.3 times as many.

See also: Automation in the Meat Industry: Cutting and Boning. Conversion of Muscle to Meat: Aging; Rigor Mortis, Cold, and Rigor Shortening. Electrical Stimulation. Meat Marketing: Cold Chain. Microbiological Safety of Meat: Prions; Viruses. Preslaughter Handling: Preslaughter Handling. Refrigeration and Freezing Technology: Freezing and Product Quality. Religious Slaughter. Stunning: Electrical Stunning

Further Reading

Aziz, M.A., 2010. Present status of the world goat populations and their productivity. Lohman Information 45, 42–52. Available at: http://www.lohmann-information.com/content/l_i_45_artikel17.pdf (accessed 26.04.14).

Barton-Gade, P.A., Chrystall, B.B., Kirton, A.H., *et al.*, 1988. Slaughter procedures for pigs, sheep, cattle, and poultry. In: Cross, H.R., Overby, A.J. (Eds.), World Animal Science, Meat Science, Milk Science and Technology, vol. B3. London: Elsevier Science Publishers, pp. 33–111.

Relevant Websites

http://www.ausmeat.com.au/industry-standards/specification/lamb.aspx
AUS-MEAT Limited.

http://www.beeflambnz.com/market/meat-specifications-and-processing/
Beef and lamb New Zealand.

http://www.grandin.com/index.html
Dr. Temple Grandin's Web Page.

http://www.dartmoor-npa.gov.uk/__data/assets/pdf_file/0014/42008/li-dhfpsheep.pdf
Meat South West.

SMOKING

Contents

Liquid Smoke (Smoke Condensate) Application

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Glossary

Carbonyls A class of compounds responsible for the browning reaction contained in smoke.
Condensable and noncondensable gases Condensable gases are those that form a liquid at room temperature; noncondensable gases do not.
Condensed smoke Cooled gaseous smoke.
Genotoxic Agents known to damage DNA.
Liquid smoke preparations These are combinations of condensed smokes.
Polycyclic aromatic hydrocarbons (PAHs) These are the cancer-causing agents.
Rapid thermal pyrolysis A pyrolysis method taking less than 1 s.
Rotary oven A slow pyrolysis reactor; pyrolysis occurs more than 1 min.
Tar fraction The non water-soluble fraction of pyrolysis that is removed from smoke.
Water fraction The gaseous phase of smoke that is condensed in water.

Introduction

Liquid smoke preparations are used extensively in meat processing. The application of liquid smoke (smoke condensates) is steadily displacing the use of traditional smoking. Research conducted in the 1960s and 1970s provided information about the basic aspects of the smoke-generating process and traditional smoking. The work dealt with the thermal degradation of wood, the physical and chemical properties of woodsmoke, the phenomena occurring in the smoking chamber, the diffusion of the smoke components into the products being smoked, and the bactericidal and antioxidative properties of woodsmoke. Studies were aimed at determining the chemical composition of woodsmoke condensate and the mechanisms in the production of polycyclic aromatic hydrocarbons (PAHs); the role of nitrogen oxides generated during thermal degradation of wood was also investigated. Many articles dealt with the species and moisture content of the wood, the degree of disintegration (wood chips or sawdust), and the sensory properties of the smoke preparations or of the smoked products. Attempts were also made to determine the relationship between the chemical composition of the smoke preparation and its sensory properties.

The results of these studies were used in the development of the first generation of liquid smoke preparations. Usually these preparations did not have the required stability and reproducibility from batch to batch. Many preparations produced at that time contained considerable quantities of PAHs. By the mid-1970s, progress in the manufacturing technology had largely eliminated the problems with smoke condensates. The smoke condensates made today are characterized by a high and repeatable quality level. The great increase in the application of smoke condensates is based on numerous factors: the development of new technological methods for producing smoke condensates; the development of technical solutions for application of the smoke condensates; environmental restrictions on the use of traditional smoke; and requirements for health and safety at work.

Methods for Smoke Condensate Production

The predominant raw material for production of smoke condensate is wood, typically of one or more of the following tree types: beech, oak, hickory, maple, eucalyptus, ash, apple, cherry, birch, mesquite, and pecan. Wood to be used for smoke condensate manufacture may be reduced to sawdust or chips, dried to approximately 5% moisture content and thermally decomposed. In some processes batch retorts are still in use. The thermal decomposition of the wood is carried out

under controlled conditions. Usually the process is a slow pyrolysis performed in externally heated retorts or in rotary ovens. In recent years, a so-called rapid thermal pyrolysis has been introduced. This enables the process of wood decomposition to be carried out in less than 1 s. The sawdust is introduced into a reactor in which heated sand is being circulated. When the hot sand comes in contact with the sawdust particle, the sawdust is pyrolyzed, forming the smoke components. The smoke is quickly transferred to a recirculation column where it is condensed. This ensures that a larger portion of the carbonyls is retained, forming a higher-browning capability smoke.

Of primary importance in the pyrolysis process is the temperature of wood decomposition. The temperature of decomposition has a fundamental influence on the quantity of condensate and its chemical composition. Most often the temperature is between 450 and 500 °C. The use of lower temperatures for wood decomposition considerably increases the content of carbonyl compounds and tar in the smoke condensate. The temperature depends on the reaction method used and which fraction is more desirable to the producer. The condensable and noncondensable gases created during the thermal decomposition of wood are contacted with a recirculating water phase of smoke condensate. Noncondensable gaseous components of wood pyrolysis contain carbon monoxide, methane, and hydrogen, resulting in a flammable gas stream that may be burned to generate heat utilized in further manufacturing steps. After cooling, concentration, and normalization, the smoke condensate is separated into a water fraction and a tar fraction. For manufacture of commercial condensed smoke preparations, both fractions may be used individually, although the tar fraction must be further processed to remove the high levels of genotoxic polycyclic aromatic hydrocarbons. The water fraction is subjected to storage for some time to isolate the slowly precipitating fraction of tar that continues to form at decreasing rates over the lifecycle of the condensate. Before further processing, the water fraction is subjected to multiple filtration steps to purify the liquid. The water fraction may then be concentrated by distillation or extraction. To improve its stability when stored, emulsifiers, such as Polysorbate 80, are added.

The filtered water fraction of smoke condensate, while useful in its native form, is best suited as an intermediate product to be used in the manufacture of many commercial forms of condensed smoke preparations. Each manufacturing batch is examined for its content of basic components. Usually, determinations of total organics content, acids (expressed as acetic acid), phenols, and carbonyls are carried out. For the preparations used for coloring of food products, methods that assess the reaction of the condensed smoke product with glycine are usually employed to determine the Maillard reaction potential of the condensed smoke.

Commercial condensed smoke preparations are normally offered in the following forms:

- Concentrated liquids for atomizing, or smoke regeneration, into smoking/cooking chambers, which is done nearly exclusively in batch processes.
- Extracts to be incorporated into food products by injection or mixing.
- Water-miscible solutions for showering products in batch and continuous processes.
- Powder on carriers such as maltodextrin, salt, yeasts, flours, spices, and seasonings to provide smoke flavor.

High-strength liquid smoke preparations are obtained from the tar fraction by distillation and/or extraction. These are highly enriched phenolic products that have far smaller amounts of the water, acids, and carbonyls that are present in seminal woodsmoke. Although smoke preparations of this type have use as is, typically they are more likely to be used commercially on carriers or in emulsions. These preparations possess only the smoke flavor without the coloring ability.

Chemical Composition of Liquid Smoke Preparations

The chemical composition of condensed smoke preparations varies as much as the composition of natural smoke, but the condensed smoke preparations do not contain the gaseous components of traditional smoke. The chemical composition of condensed smoke depends on the raw material used, the method and conditions for wood decomposition, and the methods for purification, condensation, and stabilization. During pyrolysis, the cellulose and hemicelluloses contained in wood generate organic acids, aldehydes, aliphatic and cyclic ketones, furans and pyrans plus derivatives, lactones, aliphatic alcohols, and anhydrous sugars. From the 25% or so lignin fraction of wood, a full range of phenolic compounds is produced. Most of the phenolics fall into the general classes of guaiacols, syringols, dihydroxybenzenes, and methylated phenols.

Condensed smoke preparations to be used for topical application contain the following key components:

Water	40–75% (w/w)
Acetic acid	4–12%
Formic acid	0.5–3.5%
Glycol aldehyde	1.78–5%
Formaldehyde	0.5–1.2%
Glyoxal	up to 1.2%
Acetol	2–5%
Levoglucosan	1.5–5.5%
Water-insoluble tar	up to 7%

The preparations also contain several minor components, which are present at low levels ($1.0\ mg\ g^{-1} = 0.1\%$).

- Furans (furfural, furan, and derivatives): 2–8 $mg\ g^{-1}$.
- Phenols (phenol, cresols, dimethylophenols, and derivatives): 0.4–2 $mg\ g^{-1}$.
- Dihydroxybenzenes (catechol, hydrochinone, and derivatives): 2–7 $mg\ g^{-1}$.
- Guaiacols (guaiacol and derivatives): 1–7 $mg\ g^{-1}$.
- Syringols (syringol and derivatives): 0.8–10 $mg\ g^{-1}$.
- Aromatic aldehydes (vanillin, syringol aldehyde, and derivatives): 0.2–2 $mg\ g^{-1}$.
- Other components (cyclotene, maltol, aliphatic aldehydes and ketones, etc.): 3–25 $mg\ g^{-1}$.

In addition, some preparations contain several hundred components at levels of micrograms per gram.

There is a wide variety of preparations of condensed smoke to be incorporated into food products. They can contain lower levels of the chemical compounds listed above because they are produced either by mixing the water fraction with carriers or extraction of certain components, or they may be converted into dry forms. The levels of phenolic compounds may vary by a factor of ten or more in the different preparations. The ratios between the different groups of compounds can also vary considerably.

Products made from the tar fraction of the smoke condensate have different chemical compositions. These preparations contain much greater quantities of phenolic compounds with considerably lower levels of other constituents. The primary components of these condensed smoke preparations from the tar fraction are syringol, 4-methylsyringol, 4-isopropenylsyringol, 4-ethylsyringol, isoeugenol, eugenol, guiacol, 4-methylguaiacol, phenol, and cresols. The total content of phenolic compounds may be a third of the mass.

The PAH content in condensed smoke preparations depends primarily on the production method and degree of purification. Preparations manufactured by smoldering wood might contain several hundred milligrams of PAHs per kilogram. The method of wood decomposition used today, which utilizes lower and precisely controlled temperatures, has made it possible to reduce the level of PAHs considerably from that of the past.

Thirty-four PAHs have been identified in condensed smoke preparations, and are present in highly varying quantities. Naphthalene and its derivatives are present at the highest level. The total quantity may be as high as 1800 mg kg^{-1} with a total PAH content of 3195 μg kg^{-1}. In Europe, regulation EC 2065/2003 limits benzo[a]pyrene to 10 μg kg^{-1} and benzo[a]anthracene to 20 μg kg^{-1} in smoke condensates that are used in foods. In the water phase of smoke condensates, the level of benzo[a]pyrene may be below 1 μg kg^{-1} when the tar component in the smoke condensate is below 1%.

Smoke Condensate Application Methods

The manufacture of smoke condensates has made it possible to replace traditional smoking and has created new technological possibilities. The application of smoke condensates to meat products may be classified into three basic groups: smoke regeneration (atomization), drenching or showering, and internal addition. The first and second include methods for surface application; the third group covers incorporating the smoke into the products. Applying smoke condensates to the surface of products is most frequently done in normal smoke application chambers used for traditional smoking or in specially designed units. Numerous techniques are used for applying smoke condensates to products in smoke application chambers including pneumatic or hydraulic nozzles, thermal spraying, electrostatic deposition, and ultrasound or centrifugal atomizers to form a regenerated smoke cloud (Figure 1; Table 1).

Color and flavor achieved are dependent on the smoke used, time smoked, meat block formulation, and processing schedule. In general, any color from light tan to very dark brown and very light smoke to heavy smoke flavor can be obtained.

Figure 1 Smoke application in a smoke chamber is carried out with low-velocity circulating air or air movement derived only from the application mechanism itself. The amount of smoke applied is controlled with metering pumps or by air pressure. Optimal smoke regeneration is obtained by sequential steps that include an atomization step followed by a circulation step while the dampers of the smoke house are closed. This also optimizes the use of the smoke. Multiple cycles of smoking and circulating may be utilized to provide the desired flavor and color (see **Table 1** for an example of a frankfurter processing schedule with condensed smoke regeneration).

The volume of smoke used depends on many factors. In most cases the quantity used for a manufacturing cycle is determined by the desired color intensity and flavor of the smoked product. A use of 1.5–3 kg of condensed smoke per ton of product is usually sufficient. Applying smoke condensates via smoke regeneration in smoking chambers provides a result very similar to a traditional smoking process (Figure 2; Table 2).

The color intensity and the degree of smoke flavor can be controlled by the concentration of the smoke condensate and by the contact time between the smoke solution and the product.

Another group of smoke condensates are designed for internal addition. This method is utilized to add flavor to the meat without affecting the color, and for adding a level of antimicrobial and antioxidant protection to meats. The level and type of smoke used are determined by the application method and flavor desired. To ensure a uniform distribution of this type of application in the product, smoke is normally applied in a bowl chopper or added to an injection marinade. Usage rates can be quite low depending on the smoke product used, but usually range from 0.05 to 1 g kg^{-1} of the meat product.

The process of curing meat should not be combined with the addition of a high-acid smoke concentrate. Several smoke condensates, however, are available with higher pH and low acid levels that can be added to brines. The reason is that

Table 1 Example of Frankfurter manufacturing process utilizing smoke regeneration

Chamber conditions	*Time*	*Dry bulb temperature (°C)*	*Humidity*
Reddening	10 min	50	90%
Drying	20 min	55	0%
Smoking	19 min 3 min smoking 4 min circulating 2 min smoking 4 min circulating 2 min smoking 4 min circulating	55	0%
Drying	10 min	60	0%
Cooking		78	100% to core temperature of 71 °C
Dry	5 min	75	0%

Figure 2 Showering or drenching the surface of the meat product or immersion in smoke condensate solutions is widely used. In drenching, a solution of the smoke condensate is showered over the product for a specific amount of time. Normal solution concentrations range from 5% to 50% smoke. The smoke condensate solution is recirculated through a filter system and back over the product to get the highest usage out of the condensate. The meat products are showered for 15–90 s to obtain the desired results (see **Table 2** for an example of a showered frankfurter processing schedule).

Table 2 Example of a showered Frankfurter processing schedule

Chamber conditions	*Time (min)*	*Dry bulb temperature (°C)*	*Humidity*
Reddening	10	55	90%
Drying	15	60	0%
Drying	15	65	0%
Cooking		78	100% to core temperature of 71 °C
Dry	5	75	0%

nitrites used in the curing process may react with the acid in smoke condensates forming nitrogen dioxide if a low-acid smoke is not used. Smoke condensates for brine addition allow for injection into bacon, ham, loins, and other larger meat cuts to allow for even flavor distribution and better end product uniformity.

Use of Smoke Condensates

Generally, smoke condensates can completely replace traditional smoking. Their use is especially common in automated and highly productive manufacturing lines where smoke condensates are widely used for manufacture of popular smoked meat products produced in large quantities. They are especially useful in continuous manufacturing lines using special tunnels and a series of smoking chambers. For smaller-scale production units, equipment has been developed for showering the products with smoke condensates immediately after stuffing of the casings. Smoke condensates are also used in the coextrusion process, in which a collagen dough is extruded around the meat batter. This can provide flavor and structure to the sausage. The following advantages can be achieved by using smoke condensates instead of traditional smoking:

- Eliminating the emission of harmful and undesirable chemical substances to the surrounding atmosphere.
- Avoiding the fire risk associated with traditional smokehouses.
- Reducing the processing time and weight loss of traditional smoking.
- Reducing labor costs.
- Increasing product throughput.
- Reducing smoke chamber clean-up time and expense.
- Producing a healthier finished product with little or no PAH.

In the opinion of many consumers, use of smoke condensates in meat products, even at very low quantities, improves the flavor and aroma of the products. Although according to expert panels, smoke condensates do not usually achieve a smoke flavor identical to that achieved with traditional smoking, testing has shown that consumers do not have a preference for traditional flavor. Use of smoke condensates requires knowledge of their properties and experience and understanding of consumer preferences. The application of smoke condensates for meat products should be preceded by consultation with the smoke condensate manufacturers and

technical tests in the manufacturing facility. Exceeding the optimal level for a given product can result in off-flavors in the treated product, most often in the form of an acid, sharp, acrid, chemical, or medicinal aftertaste.

Properties of Smoke Condensates

Smoke condensates possess, like traditional smoke, coloring, bactericidal, and antioxidant properties. Aldehydes and aliphatic ketones are responsible for the coloring effect. The main aldehyde responsible for the coloring is hydroxyacetaldehyde. The aldehydes react with nitrogen in protein to begin the Maillard reaction. As the reaction progresses, it forms cross-linking of the proteins and color bodies. The color is then set or stabilized in the meat matrix by proper drying. Without proper drying, the color can fade or migrate, leaving a product that is much lighter in color than when first produced. Testing can determine the proper process to use to ensure that the color is properly set.

Acetic acid lowers the pH value on the surface of the product. Acid is important for skin formation on sausages and providing the 'tang' in other meats. Acid level is also used quite extensively as an easy control measure for maintaining showering and drenching systems. Phenols are mainly responsible for the typical flavor of the smoked product. Even with the extensive knowledge about the composition of smoke condensates, it is still not possible to fully predict the quality of the smoke flavor. This places a certain amount of pressure on integrators to achieve the best possible outcome in a given situation. Among the phenolic compounds, syringol and its derivatives are the most desirable for creating a good smoke flavor. Other desirable compounds are guaiacol and its derivatives. Furfural and its derivatives, cyclotene, maltol, and aromatic ketones and aldehydes also play important roles in composing the overall smoke flavor.

Smoke condensates also have bacteriostatic and bactericidal properties. The bactericidal activity is related to the concentration of the smoke condensate in the product. The minimum inhibitory concentration (MIC) has been determined for some smoke condensates. For *Bacillus* spp., *Staphylococcus aureus*, *Listeria* spp., *Lactobacillus* spp., *Escherichia coli*, *Salmonella* spp., *Yersinia* spp., and *Pseudomonas* spp., the MIC is 0.4% for the most active smoke condensates. For less active preparations, the MIC is 1–8%. Published studies indicate that the levels of phenolic compounds and acetic acid in liquid smoke preparations are most important for their bacteriostatic properties. Aldehyde compounds are also very good antibacterial agents in smoke, making all three main groups of smoke compounds important in antimicrobial effects. However, the great variation in the chemical composition of commercial smoke condensates means that their bacteriostatic properties can only be considered a support to the basic preservative concept for a given food product.

The antioxidative property of smoke condensates is related to the content of phenolic compounds. The antioxidative activity of smoke is mainly controlled by the following compounds: *cis*–*trans*-4-propenylsyringol, 4-isopropenylsyringol, 4-propylsyringol, 4-ethylsyringol, 4-methylsyringol, syringol, and the 4-derivatives of guaiacol. It is also assumed that polyphenols in the preparations are active antioxidants and may provide some beneficial health effects.

Health Aspects Relating to the Use of Smoke Condensates

Smoke condensates have been classified by the Food and Drug Administration (FAO) as generally recognized as safe (GRAS) in the US since 1980. In 1987 the FAO/World Health Organization Expert Committee on Food Additives set recommended maximum levels for a number of risk contaminants in smoke condensates including benzo[a]pyrene at 10 $\mu g\,kg^{-1}$ and benzo[a]anthracene at 20 $\mu g\,kg^{-1}$. Subsequently, the Council of Europe followed by the European Union took up the assessment of smoke condensates. As a result, the European Food Safety Authority (EFSA) evaluated dossiers and published opinions on a number of smoke condensates from both water-based and tar-based condensates. The EFSA concluded that there was no genotoxicity concern over the products presently on the market throughout Europe. At the time of writing the European Commission is determining whether limits are advisable on the use of these condensates based on applied safety factors derived from feeding studies.

It is certain that smoke condensates do not contain nitrogen oxides and that the PAH level in the food products with smoke condensates is considerably lower than after traditional smoking. The amount of tar introduced into food products during the process is also much lower than with traditional smoking. In general, the use of smoke condensates allows a controlled application of the smoke organics necessary to achieve the color, flavor, and antioxidant and antimicrobial effects of smoke in smoked food products.

See also: Bacon Production: Bacon. Chemical Analysis for Specific Components: Curing Agents. Cooking of Meat: Maillard Reaction and Browning. Processing Equipment: Brine Injectors; Mixing and Cutting Equipment; Smoking and Cooking Equipment. Smoking: Traditional

Further Reading

Baltes, W., Söchtig, I., 1979. Niedermolekulare Inhaltsstoffe von Raucharoma-Präparaten. Zeitschrift für Lebensmittel-untersuchung and -Forschung 169, 9–16.

Borys, A., 2001. The studies on the composition and properties of the selected liquid smoke used in Polish meat industy. Roczniki Instytutu Przemyslu Miesnego i Tluszczowego 38, 113–124.

Borys, A., Platek, T., Wegrowski, J., 2000. An attempt to determine the relationship between chemical composition of wood smoke preparation and its antioxidative properties. Roczniki Instytutu Przemyslu Miesnego i Tluszczowego 37, 171–185.

Council of Europe, 1998. Health Aspect of Using Smoke Flavours as Food Ingredients. Belgium: Council of Europe, Publishing and Documentation Service. ISBN: 92-871-2189-3.

Estrada-Munoz, R., Boyle, E.A.E., Marsden, J.L., 1998. Liquid smoke effect on *Escherichia coli* O157:H7, and its antioxidant properties in beef products. Journal of Food Science 63, 150–153.

Guillén, M.D., Ibargoitia, M.L., 1998. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavouring preparations. Journal of Agricultural and Food Chemistry 46, 1276–1285.

Guillén, M.D., Manzanos, M.J., 1996. Study of the components of an aqueous smoke flavouring by means of Fourier transform infrared spectroscopy and gas chromatography with mass spectrometry and flame ionization detectors. Advances in Food Science 18, 121–127.
Guillén, M.D., Manzanos, M.J., 1996. Study of the components of a solid smoke flavouring preparation. Food Chemistry 55, 251–257.
Guillén, M.D., Sopelana, P., Partearroyo, M.A., 2000. Determination of polycyclic aromatic hydrocarbons in commercial liquid smoke flavourings of different compositions by gas chromatography–mass spectrometry. Journal of Agricultural and Food Chemistry 48, 126–131.
Kim, K., Kurata, T., Fujimaki, M., 1974. Identification of flavor constituents in carbonyl, noncarbonyl neutral and basic fractions of aqueous smoke condensates. Agricultural and Biological Chemistry 38, 53–63.
Kjällstrand, J., Petersson, G., 2001. Phenolic antioxidants in alder smoke during industrial meat curing. Food Chemistry 74, 85–89.
Maga, J.A., 1988. Smoke in Food Processing. Boca Raton, FL: CRC Press.
Potthast, K., 1984. Liquid smoke, its use in the surface treatment of meat products. Fleischwirtschaft 64, 328–331.
Suñen, E., 1998. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic microorganisms associated with foods. Letters in Applied Microbiology 27, 45–48.
Suñen, E., Fernandez-Galian, B., Aristimuño, C., 2001. Antibacterial activity of smoke wood condensate against *Aeromonas hydrophila, Yersinia enterocolitica* and *Listeria monocytogenes* at low temperature. Food Microbiology 18, 387–393.

Relevant Website

www.redarrowinternational.com
Red Arrow International, LLC.

Traditional

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Glossary

Antioxidant activity Decreases the rate of oxidation of meat lipids through smoking. The smoke components that have the highest antioxidant activity are phenols.

Cold smoking The smoke temperature is in the range 12–25 °C.

Health hazards Hazards associated with smoked foods that may be caused by carcinogenic components of wood smoke and toxic effects of pathogenic microflora not eliminated in the whole manufacturing process.

Hot smoking The smoke temperature ranges from approximately 40–90 °C.

Preservative action Decreases the rate of microbial spoilage of meats through smoking that depends on the parameters of processing and the concentration of antimicrobial smoke components in the products.

Sensory properties of smoked products Desirable changes caused through smoking – predominantly in the color, flavor, and texture of meats.

Smoke composition Wood smoke consists of approximately 400 identified organic compounds, mainly phenols, aldehydes and ketones, carboxylic acids, aliphatic and aromatic hydrocarbons, alcohols, and esters.

Smoke phenols A very diversified group of wood smoke constituents, containing monohydroxy- and polyhydroxyphenols, compounds with long side chains, and phenols with additional functional groups.

Warm smoking The smoke temperature is in the range 25–45 °C.

Introduction

Smoking, drying, and salting belong to the oldest methods of food preservation. Meat hung by the fire was preserved by a combination of drying and smoking. Often the raw material was first pickled in brine. In different regions of the world various procedures have been developed, best suited for treating meats and fish for specific purposes. Smoking extended the shelf life and imparted very desirable, new sensory properties to the products. The role of its preservative effect decreased with the advent of canning, modern chilling, and freezing, whereas the aspects of flavoring and safety gained importance. Nowadays smoking is applied in many forms to treat as much as 40–60% of the total amount of meat products.

Traditional smoking involves the exposure of meat or meat products to smoke. The smoke is developed by smoldering wood either directly in the kiln below the hanging meat (Figure 1) or in an external generator. Its flow rate is controlled by natural draft depending on the construction of the smoking oven and on the weather conditions. In modern, automatic smokehouses it is forced by mechanical equipment. The temperature of the smoke is in the range 12–25 °C during cold smoking and 25–45 °C for warm smoking. In hot smoking, which should cause thermal denaturation of the meat proteins, the process may be carried out in different stages, at which the smoke temperature ranges from approximately 40–90 °C.

The progress achieved in traditional smoking of food has focused in the recent several decades on the control of the composition of smoke, application of engineering principles to heat and mass transfer to shorten the processing time and affect the weight loss of the product, rational design of the

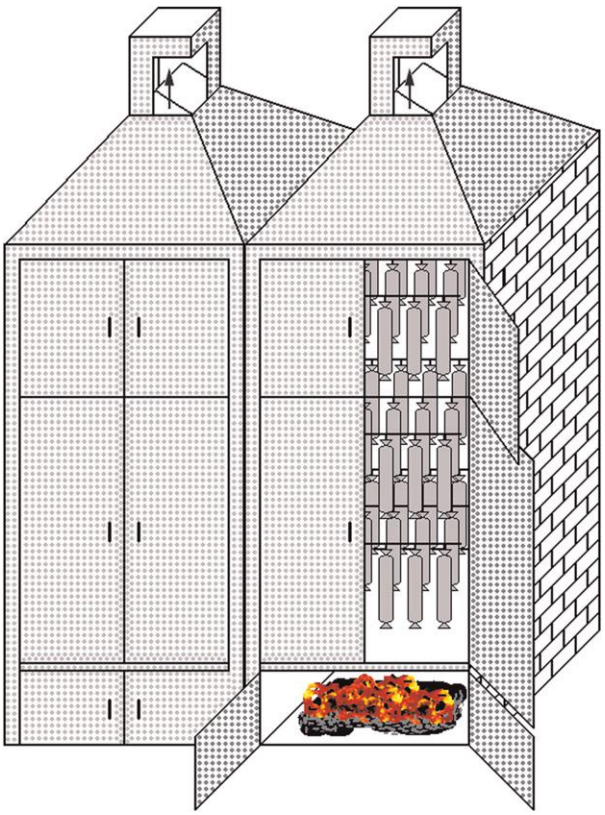

Figure 1 A traditional smoking oven. Courtesy of Łukasz Wiśniewski.

process parameters, assurance of quality including safety of the smoked goods, modernization of equipment, and treatment of the spent smoke to avoid pollution of the environment.

Wood Smoke

Generation and Composition

The curing smoke develops due to partial burning of wood or other suitable material with a controlled oxygen supply. It is composed of air, water vapor, CO_2, CO, nitrogen oxides, and a large number of different organic products of thermal degradation of hemicelluloses, cellulose, and lignin in temperature ranges of 180–300 °C, 260–350 °C, and 300–500 °C, respectively. Some of these products undergo oxidation at temperature reaching up to 900 °C. The yield and chemical composition of smoke depends more on the temperature and oxygen concentration in the zones of degradation and oxidation than on the humidity and kind of wood.

Generally, smoke is made from hardwood, mainly oak and beech. However, for imparting specific color or flavor to some products, wood of other trees that are rich in resins, including coniferous as well as heather, may also be used. According to legal requirements the wood shall be natural, and has not been subjected to any chemical treatment. In some areas, however, even bagasse and coconut husks are used.

From the large number of constituents found in different smoke condensates and extracts, approximately 400 organic compounds have been unequivocally identified. These include approximately 85 phenols, 110 aldehydes and ketones, 65 carboxylic acids, 20 aliphatic hydrocarbons, 80 aromatic hydrocarbons, and a number of alcohols, esters, and other compounds.

The phenol fraction is a very diversified group, containing among others, different compounds with long side chains, polyhydroxyphenols, as well as phenols with additional functional groups. It has been separated into approximately 240 components. The compounds present in the highest concentrations are listed in Table 1.

In the carbonyl fraction of wood smoke, the following compounds have been identified: formaldehyde, acetaldehyde, hydroxyacetaldehyde, acetone, hydroxyacetone, furfural, 5-methylfurfural, furanone, benzaldehyde, methylpropanal, and 3-methyl-2-cyclopenten-1-one. The fraction of acids consists of approximately 80% of acetic acid and includes formic, propionic, valeric, 4-oxovaleric, butyric, oxalic, malonic, maleic, fumaric, succinic acid, as well as various ketocarboxylic acids. In the alcohol fraction, methanol, ethanol, allylalcohol, *n*-amyl alcohol, 2-pentanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol, 2-hexanol, 2,4-pentadiol, and 1-heptanol have been identified. The ester fraction of wood smoke contains at least the methyl esters of formic, acetic, butyric, acrylic, propionic, 4-oksovaleric, heptanoic, and pelargonic acids, as well as ethyl and butyl acetates, ethyl butyrate, and ethyl valerate.

The fraction of hydrocarbons contains approximately 20 aliphatic compounds, mainly methane and ethene, and a large group of aromatic hydrocarbons which includes at least 61 identified polycyclic aromatic hydrocarbons (PAH). The contents of PAH can be substantially limited by keeping the temperature of smoke generation below 400 °C.

Wood smoke also contains several O-heterocyclic and N-heterocyclic components.

Table 1 The phenolic compounds present in wood smoke in the highest concentrations

Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, phenol, m-cresol, o-cresol, 3-ethylphenol, 2,5-dimethylphenol, syringol, 4-methylsyringol, 4-vinylsyringol, syringaldehyde, 1-(4-hydroxy-3,5-dimetoxyphenylo)-2-propanon, 1-(4-hydroxy-3,5-dimetoxyphenylo)-1-propanon, 1-(4-hydroxy-3,5-dimetoxyphenylo)-2-etanon, pirocatechin, 3-metoxypirocatechin, 4-methylpirocatechin, resorcinol, pyrogallol, 4-trans-propenylsyringol, hydroquinone.

Deposition on Smoked Goods

The boiling point of most smoke components is higher than the temperature in the smokehouse. Therefore, approximately 90% of the total mass of all constituents is present in the smoke in the form of small liquid droplets, approximately 0.08–0.15 μm in diameter. They are dispersed in the gaseous phase. The concentration of different components in the gaseous and dispersed phases depends on the temperature. The solid particles and liquid droplets disperse light, so that the smoke concentration can be assayed by measuring its optical density. Owing to the Brownian motion the particles and droplets coalesce and settle under the effect of gravitational and centrifugal forces on the walls of the smoking oven, the duct between the generator and the kiln, as well as on the smoked products. The electrostatic charge of the particles and absorption in the wet surface contribute also somewhat to deposition on the meat. High humidity of the smoke increases the rate of smoke deposition. On wet surfaces, the deposition of the components of the vapor phase is more effective than that of the particles and droplets. A rise in temperature of the smoke increases the concentration of some volatile components in the vapor phase and thus accelerates their sorption by the meat that is being smoked.

The quantity of compounds absorbed by the meat depends on the temperature, humidity, agitation, and composition of the smoke, the characteristics of the product's surface, and the duration of smoking. Wet surfaces adsorb approximately 20 times more phenols than dry ones. The published data on the total amount of smoke components absorbed by meat products are incomplete and vary within a broad range. The content of phenols in different smoked sausages ranges from approximately 0.02–200 $\mu g\ g^{-1}$. The composition of the fraction depends more on the conditions of processing, especially the humidity of the surface of the meats than on the concentration of individual phenols in the smoke. The quantity of formaldehyde in cold smoked goods may be as high as 20–40 $\mu g\ g^{-1}$. The amount of formaldehyde in different assortments of sausages may reach 2–50 $\mu g\ g^{-1}$; the surface layers of some products may contain approximately five times more than the inner layer.

Diffusion and Interactions in the Smoked Meats

Some compounds deposited on the humid meat surface stay there, while other diffuse into the deeper layers due to the concentration gradient. The diffusion rate is controlled also by the character of the surface, as well as by the properties of the meat and smoke compounds. Most phenols accumulate on the skin, on the sausage casing, and in the layer of the product several millimeters deep, especially in the fatty tissue. Carbonyl compounds and acids are rather equally distributed throughout the mass of some smoked meat products.

The chemically reactive phenols and carbonyls may be involved in polymerizations and react with amino and thiol groups in proteins and peptides. Furthermore, the phenols absorbed by the meat can be oxidized. The contents of guaiacol and phenol in smoked sausages stored for one month may decrease by approximately 35%. Because of comparatively low concentration of these absorbed components their reactions with meat proteins do not have any significant impact on the nutritive value of the products. However, the carbonyl-amino reactions and oxidation add to the desirable color formation.

The Effect of Smoking on the Shelf Life of Meat Products

Antimicrobial Activity

The shelf life of smoked products depends on the effect of heat pasteurization, level of water activity, as well as on the antibacterial and antioxidant properties of smoke components. Thus the preservative effect is obviously related to the concentration of salt due to presmoking treatment, the time–temperature regime and loss of water during processing, as well as the composition and quantity of smoke deposited on the meat. Cured and heavily smoked products are stable for several months at room temperature. Mild conditions of processing as applied in modern manufacturing of frankfurters do not exert such a high preservative effect. Smoking of frankfurters at internal temperature of 60–76 °C for 30 min reduces the total number of aerobic bacteria by approximately two log cycles, higher temperature and longer processing time being slightly more effective. Smoke components retard the proliferation of bacteria in cold stored frankfurters. Natural smoking can delay the onset of greening of frankfurters caused by *Leuconostoc mesenteroides* during storage.

Various smoke constituents in concentrations similar to those in smoked goods prevent the proliferation of microorganisms. To the most active antimicrobial agents of wood smoke belong guaiacol and its methyl and propyl derivatives, creosol, pyrocatechol, methylpyrocatechol, and pyrogallol and its methyl ether. The presence of an aldehyde group in a phenolic compound increases the antimicrobial activity. Formaldehyde arrests the growth of *Clostridium botulinum* in the concentration of 40 $\mu g\,cm^{-3}$. However, the smoke components present in lightly smoked foods stored under vacuum are not effective enough to inhibit the formation of *C. botulinum* toxin. Adding 8% of liquid smoke containing in 1 cm^3 approximately 1.4–4.0 mg of phenols and 20–70 mg of carbonyl compounds to raw minced beef, may significantly reduce the number of viable cells of *Escherichia coli* O157 H7 after 3 days at 4 °C. Several of the most thermo-tolerant *Staphylococcus epidermis* do not survive commercial hot smoking on inoculated rainbow trout. In cold smoked salmon, the growth of *Listeria monocytogenes* was found to be inhibited proportionally to the smoking time – 12 h of smoking reduced the population by three log cycles. However, well-adapted strains may persist in the smokehouse environment, so that *L. monocytogenes* can often be found in vacuum-packaged cold smoked salmon.

The most sensitive to smoke are generally the vegetative forms of bacteria. Molds are considerably resistant, although their development may be restrained by several phenolic compounds. A large population of molds and yeasts may survive in frankfurters smoked 30 min at an internal temperature of 67 °C. Smoking has little effect on the yeast count in the early stages of manufacturing of fermented sausages. However, in stored products the population of yeasts is lower in smoked sausages than in unsmoked controls.

Antioxidant Activity

The antioxidant effect of smoking was noticed by observing that smoked meats and fish were resistant to oxidative rancidity. Among the smoke components that have the highest antioxidant activity are phenols. The true antioxidants (AH), even in very low concentration, inhibit lipid oxidation by inactivating the radicals ($R^{\bullet}$) capable of initiating the chain reaction

$$R^{\bullet} + AH \rightarrow RH + A^{\bullet}$$

or the secondary radicals produced in the process of lipid oxidation ($LO^{\bullet}$ or $LOO^{\bullet}$)

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$

AH are able to react with the free radicals faster than the polyenoic fatty acids and thus protect the acids from being pulled into the chain reaction. However, they are ineffective in inactivating the highly reactive $^{\bullet}OH$ radicals. These radicals, because of their reactivity, attack rather the abundant fatty acids instead of the antioxidants present in low concentration. The phenolic AH inactivate the free radicals by donating the hydrogen atom of their OH group (Figure 2). The phenolic radical formed in the reaction has a low reactivity due to resonance delocalization of the radical function. The most active phenolic smoke antioxidants are pyrogallol, resorcinol, 4-methylguaiacol, 4-vinylguaiacol, and 4-trans-propenylsyringol. Less active are guaiacol, syringol, 4-methylsyringol, and 4-vinylsyringol.

The Role of Smoke in Developing Characteristic Sensory Properties of Smoked Goods

The desirable sensory properties of smoked meats result from the concerted action of the components of the meat, salting or curing, predrying, smoking, and heating. The smoke compounds induce smoky color and flavor. They also interact with

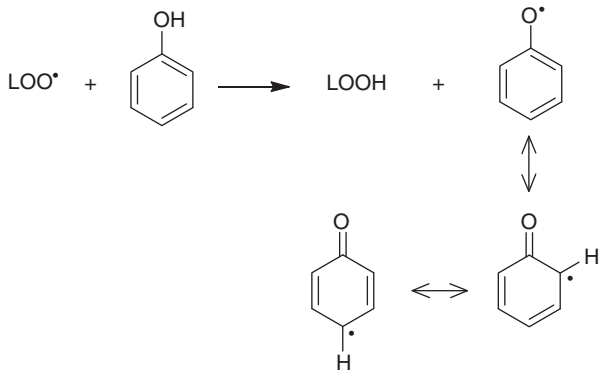

Figure 2 The inactivation of free radicals by phenolic compounds.

the nitrogenous meat constituents thus affecting some texture changes.

The color developed on the surface of the products is caused by colored smoke components and their interactions with the meat. Its intensity is primarily related to the optical density of the smoke and the duration of smoking. It increases at high smoke temperature and velocity. High temperature favors the development of dark color, because the rate of polymerization of the components, mainly phenols and of the carbonyl-amino reactions in the smoke itself and between smoke compounds and amino acid residues in the meat increases with temperature. The higher the temperature and water activity of the surface of small-calibre Brűhwurst, within limits set by other technological requirements, the darker is the color of the sausages. The kind of wood used for smoke generation also affects the color. Smoking with beech, maple, ash, sycamore, or lime tree smoke leads to golden-yellow color; yellow-brownish tint comes from oak, nut, and alder smoke. Products treated with smoke from coniferous wood have a dark coloration.

The dominant factors responsible for the smoky flavor are the smoke compounds themselves, mainly the phenols. The desirable flavor is associated with the presence of a mixture of syringol and 4-methylsyringol, although 4-allylsyringol, guaiacol, 4-methylguaiacol, and trans-isoeugenol also contribute to the typical sensory sensation. However, the multitude of variations of the smoky flavor is probably due to the contribution of different carbonyl compounds, furans, and other constituents not yet identified.

Health Hazards Induced by Smoking of Foods

The health hazards associated with smoked foods are related to the presence of carcinogenic components in wood smoke and smoked meats – PAH, N-nitroso compounds, and possibly heterocyclic aromatic amines, as well as to the toxic effects of pathogenic microflora not eliminated in the whole manufacturing process.

Wood smoke contains different PAH with a wide range of molecular weights (MW). The low-molecular members of this group, below MW 216, are not regarded as carcinogenic, contrary to many heavy MW PAH. Very mutagenic and carcinogenic is benzo(a)pyrene (BaP); until recently it was recognized as an indicator of carcinogenic PAH in wood smoke and smoked products. However, in 2008 the European Food Safety Authority stated that BaP alone is not suitable for use as the only marker of the contents of PAH in foods because, in approximately 30% of a very large number of investigated samples, no BaP could be found, although there were numerous other PAH present. The sum of the concentration of BaP, benz(a)anthracene, benzo(b)fluoranthene, and chrysene (PAH4) serve the purpose better, or else PAH8, i.e. the sum of PAH4 plus the concentration of benzo(k)fluoranthene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pirene. In the Commission Regulation (EU) of 2011, a maximum concentration of BaP in smoked meat and meat products has been maintained for the present (5 ng g^{-1} until 31 August 2014 and 2 ng g^{-1} after this date). However, additionally the upper limit for PAH4 has been introduced (30 ng g^{-1} from 1 September 2012 until 31 August 2014, and 12 ng g^{-1} after this date). Among PAH isolated from smoked products are mainly compounds with a MW less than 216. In various smoked meat products their total mass may be from approximately 30–250 times larger, whereas that of the heavy PAH approximately 10 times larger than that of BaP.

The contents of BaP in hot smoked sausages is usually below 1 ng g^{-1}, but in some black smoked products it may reach 55 ng g^{-1}. In flame-grilled sausages, BaP has been found in concentrations of approximately 20–40 ng g^{-1}. The contents of BaP in barbecued pork and beef may be in the range 1.5–10.5 ng g^{-1}, and in charcoal-broiled steaks 5–8 ng g^{-1}. In various foods nitropolycyclic aromatic hydrocarbons have also been identified. In smoked sausages the contents of 1-nitropyrene, 2-nitronaphtalene, and 2-nitrofluorene has been found to be approximately 4, 8, and 20 ng g^{-1}. This is comparable to the contents of these compounds in roasted coffee beans of approximately 2, 4, and 30 ng g^{-1}.

Smoked cured meat products contain a number of volatile and nonvolatile N-nitroso compounds, most of which are carcinogenic in laboratory animals. The aldehydes of smoke can react with cysteamine and with cysteine yielding various thiazolidine precursors that can be easily nitrosated. The reaction of formaldehyde with cysteamine and cysteine leads to thiazolidine and thiazolidine-4-carboxylic acid, respectively, which, on nitrosation turn into N-nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid. Minute amounts of these compounds occur in smoked meats. In the presence of glycolaldehyde from smoke 2-(hydroxymethyl)-N-nitrosothiazolidine and 2-(hydroxymethyl)-N-nitrosothiazolidine-4-carboxylic acid (HMNTCA) may be formed. In smoked ham, sausages, salami, pepperoni, and poultry products the contents of HMNTCA may be from approximately 10–260 ng g^{-1}. Generally the concentration of these compounds is higher in meats smoked in traditional ovens than in products processed in modern smokehouses supplied with smoke from external generators. The total amount of various N-nitroso compounds in smoked fried bacon, some of which still unidentified, has been reported to be 430–6800 ng g^{-1}.

Heterocyclic aromatic amines, known to be generated in pyrolytic reactions of amino acids and proteins and in nonenzymatic browning, are present in very heavily smoked foods in amounts lower than 1 ng g^{-1}.

The hazards associated with the effects of pathogenic microflora in smoked foods depend on the initial bacterial contamination of the raw materials, on the bacteriostatic and bacteriocidic action of all processing steps, and on the effectiveness of the applied systems of quality assurance, e.g., the hazard analysis and critical control points.

The Equipment

Smoke Generators

Traditional smoking is carried out in different countries using a variety of smokehouses. The traditional kilns, as shown earlier in Figure 1, are used only in artisan meat processing. In an advanced type of kiln extending over two storeys of a building distinct zones with different temperatures are created. Heat is generated by burning gas, but smoke is still produced in the lower section of the kiln. Modern smokehouses are supplied with smoke produced in conditions that favor the formation of desirable components and minimize the generation of PAH.

In contemporary smoldering-type generators, the wood chips, shavings or sawdust are fed automatically on a grated fire bed or electrically heated plate. The sawdust or chips of wood of standardized water content and mesh size are available commercially. The temperature of the glowing pile is controlled by the humidity of the sawdust and the rate of air flow; by using material of approximately 50% humidity and limiting the air flow, the temperature may be reduced to approximately 600 °C. Because of the relatively high temperature of generation, the smoke is rich in phenols and has fully developed aroma. However, formation of PAH cannot be avoided if the temperature of the process exceeds 400 °C.

In a friction-type apparatus, the heat necessary for thermal degradation of wood is due to pressing of a log against a rotating drum or disk. The temperature at the friction interface can be controlled by varying the pressure exerted on the log or the rotation rate of the rotor. It is generally below 400 °C. In such conditions the thermal degradation of lignin and the oxidation of the reaction products is not as advanced as in a smoldering pile of sawdust, thus the aroma of the smoke may be slightly different. These conditions, however, minimize the development of PAH. Various friction generators are available, especially for rather small smoking chambers.

Wood smoke can be also produced in a hydropyrolytic process by treating sawdust or chips with superheated steam at 250–390 °C. The smoke developed at this temperature is rich in carboxylic acids and carbonyl compounds, but relatively poor in phenols. Its composition can be controlled by adjusting the temperature of the steam, the flow of air, and the rate of feeding the sawdust.

Smokehouses

In a traditional kiln, the operator controls the processing parameters under different weather conditions by appropriate feeding of the fireplace with wood logs, chips, and sawdust, opening or closing the doors and vents, and reversing the rods carrying the smoked sausages. Modern smokehouses are built to utilize the full application of the engineering principles of heat and mass transfer. They are heated by steam, gas or electricity and equipped with devices for forced air and smoke circulation at controlled velocity. The circulation of smoke provides uniform thermal and flow conditions in all parts of the kiln. The temperature, humidity, and density of the air/smoke, as well as the process time are adjusted to requirements depending on the desired properties of the products. Smokehouses used for processing cooked sausages are equipped with a hot water or steam injection systems and a cooling water spray, so that smoking, cooking, and cooling can be carried out in the same kiln. The smoke supplied from a generator is often conditioned by a water spray to control its temperature and humidity, and to separate out some tar fractions and soot. The material to be smoked is usually hung on rods and introduced by trolleys into the kiln or tunnel (Figure 3). Some kilns can be loaded through the front door and unloaded from the back side – this is convenient for a rational organization of the process.

The exhaust gases, after leaving the smokehouse, are cleaned before entering the atmosphere. Some installations comprise three stages – an electrofilter, a fibrous filter, and activated charcoal. Other systems use afterburners to oxidize the components of the spent smoke at 800–1500 °C and some use temperature approximately 600 °C in the presence of a catalyst. Many smokehouses are equipped with installations for automatic cleaning.

Smoking Procedures

Various procedures are used in the industry and in artisan meat processing to produce smoked foods of desired sensory properties and shelf life. Traditional procedures are based on practical experience of generations of meat processors. Skilled, experienced personnel are required to run the processes in a smoking oven at different weather conditions. In mass production of popular items, like frankfurters, the process is carried out in automatic smokehouses; the parameters are based on results of research and are computer-controlled. Some selected examples of different procedures are given below.

Cold smoking is used mainly in manufacturing of raw, fermented sausages, made from cured meats. The smoke at 12–25 °C and controlled humidity is applied for several hours or days, depending on the assortment of produce. When smoking salami, the sausage links are first surface-dried for 1 day at 12 °C in low-density smoke. This is followed by 5 days of smoking in dense smoke at 15–22 °C, and finished within 2 days in a somewhat colder and less dense smoke. For smoked bacon, the cured cuts are soaked for 3–4 h in cold, running water, washed in water at 30–40 °C, hung on hooks for 12–24 h to drip and for surface drying, smoked for 24–48 h at 25–35 °C until the skin and meat surface turns dark brown, and cooled to below 18 °C. The product yield is approximately 90% in relation to the weight of the cured bacon.

When smoking frankfurters, the first phase is tempering at 32–38 °C, which is intended to remove the surface moisture to ensure uniform color development of the product. Smoking properly in dense smoke of controlled humidity brings the

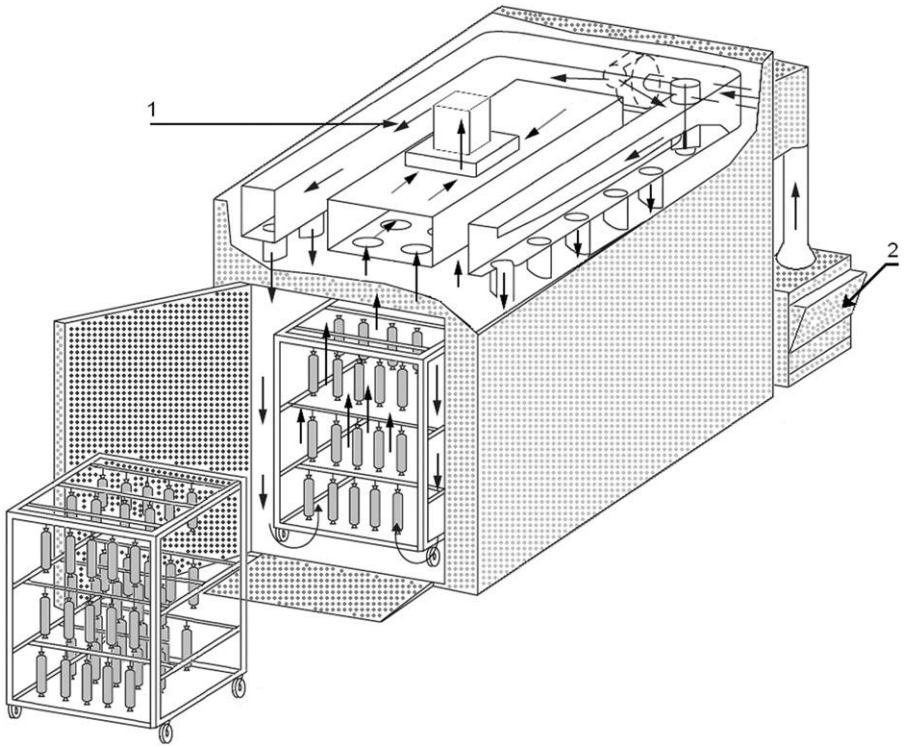

Figure 3 A modern smokehouse: (1) flow direction of the smoke and (2) smoke generator. Courtesy of Łukasz Wiśniewski.

internal temperature of the sausages to 60–68 °C and imparts the desirable smoky color and flavor. It usually lasts for approximately 1–1.5 h. This is followed by cooking in hot water or steam and by chilling. Smoking cooked sausages at a too high temperature may cause excessive fat and weight loss. This may lead to development of creased surfaces of the sausages and loss of uniformity of color.

In manufacturing jagdwurst the sausage batter made of cured pork, beef meat (9:1), and spices is stuffed into natural casings with a diameter of up to 32 mm. The links, 18–20 cm long, are hung for 12 h at 2–6 °C or 2–3 h at room temperature for setting, smoked for 80–90 min at 80 °C, dry-heated at 85 °C during 25 min to reach an internal temperature of 68–70 °C with a brown surface color, chilled to 18 °C, smoked again at 24–32 °C for 12 h to a darker-brown color, dried at 14–18 °C at a relative humidity of 75–80% during 6–8 days to a final water content of 55–57%, exposed to final smoking at 24–32 °C for 2–3 h, and cooled to below 18 °C. The yield of the sausage is approximately 59% in relation to the weight of the cured meat.

Kabanos, a delicatessen-type, spicy sausage, is produced by stuffing the sausage batter made of cured pork and spices into natural casings of 22 mm in diameter, setting the 60–70 cm long links for 12 h at 2–6 °C or for 30–60 min at room temperature, followed by 50–60 min of hot smoking, 20 min of dry heating to a core temperature 69–70 °C and dark brown color, cooling, and drying at 12–18 °C and 75–80% relative humidity for 5–7 days. The yield of the sausage is 55% relative to cured meat.

Traditional Xinjiang smoked horsemeat is made by soaking specially selected parts of the carcass for 1 h in cold water, adding 3% salt, 1% sugar, and 0.04% $NaNO_3$, holding for 3–5 days at 2–4 °C, smoking for 5–6 h at a smoke temperature of 60 °C, and steaming for about 1.5 h at a meat temperature 90–100 °C. The smoked product is packed in cans, sterilized, and cooled. It has a dark brown surface and is dark red inside.

See also: Human Nutrition: Cancer Health Concerns. Microbiological Safety of Meat: *Clostridium botulinum* and Botulism; *Listeria monocytogenes*; Pathogenic *Escherichia coli*; Yeasts and Molds. Processing Equipment: Smoking and Cooking Equipment. Sausages, Types of: Dry and Semidry; Emulsion. Sensory and Meat Quality, Optimization of. Smoking: Liquid Smoke (Smoke Condensate) Application. Spoilage, Factors Affecting: Oxidative and Enzymatic

Further Reading

Andrée, A., Jira, W., Schwind, K.-H., Wagner, H., Schwägele, F., 2010. Chemical safety of meat and meat products. Meat Science 86, 38–48.

Bartoszek, A., 2007. Mutagenic, carcinogenic, and chemopreventive compounds in foods. In: Sikorski, Z.E. (Ed.), Chemical and Functional Properties of Food Components, third ed. Boca Raton, FL: CRC Press, pp. 451–486.

Doe, P., Sikorski, Z., Haard, N., Olley, J., Pan, B.S., 1998. Basic principles. In: Doe, P.E. (Ed.), Fish Drying and Smoking. Production and Quality. Lancaster, PA: Technomic Publishing Co. Inc, pp. 13–45.

Kołakowski, E. (Ed.), 2012. Technology of Food Smoking (in Polish). Warszawa: Powszechne Wydawnictwo Rolnicze i Leśne.

Miler, K.M.B., Sikorski, Z.E., 1990. Smoking. In: Sikorski, Z.E. (Ed.), Seafood: Resources, Nutritional Composition, and Preservation. Boca Raton, FL: CRC Press, pp. 163–180.

Potthast, K., 1978. Verfahren des Räucherns und ihr Einfluss auf den Gehalt an 3,4-Benzpyren und anderen Inhaltsstoffen des Räucherrauches in geräucherten Fleischerzeugnissen. Die Fleischwirtschaft 58 (3), 340, 342–348.

Potthast, K., 1979. Einfluss der Räuchertechnologie auf die vollständige Zusammensetzung der polycyclischen Kohlenwasserstoffe in geräucherten Fleischwaren, in Rauchkondensaten und in den Abgasen von Räucheranlagen. Die Fleischwirtschaft 59 (10), 1515–1523.

Sikorski, Z.E., Kołakowski, E., 2010. Smoking. In: Toldra, F. (Ed.), Handbook of Meat Processing. Ames, IA: Wiley-Blackwell, pp. 231–245.

Tóth, L., Potthast, K., 1984. Chemical aspects of the smoking of meat and meat products. In: Chichester, C.O., Mrak, E.M.K., Schweigert, B.S. (Eds.), Advances in Food Research, vol. 29. New York: Academic Press Inc., pp. 87–158.

SPECIES OF MEAT ANIMALS

Contents

Cattle

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Glossary

Biological type The commercially important physical characteristics of a beef animal.
Carcass The primary commercial portion of a beef animal after dressing (removal of the blood, head, feet, hide, and internal organs).
Cutability The proportion of saleable meat in the carcass.
Dressing percentage The weight of the carcass expressed as a percentage of the live weight of the animal.
Finishing Feeding to achieve the appropriate level of subcutaneous fat or finish for the target market.
Heterosis The advantage conferred by crossbreeding measured as the difference between the offspring and the midparent mean for the trait; it is also known as hybrid vigor.
Inbreeding Mating of animals that are more closely related than average in the population.
Marbling The fat within the muscles.
Net feed efficiency, also known as residual feed intake The difference between expected feed intake and actual feed intake based on an animal's live weight and growth rate.
Offal All parts of the animal other than the carcass.

Introduction

All types of cattle, irrespective of whether their primary purpose is meat, milk, draft power, or some combination of these, are used ultimately in beef production. Consequently, a wide variety of shapes, sizes, body compositions, and ages of cattle consigned to abattoirs are found around the world. Some of them have been bred and managed specifically for beef production, whereas others, including culled breeding stock, produce beef as a by-product. This article is concerned mainly with the wide variety of breed types of cattle used for beef production in the developed world. In much of the developing world, cattle are not used primarily for beef production, and many different regional types have evolved that are suited to their particular local functions and management systems.

Biological Types of Beef Cattle

Although the word 'breed' is used in common parlance to describe distinct types of cattle, it is not a completely useful or biologically meaningful term. In the past few centuries in particular, livestock (and pet) owners had gathered together groups of their animals with similar phenotypes and kept a formal registry of their progeny. This group of animals was then referred to as a 'breed,' and members of the breed were normally descendants of the original group. A deliberate policy of inbreeding close relatives was often followed to fix the 'type,' i.e., to ensure that the offspring conformed to the distinctive phenotype of the breed. An unavoidable by-product of this process was inbreeding depression whereby the purebred animals were less fit in an evolutionary sense and tended to have poorer reproductive and growth performance than their non-inbred relatives. Crossbreeding nullified this inbreeding depression through heterosis or hybrid vigor, which removed the constraints of inbreeding.

Today, several species of cattle (genus *Bos*), comprising hundreds of major and minor breeds, are owned and used by farmers around the world. Commercial crossbreeding of these cattle can result in a wide variety of sizes and shapes, and the use of four genders (male, female, castrated male, and spayed female) and a broad range of ages and weights at harvest

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Figure 1 A pair of Hereford bulls. Reproduced with permission from the Canadian Hereford Association.

Figure 3 A Simmental cow and calf. Reproduced with permission from the Canadian Simmental Association.

Figure 2 A Black Angus cow and calf. Reproduced with permission from the Canadian Angus Association.

Figure 4 A Maine-Anjou cow and calf. Reproduced with permission from the Canadian Maine-Anjou Association.

further exacerbates the complexity of raw material entering the beef chain.

Clearly, in trying to categorize the production traits of cattle, 'breed' is far too unwieldy a descriptor. Instead, the term 'biological type' is preferred and is used here to indicate phenotype in terms of temperament, mature body size, muscularity, and propensity to fatten, which are the most obvious traits of market-ready beef animals. Many other traits, such as libido, fertility, calving ease, and lactation, are of enormous importance to a beef producer, but they are not considered here. A number of very broad 'biological type' descriptors are commonly used in the beef industries of the developed world, including the following:

- 'British' refer to cattle originating in Great Britain, developed specifically for beef production off pasture, and typified as Hereford (Figure 1), Angus (Figure 2), and Shorthorn. Traditionally, they tend to be of medium body size and muscling with a relatively high propensity to fatten and a usually docile temperament.
- 'Continental' or 'European' refer to the multipurpose cattle from the European continent, typified by breeds, such as Simmental (Figure 3), Maine-Anjou (Figure 4), Charolais (Figure 5), and Gelbvieh. They are generally larger than the

Figure 5 A Charolais bull. Reproduced with permission from the Canadian Charolais Association.

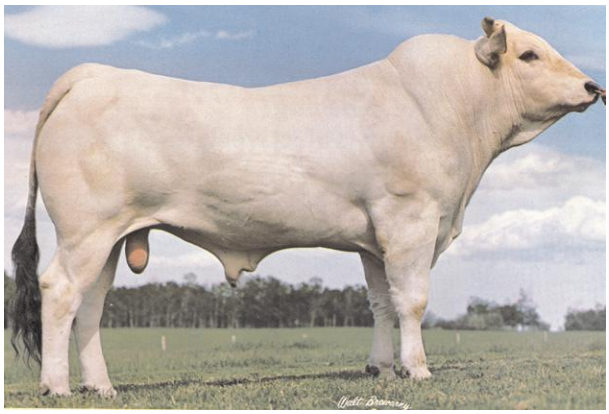

Figure 6 A Chianina bull. Photograph: Walt Browarny.

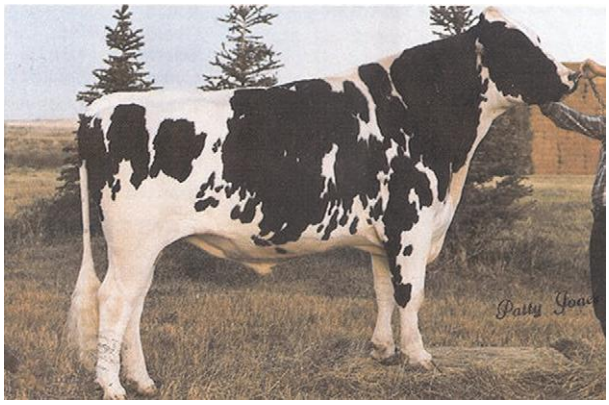

Figure 7 A Holstein bull. Photograph: Patty Jones.

Figure 8 A Zebu bull. Photograph: Mick Price.

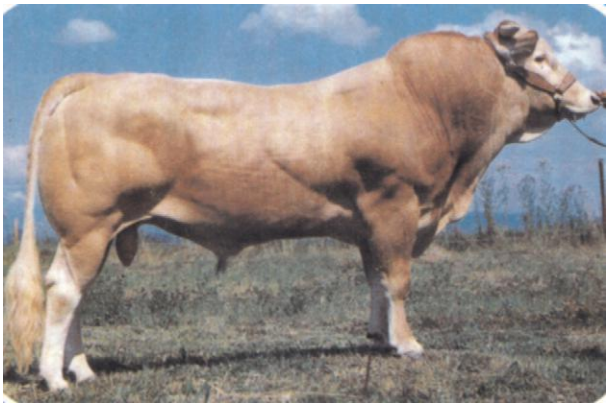

Figure 9 A Blonde d'Aquitaine bull. Photograph: Walt Browarny.

British cattle, with heavier muscling, a lower propensity to fatten, and less docile temperament. A subset of this group, sometimes referred to as the Italian White breeds (e.g., Romagnola, Marchigiana, and Chianina (Figure 6)), consists of very large cattle with a very low propensity to fatten.

- 'Dairy type' refers to cattle that have been bred primarily for milk production. They are characterized by light muscling and a low propensity to deposit subcutaneous fat, but they vary widely in mature body size from small (Jersey) to large (Holstein-Friesian). They are also characterized by a higher propensity than beef-type cattle to deposit intramuscular (marbling) and internal fats, including body cavity and kidney fat. Dairy bulls (Figure 7) are commonly described as having unpredictable and mean temperaments.
- 'Dual purpose' is a term applied to cattle bred to produce both milk and beef. They tend to be of medium to large body size (Brown Swiss, British and Dutch Friesians, Salers, and Normande) with moderate muscling and propensity to fatten. Temperament depends on the biological type of cattle involved.
- 'Zebu' is a general term applied to cattle of the *Bos indicus* species (Figure 8). They vary widely in mature body size and muscling but tend to have a lower propensity to fatten than taurine (*Bos taurus*) cattle and a more volatile disposition than British and Continental cattle. Their strengths include heat and insect tolerance, but their meat is slightly less tender than that from taurine cattle. Maximum heterosis can be obtained by crossing *B. indicus* and *B. taurus* cattle.
- 'Double muscled' (muscularly hypertrophied) is a term used to describe cattle of any breed type exhibiting a particular genetic syndrome characterized by extremely heavy muscling. Although it results from a mutation in a single gene (a deletion in the 'myostatin' gene on bovine chromosome 2), this condition has very wide-reaching effects in terms of body composition and temperament. These cattle might be of any mature body size, depending on their breed type, but always show extremely high muscle-to-bone ratios and a very low propensity to fatten. They also typically exhibit fine bones, thin skin, and a nervous disposition. Some extremely heavily muscled breeds (e.g., Belgian Blue, Blonde d'Aquitaine (Figure 9), and Piedmontese) commonly carry the gene for this condition.

Cattle producers throughout the developed world have been slow to adopt crossbreeding and hybrid breeding systems. Although these are considered essential practices for most other species of livestock and poultry, beef and dairy producers still commonly use purebred or first cross (F1) cattle. However, in recent decades, two- and three-way crosses of cattle breeds have gained in popularity (sometimes resulting in a new 'breed') but hybrids, and particularly the use of hybrid bulls, are still considered a risky venture. Beef producers

have also relied to a greater extent on subjective ('eyeball') assessment to select sires and to cull dams than other livestock breeders.

However, leaders in the beef industry have, over the past half century, increasingly incorporated objective methods of assessment into their genetic selection programs and have adopted crossbreeding to capitalize on heterosis and complementarity of traits. Measurements now include not only live weight and feed consumption but also ultrasonically assessed fat and muscle thickness. The cattle industries have also been quick to adopt the new sciences of genomics and proteomics to help identify and select genes associated with traits, such as fertility, residual (or net) feed efficiency, and carcass and meat quality, that have been difficult to improve using traditional methods.

As technology and computing power have advanced and become more affordable, it has become possible to make comparisons among bulls at very young ages, which would have been impossible a few decades ago. These include the statistical assessment of expected progeny differences (EPDs) and deoxyribonucleic acid (DNA)-based genotyping and quantitative trait loci mapping to discover chromosomal regions and ultimately single-nucleotide polymorphisms associated with traits of interest to the breeder. Modern beef producers are very much aware of the importance of EPDs for a variety of traits in selecting bulls and culling heifers and cows and are increasingly requesting the incorporation of DNA-derived genomic information to assist in sire selection.

Traits of Importance in Finished Beef Cattle

Temperament

Although temperament clearly has an environmental component (even the wildest of cattle can be tamed and the tamest of cattle made wild by the way they are handled), it is in part a genetically determined trait. Cattle handlers are very familiar with the 'typical' temperament of specific breeds of cattle and learn management techniques appropriate to those breed types. It can be generalized that zebu cattle and dairy breeds of bulls are more temperamental than the taurine beef breeds, with British beef breeds being more docile than continental cattle. Cattle exhibiting the 'double-muscled' trait are commonly stress susceptible. In management systems that involve frequent interactions between cattle and people, poor temperament is not likely to be a problem, partly because the animals become tamed through frequent handling and partly because only docile (or at least tamable) cattle are kept. In systems where there is minimal interaction with humans, it is possible for problem temperaments to remain unrecognized until animals are marketed, at which time nervous or aggressive temperaments can have important negative consequences. These cattle can be dangerous to handle and can injure or bruise themselves or other cattle during marketing or processing, resulting in removal and condemnation of the damaged tissue from the dressed carcass and possibly condemnation of the whole carcass. Even if physical damage is avoided, the stresses of marketing and transportation might be severe enough to result in dark, firm, and dry (DFD) or even pale, soft, and exudative meat.

Mature Body Size

Mature body size varies quite widely among the various biological types and genders of cattle. The lower range of sizes among traditional cattle is represented by the Dexter (a taurine breed originating in Ireland), with mature bulls typically less than 450 kg and mature cows approximately 100 kg less than the bulls. Although some specialty miniature cattle have been developed that are considerably smaller than the Dexter; they are not discussed in this article.

At the extreme upper end of mature body size is the Chianina, also a taurine breed, from Italy, with mature bulls sometimes exceeding 1800 kg and cows reaching 1100 kg. It should be noted that the castrated male (steer) of all breed types would typically grow to be larger than the entire male, particularly in linear dimensions (height and length), but only at a very advanced age. Cattle that are raised specifically for beef production are usually marketed at a live weight considerably below their mature size, with each market having a preferred live weight and fatness. When cattle are marketed as culls from the dairy- or beef-breeding herd, they are more likely to have reached their mature body size.

Muscularity

At a common live weight, different biological types of cattle can vary widely in muscularity as a result of genetically determined differences in the muscle-to-bone ratio. In a 'typical' finished beef steer carcass, the ratio of muscle weight to bone weight is approximately 4:1, and almost all steer carcasses fall within the range of 3.5:1 ('dairy type,' e.g., Jersey) to 5:1 ('heavily muscled' type, e.g., Piedmontese). Gender also has an effect on muscle-to-bone ratio; bulls are more heavily muscled than steers, which are, in turn, more heavily muscled than heifers or cows. An extreme of muscle-to-bone ratio is found in cattle with the 'double-muscled' genotype. Bulls with this syndrome have been known to reach a carcass muscle-to-bone ratio as high as 9:1.

The muscle-to-bone ratio is lowest at birth and gradually increases as animals mature. In an animal that is continually gaining weight, no matter how quickly or slowly, environmental effects, including nutrition and management, have little influence on muscle-to-bone ratio, because it is a genetically controlled function of live weight. However, if an animal loses weight, it is lost from muscle and fat in approximately equal amounts, rather than from the skeleton; therefore, weight loss results in a reduction in muscle-to-bone ratio.

Although cattle of some genotypes contain more total muscle than others, biological-type differences in the distribution of muscles, for example, between high- and low-priced cuts of meat, are too small to be of any commercial importance. Traditional livestock judges have placed emphasis on this trait and it is most likely that a great deal of selection pressure, which should have been applied to truly important traits, has been wasted in its pursuit.

Propensity to Fatten

The propensity to fatten is also a genetically determined trait, but its expression is heavily dependent on environmental effects, particularly nutrition. In general, the types of cattle that have been selected strictly for beef production (e.g., the British beef types) have a greater proportion of their fat in the subcutaneous depot, and those that have been selected for dairy production have a greater proportion in the internal fat depots. Dairy types typically deposit more marbling fat than beef types. Some cattle, notably the Wagyu or Japanese Black breeds, have been selected specifically for their propensity to deposit intramuscular (marbling) fat without excessive amounts of fat in the other depots. In most cattle, however, marbling fat is the last to be deposited and reaches high levels only in cattle fed high-energy diets for prolonged periods. These cattle also exhibit high levels of fatness in the other depots.

Traits Determining the Value of Commercial Beef Animals

The value of commercial beef animals depends ultimately on the quantity and quality of meat they contain. In general, biological type has a major influence on meat quantity (size and body composition) but considerably less direct influence on meat quality. Biological type may, however, have a major indirect influence on meat quality through, for example, its effect on temperament leading to DFD beef. Biological type can also influence the thickness of muscle and fat in the carcass, and these together influence the rate of cooling following slaughter. Rapid cooling is a major cause of toughening, through cold shortening, in beef.

Quantity

The quantity of beef in animals is determined by three factors: market weight, dressing percentage, and cutability. Dressing percentage is the weight of the carcass expressed as a percentage of the live weight of the animal and cutability is the weight of saleable meat expressed as a percentage of the weight of the carcass. In general, 'meat' constitutes approximately 35–40% of a beef animal's live weight, but a number of factors can influence this.

Live weight and fatness

The size and fatness of market-ready cattle entering the beef chain is determined by local preference. The challenge for all beef producers is to breed and manage their cattle to meet these demands, as failure to do so results in discounted prices. The biological type of cattle needs to be the one that reaches the optimum subcutaneous fat thickness at the optimum carcass weight in the particular management system. Beef from cattle marketed as culls does not have to meet these standards because it is used mainly in processed meat products. Because of the wide range in size and propensity to fatten among the different biological types of cattle, it is comparatively easy for producers to select types of cattle that quickly and efficiently reach the appropriate live weight and fatness in their particular management system. Unfortunately, this flexibility is sometimes reduced by a producer's loyalty to one particular breed.

The increasing popularity of grain feeding in North America following World War II gives an interesting insight into the ways in which beef producers tailor their product to suit the market. Straight-bred (i.e., purebred, but not registered) British-type beef cattle were the norm in most areas of North America in the early and middle parts of the twentieth century. These cattle had a high propensity to fatten, making them very suitable for grass finishing. Consequently, feeding them on high-energy (grain based) diets led to overfat carcasses at the desired carcass weight or underweight carcasses at the desired fatness. Because producers saw a strong economic advantage in feeding grain, the solution to this problem was to change the biological type of cattle to a larger, later-maturing (lower propensity to fatten) biological type. This led to a wide-scale importation of the larger, leaner, and faster-growing Continental cattle, such as Charolais, Simmental, and Limousin, which could be fed high-energy grain-based diets without becoming excessively fat at the target carcass weight.

Dressing percentage

From the point of view of meat production, live animals consist of two parts: carcass and offal. These are not biological divisions and their definitions vary from time to time and place to place, but the carcass generally consists of the major muscles in the body and their associated bones and fat. In some places the tail, kidneys, and the kidney fat are defined as part of the carcass; in other places they are not. Regardless of how a 'carcass' is defined, 'offal' is always defined as everything in animals other than the carcass. The offal is further subdivided into edible and inedible components.

Many factors affect dressing percentage; they can be conveniently categorized into those that affect the carcass weight and those that affect the liveweight.

$$\text{dressing percentage} = \frac{\text{carcass weight} \times 100}{\text{live weight}} \qquad [1]$$

An important factor affecting the numerator (carcass weight) is the definition of 'carcass.' Clearly, if the tails, kidneys, and kidney fat were defined as part of carcasses, then dressing percentage would be higher than it would be if they were defined as part of the offal. Also, the carcass itself can be weighed 'hot' (immediately after slaughter) or 'cold' (after cooling overnight at 1–3 °C). Before the advent of spray chilling (misting with cold water), a typical carcass used to lose approximately 2% of its weight during cooling; weight loss from spray-chilled carcasses is close to zero.

Other major factors affecting carcass weight are fatness and muscularity (muscle-to-bone ratio): the fatter or more muscular a carcass is (all other things being equal), the higher is its dressing percentage. Both muscularity and propensity to fatten are under genetic control, so biological type influences dressing percentage. In practice, the range in muscularity in market-ready cattle (apart from double-muscled animals) is not nearly as great as the range in fatness, and the majority of body fat is located in the carcass, so it can be further generalized that carcasses with greater dressing percentages are usually fatter.

Factors influencing the proportion of the offal are also direct contributors to dressing percentage. These factors

include gut fill (the amount of undigested feed in the gastrointestinal tract), the weight and condition of the hide (wet/dry, hairy, and mud and manure caked), the proportion of bone, and, in case of females, pregnancy. Gut fill depends more on immediate preslaughter feeding than on biological type, but it might be that some cattle have greater appetites and can be expected to be 'paunchier' given the opportunity. This would tend to include the dairy types. Type of feed, particularly high energy versus low energy (e.g., grain vs. grass), influences gut fill because animals allowed to eat according to appetite consume a greater weight of low-energy feeds in an effort to optimize their daily energy intake. As a consequence, grass-finished cattle typically exhibit lower dressing percentages than grain-finished cattle. The weight of hide is a major contributor to the weight of the offal, and some biological types (e.g., zebu and double muscled) are known to have lighter hides and consequently higher dressing percentages. Geographical area and management system, particularly feedlot versus pasture finishing, might have a major influence on the amount of mud and manure caked on the hide. The presence of large horns, another trait of biological type, influences dressing percentage, but in practice it is common for horned cattle to be dehorned if they are to be finished in feedlots.

The dressing percentage of a 'typical' steer (tail on the carcass, kidneys, and kidney fat in the offal) is in the order of 57–60% or 60–63% when kidneys and kidney fat are left in. Very fat or very muscular cattle could dress as high as 65%, but that would be exceptional. At the other extreme of normal cattle, culled cows typically have dressing percentages of approximately 48–53%, with the lower values being achieved by very lean, sick, or emaciated cattle, particularly of dairy type.

Cutability

Carcass cutability can be defined in a number of ways, but it means the amount of saleable meat in the carcass generally expressed as a percentage of the carcass weight. It is largely determined by the amount of bone and excess fat that are removed from the carcass before retail. The actual value is strongly influenced by the cutting processes used. In some countries, all bones are removed before retail, but in most countries some cuts, for example, T-bone steak and standing rib roast, are marketed bone-in. Similarly, the amount of fat left on retail cuts is a matter of national or regional preference. Thus, to put absolute values on cutability would be misleading because the absolute value varies widely depending on geographical location. It is clear, however, that the influence of biological type on cutability can be profound because a carcass with a higher muscle-to-bone ratio or lower level of fatness has a correspondingly higher cutability. This biological-type effect is further influenced by the effects of age and slaughter weight on muscle-to-bone ratio and nutrition and management effects on carcass fatness. Some carcass grading systems include a 'cutability estimate' as a criterion of carcass merit. This figure is usually based on selected prime cuts, rather than on the whole carcass and therefore cannot be taken to indicate the total amount of meat available for retail sale. In general, 55–60% of the weight of beef carcasses is composed of muscle, with exceptionally muscular or lean carcasses exceeding this range and fat or lightly muscled carcasses falling short.

The term 'conformation' has been widely used in the livestock industries but has no precise and generally accepted definition. It is derived from the concept of conforming to breed type, which was a criterion traditionally used to assess the acceptability of an animal. In modern parlance, it has to do with visually assessed shape, particularly in relation to muscle, but may in fact be greatly influenced by fatness and fat distribution. Muscularity, meatiness, and conformation are all subjective terms that attempt to convey how much 'meat' an animal carries. Unfortunately, meatiness – like beauty – may be in the eye of the beholder, and there is a poor relationship between visual assessments of conformation and actual cutability.

Meat Quality

Although dressing percentage and cutability can be precisely defined (though the definitions vary from market to market), quality is a somewhat ethereal trait and must be subdivided into appearance and eating quality factors. Appearance factors include marbling, color, and texture of the meat and fat, and consumers naturally hope to use these as predictors of wholesomeness and eating quality. Unfortunately, there is little correlation between what can be seen and the ultimate taste and texture of the cooked product. Taste characteristics, particularly tenderness, juiciness, and flavor, are the ultimate measure of meat quality, but, because of their obviously subjective nature, they are very difficult to assess accurately and with repeatability and in any case cannot be known with certainty until the meat is eaten.

Appearance

Many of the components of appearance, such as marbling, thickness, color, and texture of muscle and fat, are known to have little or no relationship to eating quality. Nevertheless, they are considered by consumers, or the retailers who often act in proxy for them, to be important quality traits in their own right, because they influence the perceptions and thus the buying decisions of consumers. A bright red lean color is perceived to be fresh and from young animals.

Marbling – the flecks of fat that appear within meat – is the only appearance trait that gives any repeatable guide to eating quality and hence it has become an important determinant of value. Unfortunately, it is not a very reliable predictor because it accounts for only approximately 10–15% of the variation in tenderness and juiciness. It is also difficult to quantify precisely and is not consistently distributed within the carcass. Marbling is a late-maturing fat depot, meaning that fat is not deposited in this depot in appreciable amounts in very young cattle. The amount of marbling present in a carcass is also related to the total amount of fat: well-marbled carcasses tend to be fatter carcasses. However, there is a discernible breed effect, with the 'dairy-type' cattle having higher levels of marbling at constant total fatness and some specialty breeds, such as the Wagyu or Japanese Black, exhibiting very high levels of marbling. Breeds with consistently lower levels of marbling (Limousin, Piedmontese, and Blonde d'Aquitaine) tend to generally lack fat. Although marbling is not a reliable guide to eating quality, it has been noted that greater amounts of marbling result in less

toughening in meat cooked to higher temperatures, giving rise to the view that well-marbled beef is more forgiving of a poor cook.

Color and texture of muscle tend to be functions of age and immediate preslaughter stress levels, and these dwarf any genetic effects; however, the yellow color in beef fat has a genetic component as well as a nutritional component. The most common cause of yellow fat, other than liver disease, is the presence of β-carotene in solution in the fat. β-carotene is red in color (carrots and tomatoes) but appears yellow in dilute solution. It is found in green forage and is converted into the much paler vitamin A at the intestinal wall during absorption as well as in the liver. Some cattle, notably the 'Channel Island' breeds, Jersey and Guernsey, and the zebus, have yellower fat, probably through a reduced capacity to convert carotenoid pigments in the feed into vitamin A before storing it in the fat. It is notable that the milk of these cattle is also yellower, for the same reason. As both β-carotene and vitamin A are tasteless, their presence or absence has no direct effect on taste, although consumers may subconsciously ascribe superior taste to meat having fat of a preferred color.

The concentration of β-carotene in the fat increases with age, particularly in an animal that has experienced cyclical storage and removal of fat during its life. Consequently, cull cows, which tend to be older and have experienced annual fluctuations in fatness, are associated with yellow fat. Their meat is not preferred, enhancing the myth that yellow fat causes poor eating quality.

Taste

The eating quality of beef is extremely complex. It is sometimes said of taste that 'quality goes in after the hide comes off,' in recognition of the fact that processing and postslaughter handling, including preparation and cooking, are far more important in determining eating quality than the biological type or management of the animal itself. Differences in eating quality among different muscles (retail cuts) and even within a single muscle completely transcend any differences found among live animals, irrespective of whether these differences result from biological type, age, gender, nutrition, or management factors. Eating quality is generally defined as a combination of tenderness, juiciness, and flavor, although each of these is, in turn, a complex of traits.

Tenderness is generally accepted as the most important eating quality trait, and there are documented biological-type effects on certain aspects of tenderness. In particular, the zebu breeds have been shown to produce tougher beef than the taurine breeds, perhaps because of genetic differences in proteolytic enzyme systems that affect the progress of postmortem tenderization. It is known, for example, that zebu cattle have higher concentrations of the inhibitor calpastatin, which is related to toughness. However, it should be clearly recognized that these differences are much smaller than the differences found among muscles in the same animal or between carcasses that have been subjected to different postmortem handling. Rapidly cooled muscles experience cold toughening, whereas aging is a process that progressively tenderizes muscle. The heritability of tenderness is moderately high.

Conclusions

All cattle ultimately produce beef, and cattle come in a wide variety of shapes and sizes. Specialist beef breeders are increasingly moving away from purebreds and are using crossbreeding combined with sophisticated assessments of the genetic determinants of important traits. Breed or biological type can influence carcass and meat quality in a number of ways. Biological types differ in size and body composition (proportions of muscle, fat, and bone), and hence both the absolute and relative (through dressing percentage and cutability effects) amount of meat they produce. However, there is less intrinsic difference in the taste or texture of their meat, and a factor, such as carcass fat covering (insulation) could have a major indirect effect on tenderness through its effect on the rate of postmortem cooling and hence the extent of cold shortening. Fatter carcasses also tend to have more marbling, which might make the (admittedly weak) relationship between marbling and tenderness more of a coincidence rather than a cause-and-effect relationship. However, marbling does tend to ameliorate the deleterious effects of high endpoint temperature on meat tenderness. Feeding and management, which may be breed related, could also have minor influences on taste and texture; for example, in North America, British-type cattle are more likely to be finished extensively on grass, whereas European cattle are more likely to be finished intensively on grain. In general, biological type can have a major influence on the quantity and appearance of meat in bovine carcasses but considerably less direct influence on its taste and texture.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. By-Products: Edible, for Human Consumption; Hides and Skins; Inedible. Chemical and Physical Characteristics of Meat: Palatability. Classification of Carcasses: Beef Carcass Classification and Grading. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Rigor Mortis, Cold, and Rigor Shortening. Modeling in Meat Science: Meat Quality. Slaughter-Line Operation: Cattle; Poultry

Further Reading

Basarab, J.A., Price, M.A., Aalhus, J.L., *et al.*, 2003. Residual feed intake and body composition in young growing cattle. Canadian Journal of Animal Science 83, 189–204.

Berg, R.T., Butterfield, R.M., 1976. New Concepts of Cattle Growth. Sydney: Sydney University Press.

Cundiff, L.V., 2005. Beef cattle: Breeds and genetics. In: Pond, W.G., Bell, A.W. (Eds.), Encyclopedia of Animal Science. New York, NY: Dekker, pp. 74–76.

Porter, V., 2002. Mason's World Dictionary of Livestock Breeds, Types, and Varieties, fifth ed. Wallingford: CABI Publishing.

Price, M., 2009. Meat science. In animal and plant productivity. In: Robert, J.H. (Ed.), Encyclopedia of Life Support Systems (EOLSS), Developed under the auspices of UNESCO. Oxford: Eolss Publishers. Available at: http://www.eolss.net (accessed 07.01.14).

Swatland, H.J., 1994. Structure and Development of Meat Animals and Poultry. Lancaster, PA: Technomic.

Thonney, M.L., 2005. Body composition: Breed and species effects. In: Pond, W.G., Bell, A.W. (Eds.), Encyclopedia of Animal Science. New York, NY: Dekker, pp. 155–158.
Warriss, P.D., 2010. Meat Science: An Introductory Text, second ed. Wallingford: CABI Publishing.

Relevant Website

http://cowcalf.cattle.ca/beef-cattle-breeds
The Canadian Cattlemen's Association.

Finfish

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Introduction

Each of four production division groups is identified in a separate table that lists the fish by name (scientific and common), major location, maximum measurement (length, weight, and age), use, production (rank and total), and cooking method.

According to the Food and Agriculture Organization of the United Nations (FAO), the estimated total world production of fishery commodities (e.g., fish and crustaceans) in 1000 metric tones (kt) was 98 627 in 1990; 116 412 in 1995, and 130 434 in 2000. These live weight-based production estimates excluded whales, seals, and other aquatic mammals and plants, but included aquaculture products.

The 2000 world fisheries production (e.g., fish and crustaceans) by capture (wild) and aquaculture revealed that of the total of 130 434 kt, wild harvest accounted for 72.7% and aquaculture contributed 27.3%. In 2000, the top 10 world fisheries production countries, in decreasing order, were China, Peru, Japan, India, USA, Indonesia, Chile, Russian Federation, Thailand, and Norway. During this timeframe, China's total production was 41 568 kt, of which wild harvest and aquaculture accounted for 40.9% and 59.1%, respectively.

An examination of the 2000 international trade in fisheries commodities by principal importers and exporters shows that the top 10 importers, in decreasing order, were Japan, USA, Spain, France, Italy, Germany, UK, Hong Kong, Denmark, and China. The top 10 exporters were Thailand, China, Norway, USA, Canada, Denmark, Chile, Taiwan, Spain, and Indonesia. Interestingly, USA, Spain, Denmark, and China were the numbers 2, 3, 9, and 10 importers, respectively, and they were also the numbers 4, 9, 6, and 2 exporters, respectively.

A comparison of the disposition of the world fishery production indicates that in 1990, 1995, and 2000 the production increased from 98 627 to 116 412 and finally to 130 434 kt, in the respective years. Of the 130 434 kt, human consumption accounted for 74.1% divided by marketed fresh (53.7%), frozen (25.7%), cured (9.6%), and canned (11.0%). The remaining 3726 kt (25.9%) was for other purposes.

Definitions

- Cartilaginous fishes: A classification of approximately 700 fish species (i.e., Class Chondrichthyes) that contains sharks, skates, rays, and chimaeras.
- Finfish: This is a generic term for true fish.
- Fish: The term fish includes finfish, crustaceans, other aquatic animals (e.g., alligators (?), frogs, turtles, and jellyfish) and molluscs.
- Sashimi: Thin slices of fresh (and previously frozen), raw fish that might be served with soy sauce and wasabi (horseradish).
- Surimi: In general terms, this is minced fish tissue from which fat, off-odors, and colors have been eliminated and to which cryoprotectants (e.g., sugars and other additives) have been added before freezing of the meat.
- True fishes: Generally, these fishes are cold-blooded, gill-breathing, aquatic bony vertebrates, usually with fins.

Capture (Wild) Production by Principal Species

FAO's 2000 capture or catch (wild) data revealed production by principal species. Twenty-three of these species by the name (scientific and common), major location, maximum measurement (length, weight, and age), use, production (kt) (rank and total) in 2000, and cooking method are listed in Table 1.

World Aquaculture Production

FAO's 2000 world aquaculture data listed the production for 18 finfish species. The production for these finfish species ranged from 3473 to 137 kt and yielded 19 024 kt; however, the first 6 finfish species' production ranged from 3473 to 1045 kt and produced 13 700 kt. These 18 principal aquaculture finfish species by the name (scientific and common), major location, maximum measurement (length, weight, and age), use, production (kt) (rank and total) in 2000, and cooking method are listed in Table 2.

Nutritional Content of Selected Groups of Finfish

A review of the nutritional content of raw tissue from selected finfish was undertaken, and the finfish were divided into four groups based on their total energy content. Group 1 consisted of Atlantic mackerel, Pacific herring, Greenland halibut, eel (mixed species), and Atlantic salmon. Group 2 contained milkfish, Spanish mackerel, rainbow trout, channel catfish, whitefish (mixed species), European anchovy, carp, and bluefish. Group 3 was made up of yellowfin tuna and skipjack tuna. Group 4 comprised Atlantic cod, walleye pollock, and orange roughy. Subsequently, 11 nutrient values (i.e., water, energy, protein, total fat (lipid), calcium, iron, sodium, ascorbic acid (vitamin C), riboflavin, niacin, and cholesterol) were selected from each group of finfish and the data were averaged and reported in Table 3. A comparison of the total fat and cholesterol as well as the protein and water values was considered to be important because of the high levels of

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Table 1 Twenty-three selected finfish species including names, locations, measurements, 2000 catch (wild) production etc.

Names[a,b,c,d] *A. Scientific* *B. Common*	*Major location*[d]	*Maximum measurement* *A. Length*[d] *B. Weight*[d] *C. Age*[d]	*Use*[d]	*Production*[a] *A. Rank*[e] *B. Total*	*Cooking method*[d]
A. *Engraulis ringens* B. Anchoveta (aka Peruvian anchovy)	Subtropical, marine waters off the western coast of South America from Peru to Chile	A. 20.0 cm B. ND C. 3 years	Fish meal (animal feed) fertilizer and oil	A. 1 B. 11 276 kt	ND
A. *Theragra chalcogramma* B. Walleye pollock	Polar, brackish, and marine waters from Alaska to the Sea of Japan and to California, USA, and near Baja California, Mexico	A. 91.0 cm B. 1.4 kg C. 15 years	Roe (egg-laden ovaries of fish) and frozen; further manufactured into surimi, etc	A. 2 B. 3025 kt	Sautéed and steamed, etc.
A. *Clupea harengus* B. Atlantic herring	Brackish and marine waters of moderate temperatures in the North Atlantic (e.g., from the Bay of Biscay to Ireland, Greenland, etc., and from Labrador to South Carolina)	A. 45.0 cm B. 1.0 kg C. 11 years	Fresh or processed (e.g., smoked and canned)	A. 3 B. 2370 kt	Broiled, fried, microwaved, etc.
A. *Katsuwonus pelamis* B. Skipjack tuna	Worldwide (except for the Black and Mediterranean Seas) in marine waters of tropical and moderate temperatures	A. 1.1 m B. 34.5 kg C. 12 years	Fresh or processed (e.g., frozen and dried/salted)	A. 4 B. 1890 kt	ND
A. *Engraulis japonicus* (aka *Engraulis japonica*) B. Japanese anchovy	In big schools near the surface of warm, marine waters of the Western Pacific (e.g., Sakhalin Island and Sea of Japan)	A. 16.0 cm B. ND C. 3 years	Fresh and manufactured (e.g., fish meal)	A. 5 B. 1726 kt	ND
A. *Trachurus murphyi* B. Inca scad (aka Chilean jack mackerel)	Tropical, marine waters of the Pacific Ocean (e.g., off Chile and New Zealand) and Southwest Atlantic (e.g., Argentina)	A. 70.0 cm B. ND C. 16 years	Fresh and processed (e.g., into canned goods and fish meal)	A. 6 B. 1540 kt	ND
A. *Trichiurus lepturus* B. Atlantic cutlassfish (aka largehead hairtail)	Native to subtropical, brackish and marine waters worldwide	A. 2.3 m B. 5.0 kg C. 15 years	Fresh (e.g., for sashimi) and processed (e.g., dried/salted and frozen)	A. 7 B. 1480 kt	Grilled and fried
A. *Scomber japonicus* B. Chub mackerel	Subtropical, marine waters of the Indo-Pacific area	A. 64.0 cm B. 2.9 kg C. 18 years	Fresh and processed (e.g., canned, smoked, and salted), and as a medicine	A. 8 B. 1456 kt	Baked, fried, etc.
A. *Mallotus villosus* B. Capelin	Native to polar, marine waters near the Arctic pole; in the North Atlantic (e.g., in the Norwegian Sea and Gulf of Maine) and North Pacific (e.g., Korea)	A. 25.2 cm B. ND C. 5 years	Roe (egg-laden ovaries of fish) and processed (e.g., canned, dried, frozen, and fish meal)	A. 9 B. 1456 kt	Fried
A. *Micromesistius poutassou* B. Blue whiting (aka poutassou)	Temperate, marine waters in the Atlantic (e.g., the Barents Sea, around Iceland and Africa, and off Greenland and the USA)	A. 50.0 cm B. 0.8 kg C. 20 years	Fresh and manufactured (e.g., into fish oil)	A. 10 B. 1420 kt	ND
A. *Thunnus albacares* B. Yellowfin tuna	Brackish and marine tropical and subtropical seas worldwide except for the Mediterranean Sea	A. 2.4 m B. 200.0 kg C. 8 years	Fresh (including for sashimi) and processed (e.g., canned and smoked)	A. 11 B. 997 kt	ND

(*Continued*)

Table 1 Continued

Names[a,b,c,d] *A. Scientific* *B. Common*	*Major location*[d]	*Maximum measurement* *A. Length*[d] *B. Weight*[d] *C. Age*[d]	*Use*[d]	*Production*[a] *A. Rank*[e] *B. Total*	*Cooking method*[d]
A. *Gadus morhua* B. Atlantic cod	Native to brackish and marine waters of moderate temperatures of the North Atlantic (e.g., Cape Hatteras to Ungave Bay and off the coasts of Greenland and Iceland)	A. 2.0 m B. 96.0 kg C. 25 years	Fresh and processed (e.g., dried/salted and frozen)	A. 12 B. 945 kt	Baked, broiled, steamed, etc.
A. *Sardina pilchardus* B. European pilchard (aka sardine)	Native to the subtropical, fresh, brackish, and marine waters of the Northeast Atlantic (e.g., North, Adriatic and Black Seas)	A. 25.0 cm B. ND C. 15 years	Fresh and processed (e.g., canned, smoked, and frozen)	A. 13 B. 943 kt	Broiled, fried, and microwaved
A. *Strangomera bentincki* B. Araucanian herring	This species is found in schools (e.g., close to the surface) of subtropical, marine waters of the Southeast Pacific (e.g., near Chile)	A. 28.4 cm B. ND C. ND	Processed (e.g., fish meal for animal feed)	A. 14 B. 723 kt	ND
A. *Scomber scombrus* B. Atlantic mackerel	Temperate, brackish, and marine waters of the Atlantic Ocean (e.g., in the Baltic, Black, and Mediterranean Seas)	A. 60.0 cm B. 3.4 kg C. 17 years	Fresh and processed (e.g., canned, smoked, and frozen).	A. 15 B. 674 kt	Baked, broiled, fried, etc.
A. *Sprattus sprattus* B. European sprat	Native to temperate, brackish, and marine waters of the Adriatic, Black, North, and Mediterranean Seas, etc.	A. 16.0 cm B. ND C. 6 years	Fresh and processed (e.g., frozen, canned, smoked, and fish meal).	A. 16 B. 660 kt	Broiled and fried
A. *Engraulis encrasicolus* B. European anchovy	Native to subtropical, brackish, and marine waters of the Eastern Atlantic (e.g., Norway to South Africa), Mediterranean, and Black Seas, etc.	A. 20.0 cm B. ND C. 3 years	Fresh or processed (e.g., canned, dried, frozen, smoked, and fish meal)	A. 17 B. 605 kt	ND
A. *Brevoortia patronus* B. Gulf menhaden	In dense schools in subtropical, marine waters (e.g., the Gulf of Mexico)	A. 35.0 cm B. ND C. ND	Fresh and processed (e.g., canned, salted, fish meal, and oil)	A. 18 B. 591 kt	ND
A. *Scomberomorus niphonius* B. Japanese seer (aka Japanese Spanish mackerel)	Native to temperate marine waters (e.g., of China, the Yellow Sea and the Sea of Japan)	A. 1.0 m B. 7.1 kg C. ND	Fresh (e.g., raw as sashimi)	A. 19 B. 539 kt	Baked, broiled, and fried
A. *Sardinops caeruleus* (aka *Sardinops sagax caeruleus*) B. California pilchard (aka South American pilchard and Pacific sardine)	Subtropical, marine waters (e.g., Indo-Pacific area) and has three lineages: (1) Southern Africa (ocellatus) and Australia (neopilchardus), (2) Chile (sagax) and California (caeruleus), and (3) Japan (melanostictus)	A. 39.5 cm B. 0.5 kg C. 25 years	Fresh and processed (e.g., canned, frozen, and fish meal)	A. 20 B. 528 kt	Fried and broiled

A. *Clupea pallasi* (aka *Clupea pallasii*) B. Pacific herring	Temperate, fresh, brackish, and marine waters (e.g., of the Arctic Sea and Pacific Ocean)	A. 46.0 cm B. ND C. 19 years	Fresh and processed (e.g., canned, dried/salted, frozen, and smoked). Pacific herring are valued for roe and eggs laid on kelp. (The latter eggs when salted are termed kazunoko-kombu.) This fish is also valued as a Chinese medicine	A. 21 B. 456 kt	Baked, boiled, and fried
A. *Thunnus obesus* B. Bigeye tuna	Originates in subtropical, marine waters of the Atlantic, Indian, and Pacific Oceans	A. 2.5 m B. 210.0 kg C. 11 years	Fresh and processed (canned or frozen). The Japanese value this fish for sashimi	A. 22 B. 433 kt	ND
A. *Euthynnus affinis* B. Kawakawa	This highly migratory species originates in tropical, marine waters (e.g., Indo-West Pacific area)	A. 1.0 m B. 14.0 kg C. ND	Fresh and processed (e.g., canned, dried, frozen, salted, and smoked)	A. 23 B. 428 kt	

[a]http://www.fao.org/fi

[b]http://www.cfsan.fda.gov/~frf/rfe0.html

[c]Randolph, S., Synder, M., 1993. The Seafood List: FDA's Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce 1993. Washington, DC: Office of Seafood, Food and Drug Administration. Superintendent of Documents. US Government Printing Office.

[d]Froese, R., Pauly, D. (Eds.), 2003. Fish Base. Available at: www.fishbase.org (version 24 Oct 2003).

[e]The original reference included production rankings (e.g., numbers 14, 18, and 21) that were nonfinfish species. For this table the production rankings were renumbered to reflect the top 23 finfish ranking.

Abbreviations: aka, also known as; ND, no data.

Source: Reproduced from Center for Food Safety and Applied Nutrition of the USA Food and Drug Administration; Food and Agricultural Organization of the United Nations.

Table 2 Eighteen finfish species including names, locations, measurements, 2000 world aquaculture production, etc.

Names[a,b,c,d,e,g] A. *Scientific* B. *Common*	*Major location*[d,e,g,h,i]	*Maximum measurement* A. *Length*[d,e,g] B. *Weight*[d,e] C. *Age*[d,e]	*Use*[d]	*Production*[a] A. *Rank* B. *Total*	*Cooking method*[d]
A. *Hypophthalmichthys molitrix* B. Silver carp	Fresh waters of moderate temperatures in Asia, China, Europe, and USA	A. 1.3 m[e] B. ND C. 10 years[e]	ND	A. 1 B. 3473 kt	ND
A. *Ctenopharyngodon idellus* (aka *Ctenopharyngodon idella*) B. Grass carp	Lake Khanka, rivers of the People's Republic of China, western portions of the former USSR, USA, etc.	A. 1.5 m B. 45.0 kg C. 21 years	Fresh	A. 2 B. 3447 kt	Baked, fried, steamed, etc.
A. *Cyprinus carpio* B. Common carp	Fresh waters of moderate temperatures worldwide, especially in the Black, Caspian, and Aral Seas, etc.	A. 1.2 m B. ND C. 24 years	ND	A. 3 B. 2718 kt	ND
A. *Hypophthalmichthys nobilis* B. Bighead carp	Fresh waters of moderate temperatures (e.g., lowland rivers) of China and worldwide for aquacultural purposes	A. ND B. ND C. ND	ND	A. 4 B. 1637 kt	ND
A. *Carassius carassius* B. Crucian carp	In fresh and brackish waters of moderate temperatures of Europe, Asia and China. The European subpopulation of this species is on the 2002 International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species[f]	A. 64.0 cm B. 3.0 kg C. ND	Fresh or processed frozen	A. 5 B. 1379 kt	Baked, fried, etc.
A. *Oreochromis niloticus* (aka *Tilapia nilotica*) B. Nile tilapia	In fairly moderate waters of the Rift Valley lakes, West African Rivers (e.g., Benue), North America (farm raised), etc.	A. 40.0 cm B. ND C. ND	ND	A. 6 B. 1045 kt	ND
A. *Salmo salar* B. Atlantic salmon	Fresh, brackish. and marine waters of moderate temperatures. This species' native habitat is the Western Atlantic (e.g., Quebec, Canada), Eastern Atlantic (e.g., Arctic Circle) and landlocked areas (e.g., Russia and North America). Atlantic salmon are pen raised (e.g., in Canada)	A. 1.5 m B. 46.8 kg C. 13 years	Fresh and processed (e.g., smoked, dried, and salted)	A. 7 B. 884 kt	Broiled, microwaved, etc.
A. *Labeo rohita* B. Rohu	Tropical, fresh and brackish waters of Pakistan, India, Nepal, etc.	A. 2.0 m B. 45.0 kg C. 10 years	Fresh	A. 8 B. 795 kt	ND
A. *Catla catla* B. Catla	Subtropical, fresh and brackish waters of Pakistan, Nepal, Myanmar, etc.	A. 1.8 m B. ND C. ND	ND	A. 9 B. 653 kt	ND
A. *Cirrhinus mrigala* (aka *Cirrhinus cirrhosus*) B. Mrigal	Tropical, fresh and brackish waters of India	A. 1.0 m B. 12.7 kg C. ND	ND	A. 10 B. 573 kt	ND
A. *Parabramis pekinensis* B. White amur bream	Tropical, fresh waters of Sungari, Liao, China, etc.	A. 55.0 cm B. 4.1 kg C. ND	ND	A. 11 B. 512 kt	ND
A. *Chanos chanos* B. Milkfish	Tropical, freshwater, brackish and marine species native to the Indo-Pacific area. Their range includes Asia, Japan, Australia, Red Sea, Africa, etc. They are also found from California to Galapagos	A. 1.8 m B. 14.0 kg C. 15 years	ND	A. 12 B. 462 kt	ND

A. *Oncorhynchus mykiss* B. Rainbow trout	Rainbow trout are native to temperate, fresh, brackish and marine waters from Alaska to Mexico as well as waters of Asia (e.g., Eastern Russia). Rainbow trout have been disseminated worldwide (e.g., Africa, Asia, New Zealand, and South America)	A. 1.2 m B. 25.4 kg C. 11 years	Fresh or processed (e.g., canned, smoked, and frozen)	A. 13 B. 448 kt	Baked, boiled, microwaved, steamed, etc.
A. *Ictalurus punctatus* B. Channel catfish	Fresh waters of moderate temperatures in Canada, Mexico, and USA	A. 1.3 m B. 26.3 kg C. 16 years	Fresh and processed (e.g., smoked and frozen)	A. 14 B. 269 kt	Baked, broiled, fried, etc.
A. *Anguilla japonica* B. Japanese eel	Tropical, fresh, brackish and marine waters of Japan, Taiwan, Korea, China, etc.	A. 1.5 m B. 0.8 kg C. ND	Fresh or further processed (e.g., smoked, canned, and frozen)	A. 15 B. 220 kt	Baked, broiled, and steamed
A. *Cirrhinus molitorella* B. Mud carp	Tropical, fresh waters of Mekong, Chao Phraya, Nanpangjiang, and Red River	A. 55.0 cm B. ND C. ND	Fresh and as prahoc (fish paste)	A. 16 B. 200 kt	ND
A. *Mylopharyngodon piceus* B. Black carp	Subtropical, fresh waters of the Amur River basin and China	A. 1.2 m B. 32.0 kg C. ND	ND	A. 17 B. 171 kt	ND
A. *Serbia quinqueradiata* B. King amberjack (aka Japanese amberjack by some sources)	Subtropical, marine waters of the Northwest Pacific (e.g., from Japan to the Hawaiian Islands)	A. 1.5 m B. 40.0 kg C. ND	Fresh for sashimi	A. 18 B. 137 kt	ND

[a] http://www.fao.org/fi

[b] http://www.cfsan.fda.gov/~frf/rfe0.html

[c] Randolph, S., Synder, M., 1993. The Seafood List: FDA's Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce 1993. Washington, DC: Office of Seafood, Food and Drug Administration. Superintendent of Documents. US Government Printing Office.

[d] Froese, R., Pauly, D. (Eds.), 2003. Fish Base. Available at: www.fishbase.org (version 24 Oct 2003).

[e] http://www.gsmfc.org

[f] http://www.redlist.org/search/details.php?species=3850

[g] http://cdserver2.ru.ac.za/cd/011120_1/Aqua/SSA/onilo.htm

[h] http://www.oceanbeauty.com/products/farm_atlantic.htm

[i] http://www.geocities.com/scott_cotter/fish3.htm

Abbreviations: aka, also known as; ND, no data.

Source: Reproduced from Center for Food Safety and Applied Nutrition of the USA Food and Drug Administration; Food and Agricultural Organization of the United Nations.

Table 3 Average nutritional data per 100 g of raw tissue from selected finfish groups[a,b]

Groups[c]	Water (g)	Energy (kJ)	Protein (g)	Total fat (g)	Calcium (mg)	Iron (mg)	Sodium (mg)	Ascorbic acid (mg)	Riboflavin (mg)	Niacin (mg)	Cholesterol (mg)
1	68.50	797.6	17.54	12.82	26.0	0.85	70.8	1.22	0.150	4.757	75.6
2	72.99	562.7	19.19	5.82	44.9	0.86	60.4	0.84	0.110	5.490	60.0
3	70.79	441.5	22.69	0.98	22.5	0.99	37.0	1.00	0.074	12.600	46.0
4	79.56	323.7	16.56	0.72	17.0	0.26	72.0	0.33	0.091	2.118	44.7

[a]The raw data for this table were obtained from the United States Department of Agriculture, Agriculture Research Service, Nutrient Data Laboratory at http://www.nal.usda.gov/fnic/foodcomp

[b]Groups 1, 2, 3, and 4 had energy ranges of 858–766, 619–519, 452–431, and 343–289 kJ, respectively.

[c]Group 1 consisted of Atlantic mackerel, Pacific herring, Greenland halibut, eel (mixed species) and Atlantic salmon (http://www.cfsan.fda.gov/~frf/rfe1at.html); group 2 contained milkfish (http://www.cfsan.fda.gov/~frf/rfe1ml.html), Spanish mackerel, rainbow trout, channel catfish, whitefish (mixed species), European anchovy, carp and bluefish; group 3 was made up of yellowfin tuna (http://www.cfsan.fda.gov/~frf/rfe1yn.html) and skipjack tuna; and group 4 was comprised of Atlantic cod, walleye pollock and orange roughy (http://www.cfsan.fda.gov/~frf/rfe1or.html).

obesity in developed countries such as USA. Further, some individuals may be concerned about consuming enough calcium for bones and iron for blood, whereas others may be interested in maintaining lower sodium intake to help decrease their potential for high blood pressure. The three vitamins were selected to show examples of those components that appeared to be important to consumers or to be significant in some fish flesh.

Processing of Finfish

Finfish products can be processed fresh, frozen, cooked, precooked, smoked, cured, canned, etc. For example, finfish might be caught and immediately transported to the processing plant in tank trucks with water. Fish may be received live at the facility and may then be stunned, headed and gutted, and washed as needed. Afterwards, fish may be cut into fillets (with skin-on or skinless) followed by a liquid cooling step. Next, fillets might be inspected (e.g., for parasites or nematodes), cut and deboned, and evaluated for size or graded. The product can then be put into packing containers and the containers passed over scales to determine the weight and labeled. The last steps might involve chilling and storage at the plant before transporting the product for sale.

Similarly, after fish have been harvested and frozen, fish can be shipped to a processing facility. At the plant, the fish can be received, thawed and examined, sorted, and passed like fresh fish through inspection. The fillets can then be individually quick frozen and glazed with water. The rest of the processing operation is as above, except that the product might be stored frozen before shipment to consumers.

The first steps of smoking fish might approximate the above processes. After the fillet is produced, the tissue might be salted and then rinsed before being placed onto a metal rack for subsequent smoking (hot or cold). After smoking, the product should be cooled before further processing (e.g., coloring and slicing). Smoked product is packaged, frozen, etc. as in the earlier processes. Cured and salted fish might be processed as in the smoked fish operation except that it has a drying step rather than a smoking cycle. These cured and salted products might be stored refrigerated or dry.

Additional fish processing operations (e.g., battered and breaded, precooked or cooked, canned, and modified atmosphere packaged) are not covered here and the reader should refer to other articles in the Encyclopedia and to the Further Reading listed at the end of this article to learn more on these topics.

Potential Health Issues Associated with Finfish

Finfish products are generally safe for human consumption. However, fish are animals and they might have parasites (e.g., anisakid nematodes) that can be killed by freezing or by cooking. In addition, they might contain toxins that are harmful to consumers.

Subjection of scombroid species (e.g., mackerel and tuna) to improper handling and chilling after harvesting can result in histamine formation by bacteria and to consumers being afflicted with scombroid poisoning or histamine toxicity. Reef fish (e.g., barracuda and snapper) might consume toxic dinoflagellates. Subsequently, when humans consume the fish tissue they could become ill from ciguatoxin. Puffer fish might contain a tetrodotoxin, which can be fatal to consumers.

Escolar is a fish that might cause consumers to have diarrhea due to gempylotoxin. Other health concerns include finfish that have been contaminated with chemicals such as heavy metals (e.g., methyl mercury), polychlorinated biphenyls , pesticides, drugs, etc.

Despite the previous comments, it is important to emphasize that finfish products are safe for human consumption in the vast majority of cases and that potential risks can be prevented with adequate processing.

Welfare Issues

Animal welfare has become increasingly important worldwide. A fundamental issue when deciding on moral duties of humans toward animals is whether they are capable of experiencing pain and other forms of suffering such as fear and distress. The welfare of fish has been much less studied than that of mammals and birds, and considerable debate with opposing views still exists on the ability of fish to suffer. The main argument used by those that deny that fish are capable of experiencing negative emotions including pain is that fish lack the extensive cerebral cortex of mammals. This argument,

however, has several problems. First, it has been suggested that emotional responses might not depend on higher forms of consciousness, but on more basic ones. Second, a given function might be served by different brain structures in different animals, and the possibility exists, therefore, that brain structures other than the cortex might support emotional experiences in fish. In this context, it is particularly relevant that the dorsomedial part of the telencephalon of fish seems to be homologous to the mammalian amygdala, which is involved in arousal and emotion, particularly fear.

Several criteria have been proposed to decide whether an animal is capable of experiencing pain. These criteria are as follows:

1. Presence of functional nociceptors.
2. Presence of nervous pathways leading to higher brain structures.
3. Activation of brain structures during potentially harmful or injurious stimulation.
4. Presence of endogenous opioids and opioid receptors.
5. Suppression of responses to noxious stimuli by analgesics.
6. Changes in behavior (including avoidance behavior and inhibition of normal behavior) associated with noxious stimulation.

Current scientific evidence suggests that fish – or at least some fish species – meet all these criteria. For example, studies in rainbow trout (*Oncorhynchus mykiss*) have shown that there are nociceptors on the face of the trout and that they are innervated by the trigeminal nerve, which projects to the thalamus as in mammals. Opioids and opioid receptors equivalent to those found in mammals have been identified in fish brains. Studies in the goldfish (*Carassius auratus*) have shown that brain activity changes during noxious stimulation. Changes in behavior (including an increase in respiration rate, a decrease in feed intake, and a decrease of fear response to a novel stimulus, supporting the idea that pain dominates fish attention) have been described in rainbow trout associated with noxious stimulation, whereas these changes do not appear if fish are given analgesics.

Taking all this evidence together, the Panel on Animal Health and Animal Welfare of the European Food Safety Authority concluded that "The balance of evidence indicates that some fish species have the capacity to experience pain. However research and developments in the area of cognition and brain imaging techniques should be carried out in fish to further our knowledge and understanding of pain perception."

Fear is another negative emotional experience with obvious effects on welfare. Fish show a variety of behavioral responses to threatening stimuli, including escaping and becoming motionless. Additionally, fish can learn to avoid threatening stimuli, suggesting that a cognitive process is involved in their fear response. A cognitive process is further supported by the analogy between the amygdala of mammals and the dorsomedial part of the telencephalon. The conclusion of the Panel on Animal Health and Animal Welfare of the European Food Safety Authority on fear in fish was that "Responses of fish, of some species and under certain situations, suggest that they are able to experience fear."

Stress physiology of fish has been studied over the past decades and has been shown to be similar to that of mammals. Chronic stress in fish, as in mammals, may have negative effects on health, for example, by impairing the immune function. Another similarity between fish and mammals is that both show individual differences in their stress response, some individuals adopting an active strategy and others a more reactive one. The Panel on Animal Health and Animal Welfare of the European Food Safety Authority concluded that "Fish possess a suite of adaptive behavioral and physiological responses that have evolved to cope with stressors. Many of these are homologous with those of other vertebrates…Prolonged exposure to stressors generally leads to maladaptive effects or chronic stress."

Nonfinfish

By definition, sharks, skates, and rays, for example, were not included in the finfish category. Several species of these animals are marketed (e.g., fresh, frozen, dried/salted, and smoked) in interstate/intrastate commerce. Further, some species are utilized to produce fish meal, fish oil, liver oil, shark fin soup, drugs or medicines, leather from hides, etc.

Disclaimer

The scientific, market, and common names identified in this document may or may not correspond with The Seafood List: FDA's Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce 1993.

See also: Canning. Environmental Contaminants. Fish Inspection. Packaging: Modified and Controlled Atmosphere. Processing Equipment: Battering and Breading Equipment. Refrigeration and Freezing Technology: Applications; Equipment; Principles. Smoking: Liquid Smoke (Smoke Condensate) Application; Traditional. Species of Meat Animals: Shellfish

Further Reading

Banister, K., Campbell, A., 1986. The Encyclopedia of Aquatic Life. New York: New York: Facts on File. pp. 3–4.

Chandroo, K.P., Duncan, I.J.H., Moccia, R.D., 2004. Can fish suffer?: Perspectives on sentience, pain, fear and stress. Applied Animal Behaviour Science 86, 225–250.

EFSA, 2009. Scientific Opinion of the Panel on Animal Health and Welfare on a request from European Commission on General approach to fish welfare and to the concept of sentience in fish. EFSA Journal 954, 1–26.

FAO, 2000. FAO Yearbook, Fishery Statistics: Capture Production. 1998 Rome: Food and Agriculture Organization of the United Nations.vol. 86(1).

FDA, 2001. Fish and Fisheries Products Hazards and Controls Guidance third ed. College Park, MD: Department of Health and Human Services, Public Health Service, Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition, Office of Seafood. Docket Number 93 N-0195.

Froese, R., Pauly, D. (Eds.), 2003. Fish Base. Available at: www.fishbase.org (accessed 31.10.13).

Fry Jr F.S., 2003. Personal communication. College Park, MD: Office of Seafood, Center for Food Science and Applied Nutrition, Food and Drug Administration.

Martin, R.E., Carter, E.P., Flick, Jr, G.J., Davis, L.M., 2000. Marine and Freshwater Products Handbook. Lancaster, PA: Technomic Publishing.

Miller, P., 1980. Collins Handguide to the Fishes of Britain and Northern Europe London: William Collins. pp. 6–7.
National Marine Fisheries Service, 1990. HACCP Regulatory Model for Raw Fish. Report of the Model Seafood Surveillance Project. Pascagoula, MS: National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Trade and Industry Services, National Seafood Inspection Laboratory.
National Marine Fisheries Service, 1991. HACCP Regulatory Model for Smoked and Cured Fish. Report of the Model Seafood Surveillance Project. Pascagoula, MS: National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Trade and Industry Services, National Seafood Inspection Laboratory.
Norman, J.R., 1963. Introductory. In: Greenwood, P.H. (Ed.), A History of Fishes, second ed. New York: Hill and Wang, pp. 1–5.
Randolph S.C., 2003. Personal communication. College Park, MD, USA: Office of Seafood, Center for Food Science and Applied Nutrition, Food and Drug Administration.
Randolph, S., Synder, M., 1993. The Seafood List: FDA's Guide to Acceptable Market Names for Seafood Sold in Interstate Commerce 1993. Washington, DC: Office of Seafood, Food and Drug Administration. Superintendent of Documents. US Government Printing Office.
Robins, C.R., Ray, G.C., 1986. A Field Guide to Atlantic Coast Fishes: North America (The Peterson Field Guide Series®). Boston, MA: Houghton Mifflin.
Rohr B.A., 2003. Personal communication. Pascagoula, MS: Mississippi Laboratories, Southeast Fisheries Science Center.
Rose, J.D., 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews In Fisheries Science 10, 1–38.
Sanders Jr N., 2003. Personal communication. Pascagoula, MS: Mississippi Laboratories, Southeast Fisheries Science Center.
Sneddon, L.U., 2006. Ethics and welfare: Pain perception in fish. Bulletin of the European Association of Fish Pathologists 26, 7–10.

Game and Exotic Animals

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Glossary

Antelope The term referring to a diverse group of even-toed ungulate species that is indigenous to various regions in Africa and Eurasia.
Bushmeat The meat derived from wild animals that are hunted for subsistence or informal trade, most often illegally.
Dressing percentage The percentage of an animal's live weight that becomes the carcass weight at slaughter, determined by dividing carcass weight by live weight and multiplying by 100.
Farmed game Land animals and birds that are not conventionally regarded as domesticated but are bred and reared in captivity.
Game birds A broad collection of birds, grouped into land fowls and waterfowls that are hunted for sport and food.
Game meat Meat derived from land animals and birds that are legally hunted in the wild.
Ungulate A mammal with hooves.
Venison The term mainly used nowadays to refer to the meat from deer species.
Wild game Free-ranging land animals and birds that are legally hunted in the wild for food.
Zoonotic disease An animal disease that can be transmitted to humans.

Introduction

Species of wildlife or game may be harvested to obtain meat, fur, skins, feathers, antlers, horns, or trophies. In particular, the use of wild animals for food is as old as humankind itself and the meat remains an important food source for many people throughout the world. With the escalating demand for animal protein from domestic animals, coupled with the decreasing supply and the high prices associated with such products, it has become inevitable for many populations to turn to meat from the wild as an alternative, including game and other exotic animals.

The conceptualization of a uniform definition for 'game' has been an issue of ongoing debate over the years and this differs widely in different parts of the world. Some sources define game as only those free-roaming land animals and birds that are hunted for food in their wild environment. According to such a definition, game meat is the result of the process of natural selection, rather than of human 'production.' Other sources, however, use the term to include both those land animals and birds – either wild or farmed – that are not generally considered to be domestic animals. The division of 'large' and 'small' game species is equally confounded, with the legal definitions of these, as well as their range and population levels, also varying from country to country. Since the production, distribution, veterinary inspection protocols, and public health risks differ vastly between wild, extensively farmed, and intensively farmed animals, it is important for concrete distinctions to be made between the latter groups. From a practical viewpoint, it might be preferable to consider 'game' as all animal and bird species that can be legally killed by hunting (recreational or commercial) and that will or may be subsequently used for human consumption. Alternatively, the term 'wild game' may be used for free-range animals/birds legally hunted in the wild, while 'farmed game' might be applied to those not conventionally regarded as domesticated but are bred and reared in captivity.

From a consumer perspective, it is also likely desirable to discriminate the meat of wild, free-roaming game from that of farmed species. Traditionally, the term 'venison' has been loosely applied around the world to describe the meat from any animal considered to be a game species. Although the English word 'venison' originated from the Latin *venari* (to hunt, pursue), today its use is largely restricted to the flesh from various cervid (deer) species, which are being increasingly farmed in the Northern Hemisphere for food. Some argue that these animals are becoming more domesticated in the sense that the farmer often decides which animals will be bred, what feed will be fed, and which animals will be slaughtered. For this reason, it is recommended that the term 'game meat' be exclusively used for the meat derived from animals/birds hunted in the wild (e.g., free-ranging antelope, wild boar, and game birds) and which can essentially be considered 'natural' or 'organic.' However, the term 'venison' should potentially be reserved for the meat from cervids, and perhaps also for that from other 'farmed game' species (in accordance with the aforementioned explanation) (e.g., rabbits and birds reared in captivity). Further, it is essential to define the term 'bushmeat,' which generally refers to the meat from wild animals that are hunted for subsistence or for the informal market, most often illegally. Although originally associated with primates, bushmeat also encompasses hippopotami, elephants, giraffe, zebra, antelope, water buffalo, wild cats, birds, reptiles, and rodents. The bushmeat trade is enormous in Africa and other developing third world countries, mainly as a consequence of rural poverty and the availability of external markets. This has not only resulted in the overexploitation and decline of a large number of wildlife species (many of which are protected by international wildlife legislation and treaties), but also poses a public health risk as these

animals may harbor diseases and would not typically undergo veterinary inspections.

One of the major drivers for the growing acceptability and consumption of game meat can be attributed to increasingly health-conscious modern consumers, as the aforementioned species are known to produce leaner meat compared to domestic livestock species. Another motivator for the increased demand lies with rising consumer concerns about the environment and there is thus the desire for organic products, as well as products produced by natural production (low-input systems) methods. There is also a new generation of younger consumers who wish to try new adventurous foods and many tourists to Africa wish to eat local wild species, ranging from springbok to crocodile as part of the 'Africa experience.' It is common to find game meat or venison on the menu of top-class restaurants throughout the major cities of the world. Furthermore, with the intercontinental immigration of diverse cultures and the fact that the world has become one large global village, many individuals are increasingly seeking access to their own traditional meats even when they are far from home. The farmed game animal industry has experienced unprecedented growth over the last few decades, largely reflecting the aforementioned consumer interests in lean and alternative food products.

Species that are discussed in this article include various ungulates (farmed and wild species) and game birds, emphasizing carcass characteristics, nutritional composition, and palatability attributes. The distribution, population, and hunting methods will not be discussed in detail as this information can be found in various literature sources and is very species-specific. Bushmeat will not be covered in depth as many species that are traded illegally are also traded legally, and are thus covered in that specific section. Since fish are excluded from the term 'game,' these are not discussed further in this section (although some fish caught for sport are termed game fish).

Game Production, Consumption, and Economics

Although it is extremely challenging to obtain reliable data relating to the extent of the global game meat trade, it is accepted that formal game production still constitutes a small share of the word's overall meat production. Africa, the two Americas, Oceania, and Europe are considered to be among the primary game producers. Records from the United Nations Food and Agriculture Organization Statistical Database (FAOSTAT) suggest that the total global game production in 2011 was more than 1 942 500 tons. The African countries that were reported to contribute substantially to the FAOSTAT figures included Nigeria (163 000 tons), Ghana (74 300 tons), South Africa (46 000 tons), and Kenya (25 100 tons). In addition, game production in 2011 in the United States (US) was estimated at 248 000 tons, whereas in the European Union (EU) this was in the order of 130 990 tons. However, it is unlikely that the magnitude of the illicit bushmeat trade was taken into account in the former calculations, neither the value of internal trade or direct sales by hunters to local consumers and establishments. In the Congo Basin alone, for instance, the bushmeat harvest is expected to be upward of one million tons per year.

In South Africa, more than 1 million animals (comprising approximately 35 different species) are harvested a year by recreational game hunters alone and the annual turnover from this industry is around ZAR 3 billion (1 ZAR = 0.095 USD (approx.)). The number of game slaughtered in Canada in 2012 exceeded 668 000 head and more than one million kg of game meat was exported from the country in the same year.

With the exception of poor communities, game meat does not constitute a high proportion of the total meat consumed. The estimated annual per capita consumption of wild and farmed game meat in Austria, France, Germany, Poland, and Switzerland is 0.6–1.0 kg. Ungulates (e.g., deer, moose, and wild boar) contribute approximately 3.3 kg per capita per annum in Norway and 1–4 kg per capita per annum in Italy. Much of the game meat consumed is either from a personal hunt or from value-added products. These range from fermented and processed products adhering to first world standards in Europe to smoked dried meat in Africa.

Birds

The ratites and game birds are among the most important meat-producing birds, being consumed and traded on markets across the globe. Their production systems are not described in this section as this has been done elsewhere.

In terms of the ratites, the raising of ostriches for meat has become well established and has been commercially successful. The birds are generally slaughtered in highly sophisticated abattoirs with strict hygiene controls rather than being hunted in the wild. Attempts have also been made to raise the emu and rhea for commercial purposes, although this has been less effective due to their low yield and the lower market value of their meat and other products. Owing to the morphological structure of ratites – with most of the meat being found on the leg – they have a low lean meat yield (Table 1). Ratites have only small localized subcutaneous fat depots, normally above the legs and then a well-developed fat pad over the belly, posterior to the keel. The low intramuscular fat and favorable fatty acid profile has resulted in an increased global demand for ostrich meat over the past years (Table 1). The fatty acid profile of the birds has been shown to be influenced by the diet.

Table 1 Summary of carcass yields and nutritional values of selected ratites

	Ostrich	*Emu*	*Rhea*
Bodyweight (kg)	85	41	25
Carcass weight (%)	59	53	61
Total lean meat (%)	39	34	36
Trimmings (%)	12	13	14
Moisture (%)	76.6	73.6	74.8
Protein (%)	20.9	21.2	22.9
Fat (%)	0.5	1.7–4.5	1.2
Energy (kJ per 100 g)	390	471–531	439
Cholesterol (mg per 100 g)	57	39–48	57

Table 2 The mean weights (±standard deviation (sd)) of the major muscles from ostriches having live weights of 85–95 kg

Muscles	*Mean weights (kg±sd) for muscles (n = 34)*	*Muscles expressed as mean percentage (%±sd) on a leg weight basis*
M. femorotibialis accessorius	0.69±0.10	4.7±0.5
M. fibularis longus	0.29±0.04	2.0±0.3
M. flexor cruris lateralis	0.30±0.04	2.1±0.3
M. gastrocnemius pars externa	0.59±0.08	4.0±0.7
M. gastrocnemius pars interna	0.84±0.13	5.7±0.8
M. iliofemoralis	0.40±0.06	2.8±0.4
M. iliofemoralis externus	0.19±0.03	1.3±0.2
M. femorotibialis internus	0.11±0.02	0.8±0.2
M. iliofibularis	1.41±0.15	9.6±1.1
M. iliotibialis cranialis	0.49±0.07	3.4±0.6
M. iliotibialis lateralis	1.08±0.15	7.5±0.9
M. obturatorius medialis	0.55±0.08	3.8±0.7

The most commonly marketed meat is derived from the posterior limbs and includes whole muscle cuts such as the Musculus iliofibularis (fan fillet), M. iliofemoralis (side strip), M. iliotibialis cranalis, M. femorotibialis accessorius, M. fibularis longus, M. flexor cruris lateralis, M. obturatorius medialis, M. gastrocnemius (big drum), and M. iliotibialis lateralis (Table 2).

Slight differences in the chemical composition and quality characteristics are found between the muscles within the ostrich carcass. Similar to other farmed livestock, the muscle composition is influenced by factors such as genetics, age, and diet. Gender has a smaller influence because most birds are slaughtered as they reach sexual maturity (10–14 months of age).

In spite of the success of the ostrich industry, the international trade in ostrich meat has been negatively influenced by outbreaks of Avian influenza, which has forced large producers in Africa to explore other avenues for meat utilization. These have included promoting consumption locally and in neighboring countries, processing the meat into various value-added products (Table 3), as well as employing a heat treatment to produce *sous vide* products that are exported.

The expression 'game bird' describes a very broad collection of birds grouped into land fowls and waterfowls, including species in the following orders: Galliformes (including guinea fowls, partridges, quails, francolins, and pheasants), Anseriformes (including ducks and geese), Columbiformes (including doves and pigeons), Pterocliformes (including sandgrouse species), and Charadriiformes (including snipes). Game birds have long been hunted for recreational reasons, but their popularity as a food source is growing and these are now obtained from the wild, as well as being farmed. The game bird industry has developed into a multimillion dollar industry, particularly in the United States and European Union, and it is also emerging in South Africa, with its success being accompanied by the ongoing management and conservation of bird populations. In the United States, more than 38 million game birds are shot each year, many of which are destined for restaurants or these are marketed directly to consumers. In the United Kingdom, approximately 19 million game birds were shot in 2004 alone, of which the majority were also incorporated into the country's food chain.

Captive-bred birds are an integral part of the international industry, chiefly in Europe, where large numbers of game birds are bred annually for release into the wild. It is estimated that more than 20 million game birds (80% are pheasant and the rest are mainly red-legged partridge) are reared and released for hunting in the UK each year, with most of the hunted birds being consumed by the hunters, farmers, or beaters. Gray partridge and ducks are also reared for this purpose.

The composition and quality of the meat from different game birds can vary quite substantially and is largely dependent on the diet of the birds. Some game birds closely resemble chicken and comprise mainly white meat, whereas others have a stronger 'gamey' flavor and can contain more dark meat. The proximate composition of selected game birds is shown in Table 4. In general, most game birds have a relatively low fat content, meaning they commonly need to be basted or larded before roasting. Wild birds are normally much leaner than the varieties reared in captivity. Older birds can be tougher and are usually best cooked with slow moist heat, or used in stews or soups.

As wild game birds are normally hunted using shot guns, the possibility of lead residues in the muscle has raised some speculation and has been the subject of considerable research. Lead from ammunition in game meat is more bio-accessible after cooking, especially when using highly acidic recipes that include substances such as vinegar.

Deer

Deer are ruminant mammals of the family Cervidae that can be found in the wild or kept in parks, but which are also increasingly being farmed both extensively and intensively in New Zealand and parts of the northern hemisphere. In particular, New Zealand (having the largest farmed deer population in the world) produced approximately 23 308 tons (carcass-weight equivalent) of venison between 2012 and 2013, of which around 95% was exported after processing. Nonetheless, the hunting of these species in the wild remains popular throughout Europe and North America. Fallow deer (*Dama dama*) and red deer (*Cervus elaphus*) are the most commonly farmed in Europe, whereas the elk or wapiti (*Cervus*

Table 3 The chemical composition of various processed ostrich products sold in retail outlets in South Africa

Chemical component (%)	*French poloni*	*Ham*	*Bacon*	*Smoked Russian*	*Smoked Vienna*	*Smoked fillet*
Dry mass	29.31	32.32	26.60	33.91	36.41	26.90
Protein	12.36	17.87	20.45	17.73	13.35	20.85
Fat	6.93	1.75	1.92	10.78	14.85	2.28
Ash	7.66	11.54	11.55	6.60	5.77	8.87
Cholesterol (mg per 100 g)	36.6	32.9	50.7	39.5	43.7	51.0
Fatty acids (% of total FA)						
C14:0	0.60	1.38	1.30	1.69	0.67	0.86
C16:0	25.79	21.97	27.65	27.30	24.31	19.84
C18:0	7.94	12.65	10.20	12.53	8.36	13.38
C20:0	0.11	0.12	0.20	0.22	0.21	0.15
C22:0	0.01	0.00	0.08	0.00	0.02	0.11
C24:0	0.01	0.00	0.35	0.00	0.02	0.11
SFA	34.46	36.11	39.78	41.74	33.59	34.44
C16:1n7	5.61	2.97	5.03	2.96	5.50	3.80
C18:1n9	37.60	46.65	28.95	44.61	43.04	32.22
C20:1n9	0.33	0.09	0.00	0.16	0.28	0.21
C24:1n9	0.04	0.00	0.00	0.00	0.27	0.19
MUFA	43.58	49.70	33.97	47.73	49.09	36.41
C18:2n6	15.91	8.20	14.78	7.94	12.92	17.99
C18:3n6	0.06	0.25	0.72	0.06	0.04	0.06
C18:3n3	4.47	1.98	2.90	1.63	3.36	2.28
C20:2n6	0.17	0.00	0.13	0.00	0.19	0.22
C20:3n6	0.08	0.19	0.20	0.00	0.11	0.55
C20:4n6	0.84	2.23	5.64	0.43	0.53	5.63
C20:3n3	0.06	0.12	0.00	0.00	0.00	0.00
C20:5n3	0.11	0.56	0.90	0.00	0.06	1.08
C22:2n6	0.00	0.00	0.00	0.00	0.00	0.00
C22:4n6	0.10	0.00	0.46	0.48	0.05	0.46
C22:5n3	0.14	0.37	0.42	0.00	0.06	0.43
C22:6n3	0.06	0.30	0.10	0.00	0.00	0.43
PUFA	22.00	14.18	26.25	10.53	17.32	29.15

Abbreviations: FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 4 Estimated proximate composition of the breast meat of selected game birds

	Guinea fowl		*Egyptian goose*	*Francolin*	*Quail*	*Wild pheasant*
	Breast raw	*Breast cooked*	*Breast cooked*	*Breast raw*	*Breast raw*	*Breast raw*
Moisture (%)	73.5	64.5	62.2	66.5	73.0	72.4
Protein (%)	23.7	31.9	30.9	28.7	19.0	25.5
Total fat (%)	1.5	3.2	5.9	3.4	0.6	1.1
Ash (%)	1.3	1.6	1.7	1.4	0.2	1.1

canadensis), fallow deer, sika deer (*Cervus nippon*), axis deer (*Axis axis*), and white-tailed deer (*Odocoileus virginianus*) are mainly farmed in the United States and Canada. The herding of reindeer is mostly carried out in the Nordic countries.

Similar to farmed domestic livestock species (cattle and sheep), various factors can influence the carcass yield and lean yield of deer species (Table 5). Adult animals will have a lower bone yield than subadults due to the fact that bone matures earlier than muscle and fat. The lean yield in mule deer, elk (wapiti), and pronghorn antelope varies from as high as 78% to as low as 57% of the skinned carcass, depending on how the lean yield is calculated. Commercial lean yield percentages vary according to the skill with which the meat is trimmed off the bone and the amount of fat and connective tissue trimmed. The lowest yields are normally obtained from the carcasses of hunted deer, as hunters seldom have the skill to trim efficiently and there is also lean meat lost due to bullet damage, fly strike, and spoilage.

Venison consumption is particularly common in the United States, central Europe, and the United Kingdom. In general, consumers are drawn to its tenderness, low fat content (but favorable lipid composition), and high mineral levels (Table 6). Only very fat carcasses will have a visible subcutaneous fat layer. The same factors that influence the fatty acid composition of ruminants will influence the fatty acid composition of cervids (Table 7).

African Ungulates

The ecosystems of sub-Saharan Africa support a wide range of wild ungulate (hoofed animals) species, including more than 70 antelope species. Antelope not only signify a vital component of the fauna attracting game-viewing tourists and hunters, but they also provide a significant source of protein for human consumption.

South Africa and Namibia are the two countries that have the most developed game meat industries in Africa. In these regions, wild ungulate species are often harvested using modern technologies and are processed according to strict EU regulations utilizing Standard Operating Procedures (SOPs) and Veterinary Procedural Notices (VPNs) to produce meat destined for the formal local and international meat trade. The game species harvested commercially are mainly the springbok (*Antidorcas marsupialis*; >80%), blesbok (*Damaliscus pygargus phillipsi*), and kudu (*Tragelaphus strepsiceros*), while the blue wildebeest (*Connochaetes taurinus*), impala (*Aepyceros melampus*), and gemsbok (*Oryx gazella*) are exported in smaller numbers. The duiker species (subfamily Cephalophinae) are most commonly targeted in the bushmeat harvest and trade throughout Africa.

A major problem faced by both these two countries as pertaining to the export of game meat is the prevalence of zoonotic diseases, particularly foot-and-mouth disease. In South Africa and Namibia, the endemic viral types are the Southern African Territories types of foot-and-mouth virus, SAT 1, 2, and 3.

In contrast to farmed deer that are harvested in commercial abattoirs, the hunted ungulates are normally eviscerated in the field and dressed in informal to formal facilities. Most of the hunted species are hunted for recreational purposes and are consumed by the hunters or landowners and their immediate families. There have been some concerns about the consumption of meat that might contain residual lead (from the bullets) although it would seem as if the levels in the muscle are low.

The dress out percentages of game meat species differ due to the influence of similar factors (age, gender, etc.) that influences the yield in other domestic species (Table 8). However, where males have horns and females have none, the former will have a lower yield.

Most game species have no subcutaneous fat layer with the exception of the zebra, which has a very thick subcutaneous fat cover. Although reliable information on the proximate composition of many African ungulate species is limited in the scientific literature, that which is available indicates that the meat from these species can be considered highly nutritious and a valuable source of protein (Table 9). The meat also has a low fat content (generally <2.5%, with the exception of blesbok), with desirable ratios of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA), as well as omega-6 to omega-3 fatty acid ratios. The fatty acid composition of game meat is like most ruminants, influenced to a lesser extent by the diet. Nonetheless, game species can be divided into grazers that only consume grass, mixed feeders that consume both grass and browse, and the browsers. The impala (*Aepyceros melampus*) is a mixed feeder and will consume the more dominant feed type found in the region, which leads to slight differences in the fatty acid profile of muscle (Table 10).

Table 5 Lean, fat, and bone percentages in carcasses of some dissected Cervid species

Species	*Percentage*		
	Lean	*Bone*	*Fat*
Nilgai antelope			
Adult	79.0	19.2	1.4
Subadult	74.2	25.1	0.1
Fallow deer adult males	73.9	15.6	9.1
Red deer adult males			
Prerut	66.0	14.9	19.0
Postrut	83.2	15.5	1.3
Mule deer adults			
Males	72.9	15.8	11.0
Females	75.7	15.7	8.2
Elk (wapiti) adults			
Males	77.8	18.2	3.3
Females	72.6	18.6	8.2
Pronghorn antelope adults			
Males	76.7	18.6	4.8
Females	77.0	19.2	3.9

Wild Suids

This section discusses various wild Suid species (wild boar, warthog, and bushpig), but will not focus on feral pigs (escaped domestic pigs).

Table 6 Nutrient composition of meat derived from wild animals (amounts in 100 g raw meat)

Species	*Energy (kJ)*	*Protein (g)*	*Fat*	*SFA (g)*	*MUFA (g)*	*PUFA (g)*	*Cholesterol (mg)*	*Iron (mg)*
Pronghorn antelope	477	22.4	2.0	0.74	0.48	0.44	52	3.2
Caribou	531	22.6	3.4	1.29	1.01	0.47	83	4.7
Deer	502	22.9	2.4	0.95	0.67	0.47	54	3.4
Elk (wapiti)	464	22.9	1.4	0.53	0.36	0.30	48	2.8
Moose	427	22.4	0.7	0.22	0.15	0.24	59	3.2
Range-grazed beef	469	21.8	2.4	0.93	0.75	0.19	49	2.2

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 7 Mean values for fatty acid composition (g per kg total fatty acids) in M. longissimus from pasture and pellet-fed reindeer (*Rangifer tarandus tarandus* L.) and red deer (*Cervus elaphus*)

Fatty acid	*Reindeer Pasture (n = 9)*	*Pellets (n = 6)*	*Degree of significance#*	*Red deer Pasture (n = 7)*	*Pellets (n = 7)*	*Degree of significance#*
Polar lipids				Polar lipids		
14:0	2.1	2.9	n.s.			
16:0	12.6	13.8	n.s	10.1	10.3	n.s.
16:1	0.6	0.9	**	1.1	0.4	**
17:0	0.4	0.2	***			
17:1	0.4	0.2	***			
18:0	12.4	13.4	*	15.8	14.1	*
18:1	3.4	2.0	***	12.3	12.4	n.s.
18:1 (*trans*)	0.4	0.3	**			
18:1 (n-9)	11.5	12.0	n.s			
18:1 (n-7)	1.0	1.7	***			
18:2 (n-6)	21.1	27.6	***	20.3	29.8	***
18:3 (n-3)	6.1	1.2	***	5.2	0.2	***
20:3 (n-3)	600	8.0	***	1.0	1.3	***
20:4 (n-6)	10.2	9.5	n.s	9.0	12.1	***
20:5 (n-3)	2.7	1.6	***	3.0	0.8	***
22:4 (n-6)	6.0	6.0	n.s.			
22:5 (n-3)	4.6	3.3	***	4.0	1.9	***
22:6 (n-3)	2.0	2.0	*	0.9	0.2	***
SFA	25.4	26.3	n.s.	25.9	24.4	n.s.
MUFA	17.3	16.0	*	13.8	12.4	n.s.
PUFA (n-6)	31.9	39.4	***	29.3	41.9	***
PUFA (n-3)	14.2	7.5	***	14.2	4.5	***
(n-6)/(n-3)	2.2	0.53	***	2.1	9.3	***
Neutral lipids				Neutral lipids		
12:0	4.5	3.5	**			
14:0	1.7	1.8	n.s.	5.0	6.1	n.s.
14:1				1.6	2.2	
15:1				0	0.1	n.s.
16:0	23.8	27.2	***	33.3	34.6	n.s.
16:1 (*trans*)	0.3	0.3	n.s.			
16:1	0.9	1.6	***	9.3	11.9	*
17:0	1.0	0.8	***	0.6	0.4	n.s.
18:0	21.4	21.0	n.s.	15.7	9.3	***
18:1				24.7	25.7	n.s.
18:1 (*trans*)	1.3	0.6	***			
18:1 (n-9)	34.1	35.6	*			
18:1 (n-7)	1.0	1.1	*			
18:2 (n-6)	2.2	2.1	n.s.	3.8	5.3	
18:3 (n-3)	1.0	0.2	***	1.5	0.3	***
20:0	0.5	0.2	***	0.1	0.1	n.s.
20:3 (n-3)				0	0.1	*
20:4 (n-6)	0.4	0.2	***	0.7	0.8	n.s.
20:5 (n-3)				0.3	0	***
22:5 (n-3)	4.0	0.1	***	0.6	0.2	***
SFA	53.0	54.6	n.s.	54.7	50.6	**
MUFA	37.6	39.2	*	36.4	39.8	
PUFA (n-6)	2.6	2.3	n.s.	4.3	6.6	**
PUFA (n-3)	1.4	0.3	***	2.5	0.6	***
(n-6)/(n-3)	1.9	7.7	***	1.7	11.0	***

Note:# n.s., Not significant, *$p < .05$, **$p < .01$, ***$p < .001$.

Abbreviations: FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Wild boar, belonging to the genus *Sus* and pig family (Suidae), is regarded as the wild ancestor of the domestic pig. The species is native to many parts of Central and Northern Europe, the Mediterranean and Asia, but has also been introduced into some regions (notably Australasia and the Americas). Although long valued for food and recreational hunting, the animals have also come to be regarded as agricultural pests and a threat to the ecosystem. The recent widespread intensifying of wild boar densities has stimulated interest in the animals as meat producers and also as a

Table 8 Mean carcass yields (±standard errors) and least significant differences (LSD) for different gender and age groups of springbok from Namibia

	Adult Male (n = 12)	*Subadult Male (n = 7)*	*Adult Female (n = 11)*	*Subadult Female (n = 9)*	*LSD (p = 0.05)*
Live weight (kg)	$40.44^{a} \pm 1.883$	$34.94^{b} \pm 2.249$	$36.61^{ab} \pm 0.495$	$29.32^{c} \pm 1.627$	4.571
Carcass weight (kg)	$24.72^{a} \pm 1.145$	$19.73^{bc} \pm 1.188$	$21.25^{b} \pm 0.416$	$16.80^{c} \pm 1.140$	9.341
Dressing (%)	$61.6^{a} \pm 1.36$	$56.0^{b} \pm 2.60$	$58.1^{ab} \pm 1.31$	$57.1^{ab} \pm 1.18$	4.539

[a,b,c]Values in the same row with different superscripts differ significantly ($p = .05$).

Table 9 Proximate composition (g per 100 g wet weight basis) of the raw meat of some wild antelope species compared to that derived from cervids

Animal species		*Sample analyzed*	n	*Moisture (g per 100 g)*	*Protein (g per 100 g)*	*Fat (g per 100 g)*	*Ash (g per 100 g)*
Ungulates, Cervidae							
Red deer	*Cervus elaphus*	M. longissimus dorsi	10	76.90	21.70	0.60	1.11
Fallow deer	*Dama dama*	M. longissimus dorsi	10	74.90	22.00	2.50	1.08
Roe deer	*Capreolus capreolus*	M. longissimus dorsi	10	74.80	23.00	1.70	1.15
Reindeer	*Rangifer tarandus*	M. longissimus dorsi	11	71.80	23.60	2.80	1.10
Ungulates, African species							
Springbok	*Antidorcas marsupialis*	Whole 9th-10th-11th rib cut	5	75.30	17.40	2.50	4.20
Blesbok	*Damaliscus dorcas phillipsi*	Whole 9th-10th-11th rib cut	4	71.10	19.30	4.60	4.00
Kudu	*Tragelaphus strepsiceros*	M. longissimus dorsi	7	75.66	22.77	1.48	1.22
Impala	*Aepyceros melampu*	M. longissimus dorsi	11	74.96	22.63	2.06	1.22
Red hartebeest	*Alcelaphus buselaphus caama*	M. longissimus dorsi	13	75.00	23.30	0.60	1.20
Oryx	*Oryx beisa*	Loin muscle	2	76.60	20.30	0.20	1.10
Duiker	*Sylvicapra grimmia*	M. longissimus dorsi	10	71.40	25.70	2.12	1.29

potential farmed species. Today, the wild boar is propagated in Canada, Japan, the United States, and the Americas.

Dressed weights (assumed to include the heads) for 3- to 4-year-old hunted wild boar have been reported to be 65–108 kg for males and 50–80 kg for females. The carcass yields of animals hunted in Poland varied from 59% to 74% (the skin contributed ~16–29% of initial weight), and increased with bodyweight. Yields of 81–83% have, however, been reported for adult and medium-sized boars hunted in Croatia and Italy, respectively. In comparison to the domestic pig, wild boar exhibits more carcass fatness and larger loin areas, while having darker, leaner, and less tender meat. The mean proximate composition of wild boar hunted in Italy was described as approximately 70.5% moisture, 25.9% protein, 1.5% fat, and 1.2% ash. Although the flavor and fatty acid composition of the wild boar may be affected by the gender and age of the animals, this is also largely influenced by the diet provided (as with other monogastric animals). The latter is largely evidenced in the depot fat of the wild boar, where unlike ruminants, the double bonds of fatty acids do not become hydrogenated during the process of digestion. An example of the fatty acid profile of hunted wild boar is shown in Table 11. The ratio of PUFA:SFA in wild boar is estimated at 0.52–0.6.

The warthog (*Phacochoerus africanus*) is a further wild member of the Suidae that has a natural distribution in the grasslands, savannah, and woodlands of sub-Saharan Africa. The species is characterized by a high fecundity (having 4–5 piglets per litter, gestation period of 167–175 days) and is frequently regarded as an agricultural pest in many farming regions. The meat has been consumed by locals in South Africa for many years and it is also being increasingly sought by tourists visiting the country's restaurants as part of a novel, uniquely African culinary experience. Recent research has focused on obtaining an enhanced understanding of the chemical composition of the meat, the development of value-added products and the promotion of its consumption based on its health and exotic qualities, with all these activities aimed at providing incentives for better management of growing warthog populations.

Mature warthogs can attain body weights of 100 kg in males and 70 kg in the females. The dressing percentage is in the order of 52%, which is somewhat lower than domestic pigs. However, in contrast to domestic pigs, carcass weight in

Table 10 Fatty acid profile (Mean ± SE) of the longissimus dorsi muscle of impala from Mara and Musina

Fatty acid (%)	*Mara (grass diet)*		*Musina (grass and browse diet)*	
	Females (n = 16)	*Males (n = 24)*	*Females (n = 13)*	*Males (n = 15)*
C14:0	0.39 ± 0.41	0.32 ± 0.77	0.30 ± 0.36	0.58 ± 0.96
C16:0	20.72^{a} ± 4.27	15.04^{b} ± 5.55	22.47^{a} ± 3.71	19.83^{a} ± 3.43
C18:0	22.07 ± 2.16	22.25 ± 3.86	21.94 ± 4.35	20.35 ± 2.92
C20:0	0.11 ± 0.04	0.14 ± 0.08	0.13 ± 0.06	0.10 ± 0.06
C22:0	0.09^{a} ± 0.06	0.16^{b} ± 0.12	0.16^{b} ± 0.10	0.19^{c} ± 0.12
C24:0	0.15 ± 0.07	0.19 ± 0.09	0.14 ± 0.07	0.19 ± 0.10
SFA	43.55^{a} ± 4.96	38.11^{b} ± 5.27	45.13^{a} ± 7.16	41.26^{a} ± 5.87
C16:1n7	0.61 ± 0.46	0.57 ± 0.33	0.67 ± 0.33	0.66 ± 0.20
C18:1n9	21.81^{a} ± 5.81	19.34^{a} ± 4.80	19.06^{ab} ± 3.72	15.98^{b} ± 4.77
C20:1n9	0.13^{a} ± 0.23	0.10^{a} ± 0.04	0.07^{b} ± 0.02	0.07^{b} ± 0.04
C24:1n9	0.11 ± 0.15	0.14 ± 0.08	0.09 ± 0.07	0.10 ± 0.11
MUFA	22.66^{a} ± 6.08	20.15^{a} ± 5.02	19.89^{ab} ± 3.72	16.80^{b} ± 4.87
C18:2n6	16.16^{a} ± 4.69	19.67^{b} ± 3.62	18.34^{a} ± 5.22	22.74^{b} ± 5.74
C18:3n6	0.20 ± 0.19	0.14 ± 0.03	0.13 ± 0.05	0.13 ± 0.04
C18:3n3	4.36^{a} ± 1.39	5.09^{a} ± 1.11	4.01^{ab} ± 1.41	3.95^{b} ± 0.70
C20:2n6	0.15 ± 0.06	0.18 ± 0.04	0.13 ± 0.04	0.15 ± 0.06
C20:3n6	0.69 ± 0.26	0.86 ± 0.23	0.75 ± 0.33	0.96 ± 0.28
C20:4n6	6.12^{a} ± 2.00	7.87^{b} ± 1.67	5.87^{a} ± 2.47	7.79^{b} ± 2.69
C20:3n3	0.06 ± 0.04	0.09 ± 0.05	0.06 ± 0.04	0.06 ± 0.05
C20:5n3	2.76 ± 1.37	3.44 ± 0.84	2.41 ± 1.02	2.78 ± 1.20
C22:2n6	0.08^{a} ± 0.07	0.14^{b} ± 0.07	0.08^{a} ± 0.06	0.14^{b} ± 0.12
C22:4n6	0.41 ± 0.55	0.43 ± 0.42	0.22 ± 0.08	0.24 ± 0.17
C22:5n3	2.02 ± 0.87	2.82 ± 0.78	2.29 ± 0.90	2.43 ± 1.17
C22:6n3	0.77 ± 0.36	1.00 ± 0.64	0.69 ± 0.34	0.56 ± 0.45
PUFA	33.79^{a} ± 10.06	41.74^{b} ± 7.02	34.98^{a} ± 10.22	41.94^{b} ± 10.31

[a,b,c]Means in the same row with different superscripts differ significantly ($p < .05$).

Abbreviations: FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

warthogs does not normally include the head, skin, and adjacent subcutaneous fat layers, which can largely account for the aforementioned factor. In warthogs, the contribution of the shoulder (37%), hind legs (32%), belly (14%), back (9%), and loin (7%) to the cold carcass weight also differs from that obtained from domestic pigs.

Findings relating to meat quality characteristics suggest that warthogs are prone to develop pale, soft, and exudative (PSE) meat when exposed to ante mortem stress, which is a similar phenomenon seen in domestic pigs under comparable conditions.

Warthog meat is of a high nutritional value and has a favorable fatty acid profile (Table 12), although the latter can be influenced by the diet as with the wild boar. The ratio of PUFA to SFA is approximately 1.33 (compared to 0.46–0.64 in domestic pigs), which is well above the minimum level of 0.4–0.5 recommended to be appropriate for human health.

The bushpig (*Potamochoerus larvatus*) is a nocturnal wild pig species found in woodlands, forests, riverine vegetation, and reed beds in parts of East and Southern Africa. Similar to the warthog, many farmers in these regions consider the bush pig as a problem animal, as it thrives on many agricultural products and unearths root crops in their masses. In spite of this, the meat of the bushpig is still relished as a delicacy. Nonetheless, there is currently little available data on its quality and composition characteristics.

Kangaroos

Kangaroos are marsupial species of family Macropodidae that are endemic to Australia, the meat from which has been consumed by the aboriginal inhabitants of this region for tens of thousands of years. Certain kangaroo species are abundant in rural Australia, being considered pests in some regions. Thus, the commercial harvest of these species from the wild is permitted in a number of Australian states, although this is under regulatory control. The red kangaroo (*Macropus rufus*), western gray kangaroo (*M. fuliginosus*), and eastern gray kangaroo (*M. giganteus*) comprise approximately 90% of the commercial harvest.

Although 70% of the Australian kangaroo harvest is currently exported, the meat also has a niche market in Australia and is sold in both restaurants and retail outlets. Kangaroo meat has a strong flavor, is high in protein, as well as iron and zinc. The total fat content ranges from 0.2 to 1.4%, depending on the species. This consists of approximately 32% SFA, 31% monounsaturated fatty acids (MUFA), and 38% PUFA. The major SFAs in the meat include palmitic and stearic acids, whereas oleic acid is the predominant of the MUFA. The main PUFA include linoleic acid, arachidonic acid, and α-linolenic acid, respectively. The cholesterol levels are low and range from 41.6 to 65.3 mg per 100 g (depending on species, geographical origin, and cut) and the meat has a favorable omega-6:omega-3 fatty acid ratio of 2.5:1.

Table 11 The lipid, cholesterol, and fatty acid profile of *M. psoas major* from wild boar hunted in Portugal

	Adult males (n = 6)	*Adult females (n = 10)*	*Youngster (n = 9)*
Carcass weight (kg)	51	43	17
Lipid (g per 100 g meat)	4.75	4.55	4.68
Cholesterol (mg per 100 g meat)	58.7	55.6	57.1
Fatty acid (% of total FA)			
C14:0	1.0	1	0.9
C16:0	20.7	20.7	20.4
C16:1 *cis*-9	2.3	2.2	1.9
C17:0	0.2	0.2	0.3
C17:1 *cis*-9	0.1	0.1	0.1
C18:0	11.5	10.5	10.4
C18:1 *trans*	0.4	0.4	0.4
C18:1 *cis*-9	36.1	39.7	39.6
C18:2 n-6	18.8	15.9	16.4
C18:2 *cis*-9 *trans 11*	0.2	0.2	0.2
C18:3 n-3	1.0	0.9	1
C20:0	0.1	0.2	0.1
C20:2 n-6	0.4	0.4	0.4
C20:3 n-6	0.5	0.4	0.4
C20:4 n-6	4.4	4.5	4.9
C20:5 n-3	0.4	0.4	0.7
SFA	34.7	34.2	33.3
cis-MUFA	38.9	42.6	42.2
n-6	24.0	21.1	22.1
n-3	1.4	1.4	1.7
PUFA	25.4	22.5	23.8
PUFA/SFA	0.6	0.52	0.55
n-6/n-3	17.0	15.5	12.8

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Rabbits and Hares

Rabbits and hares are plentiful in many regions of the world and these are included in the diets of many populations. More than 60 species are documented within the Leporidae family. All hare species belong to the genus *Lepus* and rabbits belong to eight different genera. Native rabbit and hare species occur throughout Africa, America, Asia, and Europe.

Man has used rabbits as food since 1500 BC. Today, these species can be hunted in the wild for consumption, but they are most commonly bred for meat in many parts of the world, including the United States, several European countries, Africa, and China. Rabbit breeding is particularly recognized to have potential for supplying high-value protein and for improving food security in the developing world. Since rabbits can adapt to diverse environmental conditions, have high reproductive and growth rates, as well as good feed conversion efficiencies, they are considered favorable meat producers. The Californian and New Zealand White are leading commercial breeds, with high ratios of muscle for their size.

Fresh rabbit meat is sold in butcheries and markets in certain countries, while some supermarkets also sell frozen rabbit meat. Recent reviews on rabbit meat composition have confirmed that this is of high nutritional value compared to meat from other domestic animals. Rabbit meat is very palatable and is rich in high-biological value protein (~20–22%) and bioavailable micronutrients. It is generally low in calories, fat (although this varies according to the cut) and cholesterol. The leanest cut is usually the loin (1.8% fat), whilst the foreleg is the fattiest cut (8.8% fat). The meat from wild rabbits is normally leaner than that from their domesticated counterparts, with mean total fat values of 1.05% and 5.55% in the respective animals. As rabbits are monogastric animals, the fatty acid composition of their meat is strongly influenced by the diet consumed, thus the PUFA content could be increased by supplementing diets with vegetable oils, such as linseed and rapeseed oil, or with fish oil. In rabbit meat, unsaturated fatty acids represent approximately 60% of the total fatty acids and the PUFA comprise approximately 32.5% thereof.

Bison

Although bison (*Bison bison*) was previously nearly hunted to extinction, this species is now farmed successfully in Canada and America. The farmed bison population in Alberta, Canada is estimated at >90 000 head, while approximately 500 000 plains bison are said to exist in all of North America. It is calculated that 96% of the animals are selected for anthropogenic commodity production and less than 4% of the herds are managed for conservation purposes. Canada harvests just under 20 000 bison per annum. Factors influencing the carcass characteristics are similar to those observed for cattle, such as the females tend to gain fat earlier than males and there is an increase in meat toughness with age.

In many instances, bison meat has been promoted to a niche market as a product with a strong heritage, unique flavor, dark red color and that is a healthy and nutritious red

Table 12 Means and standard deviations (SD) of the proximate and fatty acid components of warthog loins ($n=5$)

Component	*%*	
	Mean	*SD*
Moisture	74.04	0.94
Total lipid	1.69	1.39
Protein	22.14	0.30
Ash	1.29	0.03
Fatty acids		
C14:0	0.75	0.66
C16:0	19.95	2.25
C18:0	14.68	2.96
C20:0	0.14	0.02
C22:0	0.13	0.05
C24:0	0.10	0.09
Total SFA	35.75	3.01
C16:1n7	0.74	0.76
C18:1n9	15.79	11.23
C20:1n9	0.07	0.06
C24:1n9	0.10	0.19
Total MUFA	16.70	12.10
C18:2n6	26.12	9.64
C18:3n6	0.17	0.05
C18:3n3	7.26	7.97
C20:2n6	0.30	0.02
C20:3n6	1.06	0.60
C20:4n6	7.48	4.94
C20:3n3	0.94	0.35
C20:5n3	0.91	0.60
C22:2n6	0.07	0.16
C22:4n6	0.40	0.37
C22:3n3	0.00	0.00
C22:5n3	2.44	2.01
C22:6n3	0.42	0.35
Total PUFA	47.56	10.35

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, SFA, saturated fatty acids.

meat alternative. Most bison cuts have a protein content that varies from 21.0 to 23.0%. The total fat content of the meat is approximately 1.0–4.6% (depending on the cut), consisting of 0.4–1.3% saturated fat, 0.5–1.5% monounsaturated fat, and 0.1–0.2% polyunsaturated fat. The cholesterol levels found in bison muscles also varies from 25.7 to 48.3 mg per 100 g, values similar to those of other red meat species, when factors such as intrinsic variation between muscle groups, gender as well as analytical methodology is taken into account.

Camelids

Camelids of the family Camelidae comprise the genera *Camelus* (including true camel species), *Lama* (including guanaco and llama), and *Vicugna* (including alpaca and vicuña), although the term 'camel' is normally used broadly to refer to all of these camel-like animals.

Within the true camel species, the one-humped dromedary (*Camelus dromedarius*) accounts for up to 90% of all the camels found, whereas the two-humped Bactrian camel (*Camelus bacterium*) represents the remainder. Approximately 80% of the world's camels are found in Africa, with Northeast Africa having the largest population of dromedaries.

The average slaughter weight of mature, fattened desert camels is around 450 kg. The dromedary (*Camelus dromedarius*) dresses out at approximately 56% of live body and 64% of empty bodyweight, yielding 56% meat, 19% bone, and 13.7% fat. Fat partitioning differs between different body sites of the camel carcass and its distribution is quite unique when compared to other animals. The largest proportion of the camel's fat reserves occur in the hump (~30%), which accounts for up to 5% of live weight and 8% of carcass weight. A significant fat depot also exists on the abdominal floor.

In 2009, the total world camel meat production amounted to over 360 000 ton, with Saudia Arabia, Sudan, Somalia, and Egypt being among the primary producers. Camel meat is often valued in harsh, dry environments where beef is in low supply and is popular throughout the Muslim world, in parts of Africa, Australia, and China. It is, however, noteworthy that some taboos exist with regards to camel meat consumption in certain cultures and religions. For instance, camel meat is seldom eaten in Europe and North America and its consumption is prohibited in the Torah for practicing Jews, for the Raikes or Rabaris of India, and for Ethiopian Christians.

Camel meat is raspberry red to dark brown in color and is considered to be healthy compared to meat from many other animals. Compared to the meat from domestic livestock species, camel meat has a low fat content, higher moisture content, and similar protein content (**Table 13**). The hump frequently forms part of the sirloin cut and can result in the latter having high lipid content. The ratio of essential amino acids to nonessential amino acids is approximately 0.85, similar to the 0.86 reported for beef, 0.83 for lamb, and 0.90 for goat. Camel meat also has a similar mineral profile compared to domestic livestock, although it might have slightly higher sodium levels. The cholesterol content of the meat is believed to increase with the age of the camel (135 mg per 100 g fresh weight for 8 months old compared to 150 mg per 100 g fresh weight for 26-month-old animals).

Of the genera *Lama* and *Vicugna*, the llama (*Lama glama*) and alpaca (*Vicugna pacos*) are domesticated. The guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*), however, are wild, and commercial farming of the latter two remains limited. The llama is produced for both its meat and fiber, while alpaca are primarily farmed for their fiber. Male llamas generally have slightly heavier dressing percentages (approximately 56%) than females (~54%). The meat from llama appears to represent a nutritious food source, providing high levels of protein (>23%) compared with the values derived from most common domesticated animal species and fat content (0.5%) that is generally less than the latter. The fat content of guanaco meat is slightly greater than that of llamas and alpacas, but still less than that in the meat of domesticated species. A comparison of the fatty acid composition of camels and llama is depicted in **Table 14**. Both species have C16:0 as the dominant SFA, whilst llama has nearly twice as much C18:1 (the dominant MUFA) compared to camels.

Table 13 The proximate composition (g per 100 g wet weight basis) of the raw meat of camelids compared with domestic livestock species

Animal species		*Sample analyzed*	n	*Moisture (g per 100 g)*	*Protein (g per 100 g)*	*Fat (g per 100 g)*	*Ash (g per 100 g)*
Ungulates, Camelids							
Camel	*Camelus dromedarius*	Supraspinatus muscle	52	75.60	21.70	1.42	1.20
Llama	*Lama glama*	Longissimus thoracis et lumborum	20	73.90	23.10	0.50	2.40
Alpaca	*Vicugna pacos*		40	73.60	23.30	0.50	2.50
Guanaco	*Lama guanicoe*		70	73.90	20.90	1.00	1.10
Domesticated species							
Beef	*Bos* spp.	M. longissimus dorsi, without fat	3	74.84	20.83	1.61	1.04
Beef	*Bos* spp.	M. longissimus dorsi, with fat	3	67.01	19.22	9.78	0.92
Lamb	*Ovis aries*	Mean of shoulder, leg, and loin	12	71.53	18.27	9.03	2.88
Mutton	*Ovis aries*	Mean of shoulder, leg, and loin	3	73.83	20.43	8.98	1.19
Goat	*Capra hircus*	Muscle	30	75.99	18	2.51	1.38
Domestic pig	*Sus scrofa domesticus*	M. longissimus dorsi muscle	10	75.51	21.79	2.02	0.99

Table 14 Fatty acid composition (% of total fatty acids) of meat from different camelids

Fatty acid	*Camel*[a]	*Llama*[b]
Lipid (g per 100 g)	1.52	3.5
Saturated		
14:0	7.7	4.1
15:0	1.7	–
16:0	26.0	24.8
17:0	1.5	–
18:0	8.6	21.5
20:0	*	–
Total	51.5	50.3
Monounsaturated		
14:1	1.0	–
16:1	8.1	5.4
17:1	0.9	–
18:1	18.9	35.8
20:1	*	1.3
Total	29.9	42.5
Polyunsaturated		
18:2	12.1	3.1
18:3	0.5	0.8
20:2	0.1	–
20:3	0.3	–
20:4	2.8	1.8
20:5	0.3	–
22:4	0.1	–
22:5	0.5	–
22:6	0.1	–
Total	18.6	7.2
P/S	0.36	0.14

[a]*Biceps femoris*, seven 1- to 3-year-old males.
[b]*Longissimus thoracis* and *lumborum*, twenty 25-month-old males.
Note: –, not shown; *, Trace (<0.1%) or undetectable amount.

Buffalo

Water buffalo, belonging to the family Bovidae, are believed to number approximately 158 million in the world, and are important livestock species mainly in tropical and subtropical parts of Asia, as well as in South America, southern Europe, and northern Africa. The classification of water buffalo remains uncertain, with some authorities listing a single species, *Bubalus bubalis*, with three subspecies: the river buffalo (*Bu. Bubalis bubalis*), the swamp buffalo or carabao (*Bu. bubalis carabanesis*), and the wild water buffalo or arni (*Bu. bubalis arnee*). Other authorities believe that these are closely related; however, they are separate species. In 2003, the International Commission on Zoological Nomenclature ruled in favor of classifying wild buffalo as a separate taxon; consequently wild forms are now frequently referred to as *Bu. arnee* and domestic forms as *Bu. bubalis*.

Water buffalo are normally slaughtered as spent animals or for meat production at approximately 18 months of age at 300–360 kg live weight and dress out at approximately 55%. The carcass yields of water buffalo are lower than that of cattle due to a heavier head (from the horns) and skin weights. Buffalo also tend to have a thinner subcutaneous fat layer than cattle, even when reared under comparable feedlot conditions.

The meat accounts for approximately 22% of total meat produced in India, where it is gaining in popularity and there are no taboos against its consumption. There are thus opportunities for the development of the buffalo meat industry to cater for the needs of the domestic markets. Water buffalo meat is also known as carabeef or carabao meat and various value-added products including dried jerky, hamburger patties, bacon, and ham have been made from the meat of this species.

Buffalo exhibit similar meat quality characteristics as cattle as pertaining to extrinsic and intrinsic effects such as age, gender, and muscle type. The meat of the former is darker than the latter, with the myoglobin content varying from 2.7 to 9.4 mg g^{-1} depending on the type of the muscle and age. Water buffalo meat becomes darker with increasing age. It has been suggested that buffalo meat (lean) does not have any peculiar flavors and is organoleptically virtually indistinguishable from beef. Nonetheless, a consumer meat preference survey has indicated that 55.9% of the human subjects involved selected the beef, while 44.1% preferred carabeef. The color and amount of fat on the outside of the beef sample were primary responsible for buyer preference.

Crude protein, ash, fat, cholesterol, myofibrillar, sarcoplasmic and insoluble protein contents of beef and carabeef are similar. Water holding capacity, pH, muscle fiber diameter, tenderness, firmness, and marbling scores in carabeef are also comparable to beef.

See also: Meat, Animal, Poultry and Fish Production and Management: Exotic and other Species. Parasites Present in Meat and Viscera of Land Farmed Animals. Slaughter-Line Operation: Other Species. Species of Meat Animals: Meat Animals, Origin and Domestication

Further Reading

Beilken, S., Tume, R., 2008. Nutritional Composition of Kangaroo Meat. Barton, ACT: RIRDC, pp. 1–30.
Dalle Zotte, A., 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Science 75, 11–32.
Dalle Zotte, A., Szendrő, Z., 2011. The role of rabbit meat as functional food. Meat Science 88, 319–331.
Geldenhuys, G., Hoffman, L.C., Muller, N., 2013. Aspects of the nutritional value of cooked Egyptian goose (*Alopochen aegyptiacus*) meat compared with other well-known fowl species. Poultry Science 92, 3050–3059.
Hoffman, L.C., Cawthorn, D.M., 2012. What is the role and contribution of meat from wildlife in providing high quality protein for consumption? Animal Frontiers 2, 40–53.
Hoffman, L.C., Sales, J., 2007. Physical and chemical quality characteristics of warthog (*Phacochoerus aethiopicus*) meat. Livestock Research for Rural Development 19. Available at: http://www.cipav.org.co/lrrd/lrrd19/10/hoff19153.htm (accessed 09.01.13).
Hoffman, L.C., Wiklund, E., 2006. Game and venison – meat for the modern consumer. Meat Science 74, 197–208.
Kadim, I.T., Mahgoub, O., Faye, B., Farouk, M., 2013. Camel Meat and Meat Products. Oxfordshire, UK: CABI, pp. 1–248.
Lecocq, Y., 1997. A European perspective on wild game meat and public health. Revue Scientifique et Technique (International Office of Epizootics) 16, 579–585.
Mead, G.C., 2004. Poultry Meat Processing and Quality. Cambridge, UK: Woodhead Publishing, pp. 211–230.
Quaresma, M.A.G., Alves, S.P., Trigo-Rodrigues, I., *et al.*, 2011. Nutritional evaluation of the lipid fraction of feral wild boar (*Sus scrofa scrofa*) meat. Meat Science 89 (4), 457–461.
Saadoun, A., Cabrera, M.C., 2008. A review of the nutritional content and technological parameters of indigenous sources of meat in South America. Meat Science 80, 570–581.
Sales, J., Kotrba, R., 2013. Meat from wild boar (*Sus scrofa* L.): A review. Meat Science 94, 187–201.
Soon, J.M., Baines, R., 2013. Managing Food Safety Risks in the Agri-Food Industries. New York, NY: CRC Press, pp. 123–128.

Relevant Websites

www.agr.gc.ca/redmeat/rpt/tbl36_eng.htm
Canadian Food Inspection Agency.
www.daff.gov.au
Department of Agriculture, Fisheries and Forestry (DAFF), Australia.
www.nda.agric.za
Department of Agriculture, Forestry and Fisheries, South Africa.
www.fao.org
Food and Agriculture Organization of the United Nations.
www.faostat.fao.org
Food and Agricultural Organization Statistical Database.
www.helgilibrary.com/indicators/index/game-meat-production
HelgiLibrary.
www.usda.gov
United States Department of Agriculture.

Meat Animals, Origin and Domestication

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Glossary

Auroch *Bos primigenius*: ancestor of both *Bos taurus* and *Bos indicus*.
Austronesian expansion The process of historic migration and spread of people called Austronesians from Southeast Asia (most likely Taiwan) to the Pacific region.
Holocene The geological epoch which began at the end of the Pleistocene (approximately 12 000 years ago) and continues to the present.
Myostatin A negative regulator of skeletal muscle growth.
Neolithic A period in the development of human civilization, beginning around 10 000 BC in the Middle East.
Order Artiodactyla Two toed mammals, e.g., sheep pigs, and cattle.
Pleistocene The geological epoch which lasted from approximately 2.6 million to 12 000 years ago, spanning the period of repeated glaciations.
Precocial The species in which the young are mobile and feed themselves soon after birth.
Selective sweep The reduction of a local variation of the sequences of nucleotides as a result of positive natural or artificial selection.

Introduction

Domestication of plants and animals was a pivotal moment in human history. It initiated the Neolithic agricultural revolution some 10 000 years ago and underpinned the spread of human civilizations. Domestication originated in only a few areas of the world and gave inhabitants of those areas enormous advantages over other people. This ultimately transformed human demography and gave rise to the modern world.

Domestication of animals most likely had its roots in the ubiquitous habit of all people to capture and tame wild animals, and at first was unintentional. It probably originated as a practice of keeping and raising the young animals captured and spared in hunting. Domestication started at the end of Pleistocene, at the time of increasing unpredictability of climate and rapid reduction of numbers of game animals forced people to seek alternative and reliable food supplies.

The Nature of Domestication

Domesticated animals are those that were ultimately genetically modified from their wild ancestors by artificial selection for use by humans, whose breeding and maintenance is controlled and whose food is provided for the benefit of a community or society. Domestication is thus a different process from mere taming of genetically unmodified representatives of wild species and maintaining them in captivity.

The degree of suitability of wild animals for domestication depends largely on the degree of their genetic variability and the match between husbandry conditions and species-specific behavioral patterns expressed in the wild. Domestication has been restricted to surprisingly few species of mammals and birds. Particularly astonishing is the almost complete lack of domesticated mammals indigenous to subSaharan Africa, which is a homeland of the largest world populations of wild ungulates. Even African cattle probably did not evolve there but were possibly introduced from Southeast Asia. This suggests that there is a very unique suite of physiological and behavioral characteristics defining suitability of a particular species for domestication.

All domesticated mammalian species important for meat production thrive on readily available and renewable plant food that can be harvested and stored as supplies by humans, for later use beyond the main growing period. The ability to digest poor-quality plant food limited the scope of mammalian species available for domestication to large and medium-sized animals weighing 45 kg or more and belonging to the Order Artiodactyla. Most of them (including cattle, sheep, and goats) are capable of fermenting plant material in the voluminous and highly compartmentalized stomach. The domestic pig has a simple stomach and relies on fermentation in the extended morphological structures of the hindgut. The less efficient digestion of fiber-rich plant food is, however, offset by its extremely opportunistic food habits.

All ancestors of major domesticated species were precocial, that is, their offspring became mobile and able to feed themselves soon after birth, which was a prerequisite for pastoralism. Their high growth rates made them easily renewable human food resources and speeded up the process of artificial selection by promoting early sexual maturation and shortening generation time.

Equally important for successful domestication are behavioral traits. All domesticated meat animals live in herds with a well-developed dominance hierarchy. In the process of domestication, humans have essentially taken over the dominant position, which enables them to manage the herds. Many species otherwise suitable for domestication are notoriously aggressive (e.g., African buffalo), have tendency to panic in enclosure (antelopes and gazelles), or are reluctant to breed in captivity (e.g., Andean vicuña). Failure to overcome problems with any of these characteristics is the most plausible reason why only 14 out of 148 mammalian species more than 45 kg body mass, potentially suitable for domestication,

became important as locally or globally distributed domesticated animals. However, only four of them (sheep, goat, pig, and cattle) provide the bulk of world meat production.

Origins of Domesticated Meat Animals

Until recently, documentation of events of domestication in the archeological records has proved to be difficult because of equivocal discrimination of remains of domesticated animals from their wild ancestors. These difficulties have been largely overcome with the advent of analysis of the mitochondrial genome transmitted from generation to generation in maternal lineages and harbored in the egg cells. Sequences of mitochondrial DNA (mtDNA) characteristic of distinct wild populations subject to domestication events have been transmitted throughout millennia, which allow discrimination between single and multiple origins of domesticated breeds. It may also be noted that, across different species, the mutation rate of the most variable regions of mtDNA is constant and high, relative to generation time. This rate of variation constitutes the pacemaker of the so-called molecular clock and has proved to be a useful tool in reconstructing the time depth of domestication. These molecular techniques, along with other archeological evidence, have recently enabled the researchers to reconstruct fascinating histories of domestication and phylogenetic relations of major meat animals. The origins of domestic cattle, sheep, goats, pigs, and fowl, as summarized in Table 1, are briefly reviewed below.

Domestication of Cattle

Among all meat-producing domesticated animals, cattle have had the most economically important role in the evolution of human cultures. There are two major types of cattle: Western cattle (*Bos taurus*) lacking the shoulder hump and the humped Indian zebu cattle (*Bos indicus*). Both types interbreed fully and therefore their status as separate species is questionable. The continued existence of many of the 800 extant cattle breeds (of which, approximately 480 are recognized in Europe) is severely threatened by modern agricultural practices. According to a recent FAO report, 209 cattle breeds have become extinct (141 were of European origin) and more than 200 will be facing extinction in the near future.

There is little doubt that all modern cattle breeds (with the exception of mithan and Bali cattle) were derived from the auroch or wild ox (*Bos primigenius*). Three subspecies of the auroch formerly roamed over vast areas of North Africa (*Bos primigenius opisthonomus*), Asia (*B. primigenius nomadicus*), and Europe (*B. primigenius primigenius*). The auroch became extinct approximately 2000 years ago within most of its geographical range. Small populations survived in the forested parts of Central Europe, but, despite active protection, the last individual succumbed in 1627 in Jaktorowska Forest, near Warsaw, Poland.

A survey of mtDNA variation revealed that the most recent common ancestor of Western and Indian breeds of cattle lived between 330 000 and 1.7–2 million years ago – much earlier than the appearance of modern humans. Separation of African and European cattle ancestors occurred 22 000–26 000 years ago and therefore predates domestication of cattle. This suggests that each continental set of extant breeds originated as a result of separate domestication events in North Africa, the Middle East, and Southwest Asia. However, cattle domestication in Africa remains controversial, as the African mtDNA sequences differ by only few mutations from the taurine founding lineages of Southwest Asia.

The genetic affinity of European cattle breeds is much closer to the breeds from Anatolia and the Middle East (i.e., *B. primigenius nomadicus*) than to now-extinct European populations of the auroch *B. primigenius primigenius*. However, mtDNA sequencing points to the possibility of several, local introgressions from wild aurochs. In any case, the extant European breeds can be mostly considered as derivatives of cattle expanding some 5000 years ago from a center of domestication located in the region of the Fertile Crescent (the area encompassing southern Turkey, northern parts of Jordan, Syria, and Iraq). It is, therefore, unlikely that initial local

Table 1 Wild ancestors of major meat animals and poultry and approximate dates and places of their domestication

Species	*Wild ancestor*	*Date (years ago)*	*Place*
Domestic cattle[a] (*Bos taurus*) (*Bos indicus*)	Auroch (*Bos primigenius nomadicus*)	8 000–10 000	Middle East, India/Pakistan, and North Africa
Sheep[b] (*Ovis aries*)	Eastern Mouflon (*O. orientalis*) Argali (*O. ammon*)? Urial (*Ovis vignei*)?	8 000	Southwest Asia (Turkey and western Iran)
Goat[c] (*Capra hircus*)	Bezoar (*Capra aegagrus*)	9 000–11 000	Euphrates Valley, Zagros Mountains, and Eastern Anatolia
Pig (*Sus scrofa*)[d]	Eurasian wild boar (*Sus scrofa*)	9 000	Near East, China, India, and Southeast Asia
Domestic chicken[e] (*Gallus domesticus*)	Jungle fowl (*Gallus, gallus, G. sonneratii*)	8 000	Southeast Asia

[a]Beja-Pereira *et al.* (2006); Ajmone-Marsan *et al.* (2010).
[b]Tapio *et al.* (2006); Meadows *et al.* (2007).
[c]Naderi *et al.* (2008).
[d]Giuffra *et al.* (2000).
[e]Tixier-Boichard *et al.* (2011).
Source: Data compiled from various sources listed in Further Reading.

domestication contributed significantly to the establishment of European agriculture, even though separated events might have taken place in Southern Europe (Italy). A genome-wide single nucleotide polymorphism (SNP) analysis suggests that cattle were introduced into Europe sequentially, through Turkey and the Balkans. It then radiated via Central Europe and France, reaching the British Isles. Modern south European cattle breeds also carry genetic signatures of North African origin, most likely imported through the Iberian Peninsula.

Taurine cattle (*B. taurus*) were domesticated from the *B. primigenius nomadicus* 8000–10 000 years ago. The earliest, 7800-year-old, archeological evidence of *B. taurus* has been found in Anatolia (Turkey). Remains of *B. indicus* dated to be at least 4500 years old have been unearthed in Iran, Mesopotamia, and the Indus valley. The analyses of mtDNA suggest that zebu cattle must have been domesticated much earlier, some 8000–10 000 years ago. Archeological evidence of cattle herding from 7000 years ago points to Pakistan as a potential domestication center of zebu cattle.

The oldest (9000 years ago) African *Bos* remains that can be putatively associated with domestication were found in eastern Sahara, although its domestication remains controversial. In contrast to the extant humped African cattle, the earliest cattle were humpless. Although humped African cattle have the distinct morphological characteristics also present in Indian breeds, their mtDNA sequence is much closer to that of *B. taurus*. In contrast, the nuclear DNA of African breeds bears the signature of Indian zebu cattle. The apparent lack of mtDNA of zebu cattle in African breeds along with the presence of zebu cattle sequences in nuclear DNA of African breeds strongly suggests a deliberate breeding of African zebu-type females, bearing *B. taurus* mtDNA sequences, with zebu males of Asian origin. These males were most likely imported into Africa during the Arab invasions of the AD eighth century. Interestingly, as a secondary consequence of the slave trade, North African mtDNA sequences have been found in cattle from southern Portugal.

New World cattle breeds are descendants of cattle brought by Europeans as early as 1493, and bear genetic signatures of the taurine and indicine lineages, including African admixture. The latter may account for the elevated disease resistance of such breeds as Texas Longhorn.

Domestication of Sheep

Recent molecular phylogenies of the wild sheep, based on sequences of cytochrome *b* and Y chromosome (MSY), suggest that *Ovis* species consist of four phylogenetic groups, three of which (Moufloniform, Argaliform I, and Argaliform II) are native to Eurasia. Domestic sheep belongs to the Moufloniforms, along with three wild species: urial (*O. vignei*), eastern mouflon *O. orientalis*, and European mouflon (*O. musimon*). All of them produce fertile and viable offspring when bred in captivity. European mouflon is the only species to share a haplotype with domestic sheep, which agrees with its feral domesticated status, that has undergone male-mediated introgression with domestic breeds.

Eurasian wild sheep – eastern mouflon, urial, and argali (*O. ammon*) – have been suggested as potential progenitors of domestic sheep. Earlier studies indicated that one of the oldest domesticated forms of sheep probably originated some 8000 years ago from urial in the region of the Caspian Sea and was subsequently adopted by the people of the Middle East and later also by early European herders. However, recent mtDNA analysis separated the phylogenetic tree of domestic sheep into five distinct mitochondrial maternal lineages (hyplotype groups A–E), which suggest multiple, independent domestication events. Highly diverged lineages A–D are mainly found in the Caucasus, lineages A–C in Central Asia, whereas A and B in the eastern edge of Europe. Lineage C sequences have also been found in sheep from Portugal, most likely indicating gene flow from the Fertile Crescent to the Iberian Peninsula. The European mouflon is aligned to lineage B. Lineage D has been identified in a single animal sampled from the north Caucasus and therefore awaits further confirmation.

Southern European sheep breeds have higher genetic diversity and are less genetically differentiated compared with breeds from northern Europe. This most likely reflects geographic gradient with highest genetic diversity close to the center of domestication, in the Near East, which still remains a sheep genetic diversity hotspot. According to FAO estimates, 36% of the extant sheep breeds are either extinct or endangered.

Domestication of Goats

Domestication of goats (*Capra hircus*) may have played a key role in the Neolithic agricultural revolution and the spread of agriculture from its earliest homelands. The extreme ability of goats to thrive on poor-quality fodder and to cope with harsh environmental conditions makes them the most geographically widespread, domesticated herbivorous species, ranging from cold Siberian mountains to the driest parts of North Africa.

Archeological evidence suggests that the bezoar (*Capra aegagrus*), the wild progenitor of the domestic goat, was the first wild ungulate to be domesticated. Domestication most likely took place in the region of the Fertile Crescent. Recent analyses of genetic diversity of the domestic goat have revealed six distinct mtDNA lineages, with more than 90% of analyzed individuals belonging to lineage A. This lineage, as well as lineage C, most likely originated in Eastern Anatolia, where they are common in wild populations. This points to Eastern Anatolia as the major center of goat domestication. Lineages B, D, F, and G are found in less than 8% of domestic goats and were most likely integrated to the gene pool following independent, small-scale domestications events in Northern and Central Zagros Mountains. However, recent analyses do not confirm an independent domestication in the Indus Valley.

Goats must often have been human companions, both in commercial trade as well as during migrations and explorations. The geographic distribution of genetic variation of the extant lineages of goats is much less diversified than that in cattle. Intercontinental differences between goat populations account for only 10% of the total mtDNA variation, whereas genetic differences between cattle breeds on different continents explain more than 80% of the variation. This attests to an intensive intercontinental gene flow between goat populations, which resulted from long-distance transportation of goats along ancient trading routes.

Domestication of Pigs

There are two major forms of domestic pigs, European and Asian, whose distinctiveness was recognized by earlier authors including Charles Darwin. Because of marked, morphological differences, both forms were assumed to originate from different species of wild boar. However, recent mtDNA analysis revealed that wild boar originated in western islands of Southeast Asia, and then dispersed to Indian subcontinent. Subsequent radiation of *Sus scrofa* into East Asia was followed by a progressive spread across Eurasia and into Western Europe.

The time since divergence from the common ancestor of European and Asian forms of pig falls well outside the known history of animal domestication and has been estimated at 500 000 years ago. This provides strong evidence for independent domestication of pigs in Europe and Asia, approximately 9000 years ago. However, domestication was predated by a long period of wild boar management that started about the time of the Pleistocene/Holocene transition, as exemplified by the man-made introduction of this species to Cyprus 12 000 years ago. Likewise, phylogeographic structure of pig mitochondrial sequences attests to a significant human contribution to dispersal of this species across Europe (particularly the Mediterranean islands) and the Middle East.

Initially, domestic pigs managed in Europe during the Neolithic Era were of Near Eastern ancestry. By the early fourth millennium BC, local European wild boars were also domesticated. This domestication cannot be, therefore, regarded as truly independent, but rather as a consequence of the introduction of Near Eastern domestic pigs. Once domesticated, European pigs rapidly replaced the introduced Near Eastern pig lineages throughout Europe in a relatively short period of approximately 500 years and later began replacing indigenous Near Eastern pigs.

In Asia, pigs were independently domesticated in at least six locations: China, India, peninsular Southeast Asia (three locations), and off the coast of Taiwan. Some extant European pig breeds (e.g., European Large White) are characterized by high frequency of mtDNA haplotypes of Asian origin. This is most likely a legacy of well-documented European breeding practices of the eighteenth and nineteenth centuries, when Asian sows were used to improve the contemporary breeds.

Domestication of Poultry

Although the meat yield of wild birds was far lower than that of much bigger mammals, attempts to domesticate fowl have a long history and have been independently undertaken on all continents inhabited by humans. Various breeds of duck and geese species were successfully domesticated in Eurasia, turkeys in Mesoamerica, and guinea fowl in Africa, whereas the extant breeds of Muscovy duck originated in South America. The earliest remains of domestic chickens were excavated in numerous archeological sites along the Yellow River in China and dated to be at least 7500 years old. They were also found in the Indus Valley in Pakistan. The 4000-year-old remains unearthed in Spain and Ukraine attest an incredibly rapid spread of the domestic chicken. Approximately 3600 years ago, chickens were introduced to New Guinea and quickly reached Pacific islands during Austronesian expansion. This fast initial dispersion can be attributed primarily to the ease of transportation of the fowl. Another important factor could have been a religious significance attached to the chicken as a divine offering, widespread in different parts of the world.

Earlier analyses of mtDNA sequences pointed to the area of Thailand as a single location of the domestication of chickens. Recent extensive survey of mtDNA sequences from domestic chickens and four red jungle fowl subspecies (*G. g. gallus*, *G. g. bankiva*, *G. g. spadiceus*, and *G. g. jabouillei*) identified nine highly divergent lineages A–I. Seven of them consists of both wild and domestic individuals and are confined to Asia, which strongly suggests that chicken domestication took place independently in different regions of India and China. However, the ubiquitous presence of the E lineage, native to India, suggests that worldwide expansion of domestic chickens took place from there.

Changes in Species under Domestication

Behavioral Changes of Animals under Domestication

Domestic breeds diverged from their wild ancestors in many ways. Because heritabilities of behavioral traits are usually higher than heritabilities of anatomical and physiological traits, one can speculate that the development of domestic phenotypes started with changes of the behavior of animals undergoing domestication. The most obvious change was the loss of fear of humans. Equally important behavioral changes involved increasing of the threshold of within-species and between-species aggression. This has become essential for the successful maintenance of stocks of domesticated animals living under population densities far exceeding maximum densities tolerated under natural conditions, often next to large stocks of unrelated species. Perhaps the most important effect of domestication on behavior was a reduction of the sensitivity to changes in the unfamiliar environment. This stemmed from reduced emotional reactivity to handling by humans and ease of adaptation to novel conditions, which greatly contributed to the high reproductive rates essential for the success of artificial selection.

It is important to note that a successful domestication required the fulfillment of all conditions mentioned above and therefore could have been achieved only with particularly prone individual animals. For example, recent computer simulations revealed that cattle domesticated in the area of modern Iran originated from just 80 female aurochs, which attest to the difficulties of the early stages of cattle herding and breeding.

Morphological and Anatomical Changes of Animals under Domestication

Domestication has also resulted in profound changes in the morphology and anatomy of animals. Primitive breeds of domestic pigs, sheep, goats, and cattle were generally smaller than their wild ancestors, which most likely make them more manageable, as pointed out by Francis Galton in 1865. Chickens, in turn, were selected to be larger. The whole brain volume of domesticated animals is 10% less than in their wild relatives. The decrease of brain sensory centers is particularly

clear cut and corresponds well with the observed behavioral differences between domestic animals and their wild relatives.

An incredible increase of growth rate of modern meat-type strains of domestic fowl is one of the best examples of both the power of intense, directional selection and its negative side effects. During the late 1940s, broilers took approximately 90 days to grow to slaughter body mass of 1800 g. Now it takes less than half of this time to reach the slaughter mass of 2500 g. Surprisingly, most of this progress has arisen through an increase of growth rates during the first two weeks of postembryonic development. However, this impressive selection progress also incurred unavoidable costs associated with increasing incidence of metabolic diseases such as 'heart failure syndrome,' sometimes killing 10% of a broiler flock. In addition, changes in body proportions, such as that resulting from selection for large breast size in domestic turkeys, have severely impaired their mating behavior, and selection for intense egg production resulted in total loss of incubation and brooding behavior in laying hens.

Genetic Footprints of Domestication

Studies on genetics of domestication have been greatly advanced, thanks to the development of the quantitative genetics theory and molecular genetics techniques. Although still in its early stages, identification of quantitative trait loci (QTL) and whole genome sequencing have become powerful approaches that have been recently applied to detect genetic footprints of domestication. For example, one of the most important selective sweeps already identified in poultry occurred at the locus for thyroid-stimulating hormone receptor (TSHR), which underlies hormonal regulation of reproduction, photoperiod, and metabolic rates. Research on the differences in expression of polygenic traits in pigs and wild boars has resulted in the identification of loci, such as insulin-like growth factor 2 (IGF2) associated with muscularity, fat accumulation, and heart size. Domestication has also almost certainly led to near fixation of naturally occurring mutations of gene coding for GDF-8 (myostatin, a negative regulator of skeletal muscle growth) in several cattle breeds such as Belgian Blue. However, high density single nuclear polymorphism (SNP) genotyping has indicated that 50% of ancestral genetic diversity has been already lost in the extant cattle breeds.

Consequences of Domestication for Meat Composition

Ample anthropological and ethnographic evidences indicate that humans are evolutionarily preadapted to a diet that includes meat. There is also little doubt that scavenged or hunted ruminants were the main source of meat throughout early human history. Human dietary lipid requirements are, therefore, more likely to match the lipid composition of wild ruminant tissues. This composition is qualitatively and quantitatively different from the lipid profiles of meat of domesticated cattle, which may have important consequences for the health of modern consumers.

The most significant difference between meat composition of wild and domesticated ruminants is the relative amount of fat per unit mass of muscle tissue. Meat of grain-fed beef (trimmed of all adherent fat) contains 2–3 times more fat (mean 5.6 g per 100 g tissue) than the muscle of wild ungulates such as antelope, deer, or buffalo (mean 2.2 g per 100 g tissue). The high fat content of beef muscle tissues is mainly associated with the formation of intramuscular fat deposits – a phenomenon known as marbling of meat – which is largely absent in wild ruminants. This intramuscular fat is rich in triacylglycerols and resembles subcutaneous fat with respect to the profile of fatty acids.

Another important difference is that muscle tissue of wild ruminants contains a higher proportion of polyunsaturated fatty acids (PUFAs) than muscles of domestic cattle. Up to 30% of all fatty acids contained in game meat are polyunsaturated, whereas PUFAs account for only 10% of FAs in beef. Increased levels of saturated fat in beef (particularly 12:0, 14:0, and 16:0 fatty acids) have substantially contributed to increased dietary fatty acid levels in the modern westernized diet. This, in turn, may be associated with an increased risk of cardiovascular disease if not taken into account in a diet. Moreover, muscles of domesticated cattle are much poorer in long-chain PUFAs (particularly *n*-3 long-chain PUFAs), as compared with muscles of wild ruminants. It is important to note, however, that there are also significant differences in the muscle fat content and composition between pasture-fed and grain-fed cattle. Muscles of grain-fed cattle are particularly rich in saturated and monounsaturated fats, whereas the lipid profiles of pasture-fed cattle resemble those of game meat. Thus, the differences in meat composition between wild and domesticated ungulates can be largely attributed to the practice of feeding cattle grain, rather than to physiological changes incurred by the process of domestication.

The Future of Domestication of Meat Animals

The incredible progress of modern biology has made it possible not only to maintain but also to breed in captivity almost all terrestrial mammals. Some of them such as moose (*Alces alces*), red deer (*Cervus elaphus*), or American bison (*Bison bison*) have been domesticated to some extent in the past century. They are, however, generally still unsuitable for intense meat-producing farming, they cannot be herded for a long time, and it is unlikely that they will soon join a very short list of major meat-producing species. However, together with primitive breeds of already domesticated animals, they can serve as a source of meat of very desirable protein and fat profiles. Growing health concerns of consumers may paradoxically give rise to selection of meat animals: toward emulation of the meat composition of their wild ancestors.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. Chemical Analysis for Specific Components: Major Meat Components. Human Nutrition: Cancer Health Concerns; Cardiovascular and Obesity Health Concerns. Slaughter-Line Operation: Poultry. Species of Meat Animals: Cattle; Game and Exotic Animals; Pigs

Further Reading

Ajmone-Marsan, P., Garcia, J.F., Lenstra, J.A., *et al*., 2010. On the origin of cattle: How Aurochs became cattle and colonized the world. Evolutionary Anthropology 19, 148–157.

Beja-Pereira, A., Caramelli, D., Lalueza-Foxe, C., *et al*., 2006. The origin of European cattle: Evidence from modern and ancient DNA. Proceedings of the National Academy of Sciences of the USA 103, 8113–8118.

Cordain, L., Watkins, B.A., Florant, G.L., *et al*., 2002. Fatty acid analysis of wild ruminant tissues: Evolutionary implications for reducing diet-related chronic disease. European Journal of Clinical Nutrition 56, 181–191.

Diamond, J., 1997. Guns, Germs, and Steel: The Fates of Human Societies. New York: Norton.

Diamond, J., 2002. Evolution, consequences and future of plant and animal domestication. Nature 418, 700–707.

Giuffra, E., Kijas, J.M.H., Amarger, V., *et al*., 2000. The origin of the domestic pig: Independent domestication and subsequent introgression. Genetics 154, 1785–1791.

Larson, G., Liu, K.R., Zhao, X., *et al*., 2010. Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. Proceedings of the National Academy of Sciences of the USA 107, 7686–7691.

Luikart, G., Gielly, L., Excoffier, L., *et al*., 2001. Multiple maternal origins and weak paleographic structure in domestic goats. Proceedings of the National Academy of Sciences of the USA 98, 5927–5932.

McTavish, E.J., Decker, J.E., Schnabel, R.D., 2013. New World cattle show ancestry from multiple independent domestication events. Proceedings of the National Academy of Sciences of the USA. doi:10.1073/pnas.1303367110.

Meadows, J.R.S., Cemal, I., Karaca, O., *et al*., 2007. Five Ovine mitochondrial lineages identified from sheep breeds of the Near East. Genetics 175, 1371–1379.

Naderi, S., Razaei, H.-S., Pompanon, F., *et al*., 2008. The goat domestication process inferred from large-scale mitochondrial DNA analysis of wild and domestic individuals. Proceedings of the National Academy of Sciences of the USA 105, 17659–17664.

Price, E.O., 1999. Behavioral development in animals undergoing domestication. Applied Animal Behaviour Science 65, 245–271.

Tapio, M., Marzanov, N., Ozerov, M., *et al*., 2006. Sheep mitochondrial DNA variation in European, Caucasian and Central Asian areas. Molecular Biology and Evolution 23, 1776–1783.

Tixier-Boichard, M., Bed'hom, B., Rognon, X., 2011. Chicken domestication: From archeology to genomics. Comptes Rendus Biologie 334, 197–204.

Rubin, C.-J., Zody, M.C., Eriksson, J., *et al*., 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464, 587–593.

Zeder, M.A., Emshwiller, E., Smith, B.D., *et al*., 2006. Documenting domestication: the intersection of genetics and archaeology. Trends in Genetics 22, 139–155.

Relevant Websites

http://www.ansi.okstate.edu/breeds/cattle/
Breeds of Livestock.

http://www.fao.org/dad-is/
Domestic Animal Diversity Information System (DAD-IS).

http://en.wikipedia.org/wiki/Cattle
General Information on Cattle.

http://en.wikipedia.org/wiki/Jungle_fowl
General Information on Jungle Fowl.

http://www.fao.org/docrep/w7540e/w7540e0h.htm
Wildlife Farming and Domestication.

Pigs

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Glossary

Allometric growth The increase in size of different organs or parts of the same animal at differing rates.
Cutability An estimation of boneless closely trimmed retail cuts derived from a carcass.
Iodine value An indication of unsaturation levels in a fat sample. It used to be reported in units of grams of I per 100 g of tissue. In chemistry, it is the mass of iodine in grams that is consumed by 100 g of tissue.
Lairage A period of rest between the time the animal is delivered to the slaughter facility until the animal is actually slaughtered.
Metabolic modifier Compounds that are fed, injected, or implanted in animal to improve production efficiency.

Introduction

The global pork industry produces more than 100 million tons of pork annually and is the most consumed meat animal protein in the world. China is the world's leader in pork production and provides approximately 50% of the global supply. The European Union is estimated to be second at more than 20% and the US is third at a little more than 10% of the worlds pork supply. Together Brazil and Russia account for a little more than 5% of the world's pork production. Recent improvements in the efficiency and volume of pork production are largely related to increases in sow productivity and marketing of pigs at heavier slaughter weights than have been historically reported. Pig production varies greatly around the world. Some production systems, such as Australia and the UK, raise and market entire males, but slaughter them at younger ages before reaching puberty to avoid undesirable boar odor compounds. Other markets, such as the US and Brazil, generally castrate male pigs at a very young age to prevent the development of boar odors. Some parts of the world have very sophisticated vertical integration marketing systems, whereas others still rely on an open market system to buy and sell pigs for food production. Just as there are differences in how pigs are raised and sold around the world, there are differences in how the value of pig carcasses is determined. The objective of this article is to discuss growth rates and composition of pork carcasses, metabolism of early postmortem carcasses and how it influences meat quality, differences in dressing percentage, fabrication techniques, and fat quality.

Growth and Carcass Composition

Body composition changes dramatically as animals grow. Pigs have the greatest proportion of muscle at birth but it slowly declines as they grow and accumulate fat. As a rule, soft tissue comprises more than 50% water and can be as high as 90%. Protein is usually second in terms of weight, except in the cases of very fat animals. Therefore, fat is the most variable tissue in the body and can be second or third in terms of live body weight. After all, fat serves the body as an energy reserve in times of need. Additionally, fat provides insulation, protection of vital organs, and generation of heat. From a chemical element standpoint, greater than 50% of body mass is oxygen, approximately 20% carbon, 10% hydrogen, and approximately 3% nitrogen. These are the elements that make up water, protein, carbohydrate, and fat. As a rule, animals partition nutrients for tissue development in order of skeleton, muscle, and then fat. They tend to deposit fat in the mesenteric regions first followed by perirenal (mesenteric and perirenal depots are sometimes jointly called visceral fat), then subcutaneous (backfat), intermuscular (seam), and finally intramuscular (marbling) fat deposition. Even so, it is important to remember that fat accretion is not a linear process. Pigs simultaneously deposit fat in different anatomical regions of the body throughout development. Subcutaneous fat makes up the largest portion of total fat in pork carcasses and is being deposited at a faster rate than intermuscular fat toward the end of pigs' growth. Intramuscular fat is generally considered the last fat depot to develop and thus makes up the least proportion of total fat. As mentioned, after animals are born, the proportion of muscle in the body begins to decrease and the proportion of fat increases. The rate and magnitude at which fat accumulates and muscle decreases can be breed and sex dependent. Heritage pig breeds, such as Berkshire, tend to grow at a slower rate and finish with a greater proportion of fat than most high-lean, terminal crossbred pigs. Heritage breeds are often referred to as earlier-maturing breeds because of the increased proportion of fat, slower growth rate, and smaller mature body frame size relative to more commercially raised composite breeds. Differences in body composition are more pronounced when pigs of different mature frame sizes are compared at the same weight, rather than at the same age. At equal body weights, barrows will be fatter than gilts and gilts will be fatter than entire males when assessed at the area of the tenth rib. Conversely, entire males will have a greater percentage of fat-free lean than gilts and gilts will have a greater proportion of fat-free lean than barrows. During the growth period, young entire males will have a greater percentage of

lean than fat. Barrows will eventually reach a point that deposition of fat will exceed deposition of muscle. During the last few weeks of the finishing phase, barrows tend to consume more feed than entire males and gilts. Gilts tend to gain less weight per day than barrows and usually require more days on feed to reach a desired body weight than barrows.

Pigs fed a diet that is greater in protein concentration than considered necessary for growth will have a greater lean meat percentage. Additionally, seasonality can play a role in carcass composition. In warm weather, pigs tend to eat less and gain less weight per day than during colder seasons. In production systems where the flow of pigs through the system is tightly managed and feed costs are expensive, it might not be possible to allow pigs to stay on feed to achieve the desired ending live weight. Pigs raised during the hot months of the year tend to be lighter, have less backfat, and greater estimated lean percentages than pigs raised during the cold months.

Metabolic modifiers are compounds that are injected, fed, or implanted to improve growth rate, carcass cutability, or other production enhancing characteristics. There are currently two metabolic modifiers used in pork production in various parts of the world to influence carcass composition. The first is ractopamine hydrochloride (trade name Paylean™) and is commonly used in the US and Brazil pork production operations. Ractopamine is a β-adrenergic agonist that increases growth rate, feed efficiency, and carcass leanness of finishing pigs fed a diet with at least 16% crude protein for the last 20–41 kg before harvest. Feeding ractopamine will slightly reduce tenth rib fat thickness and increase loin muscle area or depth. This translates into a modification of allometric growth rates of various primal pieces and an increase in carcass cutability. The second metabolic modifier is the use of immunological castration as an alternative to physical castration. Immunological castration has been adopted in some parts of the world. It is achieved through a series of two injections that act as an immunological metabolic modifier by changing the natural hormone profile after the second injection. Immunological castration occurs later in life than physical castration and is used to suppress testicular function to reduce boar taint in intact male pigs intended for harvest. The technology was developed in Australia and is now approved for use in more than 60 countries worldwide. Immunologically castrated male pigs also have improved growth rate, feed efficiency, and carcass cutability when compared with physically castrated male pigs.

Carcass Classification

In various parts of the world, a pig's value is determined by carcass weight and an estimation of carcass leanness. In the US, pigs generally are marketed from a single barn over potentially several weeks. The majority of pigs are sold on a matrix type basis that offer premiums for carcasses meeting certain specifications and charges discounts to carcasses that do not comply with the desired carcass weight and lean meat percentage specifications. By doing this, producers are able to better manage carcass weight as well as carcass composition. This can be accomplished by marketing the heaviest pigs within a pen first, and then lighter pigs in subsequent weeks. This increases allotted space per pig in a pen, decreases competition for feeder access, and allows slower growing pigs more time to reach a desired compositional end point. This marketing approach allows producers to be rewarded for marketing pigs that have a desired carcass weight (not too heavy or too light) with minimal carcass weight variation and a desired percentage of lean meat. In many pork slaughter facilities, estimation of carcass leanness is carried out at the very end of the harvest process. Carcass leanness estimations can be accomplished using a variety of technologies. The use and application of these technologies vary greatly around the world. Some examples include: the Fat-o-Meater, Hennessy probe, animal ultrasound system, or a simple ruler to measure fat thickness. Some of these technologies are more invasive than others. So, the method used to determine carcass composition will vary among packers and regions of the world. Other technologies, such as dual energy X-ray absorptiometry, are available to determine carcass leanness, but might be prohibitive in a large-scale fast moving production facility. As fat thickness or fat content is the most variable tissue in carcasses, it plays a very influential role in estimating carcasses lean percentage. Therefore, fat thickness is included in nearly every regression equation, regardless of technology used, to estimate carcass lean percentage. Even though the value of carcasses to the live pig producer is determined by carcass weight and estimation of carcass leanness, the value of carcasses to packers is determined by the cutability of carcasses or the amount of meat products derived from those carcasses. In the US, pork carcasses are fabricated into five primal pieces (Figure 1). Those pieces are the ham (22–25% of the chilled half carcass), loin (20–22% of the chilled half carcass), picnic shoulder (ventral region of the shoulder, which accounts for approximately 9–11% of the chilled half carcass), belly (12–15% of the chilled half carcass), and Boston butt shoulder (dorsal region of the shoulder, which accounts for approximately 8–10% of the chilled half carcass). The Boston butt, picnic, loin, and ham are often referred to as the four lean cuts or lean carcass cutability. When the belly is included, the calculation is referred to as carcass cutability. Lean carcasses generally have a greater cutability than fatter carcasses because there is less fat (also less valuable) to trim away, thereby a greater percentage of carcasses can be sold as lean meat.

In Europe, carcasses are classified based on lean meat percentage using the EUROP classification system that is based on

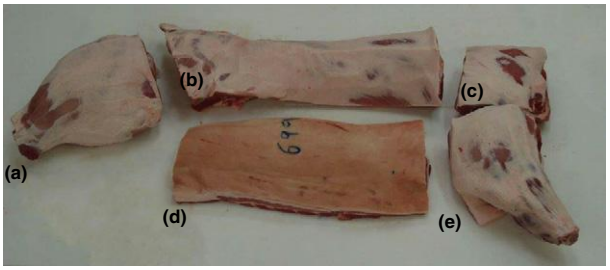

Figure 1 A pork carcass fabricated into the five US primal pieces: (a) ham (22–25% of chilled half carcass), (b) loin (20–22% of chilled half carcass), (c) Boston butt (8–10% of chilled half shoulder), (d) belly (12–15% of chilled half carcass), and (e) picnic shoulder (9–11% of chilled half carcass).

muscle and fat thickness. In that system, E has the greatest lean meat percentage (>55%), U=50–55% lean meat, R=45–50% lean meat, O=40–45% lean meat, and P is the least lean meat percentage (<40%). Similar to the US, European packers also estimate lean meat percentage with objective tools such as a caliper to determine midline fat thickness, Fat-o-Meater and Hennessy optical probes, and various ultrasonic scanners.

Dressing Percentage

Dressing percentage, or carcass yield as it is sometimes referred to, is the proportion of ending live weight yielded after animals have been stunned (desensitized), exsanguinated, skinned or scalded, and eviscerated. The average dressing percentage of pigs in the US is approximately 74%. Average dressing percentages will vary in other parts of the world depending on several factors. Sex of animals is one such factor with entire males usually having a lesser dressing percentage than castrated male or female pigs. This can be partially attributed to the presence of testicles of entire male pigs, which accounts for approximately 0.5–0.7% of ending live weight. Reduced fatness of entire male pigs also decreases dressing percentage. Diet can also impact dressing percentage. Finishing diets that are high in fiber can reduce dressing percentage. It is thought that diets rich in fiber, such as those that contain distillers dried grains (ethanol coproducts), increase intestinal mass, which is approximately 3% of ending live weight, and reduce dressing percentage due to a larger portion of live weight from intestinal mass. In addition to intestinal mass, transport distance, gut fill, and time spent in lairage also will influence dressing percentage. Lairage is the time from when animals arrive at the harvest facility until animals are slaughtered. Pigs are not usually fed during transport and lairage unless they jointly exceed 24 h. Therefore, as transport and lairage time increases, gut fill generally decreases and thereby increasing the dressing percentage. Gut fill, even after a 15-h lairage period can be as much as 5% of ending live weight. Not only can things such as sex, diet, composition, and transportation/lairage loss influence dressing percentage, but the actual dressing process itself can also greatly impact dressing percentage. Some parts of the world leave the head attached to carcasses or leave the front feet intact. The head can account for 5–7% of ending live weight and the front feet, depending on the anatomical removal location, can account for approximately 1% of ending live weight. Skin can account for approximately 4–6% of ending live weight when carcasses are skinned rather than scalded and can be much greater depending on the skill level of persons skinning the carcasses. Other visceral organs that can influence dressing percentage are the heart (~0.4%), liver (~1.7%), and the kidneys (~0.5%) and can be influenced by a variety of management practices.

Time of harvest in relation to the biological growth curve will also influence dressing percentage. Live animals that are destined for harvest can be divided into a carcass component and a noncarcass component. Early in life, noncarcass components, such as blood, viscera, and skin in some cases will make up a greater proportion of live weight than later in life. As animals reach maturity, visceral growth is completed and animals begin to accumulate fat at a more rapid rate. Carcass components begin to increase in proportion to live weight relative to noncarcass components, thereby increasing the dressing percentage. Therefore, larger, heavier, and older animals usually have a greater dressing percentage than young, growing animals. Even though noncarcass components are considered byproducts, they represent a great deal of value to the pork industry in the US and other countries. Drop value, or the value of ears, hearts, livers, tongues, snouts, salivary glands, and other noncarcass component byproducts can be worth approximately US$5 per cwt per pig or in some cases even more.

Meat Quality

Shortly after animals are stunned, they are exsanguinated. When animals are exsanguinated, the circulatory system is disrupted and homeostasis is lost. A series of metabolic and biochemical reactions take place in an attempt to regain homeostasis. Thus, exsanguination begins the conversion of muscle to meat. During the conversion of muscle to meat, tissue pH declines from approximately 7.2 in living muscle to an ultimate pH of approximately 5.7 but can range from 5.2 to 6.5 in very extreme cases. In rare cases, the magnitude may be even greater. Ultimate pH, or the pH after postmortem metabolism has concluded, usually between 12 and 24 postmortem, is often the first topic of discussion when evaluating meat quality because it shares the greatest relationship with other meat quality parameters such as color, water-holding capacity, and texture or firmness. Ultimate pH is measured as the inverse log of the $[H^+]$ ion concentration. In living animals, the circulatory system transports oxygen, dissipates heat, and removes waste from various tissues. As blood is removed during the conversion of muscle to meat, carcasses undergo transition from aerobic postmortem metabolism to anaerobic postmortem metabolism. In an anaerobic environment, the carbohydrate source used to produce adenosine triphosphate is converted from pyruvate into lactic acid rather than from pyruvate to acetyl CoA as is the case in living muscle. As there is no blood available to remove lactic acid, it begins to accumulate and results in postmortem muscle pH decline. The rate and magnitude of pH decline will have noticeable effects on meat quality. When little carbohydrate is available at the time of death, usually due to chronic stress, very little pH decline occurs. This condition is referred to as dark, firm, and dry (DFD). Water-holding capacity is inversely related to ultimate pH. So, in DFD conditions, water-holding capacity is at its greatest. Water is bound tightly within the muscle cells and makes the surface of the meat appear very dry. When the surface of the meat is dry, light is absorbed into the tissue rather than reflected or scattered from the surface. This causes the surface of the meat to appear dark (similar to a color score of 6; Figure 2). A condition opposite to DFD is known as pale, soft, and exudative (PSE). This condition occurs in part due to acute stress shortly before the animals are harvested. Excessive available glycogen at the time of death can result in a rapid rate of pH decline when carcass temperatures are relatively warm. The combination of low pH and high temperatures denatures sarcoplasmic and myofibrillar proteins. When this happens, water becomes loosely associated with muscle cells

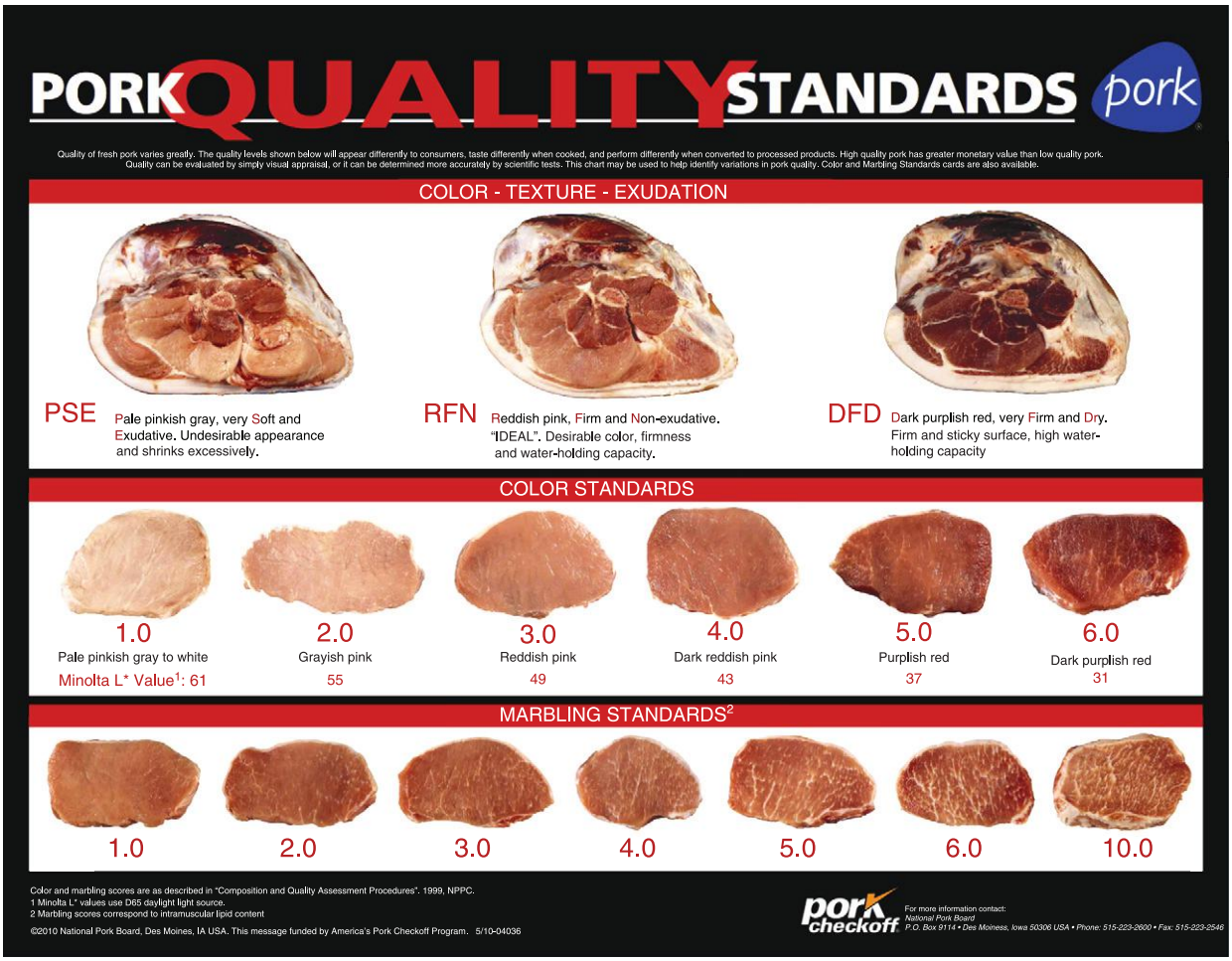

Figure 2 Pork quality standards for color–texture–exudation of hams (top), color standards of loins (middle) and marbling (bottom). Provided courtesy of the National Pork Board and Pork Checkoff.

and tends to pool on the surface. This is what is referred to as exudative. Moisture on the cut surface of meat tends to reflect rather than absorb light making the color appear light (similar to a color score of 1; Figure 2). Proper environmental conditions and handling practices can aid in preventing PSE and DFD from occurring. Another metabolic condition is referred to as acid meat. Acid meat is a condition sometimes found in the Hampshire breed where excess glycogen is stored in the muscle and results in very low ultimate pH values when animals are harvested. This condition is different from PSE, however, in that the rate of pH decline is normal but the magnitude of pH decline is greater. Pigs that store excessive amounts of glycogen in their muscles are suspect for extended postmortem pH decline and a greater concentration of lactic acid in the postmortem tissue. These pigs are referred to as having a high glycolytic potential. This phenomenon is often referred to as the 'Hampshire effect' caused by the Rendement Napole gene. The dominant allele (RN^-) is responsible for the increased muscle glycogen levels. The RN^- mutation is a single-nucleotide polymorphism of a gene that encodes for a regulatory subunit of adenosine monophosphate-activated protein kinase. New literature available now indicates that postmortem pH decline may be more complex than originally thought.

All three of these metabolic conditions are a concern for the pork industry because they negatively influence palatability. Palatability is generally referred to as the combination of tenderness, juiciness, and flavor of cooked meat. These three things together will largely impact consumers eating experiences. However, color is often cited as the primary parameter involved in consumers intent to purchase. Myoglobin is the sarcoplasmic protein that gives meat its color. Myoglobin concentration tends to increase as animals age due to a loss of affinity to oxygen. As a rule, however, pigs have approximately 2 mg of myoglobin per gram of muscle. Entire males tend to have a slightly greater myoglobin concentration than castrates or gilts.

Producers are not often directly compensated for lean muscle quality. In some cases, such as product meeting export specifications, a premium may be offered but, in general, there are no value-based premiums for meat quality parameters. This is largely because quality is subjective in nature and can be difficult to measure in real time. Tenderness is often the most influential parameter in determining the eating experiences of the consumers. A new technology is being developed to classify pork loins based on tenderness levels. Pork is generally considered tender, but the genetic selection of lean fast

growing pigs may negatively influence tenderness. Visible and near-infrared reflectance spectroscopy can allow packers to noninvasively predict tenderness on the fabrication line of plants.

Eating meat provides several important dietary nutrients. Meat consumption directly contributes to a low glycemic index because meat is low in carbohydrates. Dietary protein from meat provides necessary amino acids needed for muscle growth and maintenance of tissue. Meat consumption is an excellent source of minerals such as iron, calcium, and zinc. Meat is also a good source of all four fat soluble vitamins (A, D, E, and K). Pork, in particular, is a good source of B vitamins. Pork consumption also provides selenium, which is a natural antioxidant via glutathione peroxidase.

Fat Quality

Over the past couple of decades, pigs have become leaner. Leaner pigs tend to have greater concentration of polyunsaturated fatty acids (PUFA) than fat pigs. The type of fat (saturated or unsaturated) in various fat depots can impact consumers perceptions, shelf-life, processing capabilities, and bacon characteristics of pork products derived from those fats. Fat quality can be described using a variety of parameters, but is most commonly discussed in terms of iodine values. An iodine value is an indication of the level of unsaturation in fat samples. Iodine values are most commonly calculated using a regression equation based on fatty acid concentrations: iodine value (IV) = 16:1 (0.95)+18:1 (0.86)+18:2 (1.732)+18:3 (2.616)+20:1 (0.785)+22:1 (0.723). More recently, a similar equation has been offered that includes most of the previous coefficients, but also includes some longer chained fatty acids. A newer equation for iodine value is as follows: IV = 16:1 (0.95)+18:1 (0.86)+18:2 (1.732)+18:3 (2.616)+20:1 (0.795) +20:2 (1.57)+20:3 (2.38)+20:4 (3.19)+20:5 (4.01)+22:4 (2.93) +22:5 (3.68)+22:6 (4.64). As IV increases, fat firmness decreases and shelf-life of products becomes shorter. Lipid oxidation is one of the primary processes involved in quality losses of pork products. The oxidative breakdown of PUFA leads to rancidity and development of undesirable odors and flavors. Fat quality can also be assessed by measuring the thickness of the belly. Generally speaking, fatter pigs have thicker bellies and a greater proportion of saturated fatty acids than lean pigs. This means the bellies are firmer and easier to slice into bacon. Pork fat can range from less than 25% PUFA to greater than 35% PUFA and is dependent on things such as diet, sex, season, fat depot, and a variety of other factors. Generally, pork fat is less saturated than lamb or beef, but more saturated than fish or poultry. As mentioned above in the Section Growth and Carcass Composition, gilts tend to be leaner than barrows and thus usually have greater iodine values. It is also well known that fatty acid profiles of pigs are directly influenced by diet. Feed ingredients that are high in polyunsaturated fat will increase the polyunsaturated fatty acid concentration of pork fat and increase the iodine value of various depots. Increasing the dietary consumption of linoleic acid (an unsaturated fatty acid) during the last 6–8 weeks before harvest increases the calculated iodine value of backfat in finishing pigs. Finally, pigs tend to deposit fat anatomically from the head and tail end toward the visceral cavity. So, as pigs reach physiological maturity, they begin to deposit fat in the jowl and shoulder region earlier in their growth curve than they deposit fat in the loin and belly area.

In some parts of the world, the belly is the most valuable primal component of pork carcasses. Pigs bellies can be as much as 40% fat. So, it is important for packers to understand how diet and sex can influence fat quality and quantity. Soft, oily bellies are more difficult to slice and ultimately reduce the yields of salable products that can directly influence profits. Fat quality at all stages of pork production is quickly becoming of major interest to scientists because of its direct link to bacon processing. Leaner pigs have a greater percentage of lean, greater percentage of moisture, and a greater proportion of PUFA relative to fatter pigs. All of these things can lead to soft, oily bellies and reductions in bacon yield. Allowing pigs to become adequately fat (in markets that value bacon) will reduce slice yield variation and increase total bacon yield.

See also: Animal Breeding and Genetics: DNA Markers and Marker-Assisted Selection in the *Genomic* Era; Traditional Animal Breeding. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants. Nutrition of Meat Animals: Pigs

Further Reading

Aberle, E.D., Forrest, J.C., Gerrard, D.E., Mills, E.W., 2012. Principles of Meat Science, fifth ed. Dubuque, IA: Kendall/Hunt Publishing.

AOCS, 1998. Official Methods and Recommended Practices of the AOCS, fifth ed. Champaign, IL: American Oil Chemist Society.

Apple, J.K., Rincker, P.J., McKeith, F.K., *et al.*, 2007. Review: Meta-analysis of the ractopamine response in finishing swine. Professional Animal Scientist 23, 179–196.

Averette Gatlin, L., See, M.T., Hansen, J.A., Sutton, D., Odle, J., 2002. The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. Journal of Animal Science 80, 1606–1615.

Boler, D.D., Dilger, A.C., Bidner, B.S., *et al.*, 2010. Ultimate pH explains variation in pork quality traits. Journal of Muscle Foods 21, 119–130.

Boler, D.D., Kutzler, L.W., Meeuwse, D.M., *et al.*, 2011. Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. Journal of Animal Science 89, 2189–2199.

Briskey, E.J., 1964. Etiological status and associated studies of pale, soft, exudative porcine musculature. Advances in Food Research 13, 89–178.

Correa, J.A., Gariépy, C., Marcoux, M., Faucitano, L., 2008. Effects of growth rate, sex and slaughter weight on fat characteristics of pork bellies. Meat Science 80, 550–554.

Dikeman, M.E., 2007. Effects of metabolic modifiers on carcass traits and meat quality. Meat Science 77, 121–135.

Dunshea, F.R., Colantoni, C., Howard, K., *et al.*, 2001. Vaccination of boars with GnRH vaccine (Improvac) eliminates boar taint and increase growth performance. Journal of Animal Science 79, 2524–2535.

Hugo, A., Roodt, E., 2007. Significance of porcine fat quality in meat technology: A review. Food Reviews International 23, 175–198.

Meadus, W.J., Duff, P., Uttaro, B., *et al.*, 2010. Production of docosanhexaenoic acid (DHA) enriched bacon. Journal of Agriculture and Food Chemistry 58, 465–472.

Monin, G., Sellier, P., 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. Meat Science 13, 49–63.

Pauly, C., Spring, P., O'Doherty, J.V., Ampuero Kragten, S., Bee, G., 2009. Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac®) and entire male pigs and individually penned male pigs. Animal 3, 1057–1066.

Scheffler, T.L., Park, S., Gerrard, D.E., 2011. Lessons to lean about postmortem metabolism using the AMPKγ^{R200Q} mutation in the pig. Meat Science 89, 244–250.
Shackelford, S.D., King, D.A., Wheeler, T.L., 2011. Development of a system for classification of pork loins for tenderness using visible and near-infrared reflectance spectroscopy. Journal of Animal Science 89, 3803–3808.
Stein, H.H., Surshon, G.C., 2009. Board-invited review: The use and application of distillers dried grains with solubles in swine diets. Journal of Animal Science 87, 1292–1303.
Wood, J.D., Richardson, R.I., Nute, G.R., *et al.*, 2003. Effects of fatty acids on meat quality: A review. Meat Science 66, 21–32.

Relevant Websites

http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm117251.pdf FDA.
http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM260401.pdf FDA.
http://www.ams.usda.gov/mnreports/lswwklyblue.pdf United States Department of Agriculture (USDA).

Poultry

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Introduction

Poultry refers to birds such as chickens, turkeys, ducks, pheasants, geese, ostrich, emu, quail, and related species that are used for commercial production of meat. Many breeds of these species exist in the wild, but in commercial production many different breeds have been replaced by crossing several breeds with different desirable characteristics to produce a single breed or to develop hybrid lines with optimal meat yield and production efficiency. The focus of this article will be to review the classic and current species of poultry possessing desirable eating characteristics, and the nutritive value of poultry meat. The meat from almost all birds is commercially available. This review focuses not only on chickens, turkeys, ducks, geese, but includes ratites, which are flightless birds with rudimentary wings and without a sternum.

Chickens

Domestication of chickens began with red jungle fowl, which were raised in different regions of India and China approximately 1000 BC. However, the birth of the modern chicken industry in the US began in the early 1900s, when chicken production was characterized by small backyard flocks that were maintained to produce eggs for food or sold locally. The aged hens or roosters from the home flocks were cooked in pressure cookers and eaten only for a Sunday dinner or holiday meal. In the early to mid-1900s, there was no organized system for processing poultry, which made it impossible for poultry meat to be available for retail sale at grocery stores, but live birds could be purchased and processed at home. As the twentieth century progressed, large markets for poultry meat developed in the northeast and the poultry industry became a year-round enterprise with broiler (i.e., young meat-type chicken) production becoming concentrated in the southeast because of its warm climate, economical labor, and access to grain via rail and barge transportation. As the demand for white/breast meat increased in the 1950s, the chicken industry began to undergo vertical integration to bring control of the hatcheries, feed mills, growth facilities, and processing plants under a single corporate structure. Concurrently, the poultry companies ceased using dual-purpose (meat and eggs) breeds of chickens, and they began to breed and produce chickens specifically for meat production.

In the early days of the meat chicken production industry, it was common to grow dual-purpose breeds or to mate a dual-purpose rooster such as a Rhode Island Red with a Barred Plymouth Rock (Figure 1) hen to produce male progeny that were barred like their mothers and female progeny that were nonbarred like their fathers. The cockerel (young male chickens) could then be separated and raised for meat production and the pullets (young female chickens) could be kept as egg producers. However, to improve production, companies stopped using dual-purpose birds and developed separate lines of chickens to produce either meat or eggs. The meat chicken or broiler industry has traditionally used crosses between White Plymouth Rock and Cornish birds. Both breeds have a large body size, but Cornish birds tend to grow faster than White Plymouth Rocks. Although most commercial broilers originated from crosses between White Plymouth Rock and Cornish chickens, the broilers of the 1950s are very different from modern day broilers.

A commercial broiler chicken from a 2001 genetic background takes approximately 42 days to reach a bodyweight of 2.6 kg, a carcass weight of 2 kg, and a *Pectoralis thoracicus* (breast muscle) percentage of bodyweight of approximately 15.8%. In comparison, a commercial broiler from a 1957 genetic stock reaches a bodyweight of 1.8 kg, a carcass weight of 1.2 kg, and a *Pectoralis thoracicus* percentage of bodyweight of approximately 8.6% at 84 days of age. The most economically important chicken and turkey muscle is the *Pectoralis thoracicus*, which is composed of predominantly white (or fast twitch) muscle cells. Modern selection has decreased the time to market weight, increased the total size of the chicken, and increased the size of the *Pectoralis thoracicus* relative to bodyweight. Overall, modern selection techniques have profoundly changed the size of the chicken breast muscle (Figure 2).

Chicken has grown to be a popular meat product because the increases in production efficiency have led chicken to become a low cost, tasty alternative to traditional red meats, such

Figure 1 Barred Plymouth Rock–layer chicken. Photo Courtesy of Dr. James Petitte, North Carolina State University.

Figure 2 Broiler chickens. Photo Courtesy of Dr. Ken Anderson, North Carolina State University.

Figure 3 A 'typical' broiler chicken from a genetic background that was produced in 1957 (1957 Genetics). A 'typical' broiler chicken from a genetic background that was produced in 2001 (2001 Genetics). Both birds were killed at 42 days of age. Photo courtesy of Dr. Gerald Havenstein, North Carolina State University.

as beef and pork. Chicken has become a low cost product because modern selection and production techniques have reduced the time to produce a broiler chicken to approximately 6 weeks. More importantly, it is possible to manage large numbers of chickens on a single farm (Figure 3), lending poultry production to be very efficient and making it possible for the poultry industry to easily become vertically integrated. Almost every chicken produced in the US comes from contract growers who enter into partnerships with major poultry companies and who may supply the chicks that are grown on the farm. Therefore, the large corporation can determine the type and number of chickens grown, the feed provided to the animals, and all aspects of production. Subsequently, the same company buys the chickens from the growers, processes the chickens, and distributes the final product to the retailers. The vertical integration of chicken production almost eliminates the costly possibility of either an oversupply or shortage of chickens for the processor, and provides the opportunity for an efficient operation.

Chicken consumption in the US was approximately 9.9 kg per person (carcass weight) in 1955, whereas in 2009 it was approximately 42 kg per person (carcass weight). The increase in chicken consumption has not only occurred because of its relatively low cost but also because chicken meat tends to have consistent quality. Chicken tends to have few inherent tenderness issues because the vast majority of chickens grown in the US and other countries for fresh consumption are harvested at a young age (approximately 6 weeks) when the animals have low connective tissue levels. Furthermore, the normal rapid pH decline and rapid onset of rigor mortis (approximately 4 h) and subsequent ageing in poultry has made meat quality defects, such as cold-shortening or thaw rigor negligible issues. In particular onset of cold shortening occurs at a much lower temperature (approximately 2 °C) than for red meats, but rigor at elevated temperatures (if there is no stimulation) can still toughen meat. Electrical stimulation can be used and in fact can both enable early portioning without shortening and toughening thus enhancing tenderness, but is not commonly used. Meat quality problems in poultry tend to be related to a pink color in the normally white breast muscle or a pink color in processed meat products. Similarly, hemorrhaging during slaughter and bruising during processing also tend to be a quality problem. A pale soft exudative (PSE) condition has been described in chicken and turkey meat, but the biological basis for the PSE condition in poultry is not as well understood as PSE condition in pork.

The advent of new chicken products (chicken bologna, chicken nuggets, chicken hotdogs, and chicken wings) throughout the 1980s and the 1990s that are not only tasty but also convenient to prepare has fueled an increase in poultry consumption. The new chicken convenience foods have been successfully marketed for consumption at home and in the growing fast-food industry. However, one of the greatest reasons for the growth in chicken consumption may be the perception by health conscious consumers that chicken is a low-fat high protein source of healthy nutrition (Table 1).

Turkeys

Domestication of the turkey may have begun with the Mayas in Mexico and Central America, and there are two different subspecies of turkeys found in the wild. One subspecies is found in Mexico/Central America, whereas the other is found native to the US. The variety found in the US is large, has a characteristic bronze plumage, and it is likely that the commercial lineage of domestic turkeys arose from the turkeys native to the US. The current standard breeds of turkeys are the Broad Breasted Bronze, White Holland, Naragansett, Black, Bourban, Royal Palm, and Slate. The White Holland was the only commercial white turkey during the early twentieth century. Much of the success of the modern turkey industry lies with the Broad Breasted Bronze whose rapid growth rate made it an exceptional animal for turkey meat production. Modern turkey production uses a large white breed (Figures 4 and 5), which was likely developed from the Broad Breasted Bronze and the White Holland breeds.

Modern turkey production/consumption has undergone as great or greater increase than chicken production over the past 50 years, and the turkey industry has also undergone vertical integration. Similar to chickens, modern selection techniques have greatly changed the turkey over the last half of the twentieth century. In the late 1950s, tom turkeys were marketed at approximately 10.5–11.3 kg live weight. However, it took nearly 25 weeks for a tom turkey to reach approximately 11.3 kg in 1960, approximately 21 weeks to reach the same weight in 1974, and only approximately 28 weeks to reach 15.8 kg. In 2011, an achievable performance goal for a tom

Table 1 Nutrient composition of various poultry meats data for beef and pork are provided for comparison

Data for 100 g edible portion	*Calories*	*Protein (g)*	*Fat (g)*	*Cholesterol (mg)*	*Iron (mg)*
Chicken breast meat only, raw	114	21	3	64	0.4
Chicken breast meat and skin, raw	172	21	9	64	0.7
Chicken leg meat only, raw	120	19	4	91	0.8
Turkey breast meat only, raw, fryer-roaster	111	25	1	62	1.2
Turkey breast meat and skin, raw	157	22	7	65	1.2
Turkey leg meat, raw fryer roaster	108	20	2	84	1.8
Duck meat only, raw	135	18	6	77	2.4
Goose meat only, raw	161	23	7	84	2.6
Ostrich round, raw	116	22	2	71	3.5
Ostrich, tenderloin, raw	123	22	3	80	4.9
Pork, fresh, composite of trimmed retail cuts (loin and shoulder blade), separable lean and fat, raw	177	20	10	65	0.7
Pork, fresh, composite of trimmed retail cuts (loin and shoulder blade), separable lean only, raw	144	21	6	60	0.9
Pork, fresh, loin, tenderloin, separable lean and fat, raw	120	21	4	66	1.1
Pork, fresh, loin, tenderloin, separable lean only, raw	109	21	2	65	1.0
Beef, tenderloin, separable lean and fat, trimmed to 3.25 mm fat, Choice, raw	246	20	18	85	1.4
Beef, tenderloin, separable lean only, trimmed to 3.25 mm fat, Choice, raw	158	22	7	66	1.6
Beef, composite of trimmed retail cuts, separable lean and fat, trimmed to 3.25 mm fat, Choice, raw	243	19	18	66	1.9

Source: Reproduced from US Department of Agriculture, 2011. Agricultural research service. USDA nutrient database for standard reference, release 24. Nutrient data laboratory home page. Available at: http://www.ars.usda.gov/main/site_main.htm?modecode=12–35–45-00 (accessed 15.10.13).

Figure 4 Typical young production turkey hens. Photo Courtesy of Dr. Ken Anderson, North Carolina State University.

Figure 5 Turkeys (typical) grown on range. Photo Courtesy of Dr. Ken Anderson, North Carolina State University.

turkey to reach 11.3 kg of live weight was 13 weeks of age, and a 22 week-old tom turkey to reach 22 kg of live weight. Therefore, modern selection has significantly altered the quantity, proportionality, and muscularity of turkeys, and rate of turkey muscle development. Overall, turkey carcasses have a high muscle to bone ratio, the breast meat accounts for approximately 29% of the carcass weight, and there is a high dressing percentage (>75% live weight). However, selection for rapid growth has caused some problems for modern turkeys because the size of the breast muscle precludes mating and focal myopathy, which is a pathological muscle condition characterized by enlarged muscle cells, in the breast muscle has been reported to be associated with the rapid growth of these fascinating birds.

In concert with the improvement in the turkey production efficiency were also increases in turkey meat consumption. Till the late 1970s, turkey consumption was heavily concentrated at holiday festivals with very little consumption during the remainder of the year. However, because of aggressive marketing programs and the advent of new products, turkey has become a year-round meat product that American consumers enjoy on a daily basis. Per capita US turkey consumption was approximately 2.25 kg in 1955, but it rose to nearly 8 kg in the late 1990s, and the 2008 per capita consumption was approximately 8 kg and consumption has remained flat. The growth of the turkey industry can be tied to the relatively low cost of turkey meat, the advent of new turkey products (turkey bacon, turkey bologna, turkey hotdogs, sliced turkey breast,

and turkey ham), and the perception by health conscious consumers that turkey is a healthy food. Furthermore, turkey meat is low in fat, high in protein (Table 1) and versatile.

Ducks

Wild Mallard ducks are generally regarded as the ancestor of all breeds of domestic ducks. The Pekin is likely the most popular breed of duck that is used in commercial production. The Pekin undergoes early maturity, is hardy, and develops a good carcass. Pekin ducks originated in China during ancient times, and they were first imported to America after a lengthy sea voyage from Peking China in 1874. A young duck or duckling (usually under 8 weeks of age) has dark, tender meat, and weighs approximately 1.6–2.25 kg. The duck industry in the US was initially concentrated on Long Island, New York to supply the New York City market. However, the duck production industry in the US has shifted from its original Long Island base to be concentrated in the mid-western US, and the duck industry now enjoys a nation-wide market in the US. The single largest company producing ducks in the US has facilities in California, Wisconsin, Indiana, and Michigan producing 14 million ducks per year. Overall, the duck industry in the US is still much smaller than the chicken or turkey industries. It produces approximately 24 million ducks annually compared to approximately 8 billion chicken, and approximately 275 million turkeys. Similarly, the average American only consumes approximately 0.15 kg of duck per year making duck much less popular with American consumers than other poultry meat species. The largest duck producing nation is China (approximately 2.6 million metric tons), followed by France, Thailand, Vietnam, and the US (approximately 50 000 metric tons). Although it appears that duck meat is consumed on a world-wide basis, it tends to be less popular world-wide than chicken or turkey meat.

The appeal of duck meat to American consumers tends to be in affluent specialty markets for people who prefer the taste of duck to turkey or chicken. Duckling is an international mealtime favorite, and it is best known for the elegant dishes prepared in elite restaurants, such as Peking Duck and Duck al' Orange. However, there are a number of delicious duck recipes that are easy to prepare at home, such as Bar-B-Q duckling, roast duckling, and duckling pasta. Duck breast meat does not appear as white as turkey or chicken meat, and it is marketed as a 'red' meat. Duck meat is nutritionally similar to other poultry meats, except it appears that skinless duck meat has a higher fat content than skinless chicken or turkey meat (Table 1). A potential reason for the limited growth of the duck industry may be that duck tends to be much more expensive than chicken or turkey, or it may simply be related to the high cost of duck compared to all other food. Similarly, the convenience food, such as chicken nuggets, sliced turkey breast, chicken hotdogs, and turkey burgers, have not been produced or successfully marketed by the duck industry.

Geese

The popular geese breeds are the Embden, Toulouse, African, Chinese, Pilgrim, Egyptian, and Diepholz. The development of modern geese breeds has not followed the same path as modern chickens or turkeys, because goose production has not achieved the same corporate scale as the chicken or the turkey industry. Therefore, few industrialized breeding programs have been implemented for geese. The number of geese in Europe has dropped steadily since the introduction of modern poultry production techniques during the early twentieth century. An increases in geese production have occurred in less developed countries where geese can free range, live independently, and produce culturally acceptable, tasty meat. A major country producing goose meat is China. The barriers to geese playing a role in large-scale agriculture are the relatively poor reproduction rate, their slow growth rate compared to chickens and turkeys, and the lack of corporate marketing. Nutritionally, skinless goose meat is similar to other poultry meat species, but it contains a higher caloric fat and iron content than skinless chicken or turkey meat (Table 1). Overall, goose meat is a highly nutritious product that is an excellent source of protein for people in developing countries or consumers in more developed countries who enjoy its taste.

Ratite

The US ratite (ostrich, emu, and rhea) industry began in the early 1980s as an almost totally breeder-production system. Bird prices were very high and the ratite market quickly reached a saturation point. The present ratite industry has shifted from a breeder-based industry to be a product-based industry (meat, hide, oil, and feathers), but the limited infrastructure of ratite-processing facilities has been a major barrier to the development of the industry. There are limited statistics available for ratite production, but there are likely between 50 000 and 100 000 ostriches and between 50 000 and 100 000 emus in the US. In Canada, there were only 11 ostrich, emus, or rhea harvested in 1993, whereas in 1997, there were 13 000 birds harvested. Therefore, some growth has occurred in the North American ratite industry during the 1990s, but there has not been a strong demand for ratite meat by North American consumers. Subsequently, there has been a steady decline in the number of Canadian farms producing ostrich and emus as well as a steady decline in total Canadian ostrich/emu numbers between 1996 and 2006 suggesting that the ratite industry has failed to develop in North America.

The ostrich is indigenous to Africa, and it has been raised domestically in Africa since the 1800s. Ostriches stand up to 3 m high and can weigh 180 kg. Normally, ostriches are processed at approximately 10–14 months of age. Emus originated in Australia, and they are smaller than ostriches standing approximately 1.5 m high and weighing approximately 54 kg. Lastly, rheas are indigenous to South America, and they stand approximately 1.5 m high and weigh approximately 27–36 kg at maturity. All three species have organized associations to support the marketing of their products. The current market for ratite has been primarily for specialty meats, focusing on customers who wish to enjoy a tasty, low fat alternative to traditional red meat products.

Consumer taste panels have only found slight differences in palatability attributes between ostrich steaks and Choice beef top loin steaks; however, the slight differences in palatability

did not significantly affect overall acceptability of ostrich steaks. Ostrich meat is very high in protein, but low in lipid content (Table 1). The nutrient composition of ostrich meat is similar to other poultry meats, but the sensory attributes are similar to traditional red meat. Overall, it has been difficult to introduce new meat products to American consumers, and demand for ratite meat failed to be firmly established. Any future success of the ratite meat industry in the US may depend on an effective distribution, marketing, and promotion strategy that have been characteristic of the broiler and turkey industries.

Game Birds

The US game bird industry raises millions of birds to stock land for recreational hunting, sale to restaurants, and sale directly to consumers. Wild game legally hunted in the US cannot be sold to consumers, but the game can be harvested for personal consumption. Wild game that is raised on farms and processed under appropriate regulations can be sold to the consumers. In general, a large portion of the game bird industry is focused on hobbyists raising home flocks, and also on raising game birds to stock hunting grounds. Therefore, game birds, such as pheasants and Bobwhite quail, can be marketed as day-old-chicks for the small flock hobbyist, as young mature birds to stock hunting lands, and as meat for restaurants or the home consumer. In the US, up to 10 million pheasants, and 37 million quail are raised for consumption. Pheasants reach approximately 1.2 kg by approximately 16 weeks of age. A major obstacle to pheasant production is cannibalism, which is prevented by beak trimming, and the pheasants need their wings clipped to prevent flight. Quail are raised in similar conditions to broiler chickens, but they are very small birds making each require little floor space, and they reach market age by approximately 7 weeks of age with a carcass of 0.2 kg.

Overall, the poultry industry provides a variety of tasty products, such as ratite, duck, quail, and pheasant for consumers who wish to explore the exotic meats, but these speciality products are vastly overshadowed by the larger scale production and consumption of lower cost broiler and turkey meat.

See also: Genome Projects: Modern Genetics and Genomic Technologies and Their Application in the Meat Industry – Red Meat Animals, Poultry. Human Nutrition: Macronutrients in Meat; Micronutrients in Meat. Meat, Animal, Poultry and Fish Production and Management: Antibiotic Growth Promotants; Beta-Agonists; Poultry. Muscle Fiber Types and Meat Quality. Nutrition of Meat Animals: Pigs; Poultry. Slaughter-Line Operation: Poultry

Further Reading

Agriculture and Agri-Food Canada, 1999. Snapshot of the Canadian Ostrich, Emu, and Rhea Industries. Agriculture and Agri-Food Canada, Marketing and Industry Services Branch, Agricultural Industry Services Directorate. Animal Industry Division, Poultry Section. Ontario.

Chilliwack Agricultural Commission, 2002. Games birds. Description of specialty bird industry. Chilliwack, BC, Canada: Chilliwack Partners Commission. 23 January.

Damron, W.S., 2002. Introduction to animal science: Global, biological, social, and industry perspectives. Saddle River, NJ: Prentice Hall.

Economic Research Service (ERS), 2008. Food Availability (Per Capita) Data System. US Department of Agriculture (USDA). Available at: http://www.ers.usda.gov/Data/FoodConsumption (accessed Mar 2013).

Food Safety and Inspection Service (FSIS), 2008. US Department of Agriculture (USDA). Fact Sheets. Game from Farm to Table http://www.fsis.usda.gov/factsheets/Farm_Raised_Game/index.asp#3. Food and Agriculture of the United Nations Statistical Database, Home Page. Available at: http://apps.fao.org/ (accessed Mar 2013).

Gillipespie, J.R., 2002. Modern Livestock and Poultry Production, sixth ed. Clifton Park, NY: Delmar Publishers.

Harris, S.D., Morris, C.A., Jackson, T.C., *et al*., 1994. Ostrich Meat Industry Development. Final Report to American Ostrich Association. Texas, USA: Texas A&M.

Havenstein, G.B., Ferket, P.R., Qureshi, M.A., 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82, 1500–1508.

Havenstein, G.B., Ferket, P.R., Qureshi, M.A., 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82, 1509–1518.

Hugo, S., 1999. Geese: The Underestimated Species. FAO Report. Texas, USA: Texas A&M.

Richard, R.I., Mead, G.C. (Eds.), 1999. Poultry meat science. New York: CABI Publishing.

Sosnicki, A.A., Cassens, R.G., Vimini, R.J., Greaser, M.L., 1991. Histopathological and ultrastructural alterations of turkey skeletal muscle. Poultry Science 70, 349–357.

Statistics Canada, 2008. Alternative livestock on Canadian farms, census years 1981, 1986, 1991, 1996, 2001, and 2006. Catalogue no. 23-502-X. Available at: http://www.statcan.gc.ca/pub/23–502-x/23–502-x2007001-eng.pdf (accessed 15.10.13).

US Department of Agriculture, Agricultural Research Service, 2011. USDA nutrient database for standard reference, release 24. Nutrient data laboratory home page. Available at: http://www.ars.usda.gov/main/site_main.htm?modecode=12–35–45-00 (accessed 15.10.13).

Relevant Websites

http://aea-emu.org/
American Emu Association.

http://www.meatscience.org/
American Meat Science Association.

http://www.ostriches.org/
American Ostrich Association.

http://en.aviagen.com/tech-center/
Aviagen Group.

http://www.nationalchickencouncil.org/
National Chicken Council.

http://www.eatturkey.com/home.html
The National Turkey Federation.

http://www.uspoultry.org/
US Poulty and Egg Association.

Sheep and Goats

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Glossary

Average daily gain (ADG) A measure used to compare growth rates of animals.
Cabrito A name given to meat from milk-fed kids. It is similar in meaning to veal in cattle.
Callipyge 'Beautiful buttocks.' A genetic mutation found in sheep associated with muscle hypertrophy, typically seen in the leg.
Carcass composition The combination of, and relationship between, lean, fat, and bone present in the carcass.
Carcass quality Meat tenderness, juiciness, color, and flavor.
Chevon Generic name for goat meat.
Cold shortening A phenomenon that results in decreased tenderness of meat due to rapid heat dissipation from carcasses in the hours following slaughter.
Small ruminant A term that denotes both sheep and/or goats. Small ruminants have a multicompartment pregastric digestive system similar to that of cattle and buffalo.

Introduction

Small ruminants, such as sheep (*Ovis aries*) and goats (*Capra hircus*), were among the first animals to be domesticated, with historical evidence linking them to western Asia approximately 9000–12 000 years ago. Domesticated sheep and goats provided early humans with a supply of fiber, pelt, meat, and milk. Owing to their small stature and versatility, small ruminants were, and still are, an important food source in dry, remote regions of the world that lack electricity and have limited grain or roughage. Small ruminants are also efficient convertors of low-quality feed materials to high-quality protein. Furthermore, a small ruminant carcass can be consumed in a few days, which allows only limited time for spoilage.

Unlike sheep, which descended from four distinct types of wild sheep, including the Urial, Argali, Mouflon, and Aoudad, most goat breeds can be traced back to only one wild type, the Benzoar of Asia Minor and the Middle East. There are more than 850 breeds of sheep and more than 500 different breeds of goats worldwide. Although many believe that sheep and goats are similar in their physiological traits and behavior, they are quite dissimilar and should be treated separately. As large ruminants, such as cattle, and small ruminants are researched and managed differently, the sheep and goat should also be evaluated independently.

Since the 1960s, meat consumption has increased worldwide following the increase in human population and greater disposable income in developing countries. Production of small ruminants could be increased in developing countries, if it were not for limited feed, lack of veterinary assistance and educational resources, and climatic challenges. In many areas of the world, including the USA, sheep and goat production tends to be thought of as low-investment, low-output enterprises. However, in many countries, these animals could generate greater income for producers, if it were not for a complicated system of government subsidies and regulations.

World Sheep and Goat Inventory

Between 2004 and 2007, the areas of the world most noted for sheep and goat production included China (143.8 million; home to one-third of all small ruminants), India (182 million), Australia (99.3 million), Iran (54 million), the Sudan (47 million), and New Zealand (40 million). Total worldwide production of small ruminants was approximately 2 billion head (1.25 sheep for every 1 goat), yielding more than 11.8 million metric tons of product. More than 90% of the world's goats and almost 70% of the world's sheep are in Asia and Africa. Australia produces more than 50% of the world's exported goat meat. The total number of goats worldwide has increased 146%, whereas production has doubled since 1990; in contrast, sheep numbers have decreased 10% since 1990. Although sheep meat has increased in value, the consumption of sheep meat has increased 36% from 2010 to 2011, which is greater than both beef (18%) and poultry (16%).

The number of small ruminants worldwide has increased due to high fertility rates, frequency of multiple births, short generation interval, low-cost management, and opportunity for short-term return on investment. People living in rural areas with marginal land and poor economic opportunities can raise small ruminants, which improves income potential and the opportunity for consumption of quality protein. Since 2006, government restrictions in China and adverse weather conditions worldwide have slowed the population growth of small ruminants. Some countries, such as Australia, are trying to restock their breeding inventories, but elevated sheep and goat meat prices have encouraged the sale of live animals into the slaughter market.

Small ruminants do well in a variety of climates. In general, sheep do better than goats in colder climate, whereas goats do better in rugged areas where brush and shrubs are the predominant forage types. Both can thrive on marginal lands and in areas that are unsuitable for cattle or buffalo and are more

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efficient than larger ruminants at converting forage into useable product. Except for dairy animals, sheep and goats have lower dietary requirements than larger livestock.

Biological Types of Sheep

Domestic sheep are found in nearly every country throughout the world and are highly sought after for their wool, hides, and carcasses. Breeds, or biological types, of sheep can be broadly classified into five general types: ewe/wool, ram/carcass, dual purpose, milk, and hair breeds. Ewe/wool breeds, such as Rambouillet, Merino, and Finn, are noted for their maternal characteristics and/or high-quality wool. Ram breeds (e.g., include Suffolk, Hampshire, and Dorset) tend to excel at growth and carcass traits. Dual-purpose breeds, such as the Targhee and Columbia, are productive at two or more traits (e.g., wool and meat). The East Friesian is the world's highest-producing dairy breed, producing 500–700 kg of milk per lactation with 6–7% milk fat. Hair breeds shed, and thus do not have to be shorn, and are noted for their maternal or carcass qualities. Some hair breeds are also noted for their tolerance to parasites and overall vigor, making them popular in both tropical and temperate climates. Popular hair breeds include the St. Croix, Dorper, Barbados Blackbelly, and Katahdin.

Selection of a particular breed depends on the breed's suitability to the production environment and its ability to meet the social and economic needs of the community. In areas of the world possessing a moderate climate, sheep breeds are often medium framed, of a compact conformation, with short legs and thicker wool. The average breed type in tropical regions has longer body and legs, longer ears and tails, and possesses short hair, as opposed to wool. The biological type chosen in arid regions that have a limited or seasonal supply of forage are often described as 'fat-tailed' breeds. Fat-tailed sheep, such as the Karakul, utilize the large stores of fat in their tail and hind-saddle region to sustain them through periods when food and water are limited.

Biological Types of Goats

Goats can be sorted into fiber, milk, meat, or feral/brush types. The two most popular fiber breeds or breed types are the Angora and Cashmere (the latter of which is a breed type, not a particular breed). Although any goat can be used for milk production, popular milk breeds include the Alpine, Nubian, and Majorera. Meat breeds vary in type and kind and can include small breeds such as the Pygmy and large breeds such as the Boer. Feral/brush types include the Spanish (breed type) found in West Texas and the Kiko of New Zealand (before genetic improvements). In many areas of the world, breeds of goats are indiscernible and often exist in a feral state. Like sheep, selection of a goat breed for any one particular region of the world is based on the inherent traits of that animal in conjunction with the environment and the specialized needs of the populace.

Of particular note is the Boer, which was developed in South Africa and is being used in countries such as China, Australia, and the USA to improve meat quality of feral goats. Boer goats have superior growth and carcass quality compared with many other breeds; however, research indicates that Kikos can wean more and heavier kids than Boers.

Factors Affecting Growth, Carcass Composition, and Carcass Quality

Growth Effects of Breeds and Genetics on Growth

Growth traits such as weaning (20% heritable) and postweaning (40% heritable) weights are used for selecting carcass quality and quantity. Carcass traits are moderately to highly heritable (30–60%), thus selection for superior growth and carcass traits can be used for increasing meat production. However, in the quest for increasing growth, it is important not to overlook fertility and efficiency traits. In addition, some genetic traits, such as growth rate and wool/hair production, are negatively correlated, and as more emphasis is placed on growth, fiber production and/or quality declines. If feed or capital resources are limited, it is not economically feasible to select for maximal growth.

Sheep

Traditionally, ram breeds have been primarily used to increase growth in wool breeds. However, as wool becomes less valuable, hair breeds become more popular. Most hair breeds have decreased growth rates and average daily gain (ADG) compared with ram breeds. However, the Dorper, a hair breed originating from South Africa and comprised of the Black-Headed Persian and the Horned Dorset, offers advantages in growth as compared with other hair breed types. More research is needed to understand differences in growth between hair, wool, and ram breeds.

Goats

Goats typically produce smaller carcasses, even when slaughtered at similar ages or weights as sheep. Meat-type goats, such as the Boer, have greater ADG and yield heavier, meatier carcasses when compared with milk or feral breed types; however, Boers are not noted, at least in the USA, for possessing superior mothering or hardiness traits. Crossbreeding Boers with other breeds creates hybrid vigor, making the offspring superior than both parents in economically important traits.

Effects of management on growth

Diet can significantly affect ADG, live and carcass weights, fat quantity and composition, and meat flavor. Animals on a high-energy diet grow faster and produce fatter carcasses than those on a forage diet. Animals on high-protein diets yield carcasses that consumers perceive as having 'off'-flavors compared with animals fed high-energy diets.

Castrated sheep tend to grow slower than intact males, which is similar to results demonstrated in cattle and hogs. Studies with goats indicate that there is both a breed and time of castration effect on ADG; however, the results are conflicting with respect to age of castration. Some studies recommend early castration (less than 1 week of age), whereas others

recommend delayed castration (after 6 months) for achieving most efficient growth in goats.

Carcass Composition

Effects of breeds and genetics on carcass composition

On an average, approximately 50% of live sheep/goat weight is retained as carcass weight. The carcass dressing percentage increases with increasing levels of fatness and/or muscularity. Dressing percentages typically range from 40% to 60%; however, extreme, heavily muscled animals may yield greater than 60% of their live weight as carcass weight. The weight of muscle tissue comprises 46–65% of the total dressed carcass weight. Sheep are intermediate to pigs and cattle for the propensity to deposit subcutaneous fat. Sheep produce carcasses with more dissectible fat and lean but with less bone than goats raised under similar management and slaughtered at similar ages and weight. Hair sheep may differ in the muscle–fat ratio; however, sufficient research has not been conducted to fully understand possible breed or type differences. Generally, it is thought that hair sheep deposit fat more like goats rather than like other sheep breeds.

Small ruminants are sold for harvest as intact males, intact females, and wethers (castrated males). In some countries/cultures, castration is either not permitted or consumers prefer meat from intact males. Carcasses from intact males typically have a thicker, more muscular neck and shoulders (adding weight to low-value cuts) compared with carcasses from females or castrated males. Depending on culture, these characteristics may be considered undesirable. The pelt of intact males is also more difficult to remove and may result in tearing of the external fat cover and a reduction in carcass appeal. Overall, breed and weight at the time of slaughter may have a greater effect on dressing percentage than gender.

Goats deposit greater amounts of internal fat rather than subcutaneous fat when compared with sheep or cattle. Goat meat tends to be leaner than sheep meat and therefore has higher proportions of protein and minerals. Not much is known regarding growth or body composition of goats or hair sheep, especially when compared with wool and ram breeds of sheep, beef cattle, and swine.

Chemical profiles of sheep and goat meat

Meat from lamb and goats is frequently touted as healthy. On an average, lamb contains 22% protein, 16% fat, and 78 mg of cholesterol per g of meat. Chevon contains, on average, 23% protein, 12% fat, and 94 mg of cholesterol per gram of meat. However, all of these values differ depending on preslaughter management and the particular cut of meat from which the sample was obtained. Animals slaughtered before six months of age tend to have lesser amounts of cholesterol than older animals. Also, breed may influence cholesterol content, as meat from St. Croix lambs may contain greater amounts of cholesterol compared with other breeds and breed crosses.

Although there are species and breed differences, in general, meat from sheep and goats has a high monounsaturated to polyunsaturated fatty acid ratio. However, feeding diets with a ruminally protected source of fat can produce a more favorable polyunsaturated to saturated ratio. Also, as animals become fatter, the composition of fatty acids changes because the triglycerides, which increase with fatness, are more saturated. Diet can also affect the ratio of oleic, palmitic, and linoleic acids. In addition, some have reported that there is a difference in fatty acid profile between genders, with males having a greater proportion of palmitic acid. The predominant fatty acids isolated from lamb and goat meats are oleic (28–44%), stearic (12–25%), and palmitic (16–23%) and are roughly in the same proportions as found in beef and pork. Proportions can differ depending on diet (milk fed vs. concentrate vs. forage), age, and breed.

Effects of Management on Carcass Composition

Sheep

Across all breeds and weights, carcasses from rams are the leanest and ewes are the fattest. Ram lambs tend to have the largest and the longest carcasses and possess larger loin muscles compared with ewes. Wether lambs are intermediate. Delaying castration or slaughtering ram lambs before puberty can increase the carcass lean to fat ratio.

Goats

Few studies have evaluated the effects of gender on carcass composition in goats. Time of castration affects dressing percentage, and variations in timing may explain why there is limited agreement between the published studies. In most cases, goats are slaughtered either before or soon after puberty (4–8 months); therefore, the effects of the male sex steroids may not have had sufficient time to affect dressing percentage. The proportion of retail carcass cuts is also affected by gender; however, breed and age have greater effects on carcass composition.

Carcass Quality

Carcass quality is commonly assessed for carcass conformation and indicators of potential eating satisfaction (palatability). It should be noted that carcass conformation has little relationship with palatability. Furthermore, the definition of quality in the eye of the consumer is much broader and includes not only palatability but also product appearance, nutrient density, and wholesomeness (freedom from pathogens). Sensory characteristics, such as flavor, aroma, juiciness, and tenderness, are influenced by breed, diet, gender, and pre- and postslaughter management. Small ruminants are consumed all over the world; one should, therefore, expect the perception of quality to be extremely diverse. Factors that influence the perception of quality include (but are not limited to) muscle texture, tenderness, flavor, fat content, fatty acid profile, water content, preslaughter nutrition, postslaughter handling, processing practices, level of sanitation, meat aging, refrigeration (or absence of refrigeration), and cooking methods.

Factors affecting meat flavor

Components of meat flavor are both fat and water soluble, but the water-soluble components are relatively similar across species and are the main reason why fat-free products taste similar across species. It is the fat component of meat that primarily

contributes to species differences in flavor. The total and ratios of fatty acids present (often referred to as the fatty acid profile) in the adipose tissue of lamb and chevon influence flavor perception by consumers. Forages contain a variety of odoriferous and reactive fat-soluble components that ultimately are deposited as flavor precursors in the muscle. These compounds can accumulate within adipose tissue over time and are perceived as either positive or negative meat flavors (depending on culture or geographical origin of consumers). These flavor-influencing compounds are more prevalent in chronologically older animals and can contribute to the distinct flavor differences between young and old animals. Generally, as animal's age, flavor intensity increases, often to the point of undesirability for many consumers. In some areas of the world it is common to harvest and consume intact males, the meat from which may be perceived as less tender with stronger flavor.

In the USA, more than 80% of lambs marketed are finished on high-concentrate (predominantly corn) diets, yet, in many countries, lambs are fed 100% forage diets. Lamb consumers accustomed to corn-finished lamb perceive forage-finished lambs to have 'lamby' or 'grassy' flavors. Those accustomed to the more common worldwide production practice of pasture- or forage-finished lambs find the corn-finished lamb to be mild, lacking in traditional lamb flavor, and too fat. Little research has been conducted regarding the effects of diet on sensory characteristics of goat meat.

Effects of breeds and genetics on carcass quality

There are some notable differences between lamb and chevon with respect to the role of breed on carcass quality. Although lamb is rarely perceived as tough, biological (breed) type can influence tenderness. Hair breeds are generally regarded as yielding tougher meat compared with ewe breeds. Although it has not been evaluated, differences may exist between hair breeds. Furthermore, meat from lambs expressing the callipyge (double muscling) genotype is distinctly less tender compared with lamb from 'normal' carcasses, irrespective of whether it is from wool or hair breeds. Lambs of St. Croix breeding have been shown to produce a more palatable carcass when compared with wool or callipyge sheep. However, little research has been conducted comparing hair breeds with the other breed types in terms of overall carcass palatability.

Sheep carcasses tend to be perceived as higher quality than goat carcasses, and meat breeds of goats, such as the Boer, tend to be perceived as having higher quality carcasses, based on hindleg circumference, than indigenous or feral- and milk-type goats. However, assessment of carcass quality is influenced by local custom and preference, so it is hard to define a universal standard for carcass quality in the goat.

When similarly prepared, consumers generally prefer lamb over goat in terms of tenderness, juiciness, and overall palatability. Unfortunately, less is known concerning the effects of breed or breed type on carcass quality in goats. There are conflicting results regarding the effect of breed on carcass quality, with some reports showing small or no effects, whereas others report breed to be a significant factor in carcass quality traits. This represents another area in which additional research efforts are needed.

Goat carcasses have greater collagen content compared with lamb carcasses, which results in greater toughness. However, because chevon is leaner than lamb, mutton, or beef, consumers may overlook some of the negative palatability traits as a trade-off for a leaner protein source.

Additionally, sheep and goats have less subcutaneous fat cover than pigs and cattle have; moreover, goats deposit less subcutaneous fat than sheep and yield leaner carcasses. Lean carcasses chill faster than ones with more fat cover, which may lead to cold shortening of muscle fibers and increased meat toughness. Rapid chilling of prerigor carcasses can also induce cold shortening of the muscle fibers and thus should be avoided. Cold shortening can be reversed by applying electrical stimulation to the carcass or by increasing the postmortem aging process, which may increase tenderness. This procedure shortens the time necessary for the onset of rigor mortis by accelerating postmortem glycolysis so that carcasses may be rapidly chilled without the risk of inducing cold shortening.

Effects of management on carcass quality

Worldwide management practices favor production of small ruminants on a pastoral diet. Pasture-raised animals rarely become excessively fat. However, in countries where it is economically feasible to feed high-energy, corn-based diets, the potential for overfattening exists if animals are not properly managed. Those raised on high-energy diets tend to be tenderer, but fatter, than animals fed with low-energy diets. Little research has been conducted in goats to determine how diet affects overall carcass quality, including tenderness.

In other meat animals, age at harvest affects tenderness; however, there is no consensus as to the effect of chronological age on lamb tenderness (lamb vs. mutton). The accumulation of connective tissue and the increased maturation of muscle of older lambs increase the potential for tougher meat. Early maturing, small-framed sheep (such as the hair breeds) reach an ideal lean to fat ratio earlier, and if fed to a harvest weight similar to large-framed breeds, they will possess too much fat. Later maturing, fast-growing sheep (often black-faced breed types) and goats (Boer or Boer type) tend to be more heavily muscled and should be marketed at an earlier chronological age to increase the lean to fat ratio.

The age at which small ruminants are harvested depends on the demands of consumers. If consumers prefer larger cuts of lamb/goat, age at slaughter will increase to allow more time for increased muscle growth. However, without proper management, lambs/kids fed to heavier market weights reach a point of physiological maturity, at which increases in muscle mass slow or cease causing the animal to become excessively fat. Consumers usually avoid excess fat when purchasing lamb/goat. Animals slaughtered at an older age may also be perceived as less desirable by the consumer, in part due to darker meat color. In addition, due to enhanced proteolysis, older animals yield meat with flavor differences, which are often characterized as bitter tasting.

An additional benefit of castration is to prevent taint of the meat, which is caused by androstenone, a steroid produced by the testes. Androstenone accumulates in the fat and is released when the meat is cooked. However, the perception of an undesirable sexual odor in meat is not consistent or well understood. Factors such as breed, season of rearing, age at castration, and age at slaughter all affect palatability traits of meat from

intact males. Regardless of gender, lamb meat tends to be perceived as tender. In goats, does yield tenderer carcasses with greater marbling than intact males. Color is also affected by gender, with males generally yielding lighter lean tissue. In goats, differences between intact and castrated males have not been evaluated. However, gender does not influence muscling indicators, such as carcass or leg conformation scores, which are commonly used to assess carcass quality in goats.

Effects of postslaughter management on carcass quality

Breed, genetic type (e.g., callipyge genetic condition), subcutaneous fat cover, and pre- and postslaughter handling may be more important factors affecting tenderness than chronological age in sheep or goats. Most sheep and goats are killed before 1 year of age, which decreases the amount of connective tissue growth, thus decreasing the chance of toughness. Consumers first assess leanness when determining their intent to purchase lamb. However, the evaluation of retail cuts of lamb for freshness, as perceived by color, is an important factor for consumers. There are contradictory assessments of meat color and presumed freshness; some consumers equate a bright red color with greater quality and freshness of the product, whereas others deem a darker red color as more acceptable. Like flavor, color preference is culturally and geographically specific. Potential consumers also perceive lamb meat color differently based on the manner of packaging.

Meat color can also be influenced by factors that have little to do with the overall quality of the product, such as preslaughter management of live animals. Sheep exposed to prolonged periods of preslaughter stress, such as illness, poor nutrition, or prolonged transportation, often produce meat that is darker in color. Furthermore, these animals often yield carcasses with greater intramuscular pH that leads to an increased risk of premature spoilage.

Summary

Small ruminants have been economically important to humans for thousands of years; because of this, more than 1000 breed types have been developed to fit various management practices and environmental conditions worldwide. Meat quality (palatability, nutrient density, wholesomeness, appearance) can be influenced by various factors, such as breed, gender, feeding regimen, and chronological age. There is no universally accepted method of assessing carcass composition and quality, because of worldwide differences in perception of what constitutes acceptable lamb/goat. Generally speaking, consumers of lamb and goat prefer a lean, low-fat product, but they disagree with regard to what is considered desirable palatability or flavor. Sheep and goats are inherently different animals, and thus they should be treated and researched as such.

See also: Boar Taint: Biological Causes and Practical Means to Alleviate It. Carcass Chilling and Boning. Chemical and Physical Characteristics of Meat: Adipose Tissue; Color and Pigment; Palatability; pH Measurement; Water-Holding Capacity.

Conversion of Muscle to Meat: Aging; Rigor Mortis, Cold, and Rigor Shortening. Double-Muscled Animals. Growth of Meat Animals: Adipose Tissue Development; Growth Patterns. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Modeling in Meat Science: Meat Quality. Nutrition of Meat Animals: Ruminants. Physical Measurements: Temperature Measurement. Sensory and Meat Quality, Optimization of. Slaughter-Line Operation: Sheep and Goats. Species of Meat Animals: Meat Animals, Origin and Domestication

Further Reading

Bailey, M.E., Suzuki, J., Fernando, L.N., Swartz, H.A., Purchas, R.W., 1994. Influence of finishing diets on lamb flavor. In: Ho, C.T., Hartman, G.T. (Eds.), Lipid in Food Flavors. Washington DC: American Chemical Society, pp. 171–185.

Bayraktaroglu, E.A., Akman, N., Tuncel, E., 1988. Effects of early castration on slaughter and carcass characteristics in crossbred Saanen × Kilis Goats. Small Ruminant Research 1 (2), 189–194.

Berg, E.P., Neary, M.K., Forrest, J.C., 1998. Methodology for identification of lamb carcass composition. Sheep and Goat Research Journal 14 (1), 65–75.

Beserra, F.J., Madruga, M.S., Leite, A.M., da Silva, E.M.C., Maia, E.L., 2004. Effect of slaughter age on chemical composition of meat from Moxotó goats and their crosses. Small Ruminant Research 55, 177–181.

Burke, J.M., Apple, J.K., Roberts, W.J., Boger, C.B., Kegley, E.B., 2003. Effect of breed-type on performance and carcass traits of intensively managed hair sheep. Meat Science 63, 309–315.

Cooper, S.L., Sinclair, L.A., Wilkinson, R.G., *et al.*, 2004. Manipulation of the *n*-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. Journal of Animal Science 82, 1461–1470.

Guiterrez, A.N., 1986. Economic constraints on sheep and goat production in developing countries. In: Timon, V.M., Hanrahan, J.P. (Eds.), Proceedings of an Expert Consultation Small Ruminant Production in the Developing Countries. Sofia, Bulgaria: Agriculture and Consumer Protection. FAO Corporate Document Repository, pp. 138–147. Available at: http://www.fao.org/docrep/009/ah221e/AH221E13.htm (accessed 19.12.11).

Lawrie, R.A. (Ed.), 1998. Factors influencing the growth and development of meat animals. Lawrie's Meat Science, sixth ed. Lancaster, PA: Technomic, pp. 11–30.

Lind, V., Berg, J., Eilertsen, S.M., Hersleth, M., Eik, L.O., 2011. Effect of gender on meat quality in lamb from extensive and intensive grazing systems when slaughtered at the end of the growing season. Meat Science 88, 305–310.

Melton, S.L., 1990. Effects of feeds on flavor of red meat: A review. Journal of Animal Science 68, 4421–4435.

Mitcham, S., Mitcham, A., 2000. Meat Goats: Their History, Management and Diseases. Sumner, IA, USA: Crane Creek Publications.

Mottram, D.S., 1998. Flavour formation in meat and meat products: A review. Food Chemistry 62 (4), 415–424.

Nsoso, S.J., Young, M.J., Beaton, P.R., 2000. A review of carcass conformation in sheep: Assessment, genetic control, and development. Small Ruminant Research 35, 89–96.

Sande, D.N., Houston, J.E., Epperson, J.E., 2005. The relationship of consuming populations to meat-goat production in the United States. Journal of Food Distribution Research 36 (1), 156–160.

Seideman, S.C., Cross, H.R., Smith, G.C., Durland, P.R., 1984. Factors associated with fresh meat color: A review. Journal of Food Quality 6, 211–237.

Tshabalal, P.A., Strydom, P.E., Webb, E.C., de Kock, H.L., 2003. Meat quality of designated South African indigenous goat and sheep breeds. Meat Science 65, 563–570.

Relevant Websites

http://www.fao.org/docrep/014/al981e/al981e00.pdf
Breeds of Livestock Website Maintained by the Department of Animal Science at Oklahoma State University.

http://www.ansi.okstate.edu/breeds/
FAO, November 2011. Food Outlook.

http://www.sheepandgoat.com/
Maryland Small Ruminant Page.
http://www.lsuagcenter.com/NR/rdonlyres/B8FE3706-64DC-417F-A592-B8DEC14B4D9F/43291/pub2951MeatGoatJanuary2008HIGHRES.pdf
Meat Goat Selection, Carcass Evaluation & Fabrication Guide.
http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3060365
United States Standards for Grades of Lamb, Yearling Mutton, and Mutton Carcasses.
www.wri.org
World Resources Institute. 2002–2004 Earth Trends – The Environmental Information Portal Livestock Production Management – Demography of Sheep and Goat Population and Their Role in Economy.

Shellfish

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Introduction

Shellfish are edible aquatic invertebrate animals, usually with a shell, including molluscs such as oysters, clams, mussels, and cephalopods and crustaceans such as shrimp or lobsters. This article presents the major species of shellfish consumed worldwide. It is divided by scientific classification and presented with the market and scientific names as well as a brief description of each. Those species of major economic importance and those of popular or regional consumption such as, 'Louisiana crawfish,' are described in more detail.

Nutritionists value shellfish as a quality protein source. Seafood contains all of the essential amino acids, and with 17–25% protein is an excellent source to meet our daily protein needs. Although shellfish tend to contain slightly higher amounts of cholesterol than finfish, the amounts for crab and lobster are similar to that in the dark meat of chicken. Cholesterol levels vary with shrimp species, but are generally 1.5–2 times higher than in other shellfish. Previously, shellfish were excluded from low-cholesterol diets because they were believed to be high in cholesterol. Today, with modern sophisticated measuring, it is known that cholesterol levels are lower than previously reported. Molluscs, such as clams, oysters, scallops, and mussels, have been found to have a large percentage of noncholesterol sterols that appear to have a positive effect on cholesterol levels. Shellfish are a rich source of essential minerals. Oysters and crustaceans are rich in zinc, iron, and copper; mussels, scallops, and clams are rich in potassium. All shellfish are good sources of iodine, phosphorus, and selenium. Shellfish eaten raw or cooked without added fat are low in fat ($<5\%$) and calorie content.

Crustaceans or Crayfish

Crustacea, a Subphylum of Arthropoda, contains mostly marine arthropods, though many of its members, like crayfish, have invaded fresh water. In the sea, large crustaceans such as crabs and shrimp are common bottom-dwellers. Many minute species of crustaceans are important components of the zooplankton (floating or weakly swimming animals) and serve as food for other invertebrates, fishes, and even whales.

Shrimp/Prawn

One of the world's most abundant and popular seafoods, shrimp contribute to the diets of cultures around the world. Shrimp and prawn are vernacular or colloquial terms and are terms of convenience, but one should not confuse them with the names or relationships of actual taxa (Figure 1). Shrimp are caught or cultured in temperate and tropical salt waters and fresh waters, especially in China, Thailand, Ecuador, Indonesia, India, Bangladesh, and the Gulf of Mexico. World shrimp consumption has increased steadily since 1970. Wild-caught shrimp provided most of the world supply until the mid-1980s. The world catch is now more than 2 million tones, with 30% supplied from aquaculture. Common edible shrimp are presented in Table 1.

Lobster and Spiny Lobster

Lobster is the common name for marine decapod crustaceans. The American and European lobsters are characterized by an enlarged pair of pincers or claws. Spiny lobsters (Figure 2) are not closely related to true lobsters and are distinguished from American and European lobsters by their long antennae and hard shell and are clawless. Spiny lobsters are also, especially in Australia, New Zealand, and South Africa, are sometimes called crayfish, sea crayfish, or crawfish, terms which elsewhere are reserved for freshwater crayfish. Lobster is considered a luxury seafood. Species of the common lobster are included in Table 2.

Crab

Crab meat and claws are among seafood lover's favorite seafood. Crabs are found both on the Atlantic and Pacific coasts of North and South America and the western coasts of north and central Europe, with each region having a local favorite. Several of the world's favorite crab species are presented in Table 3 (Figure 3).

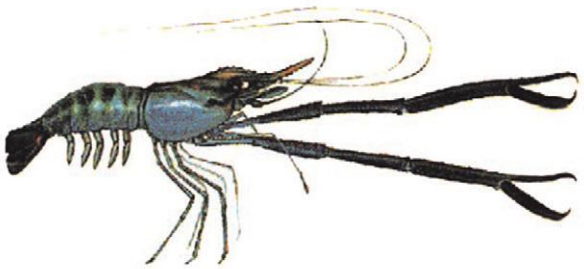

Figure 1 Blue prawn. Reproduced with permission from L&S Farms, Photographer: Cortney Ohs.

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Table 1 Common edible shrimp

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attributes*
Penaeus monodon	Black tiger	Central and SE Asia	Black with yellow striped tails	Mild flavor
Penaeus stylirostris	Blues or Mexican whites	Pacific coast of Mexico	Similar to Gulf of Mexico Whites	Firm texture and full, nutty flavor
Penaeus aztecus	Browns	SE USA; Gulf of Mexico	Abundant, low cost	Softer texture than others
Pandalus borealis	Deep sea shrimp	Coast of northern Europe	Most are sold at local markets	Firm texture
Hymenopenaeus robustus	Gulf shrimp	Gulf of Mexico	Large shrimp weighing ≤40 g	Firm texture
Penaeus japonicus	Kuruma or Japanese prawn	Indo-Pacific area; Red and Mediterranean Seas	Large prawns	Firm texture
Aristeus antennatus	Mediterranean prawn	Mediterranean Sea	Blue or red varieties	Sold as appetizer or 'starter' in local markets
Penaeus duorarum	Pink shrimp	Coastal USA in wide bottom sand or mud shelves	Premium domestic US shrimp	Firm texture and mild sweet flavor
Palaemon serratus	Prawn/pink shrimp; sword shrimp; crevette rose; camaron or gamberellon	Deep water Atlantic Ocean and Mediterranean Sea	Premium shrimp of France and Italy	Firm texture and mild sweet flavor
Sicyonia brevirostris	Rock shrimp	Tropical variety found off the coast of Florida	Small with a thick shell	Texture and flavor similar to lobster
Crangon crangon	Sand shrimp	Coastal Europe	Often served with oysters	Cooked whole
Panaeus setiferus	Whites	Gulf of Mexico and SE USA	30% of total US harvest	Firm texture with nutty flavor

Figure 2 Spiny lobster. © Avril Bourquin.

Crayfish/Crawfish

More than 400 species of crayfish are found worldwide. Only 3 of the 250 edible species are available commercially in North America. Crayfish live in freshwater rivers and streams, mostly in temperate climates. In North America, the crayfish is commonly called 'crawfish.' Most crawfish processing occurs in Louisiana, where they are caught wild and pond-raised. Crawfish are sold live for 'crawfish boils' or processed for frozen tail meat. Crawfish, like crabs, replace their hard shell during growth. During the 'softshell' period, the whole crawfish can be eaten and is considered a delicacy.

Procambarus clarkia (Red Swamp Crawfish)

Louisiana red swamp crawfish are harvested in the delta region. They are the most popular because of their larger size than other commercial species and they turn a beautiful bright red when cooked. The meat has a firm texture and is generally considered more flavorful than shrimp. The hepatopancreas (called 'fat' by local consumers) is often consumed along with the meat and is used as a flavor ingredient in a variety of local recipes.

Procambarus zonangulu (White River Crawfish)

White river crawfish are primarily harvested from northern and central Louisiana.

Pacifastacus leniusculus (Pacific Crayfish)

Harvested in California and Oregon, they are consumed by local markets.

Krill (*Euphausia superba*)

Krill is a tiny shrimp-like crustacean and is considered the most important zooplankton species associated with sea ice, playing a key role in the Antarctic food web. Krill travel in large swarms. Commercial fishermen harvest krill as a high-protein ingredient for value-added products and krill has recently been featured at various seafood shows throughout the world.

Molluscs

Any member of the Phylum Mollusca, an invertebrate with a soft unsegmented body, usually protected by a shell in one, two, or three pieces. The molluscs include oysters, clams,

Table 2 Common edible lobster

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attributes*
Jasus edwardsii and jverreauxi	Australian rock lobster	Coastal Australia and New Zealand	Major import to the USA	Marketed frozen for broiling
Panulirus llaevicauda	Brazilian spiny lobster	Caribbean Sea; East coast of S America	Harvested for tail meat	Marketed frozen for broiling
Palinurus interruptus	California spiny lobster	Southern coast of California	Mainly supplied local markets	Boiled whole
Homarus americanus	Canadian/American lobster	Western Atlantic Ocean from Labrador to North Carolina	Weight range 0.9–2.2 kg; bright red shell	Considered a delicacy in American and European markets; boiled live
Homarus gammarus	European lobster	Coasts of Great Britain, Norway and Brittany	Darker, bluish shell compare to American	Flavor and texture same as American
Palinurus mauritanicus	European spiny lobster	Mediterranean and E Atlantic	Thorny shell	European delicacy
Palimurus argus	Florida spiny lobster, Caribbean lobster, rock lobster, or langouste	Western tropical Atlantic from Florida to S America	Rough, hard shell. Sold for local markets only	Flavor and texture similar to American and European lobster
Panulirus marginatus	Hawaiian spiny lobster	NW Hawaiian Islands	Season limited by supply	Firm texture and sweet flavor
Nephrops norvegicus	Langoustines, Dublin Bay prawns, scampi	Norway, E Atlantic coast of Europe and Adriatic and western Mediterranean Sea	Smaller than lobsters, with long, smooth bodies	Boiled whole
Scyllarus arctus	Slipper/squat lobster, or Australian 'bug' lobster	Tropical seas	Small	Boiled whole
Jasus lalandii	South African rock lobster	South Africa	Cape crayfish	Premium lobster

Table 3 Common edible crab

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attributes*
Callinectes sapidus	Blue crab	East Coast USA and S America, Gulf of Mexico, France, Holland, and Denmark	Largest commercial crab fishery in the USA	Cooked and eaten whole or processed for meat and claws. Strong distinct flavor
Cancer magister	Dungeness crab	Oregon and Washington states	Niche market	Cooked for local markets
Paralithodes camtschaticus	King crab or 'red' king crab	Alaska	Coldwater crabs. Have 6 legs and 2 claws, unlike other crabs with 8 legs and 2 claws	Firm texture; meat like lobster; legs are meatier and are preferred over the claws
P. brevipes	Brown or deep water king crab	Deep water Alaska		
Lithodes antarcticus	Southern king crab	Chile to Antarctica		
Chionoecetes oplio, C. bairdi, and *C. tanneri*	Snow crab	Alaska	Smaller and less expensive than king crab	Legs are steamed or boiled
Maia squinado	Spider crab, thomback crab	Europe and Japan	Giant crab may reach 40 cm	Steamed or broiled
Menippe mercenaria	Stone crab	Florida west coast	Stone crab claws are snipped off the live crab and will regenerate	Claws are steamed and served cold
Portunidae spp.	Swimming crab, mud crab, shore crab, velvet crab	Italy, Portugal, Australia, and SE Asia	Distinguished by their extra pair of 'paddlelike' appendages	Soft shell are a delicacy in China; used for Thai crab cakes and Scottish crab soup

snails, slugs, squid, and octopuses. Most molluscs are aquatic but some land snails like *Helix aspersa* are also eaten. It is the snail most cultivated for gourmet food and is known as petit gris.

Bivalves (Two Piece Shells)

Oysters

Oysters have a rough, irregularly shaped shell and live mainly in temperate or warm coastal or estuarine waters. Oysters (often eaten raw) are considered a seafood delicacy. Raw oyster consumption is occasionally associated with gastrointestinal disease. Oyster beds, often located adjacent to rural communities, must be carefully monitored to ensure that they have not been contaminated with polluted water, especially during periods of heavy rain. Many species of oysters are harvested in small numbers and marketed for retail as exotic speciality oysters. The more important edible species are presented in Table 4 (Figure 4).

Clams

These burrowing shellfish are freshwater or marine molluscs having a muscular foot with which they can burrow into sand. More than 20 000 species are edible, but only approximately 50 species are harvested commercially. A few of these commercially available clams are presented in Table 5 (Figure 5).

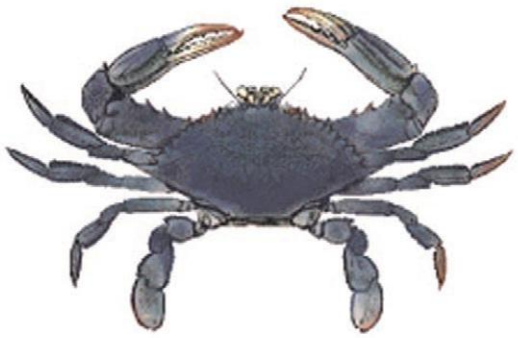

Figure 3 Blue crab. © Avril Bourquin.

Figure 4 True oysters. © Avril Bourquin.

Table 4 Edible oysters

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attributes*
Crassostrea virginicus	Eastern Atlantic or American oyster	Eastern USA, Southern Canada, Gulf of Mexico	Thick rough shells; the most popular and plentiful oyster in the USA	Mild delicate flavor; often consumed raw
Ostrea edulis	Native or 'flat' oyster; French Belon, the English Whitstable, Colchester and Helford, the Irish Galway and the Belgian Ostendes	Europe		Fine texture and rich flavor. The Belons, grown in cold water, have a briny, metallic flavor
Crassostrea gigas	Pacific or Japanese oyster	Pacific coasts of USA and Japan	Most widely farmed oyster in the world	Very large size makes them highly suitable for cooking
Crassostrea angulata	Portuguese cupped oyster	France and Portugal	Farm raised	Considered a delicacy, spéciales claires
Crassostrea commercialis	Sydney rock oyster	New South Wales	Farm raised	Rich, fresh flavor
Ostrea lurida	Western Olympia oyster; American native	Pacific coast USA	Small oyster less than 5 cm in length	Sold at local markets
Ostrea chilensis	Bluff oyster	Native to Chile and New Zealand	Dredged	Sold at local markets
Saccostrea commercialis	Rock oyster	New Zealand	Commercially grown originally but now supplanted by Pacific oyster	Exported

Table 5 Common edible clams

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attributes*
Panopea generosa	Geoduck clam	Pacific coast USA	Large clam attaining an in-shell weight of 4 kg	Steamed for delicate texture and flavor
Mercenaria mercenaria and *Mercenaria campechiensis*	Hard clam; quahog	New England shores of USA	Popular recreational fishery	Marketed for steaming as chowder clams (large), cherrystone (medium), and 'little neck' (small)
Tapes philippinarium	Manila clam	Philippines	Exotic species to USA	Satisfy a 'niche' market
Arctica islandica	Ocean quahog or 'black clam'	N Atlantic Coasts from Europe to USA and Canada	Harvest estimates are 46 million annually	Comprise 38% of US clam market; light flavor with crisp texture
Venerupis decussate	Palourde/carpet shell clam	Southern Europe	Small clam	Consumed raw in local markets
Venus verrucosa	Praire/warty Venus clam	Europe to Africa	Small clam	Consumed raw in local markets
Ensis directus, Solen marginatus and *Siliqua patula*	Razor or jackknife clam	Europe, California, Aleutian Islands	Long shape up to 25 cm	Popular for 'clam diggers;' steamed in sandy pits
Mya arenaria	Soft-shell clam	North America, Europe, and Pacific coast of the USA	A soft-shell clam with a long neck	Usually steamed
Spisula solidissima	Surf clam	Atlantic coast of N America from S Carolina north to the St Lawrence Gulf	A larger clam species	Processed as clams strips for breading and frying

Figure 5 Quahog clam. © Avril Bourquin.

Scallops

Marine bivalve molluscs have a distinctive fanshaped shell with radial ribs and wavy edges. Scallops move by opening and closing their valves. Near the hinge where the two valves (shells) meet is the eye, or adductor muscle, which is the part of the scallop eaten in North America. In Europe, the entire scallop is eaten. More than 300 species of scallop occur worldwide, with varying shell color including beige, pink, salmon, and yellow. The more commonly consumed scallops are presented in Table 6 (Figure 6).

Mussels

Mussels are distinguished by a blue–black shell and live attached to objects in the sea. The many varieties of mussels are harvested from cold Atlantic waters in both Europe and the US and off the coast of New Zealand.

Mytilus edulis *(blue mussel)*

The blue mussel represents the dominant mussel species in North America. They are found in Atlantic waters from Canada to North Carolina. They have a smooth, bluish-black elongated shell. The inside of the shell is pearly violet or white. Between the shells on one side is a bundle of tough, brown fibers called the byssal threads or 'beard.' These fibers are used to anchor the mussel to rocks, pilings, and other mussels. As demand for consumption increases, wild populations are being supplemented by aquaculture to prevent depletion of natural beds. In Europe, blue mussels have been cultured for over 300 years. Mussels are efficient feeders compared to other shellfish. They have a third more protein than oysters. Orange-tinted meats represent mature female mussels, whereas the ivory meats are males and immature females. Connoisseurs maintain that mature females have the best flavor.

Perna canaliculus *(greenshell mussel)*

Greenshell mussels are harvested off the coast of New Zealand and may grow up to 23 cm.

*Cockles (*Cardium edule*)*

Cockles are found worldwide but are traditionally thought of as a British speciality. In North America, they are known as 'heart clams.'

Table 6 Common edible scallops

Zoological name	*Common name*	*Location*	*Properties/uses*	*Culinary attribute*
Argopecten irradians	Bay scallop	N America: New England to Gulf of Mexico	Small scallop only 2 cm	Firm, white meat
Argopecten gibbus	Calico scallop	Tropical and subtropical Atlantic from N Carolina to Brazil	Important commercial species; 465 t annually	Firm, white meat
Chlamys opercularis	Queen scallop	SE Asia	Small scallop	Firm, white meat used in soups
Placopecten magellanicus	Sea scallop	Atlantic coast of N and S America	Largest species, market size is 1.5–8 cm	Distinct sweet odor when fresh
Patinopecten cauimus	Weathervane scallop	Alaska	Short harvest season; 2–3 weeks in late summer	Sweet, crisp flavor and texture
Pecten novaezelandiae	Scallop	New Zealand	Important commercial species; 747 t annually in 2004. Seeded from spat	Firm, white meat

Figure 6 Scallop. Reproduced with permission from Shells Database.

Single-Shell Molluscs

Gastropods

Gastropods have a head with eyes, a large flattened foot, and often a single shell. Gastropods are the largest class in the Phylum Mollusca and are the most diverse. Nearly 35 000 living species and 15 000 fossil species have been identified, including spirally coiled snails, flat-shelled limpets, shell-less nudibranchs, whelks, abalones, pteropods, and terrestrial snails and slugs.

There are several varieties of Abalone (*Haliotis cracherodii* (black), *Haliotis rufescens* (red), and *Haliotis iris* (white)).

Of all the gastropods, abalone is the most popular for human consumption. The red and black abalone are found along the coastlines of California, Mexico, and Japan. Approximately 100 species exist worldwide. The edible portion is the adductor muscle, by which it clings to rocks. Abalone, used widely in Chinese and Japanese cooking, can be purchased fresh, canned, dried, or salted. The iridescent shell is a source of mother-of-pearl. The wild fishery is carefully managed and abalone farms have been established to meet consumer demand.

Conches (Strombidae *sp.*)

Of the numerous species of conches, the queen conch is the most popular edible species. They are mostly found near the breaking surf of barrier reefs because of their high oxygen requirement. Conches are harvested from Belize, Turks, and Caicos and the Bahamas for restaurant trade. The meat is white with a tough rubbery texture and is pounded to tenderize it. Because of their rarity, conches bring a good price in restaurants.

Whelks (Subclass Prosobranchia, Buccinum undatum*)*

Prosobranchia (limpets, winkles, whelks, etc.) are familiar creatures, often found in rockpools on the seashore. The common whelk is distributed along Atlantic coasts, the English Channel, the North Sea, and the Baltic Sea. They inhabit sand and mud from shallow water to a depth of 100 m. Fully grown, whelks have a shell up to 10–15 cm high with a pointed spire and well-defined ribbed whorls. They are usually pale brown in color. The largest whelks (like *Busycon carica)* are found on the American side of the Atlantic and may grow up to 30–35 cm.

*Periwinkles or sea snails (*Littorina littorea*)*

Nearly 300 species are known throughout the world, but few reach edible size. Periwinkles are found among seaweed on the rocky shores of the eastern coast of North America from Canada to lDelaware. Usually dark brown in color, the shell is rounded with concentric ridges and dark lines. Periwinkles are a common food in Europe but are not harvested in large numbers.

Cephalopods

Cephalopods are molluscs closely related to snails and include squid, nautilus, cuttlefish, and octopus. Cephalopods are found in most of the world's oceans in plentiful supply.

Squid (Loligo pealei, Illex illecegrosus, *and* Loligo opalescens)

Squid, commonly called calamari, are found in the northwest Atlantic Ocean and on the Pacific coast. There are marine cephalopods with two long tentacles and eight shorter arms, a long tapered body, two triangular fins, and an internal shell (Figure 7). Squid sizes range from 7.5 cm to as large as 17 m for the giant squid. Squid landings for edible food have greatly increased over the past 25 years; most are from Pacific harvest and more than half are exported to Asian markets, especially Japan. *Loligo vulgaris*, found throughout Europe, weigh up to 2 kg and are noted to propel themselves out of the water, like a missile.

Octopus (Octopus spp.)

Unlike the cuttlefish and squid, the octopus has no internal shell. There are several species of edible octopus. The giant octopus ranges the coastal waters off northern California through the Gulf of Alaska and around the Pacific Rim to Japan and Korea. Typically they are found in waters shallower than 180 m. The octopus has a large head, a soft oval body, well-developed eyes, and eight arms containing rows of suckers (Figure 8). Edible species range in size from 1 to 3 m in length. Octopus is available live, fresh, frozen, and cooked. Spiced and boiled octopuses are prepared with the viscera and eyes removed. Pickled octopuses are primarily available in Mediterranean and Asian fish markets.

Figure 7 Squid. © Avril Bourquin.

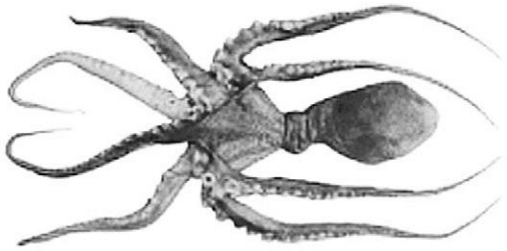

Figure 8 Octopus. © Avril Bourquin.

Cuttlefish (Sepia spp.)

Cuttlefish are caught for food in the Mediterranean, East Asia, the English Channel, and elsewhere. Squid is more popular as a restaurant dish all over the world. Cuttlefish ink was formerly an important dye, called sepia.

Welfare Issues

Animal welfare has become increasingly important worldwide. A fundamental issue when deciding on our moral duties toward animals is whether they are capable of experiencing pain and other forms of suffering. The welfare of shellfish has been much less studied than that of mammals, birds, and even finfish. A summary of findings relevant to the welfare of cephalopods and decapods follows.

There is evidence that cephalopods have a nervous system and relatively complex brain similar to many vertebrates, with good learning ability, and memory retention. They release adrenal hormones in response to situations that would elicit pain and distress in humans, can learn to avoid painful stimuli and have nociceptors in their skin.

Research has found the presence of opioids and opioid receptors in crabs. Also, in an avoidance learning experiment, crabs showed memory of aversive stimuli and learned to avoid them. In another experiment, noxious stimuli (irritating chemical solutions and physical pinching) applied to antennae caused prawns to display immediate reflex tail-flicking responses and also two prolonged activities, grooming of the antenna, and rubbing of the antenna against the side of their enclosure. These responses were blocked with the application of a local anesthetic.

According to some authors, the previous findings suggest that at least some groups of shellfish (in particular, cephalopods and decapods) are capable of experiencing pain and suffering. This would have obvious implications for the industry, as some of the methods used to catch and kill these animals would then be questionable on ethical grounds. Others, however, think that one does not know enough yet to decide whether shellfish are sentient, i.e., capable of experiencing emotions such as pain. Even in this case, however, whether they should be given the benefit of doubt is still an open question so one should aim for procedures that ensure humaneness.

See also: Meat, Animal, Poultry and Fish Production and Management: Disease Control and Specific Pathogen Free Pig Production

Further Reading

Britannica (2003). Deluxe edition CD-ROM. Shellfish. In: Kennedy, V.S., Newell, R.I., and Eble, A.F. (Eds.), The Eastern Oyster: *Crassostrea virginica*. College Park, MD: The University of Maryland Press.

EFSA, 2005. Scientific Opinion of the Panel on Animal Health and Welfare on a request from European Commission on Aspects of the biology and welfare of animals used for experimental and other scientific purposes. The EFSA Journal 292, 1–136.
Elwood, R.W., Barr, S., Patterson, L., 2009. Pain and stress in Crustaceans? Applied Animal Behaviour Science 118, 128–136.
Martin, R.E., Carter, E.P., Flick, G.J., Davis, L.M., 2000. Marine and Freshwater Products Handbook. Boca Raton, FL: Technomic.
Mather, J.A., 2007. Cephalopod consciousness: Behavioural evidence. Diseases of Aquatic Organisms 17, 37–48.
Negedly, R., 1990. Elsevier's Dictionary of Fishery, Processing, Fish and Shellfish Names of the World. Amsterdam: Elsevier.
Oberrecht, K., 1997. Fish and Shellfish, Care and Cookery. New York: Stoeger Publishing.
Peterson, J., 1996. Fish and Shellfish. New York: William Morrow.
US Department of Commerce ,2002. Fisheries of the United States, 2001. Silver Springs, MD: National Marine Fisheries Service, Office of Science and Technology, Fisheries Statistics and Economics Division.
Whiteman, K., 2001. The World Encyclopedia of Fish and Shellfish. Singapore: Anniss Publishing Limited.

Relevant Website

http://en.wikipedia.org/wiki/
Separately for Oysters, Mussels, Lobsters, Spiny lobsters crayfish, Crab, Shrimp prawn, Krill, Squid, Cuttlefish, Octopus.

SPOILAGE, FACTORS AFFECTING

Contents
Microbiological
Oxidative and Enzymatic

Microbiological

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Glossary

Catabolite repression The quick adaptation of bacteria to a preferred carbon and energy source through inhibition of synthesis of enzymes involved in catabolism of carbon sources other than the preferred one.
Gram-negative bacteria Bacteria that are decolorized when stained with crystal violet dye after treatment with ethanol according to Gram's procedure.
Gram-positive bacteria Bacteria that retain the crystal violet dye on treatment with ethanol according to Gram's procedure.
Partial pressure The pressure due to an individual gas in a mixture of gases.
Spoilage flora (plural flora) A group of microorganisms that grow on food with ultimately deleterious effects.
Water activity (a_w) The ratio of vapor pressure of water in a food or substance to that of pure water at the same temperature.
Wiltshire bacon The pork cured by immersion in brine.

Introduction

Meats are spoiled by microorganisms when microbes on or in the product cause changes to meat qualities that consumers perceive as being undesirable or frankly offensive. Undesirable or offensive changes can involve the appearance, odor, and/or flavor of the meat. Visible changes include the appearance of visible colonies or a layer of slime on the product surface; or changes in the color of meat, from red to brown, gray, or green. Changes in the odor of meat can range from strong, putrid, or sulfurous odors to mild, stale, aromatic, or acid odors. Flavor changes can be similarly variable, but during the development of microbial spoilage flavor changes can often be detected before spoilage odors are apparent.

The spoilage conditions that develop will depend on the types of organisms that are present in the spoilage microflora. The composition of the spoilage flora will be affected by intrinsic properties of the meat, such as the pH and the water activity (a_w) of the product; and by extrinsic factors, such as the atmosphere around the product and the temperature at which it is held. In addition, the form of spoilage that is manifest can be affected by the amounts of specific nutrients for some elements of the microflora that are present in the product. As meats are not necessarily homogeneous products, spoilage need not be uniform over all parts of an item of meat. For example, spoilage of moist fat tissue of a meat cut may precede spoilage of the muscle tissue. Moreover, the environment around an item of meat need not be homogenous. For example, meat in clipped chub packs may be exposed to an aerobic environment in the regions of the clips but be anaerobic elsewhere. However, spoilage of one part of an item will usually render the whole item unacceptable.

Aerobic Spoilage of Raw Muscle Tissue

Muscle tissue in the carcass immediately after slaughter is essentially sterile. The tissue is contaminated with bacteria from the hide and from the packing plant equipment and environment during the dressing and breaking of carcasses. Consequently, meat surfaces are contaminated with a variety of organisms that include psychrotrophs from environmental sources, which can grow at chiller temperatures, as well as mesophiles derived from flora associated with animals, which cannot grow on chilled meat. Initial numbers of bacteria on the surfaces of meat can exceed 10^4 cfu cm^{-2}. However, improvement of processing hygiene at packing plants in recent

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years has resulted in some plants at least routinely producing carcasses and cuts with initial numbers of $< 10^2$ cfu cm^{-2}.

Postrigor muscle tissue provides a rich medium for the support of bacterial growth. Although the major potential nutrient for bacteria is protein, most bacteria do not elaborate enzymes to attack complex compounds when simple compounds are readily available to support their growth. As lactic acid, amino acids, and glucose are readily utilized by most bacteria, and are generally abundant in muscle tissue, such simple substances, not proteins, are the initial nutrients for the spoilage flora (Table 1).

The accumulation of lactic acid in the muscle tissue during the development of rigor can reduce the tissue pH to 5.5 or a little lower. The aerobic growth of many bacteria is not affected by such pH values, and the concentrations of solutes do not reduce the water activity to values that inhibit bacteria growth. Thus, aerobic growth of many bacteria on muscle tissue is initially constrained by temperature alone. In these circumstances, the organisms that can grow most rapidly at the prevailing temperatures will tend to overgrow competitors, to predominate in the spoilage flora.

The extent to which the fastest-growing species dominate the flora will depend not only on the extent to which their rates of growth exceed those of competitors, but also on the absolute numbers of the initial flora and the initial fraction of the potentially dominant organisms. If the initial numbers of the flora are low, then the relatively large number of generations required before maximum numbers are attained will allow extensive expression of a growth rate advantage. However, if the initial numbers are high, but the numbers of the faster-growing organisms are relatively low, then the final flora may contain relatively large fractions of slower-growing organisms.

Under aerobic conditions, the organisms that grow best on muscle tissue at chiller temperatures are Gram-negative, strictly aerobic pseudomonads and moraxellaceae (Table 2). The latter group includes acinetobacteria, moraxellae, and psychrobacteria. Although organisms of the latter groups are usually found in aerobic spoilage flora, they generally do not produce offensive metabolic by-products; pseudomonads, which do produce offensive by-products, are generally major components of the flora at spoilage onset, and often predominant. Consequently, aerobic spoilage at chiller temperatures is largely the result of the activities of pseudomonads.

The pseudomonads are nutritionally versatile but generally exhibit strong catabolite repression during the utilization of substrates from complex media. Catabolite repression ensures that while a preferred substrate is available, metabolic pathways for the utilization of other substrates are suppressed. For pseudomonads, glucose and related substances are the preferred substrates. When these are metabolized, no by-products that impart objectionable odors or flavors to meat are produced. However, when such substrates are exhausted amino acids are utilized, with the production of ammonia and other by-products, such as organic sulfides, esters, and acids, which impart strong, putrid odors and flavors to meat.

The amounts of glucose present in muscle tissues are limited. When glucose diffusing from within a piece of muscle can no longer meet the demand of bacteria proliferating at the surface, then pseudomonads in the flora will attack amino acids. When glucose is at concentrations in the tissue of approximately 0.1 mg g^{-1}, as is typical for beef from pasture-fed animals, this will occur when the aerobic flora numbers approach 10^8 cm^{-2}. With meat from feed-lotted cattle, glucose concentrations may exceed 1 mg g^{-1} and overt spoilage may not occur until numbers are $> 10^8$ cm^{-2}. At these high numbers, offensive by-products are rapidly generated in organoleptically detectable quantities from amino acids. Thus, in these circumstances the onset of spoilage is abrupt, with the tissue being wholly unspoiled when glucose is available at the surface even though bacterial numbers are high.

The abundance of nutrients other than glucose precludes growth of the aerobic spoilage flora being limited by the availability of nutrients. Instead, numbers increase to exceed 10^9 cm^{-2}. At these numbers, putrid spoilage is visibly augmented by a layer of slime on the tissue surface. Growth of the aerobic flora is then limited by the rate at which oxygen can diffuse from the atmosphere into the slime layer. As catabolic activities decline because of the increasingly limited availability of oxygen, catabolite repression is relieved, and exoenzymes that degrade proteins and other complex substrates are synthesized. Such enzymes degrade structural elements of muscle tissue, which allows bacteria to move from the surface into the deeper tissues, between muscle fibers.

If muscle tissue is deficient in glycogen at the time an animal is slaughtered, then the amount of lactic acid formed will be lower than usual, the pH of the tissue will remain high, and little or no glucose will be present in the postrigor muscle. The high pH does not affect the composition of the spoilage

Table 1 Typical concentrations of low-molecular weight soluble components of beef muscle tissue of normal pH from pasture-fed animals

Substance	*Concentration (mg g^{-1})*
Lactic acid	9.0
Creatine	5.5
Amino acids	3.5
Dipeptides	3.0
Inosine monophosphate	3.0
Nucleotides	1.0
Glycogen	1.0
Glucose 6-phosphate	0.2
Glucose	0.1

Table 2 Rates of growth of Gram-negative bacteria from aerobic spoilage flora on muscle tissues of normal and high pH stored in air at 2 °C

Organisms	*Growth rate (generations/day)*	
	pH 5.6	*pH 6.4*
Pseudomonas spp.	2.03	2.11
Moraxella spp.	1.85	1.82
Acinetobacter spp.	1.58	1.55
Flavobacterium spp.	1.18	1.14
Enterobacteriaceae	1.13	1.20
Aeromonas spp.	0.96	1.36

flora. However, in the near or total absence of glucose, the pseudomonads will degrade amino acids at an early stage of spoilage flora development. At first, the amounts of offensive by-products produced by the relatively few bacteria are undetectable organoleptically. However, as the flora increases, offensive by-products accumulate until putrid spoilage is apparent when numbers are approximately 10^6 cm^{-2}. Therefore, the deficiency of glucose in muscle tissue of high pH results in the meat being prone to early spoilage.

Psychotrophic pseudomonads usually dominate aerobic spoilage flora when meat is held at temperatures $\leq$20 °C. At higher temperatures, mesophilic enterobacteria will predominate on moist meat surfaces. However, the enterobacteria also utilize glucose preferentially, and thus the course of spoilage with these organisms is similar to that resulting from the activities of pseudomonads.

Aerobic Spoilage of Fat and Organ Tissues and Minced Meats

As moisture that evaporates from fat tissue surfaces cannot be replenished from within the tissue, fat tissue surfaces can dry and hence preclude the growth of bacteria. However, if fat tissues remain moist because the meat is held in a humid environment, then an aerobic flora will develop on the fat as on muscle tissue. Fat tissue surfaces are contaminated with exudate, from cut blood vessels and/or muscle tissues, that contains bacterial nutrients. However, the concentrations of bacterial nutrients on fat surfaces are generally low and the nutrients cannot be replenished from the underlying tissues. Thus, glucose is rapidly exhausted, and growth continues with the utilization of amino acids, and then lactic acid when preferred amino acids are depleted. Consequently, putrid spoilage becomes evident when numbers approach 10^6 cm^{-2}, as with high-pH meat. However, the total amount of nutrients available may be inadequate for the flora to grow to numbers at which a visible slime layer is formed. That is, growth may be nutrient-limited rather than oxygen-limited.

Organ tissues are generally of pH$>$6 but can contain substantial concentrations of glucose. For example, liver can contain glucose at concentrations up to several milligrams per gram. Unlike muscle tissue, the tissue structures of liver and other organs allow bacteria from the surface to invade the deep tissues. However, the deep tissues are anaerobic, and thus growth of bacteria within the tissues is slower, and the bacteria growing these do not include the strictly aerobic organisms that predominate in the flora on the surface. Spoilage at surfaces exposed to air will then precede spoilage of deep tissues. Aerobic spoilage will proceed as with muscle tissue, but the formation of visible colonies or slime may precede or be contemporaneous with the development of spoilage odors when glucose concentrations are high. In addition, the tissues may be acidified, because when glucose is abundant it is converted extracellularly by pseudomonads to gluconic and 2-oxogluconic acids.

For minced meat products that are not preserved by high concentrations of solutes or acidification, spoilage at surfaces exposed to air will also precede deep spoilage, and proceed as for muscle tissue. Again, colony or slime formation may be among the first symptoms of spoilage, if carbohydrates preferentially utilized by pseudomonads have been added to a product.

Anaerobic Spoilage

The pigments in muscle and organ tissues – myoglobin and hemoglobin – react readily with oxygen at all partial pressures. Thus, when meats are sealed, with little or no headspace, in packs composed of materials that are nearly or wholly impermeable to oxygen, the residual oxygen will be removed from the meat environment quite rapidly. With raw tissues packaged in an essentially gas-impermeable material, such as laminated plastic films that include two layers of metalized film, anaerobic conditions will develop and be maintained. However, vacuum packages for meats are usually composed of plastic films with various low, but measurable, oxygen transmission rates at temperatures above 0 °C. In addition, some raw tissues, such as fat, and some meat products have only limited oxygen-scavenging capabilities. Thus, in many circumstances, the environment at the surface of vacuum-packed product can be microaerobic rather than anaerobic. In either environment, growth of the strictly aerobic organisms that predominate in aerobic spoilage flora will usually be suppressed. Instead, spoilage flora dominated by anaerobic or facultatively anaerobic organisms develop. Unlike with aerobic spoilage, the types of organisms that contribute to the spoilage flora are determined by the pH of the meat as well as the storage temperature.

Many spoilage organisms are unable to grow under anaerobic conditions on muscle tissue of normal pH (5.5) held at chiller temperatures. Under these conditions, the flora that develops is composed of Gram-positive lactic acid bacteria such as lactobacilli, carnobacteria, and leuconostocs, with organisms of the last group tending to predominate. The lactic acid bacteria are metabolically anaerobic, although they are aerotolerant. The substrates they can ferment to support growth on muscle tissue are limited to glucose and some other carbohydrates available in lower amounts. Thus, growth of the lactic flora ceases when the concentration of glucose at the tissue surface is depleted. This substrate limitation of the flora growth typically occurs when numbers are approximately 10^8 cm^{-2}.

When fermenting glucose, the lactic acid bacteria do not produce offensive by-products. Although most lactic acid bacteria cannot utilize amino acids to support growth, some amino acids, notably valine and leucine, may be metabolized with the production of volatile fatty acids as byproducts. The slow accumulation of such substances can impart acid/dairy flavors and, ultimately, odors to meat. Such flavors and odors are unusual for meat rather than grossly offensive, but they finally render the meat unacceptable to consumers some time after the flora has reached maximum numbers. Some strains of lactic acid bacteria can metabolize sulfur-containing amino acids, with slow production of hydrogen sulfide. Hydrogen sulfide can react with muscle or blood pigments to spoil the meat by green discoloration. Hydrogen peroxide produced by some lactic acid bacteria under microaerobic conditions can also cause the green discoloration of fresh and cured meats.

If the pH of the meat is above 5.8, facultative anaerobes of high spoilage potential can grow on products held under

anaerobic conditions at chiller temperatures. Such organisms include psychrotophic enterobacteria, *Shewanella putrefaciens*, and *Brochothrix thermosphacta*.

Under anaerobic conditions, the enterobacteria will ferment glucose while it is available, and then utilize amino acids when glucose is exhausted. Some amino acids are decarboxylated to give malodorous amines, while hydrogen sulfide as well as organic sulfides may be produced from others. All such by-products grossly affect the odor and flavor of meat, and hydrogen sulfide can cause green discoloration.

Shewanella putrefaciens can form part of an aerobic spoilage flora, in which its behavior is similar to that of the pseudomonads. However, unlike the pseudomonads, it utilizes the amino acids serine and cysteine even when glucose is available. Hydrogen sulfide and organic sulfides derived from the latter substrate contribute to spoilage odors and flavors. The organism is not fermentative, but under anaerobic conditions it can utilize a variety of terminal electron acceptors other than oxygen to maintain respiratory metabolism. Under anaerobic conditions, hydrogen sulfide is produced in abundance, with consequent degradation of the color, odor, and flavor of product.

Brochothrix thermosphacta ferments glucose to lactic acid, and therefore under strictly anaerobic conditions its spoilage potential is limited. Under aerobic conditions, glucose is metabolized to acetoin, diacetyl, and a variety of fatty acids and alcohols. These products of aerobic metabolism impart strong, stale, and sour odors and flavors to meat. Thus, under conditions where some aerobic metabolism is maintained, these by-products can spoil meat.

Unlike pseudomonads, enterobacteria, and lactic acid bacteria, which produce offensive by-products only when preferred carbohydrate substrates are unavailable, *B. thermosphacta* and *S. putreficiens* can produce offensive by-products at all times during their growth on meat. Therefore, even when the numbers of these organisms are less than $10^6\,cm^{-2}$, offensive by-products may be produced in detectable quantities to spoil the meat, irrespective of the state of growth of the spoilage flora as a whole.

However, the maximum numbers of the potent spoilage organisms in an anaerobic flora are usually constrained by the lactic acid bacteria, which sequester nutrients and produce inhibitory bacteriocins. The inhibition of other organisms by lactic acid bacteria occurs only as the flora approaches its maximum numbers. Before that, the various types of bacteria grow at rates that are determined by the temperature and the environment provided by the meat, without any obvious interactions between types of bacteria. Thus, whether or not the potent spoilage organisms contribute to the spoilage of anaerobically stored meat, when pH does not inhibit their growth, will depend on their numbers in the initial flora. If their numbers are low relative to the numbers of lactic acid bacteria, growth of the potent spoilage organisms can be suppressed before they reach numbers sufficient to elaborate offensive by-products in detectable quantities; but if their numbers are relatively high, they will reach numbers sufficient to cause spoilage before growth ceases.

Although lactic acid bacteria, particularly leuconostocs, have a growth rate advantage at chiller temperatures, that advantage reduces at increasing temperatures (Table 3). At abusive temperatures, enterobacteria and *B. thermosphacta* can compete effectively with the lactic acid bacteria; and at warm temperatures, anaerobic flora can be dominated by enterobacteria.

Table 3 Rates of anaerobic growth of bacteria from anaerobic spoilage flora at abusive and warm temperatures

Organism(s)	*Growth rate (generations/day)*		
	10 °C	*20 °C*	*30 °C*
Leuconostoc spp.	3.9	10.4	15.0
Enterobacter spp.	2.8	10.9	12.6
Brochrothrix thermosphacta	2.5	14.1	15.7
Escherichia coli	1.9	11.4	17.1

In addition to the usual microbial spoilage conditions of vacuum-packed meats, both raw and cooked meats may be spoiled by psychrotrophic clostridia. A number of species can apparently be involved in such spoilage, which often involves some swelling (blowing) of packs. However, gross swelling of vacuum-packed chilled meats stored for short times at non-abusive temperatures appears to be due largely to the fermentation of lactic acid by *Clostridium estertheticum*. Other clostridia can cause softening of meat, with the release of large volumes of exudate and the development of putrid and sulfurous odors. The organisms responsible for the production of the large volumes of gas and the proteolytic degradation of the muscle tissue are often difficult to recover. Usual methods for enumerating and isolating bacteria from meat generally recover a flora of mainly lactic acid bacteria from meat spoiled by psychrotrophic clostridia. In view of the difficulties with their recovery and the limited understanding of the circumstances under which they appear in meat spoilage flora, it is possible that psychrotrophic clostridia are involved in meat spoilage more often than is now recognized. Studies aimed at characterizing meat spoilage flora by molecular methods, and identifying the metabolic activities of psychrotolerant clostridia growing on meat, may resolve the current uncertainties about their roles in meat spoilage.

Spoilage in Modified and Controlled-Atmosphere Packagings

Modified-atmosphere packagings are filled with aerobic atmospheres that are usually rich in oxygen, and which have concentrations of carbon dioxide (CO_2) sufficient to inhibit growth of pseudomonads. As large volumes of CO_2 can dissolve in meat, and CO_2 and oxygen can exchange across packaging films, the atmosphere can change during storage. Controlled-atmosphere packagings are those in which a stable atmosphere is maintained throughout storage of the product. The only packagings of this type used with meats employ pouches made of gas-impermeable films that are filled with an atmosphere of CO_2 to obtain an anaerobic atmosphere.

The growth rates of pseudomonads decrease with increasing CO_2 concentrations up to approximately 20%. Increases in CO_2 concentrations beyond that do not greatly reduce the rate of growth provided the atmosphere is aerobic. The maximum reduction in the rate of growth of pseudomonads is

Table 4 Examples of conditions tolerated by spoilage organisms growing on meats

Organisms	*Conditions*			
	Minimum a_w	*Minimum pH*	*Maximum salt concentration (%)*	*Maximum sorbate concentration (ppm)*
Gram-negative bacteria	0.95	4.4	10	100
Gram-positive bacteria	0.90	3.8	15	700
Yeasts	0.80	2.0	20	400
Molds	0.75	1.7	<20	1000

approximately 50%. A reduction of that order is sufficient to allow lactic acid bacteria, which are not affected by CO_2 at such concentrations, to outgrow pseudomonads and dominate the spoilage flora. However, *B. thermosphacta* and enterobacteria are also unaffected by the CO_2, and thus can form large fractions of the spoilage flora. In such circumstances, *B. thermosphacta* can cause early spoilage of a product as the flora develops. Enterobacteria cause spoilage as glucose is exhausted by the activities of the total flora.

As a controlled atmosphere of carbon dioxide is anaerobic, growth of pseudomonads is totally inhibited. Such an atmosphere also inhibits the growth of enterobacteria, raises the minimum temperature for growth of *B. thermosphacta*, and probably affects some elements of the lactic flora. Consequently, meat in a carbon dioxide atmosphere generally supports a lactic flora and develops acid/dairy flavors only well after the flora attains maximum numbers.

Bacterial Spoilage of Preserved Meats

Meats are preserved by drying; by the addition of salt or other solutes in quantities sufficient to reduce the water activity to levels at which growth of spoilage bacteria is affected; by fermentation of added carbohydrates or addition of acidulants, to reduce the pH; or/and by addition of antimicrobial agents, such as curing salts (nitrate/nitrite), sulfite, and benzoate. The Gram-negative organisms that spoil products rapidly are mostly susceptible to relatively mild preservative treatments (Table 4). Inclusion of preferentially utilized carbohydrates in preserved meats also tends to inhibit the production of ill-tasting and malodorous byproducts. Bacterial flora of preserved meats is then commonly dominated by Gram-positive organisms of low spoilage potential, with microbial spoilage being first manifest as slime or visible colonies, or discoloration of cured products. However, certain preserved meats tend to develop a flora enriched for specific spoilage organisms and thus undergo spoilage in a product-typical fashion. Examples of such products are raw sausages preserved with sulfite, which are usually spoiled by *B. thermosphacta*, as that organism is tolerant of the preservative, and Wiltshire bacons, which can be spoiled by the activities of salt-requiring vibrios.

Spoilage by Yeast and Molds

Yeast and molds grow far more slowly than the spoilage bacteria and thus will cause meat spoilage only when conditions prevent bacterial growth. Most yeasts and molds can grow only aerobically and are inhibited by relatively low concentrations of carbon dioxide, and therefore atmospheres other than air do not favor their growth. However, yeasts and molds can commonly tolerate lower water activities and more acidic conditions than spoilage bacteria, and some can grow at lower temperatures or are less affected by preservatives than the bacteria. Yeasts generally grow more rapidly than molds, and hence when conditions allow the growth of both, but prevent the growth of bacteria, spoilage will likely be caused by yeasts. Spoilage by molds or yeasts is usually due to the development of visible colonies on product surfaces.

Table 5 Growth rates of bacteria, yeasts, and molds at 0 °C and subzero temperatures

Type of organism(s)	*Name of organism(s)*	*Growth rate (per day)*[a]		
		0 °C	*−2 °C*	*−5 °C*
Bacteria	*Pseudomonas* spp.	1.75	1.00	–
	Leuconostoc spp.	0.48	0.19	–
Yeasts	*Cryptococcus infirmo-miniatus*	1.03	0.57	0.11
	Cryptococcus laurentii	0.60	0.43	0.09
Molds	*Thamniduim elegans*	0.67	0.50	0.03
	Cladosporium herbarum	0.31	0.18	0.03
	Penicillium hirsutum	0.35	0.17	–
	Penicillium corylophilum	0.19	–	–

[a]Growth rates of bacteria and yeasts are generations/day. Growth rates of molds are increase in length per unit length per day of hyphae from newly germinated spores.

On raw meats, mold spoilage occurs when desiccation of the surface prevents the growth of spoilage bacteria. The surfaces of chilled carcasses can become desiccated and develop mold colonies when circulating air of low humidity prevents the dry surfaces of muscle tissues being rehydrated by the water that moves from within the muscle. Fat surfaces will obviously remain dry under such conditions and may also support mold growth.

Mold spoilage also occurs on frozen raw meats that experience prolonged periods of temperature abuse. The minimum temperature for growth of spoilage bacteria is approximately −3 °C, whereas that for molds and yeast is approximately −5 °C. It has therefore been assumed that temperature alone can dictate mold instead of bacterial spoilage. In fact, molds grow very slowly at −5 °C compared with yeasts (Table 5), so when only temperature and the depression of water activity associated with freezing affect microbial growth, visible yeast colonies can be formed long before mold colonies appear. Mold colonies are the main

manifestation of spoilage when substrate limitation precludes the formation of yeast colonies, or when surfaces desiccate to give water activities below those tolerated by yeasts. Such desiccation can occur by sublimation of ice from frozen tissues, but in practice the appropriate conditions for mold spoilage of frozen meat seem to arise when surfaces thaw, perhaps cyclically, and water evaporates into the dry, refrigerated air.

Yeasts and mold colonies can also cause spoilage of cured and preserved meats of low water activity, whereas raw meats preserved by the addition of sulfite can be spoiled by fermentative yeasts.

See also: Bacon Production: Bacon; Wiltshire Sides. Chemical and Physical Characteristics of Meat: Adipose Tissue; Chemical Composition; pH Measurement. Curing: Brine Curing of Meat; Dry. Meat Marketing: Cold Chain. Microbiological Safety of Meat: Hurdle Technology. Packaging: Equipment; Modified and Controlled Atmosphere; Overwrapping; Vacuum. Refrigeration and Freezing Technology: Applications; Principles; Thawing. Sausages, Types of: Cooked; Dry and Semidry; Fresh

Further Reading

Corry, J.E.L., 2007. Spoilage organisms of red meat and poultry. In: Mead, G.C. (Ed.), Microbiological Analysis of Red Meat, Poultry and Eggs. Cambridge, UK: Woodhead Publishing, pp. 101–122.

Davies, A., Board, R., 1998. The Microbiology of Meat and Poultry. London: Blackie Academic.

Gill, A.O., Gill, C.O., 2010. Preservative packagings for fresh meats, poultry and fin fish. In: Han, J.H. (Ed.), Innovations in Food Packaging. Amsterdam: Elsevier, pp. 204–226.

Gill, C.O., 1988. Microbiology of edible meat by products. In: Pearson, A.M., Dutson, T.R. (Eds.), Edible Meat By-Products. London: Elsevier, pp. 47–82.

Nychas, G.J.E., Douglas, L.M., Sofos, J.N., 2007. Meat, poultry and seafood. In: Doyle, M.P., Beuchat, L.R. (Eds.), Food Microbiology. Fundamentals and Frontiers. Washington, DC: ASM Press, pp. 105–140.

Pennacchia, C., Ercolini, D., Villani, F., 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiology 28, 84–93.

Samelis, J., Kakouri, A., Rementzis, J., 2000. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4 °C. Food Microbiology 17, 329–340.

Yang, X., Balamurugan, S., Gill, C.O., 2011. Effects on the development of blown pack spoilage of the initial numbers of *Clostridium estertheticum* spores and *Leuconostoc mesenteroides* on vacuum packed beef. Meat Science 88, 361–367.

Relevant Websites

http://www.meatupdate.csiro.au/data/Chilled_meat_for_export_02-91.pdf
Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia.

http://www.ime13.org/programs/presentations/mhardin%20presentation-mprc.pdf
IEH Laboratories.

http://www.redmeatinnovation.com.au/innovation-areas/food-safety/food-safety-publications
Meat and Livestock Australia.

http://www.beefresearch.org/CMDocs/BeefResearch/Beef%20shelf-life.pdf
National Cattlemen's Beef Association.

http://www.scientificamerican.com/article.cfm?id=how-do-salt-and-sugar-pre
Scientific American.

Oxidative and Enzymatic

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Glossary

Anserine An imidazole dipeptide of the amino acids *β*-alanine and *N*-methylhistidine, found in skeletal muscle, which acts as an antioxidant due to its chemical structure.
Carnosine An imidazole dipeptide of the amino acids *β*-alanine and L-histidine, found in skeletal muscle, which acts as an antioxidant due to its chemical structure.
Ferrylmyoglobin Metmyoglobin (Fe^{3+}) activated with hydrogen peroxide to yield a relatively stable hypervalent (Fe^{4+}) heme protein (without a protein-based radical) with prooxidant activity.
Free radical An atom or group of atoms that has at least one unpaired electron and is therefore unstable and highly reactive.
Glutathione peroxidise An enzyme family found in muscle, whose preferred substrate is hydrogen peroxide and main biological role is to protect the organism from oxidative damage.
Malondialdehyde A compound produced by oxidation of unsaturated fatty acids.
Metmyoglobin A brown-colored pigment found in meat, formed from myoglobin by oxidation of iron from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state.
Myoglobin An iron-containing protein (pigment) found in meat, consisting of heme (iron) connected to a single peptide chain. Myoglobin's role in muscle is to transport oxygen released by red blood cells to the mitochondria, where the oxygen is used to produce energy.
Oxymyoglobin The form of myoglobin that is complexed with an oxygen molecule – the color of meat most acceptable to consumers.
Perferrylmyoglobin Metmyoglobin (Fe^{3+}) activated with hydrogen peroxide to yield a transient hypervalent (Fe^{4+}) heme protein (with a protein-based radical) with prooxidant activity.
Reductase Any enzyme that catalyzes a biochemical reduction reaction.
Superoxide dismutase An enzyme that catalyzes the conversion of superoxide into hydrogen peroxide and oxygen.
Thiobarbituric acid (TBA) A widely used test for determining the extent of lipid oxidation by measuring the concentration of relatively polar secondary reaction products such as aldehydes.
Warmed-over flavor (WOF) An unpleasant flavor (rancid, stale) arising from heme-mediated lipid oxidation that develops in cooked meat that is subsequently refrigerated before reheating.

Introduction

Spoilage of meat occurs when there is deterioration of its odor, flavor, color, texture, and/or nutritive properties. These changes may result from chemical, physical, enzymatic, or microbiological processes. Oxidation, or the process of loss of electrons, hydrogen abstraction, or flow of unpaired electrons, may occur in all the chemical constituents of muscle foods. Peroxidation of lipids will become apparent to consumers by the development of rancid odors or flavors, and 'warmed-over' flavor (WOF) in previously cooked meats. Oxidation of meat pigments is recognizable by the development of brown discoloration replacing the normally acceptable bright red meat color. Oxidation of meat proteins leads to the loss of functional properties such as gel-forming ability, meat-binding ability, emulsification capacity, solubility, viscosity, water-holding capacity, and nutritive value. Changes to functional properties may result in a loss of texture in further processed meats or, in some cases, an increase in toughness/hardness and decrease in juiciness in whole muscle foods. Because of these deleterious changes associated with oxidation, much of the applied research in the meat industry is directed toward slowing the rate of oxidative reactions. Endogenous catalysts, antioxidants, and enzymes can all affect oxidation rates. In addition, oxidation rates can be affected by numerous interventions throughout the production chain, such as incorporation of antioxidants into animal feed, packaging systems, temperature control, exposure to light, or direct addition of antioxidants, metal chelators, dipeptides, etc. during processing. In the context of the present review, only enzymes that affect rates of lipid oxidation are considered. In addition, recent research on the oxidation of enzymes associated with changes in texture (proteolysis), which can contribute to decreased tenderization postmortem, will be overviewed. However, the mechanism of action of these enzyme systems and their postmortem activity, which may result in spoilage due to overtenderization, are discussed elsewhere.

Peroxidation of Lipids

Lipid peroxidation is the major form of quality deterioration, including flavor, odor, taste, color, texture, and appearance, leading to spoilage in meat and fish products, even when lipid content is fairly low. In lean beef, triacyglycerols and phospholipids comprise 2–4% and 0.8–1% of the meat weight,

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respectively. Only oleic, palmitic, and stearic fatty acids are present in substantial amounts in the fat of meat animals, combined with glycerol to form the triacylglycerols. The phospholipids, the so-called 'structural lipids,' are located in the membranes and contain over 40% polyunsaturated fatty acids (PUFA; 22% 18:2n−6, linoleic acid; 2% 18:3n−3, linolenic acid; 15% 20:4n−6, arachidonic acid; 1% 20:5n−3, eicosapentaenoic acid (EPA); and 2% 22:6n−3, docosahexaenoic acid (DHA)). Owing to their high level of unsaturation and their proximity to the heme catalysts of the mitochondria and microsomes, the initial oxidation reactions in meat generally involve the phospholipids. Chicken and turkey muscle are more susceptible to oxidation than beef due both to the higher levels of polyunsaturated phospholipids and lower levels of antioxidants in the poultry meats. Fish muscle is even more susceptible to oxidation due to the high degree of unsaturation, including enrichment with the characteristic omega-3 fatty acids, EPA, and DHA. Recent applied research efforts to shift the fatty acid composition of meat animals (e.g., beef and pork) toward increased long chain omega-3 PUFA to improve human dietary health may result in increased rates of oxidation, particularly in high-fat muscles and meat products. In this context, Australia has recently recognized the potentially important contribution to human dietary health of the long chain omega-3 PUFA, docosapentaenoic acid (DPA), which is present in quite high levels in ruminant animals (e.g., beef and lamb).

Refrigerated, whole, raw meat is relatively resistant to lipid peroxidation. Under the appropriate conditions, frozen beef has been found to maintain an acceptable quality for 10 years or more, provided desiccation (freezer burn) is avoided. However, oxidation of the tryacylglycerol fraction does proceed slowly and is referred to as 'normal oxidation,' as opposed to WOF, which is a rapid, heat-activated oxidation affecting mainly the phospholipids.

Normal Lipid Peroxidation

In whole meat, fats are compartmentalized away from propagators of oxidation. However, prolonged storage under unfavorable conditions can create rancid odors described as tallowy for beef; muttony for mutton; stale, cheesy, acrylic, fishy, or oily for pork; and rancid, painty, fishy, and cod-liver oil-like for fish. These odors develop from the products of autoxidation of unsaturated fatty acids, such as oleic, linoleic, linolenic, and arachidonic. Three stages have been proposed to describe the autoxidation process, namely, initiation, propagation, and termination (Figure 1). Initiation occurs when an unsaturated fatty acid reacts with O_2 to produce a free radical. The actual formation of the free radical occurs when a labile hydrogen is abstracted from the carbon atom adjacent to the double bond. The free radical then reacts with oxygen to form a peroxyl radical, which in turn can abstract another hydrogen from a different fatty acid, resulting in the propagation of a chain reaction. Termination occurs when two free radicals react together, when a peroxyl radical reacts with a free radical, when two peroxyl radicals react, or when radicals react with other meat constituents (e.g., vitamins, amino acids, dipeptides, etc.). The hydroperoxides formed during

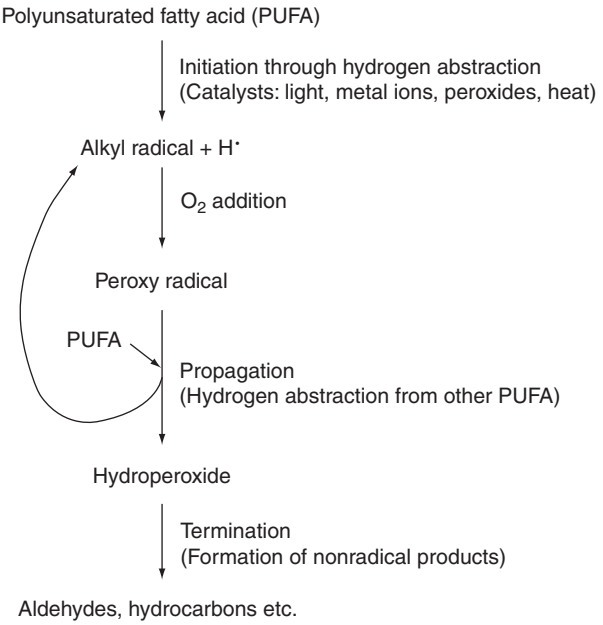

Figure 1 Fatty acid oxidation showing initiation, propagation, and termination steps.

propagation decompose and form secondary products that include aldehydes generated from the methyl ends of the fatty acids. Aldehydes, the types of which depend on the structure of the parent fatty acids, are largely responsible for rancid flavor development in meats. The degree of rancidity in fats has been traditionally measured using an assay for the determination of malondialdehyde by its reaction with thiobarbituric acid (TBA). However, the TBA assay is not specific to malondialdehyde and in some cases has been found to overestimate malondialdehyde content up to ninefold. Hence, assays specific to malondialdehyde or other specific aldehydes may be preferable for determining the development of rancidity in meats.

Rancidity in refrigerated and frozen whole meat products can be prevented through appropriate handling and packaging. Although some inherent factors (Vitamin E levels and animal age) can be controlled through management practices, others can be implemented in the postmortem period. Packaging under atmospheres of low-oxygen partial pressure and vacuum packaging are useful means of prolonging the oxidative stability of meat products. Packaging under oxygen-free atmospheres of nitrogen or carbon dioxide is an even more effective means of increasing the stability of meat products. The use of opaque packagings reduces exposure to light and this can further reduce the rate of oxidation. Grinding of meat, as in hamburger manufacture, disrupts membrane integrity and exposes the lipids to metal catalysts, and thus accelerates oxidation. Appropriate temperature control will minimize oxidation. Fish is particularly susceptible to temperature abuse as lipid peroxidation can continue even in the frozen muscle. At −4 °C, rancidity in fish is accelerated because freezing of a large fraction of free water as pure ice causes concentration of the catalytic metals in the unfrozen fraction. Frozen storage of meat at steady temperatures (−18 °C or lower) in tight-fitting, moisture-proof packaging is required to minimize lipid

peroxidation and prevent freezer burn. Unfortunately, consumers' need to assess the appearance of fresh meat products leads to nonoptimum conditions for preserving oxidative stability during retail display and home storage.

Warmed-Over Flavor (WOF)

WOF is one of the major causes of quality deterioration in cooked, refrigerated, and precooked meat products. WOF includes both the development of undesirable flavors and the loss of desirable meat flavor characteristics. The odors and flavors associated with WOF are commonly described as painty, rancid, stale, and cardboard-like. WOF can develop rapidly: in 48 h or less in reheated, refrigerated meat, and within a matter of days in precooked, frozen meats.

WOF results from the oxidation of PUFA located mainly in the cell membrane as phospholipids. The highly reactive sites next to the double bonds readily lose hydrogen atoms, resulting in the formation of lipid free radicals. Free radicals rapidly react with oxygen, to yield aldehydes such as pentanal, pentenal, hexanal, hexenal, and 2,4-decadienal. These compounds are volatile and perceptible as WOF at low concentrations (ppb). Unsaturated aldehydes are perceptible at lower concentrations than saturated aldehydes. Hence, muscles that are high in PUFA content are also the most susceptible to WOF development. This translates into a species difference in WOF development, with the problem for meats in the order fish > poultry > pork > beef > lamb. With the addition of advanced analytical technologies (e.g., gas chromatography–mass spectrophotometry with an olfactory port (GC–MS-O) and the electronic nose), progress in identification of the compounds directly related to the sensory perception of WOF continues to be made for different species, muscles, and types of muscle foods.

The rate of oxidation of PUFA can be influenced by catalysts that reduce the energy required for oxidation (metals, high-energy oxygen, or enzymes) or that add energy to drive the reaction (heat, light, oxidizing enzymes). During cooking, heat causes extensive protein coagulation and loss of functional properties. Of particular importance for lipid stability is the loss of iron-binding capacity in hemoglobin and myoglobin. On heating, free iron released from the globin proteins can come into contact with oxidizable substances such as PUFA. Free iron in the reduced, ferrous (Fe^{2+}) state readily converts into its oxidized, ferric (Fe^{3+}) state, assisting in the generation of lipid free radicals that then propagate a lipid peroxidation chain reaction. Hence, once heating occurs, oxidation proceeds very rapidly. Salt has been shown to enhance iron-catalyzed oxidation. Transition metals other than iron can also play a role in lipid peroxidation and are often added during processing through the addition of water and spices. Similar to iron, they undergo the loss of a single electron (e.g., Cu^{2+} to Cu^{3+}) during oxidation, and catalyze the formation of free radicals from PUFA.

Certain wavelengths of light (particularly blue-purple fluorescent and ultraviolet) are able to promote the oxidation reaction. Light energy elevates the energy states of oxygen and meat pigments, increasing their ability to participate in oxidation. Hence, the quality of light used during retail display of meat products and precooked frozen items is of concern.

Because oxygen is integral to the formation of WOF, any process that increases oxygen content in the muscle increases the problem. Mechanical manipulation through grinding, chopping, deboning, mixing, and tumbling introduces air into the normally anoxic interior of whole muscle cuts.

Although a number of factors can trigger oxidation and add to the development of WOF, there are also a number of ways of preventing or delaying its development. In the raw product, it is important to use fresh materials that have had little time to undergo extensive enzymatic oxidation preventing the production of autocatalytic substances that can cause oxidation even after the enzymes themselves have been inactivated by heat.

Antioxidants protect PUFA from oxidation by undergoing oxidation themselves, thus delaying the development of WOF. However, the protective effect of an antioxidant is dependent on its concentration and fat solubility, and on the number of antioxidative sites on the molecule. Dietary supplementation of the naturally occurring vitamin E (α-tocopherol) has been shown to reduce the susceptibility of meat to oxidation. α-Tocopherol is readily stored in the cell membrane, preventing the oxidation of nearby PUFA. Carotenoids are another group of fat-soluble antioxidants that can be obtained from the diet and can react with singlet oxygen to block the formation of lipid peroxides. However, their inclusion at high concentrations can lead to discoloration of fats, skeletal muscle, and associated skin. Although this can be detrimental in some muscle foods, carotenoids are purposefully added to both salmon and poultry diets to enhance the color of the final product.

Various synthetic phenolic substances, including butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyquinone (TBHQ), and propyl gallate (PG), can be used as antioxidants in muscle food systems. In addition, some common herbs and spices such as rosemary, marjoram, sage, thyme, mace, allspice, and clove have antioxidant properties. In cured meats, nitrite is known to have a powerful antioxidative effect, although the mechanisms are not clearly understood. Mechanisms that may be involved include prevention of ferrous iron release via complex formation with heme pigments, stabilization of PUFA in cell membranes, and chelation of metal ions.

The histidine-containing dipeptides found in muscle tissue, carnosine, and anserine have been found to have antioxidant activity in addition to their pH-buffering capacity. Species differences exist, with pigs, beef, and turkey having higher concentrations of carnosine than anserine, and the reverse being the case for salmon, rabbit, and chicken. Primarily anaerobic muscles have higher carnosine and anserine concentrations than aerobic muscles. The antioxidant mechanism of carnosine and anserine may be due to metal chelation or free radical scavenging. Many of the lipid peroxidation catalysts and free radicals are found in the cytosol, and hence the hydrophilic nature of carnosine and anserine is probably significant for their antioxidative activity.

Chelating agents such as citric acid, ethylenediaminetetracetic acid (EDTA), sodium tripolyphosphate, pyrophosphate, or hexametaphosphate are effective in reducing

oxidation that is initiated or propagated by metal ions. These agents form stable complexes with metals, thereby preventing their involvement in the oxidation of PUFA.

Oxygen scavengers such as ascorbic acid and erythorbic acid are added to cured meats to prevent nitrosamine formation, but also to prevent lipid oxidation. They act alone as reducing agents in low concentrations, and synergistically with other antioxidants. Ascorbic acid can form a stable complex with metals, thereby raising the energy required to initiate oxidation.

Exclusion of oxygen through physical means such as vacuum tumbling, vacuum stuffing, and vacuum packaging can delay the onset of WOF. Oxygen can also be excluded by covering precooked products with liquids or sauces. Owing to the antioxidant nature of Maillard (browning) reaction products (particularly histidine-glucose reaction products), covering pork with drippings or gravy made from the drippings can also increase the acceptable frozen storage life in precooked products. The use of red-orange tungsten halogen lights for illumination may also be beneficial, and the complete exclusion of light may be a necessity for some products.

Oxidation of Pigments

Muscle color is a major factor in consumer selection of meat on retail display and is, in some cases, mistakenly relied on as an indicator of freshness. Hence, consumers have firm expectations about fresh meat color, and deviations, particularly browning, are thought to indicate spoilage.

The pigment primarily associated with meat color is myoglobin, which in the living animal functions in the transfer of oxygen from hemoglobin in the blood to the mitochondrial cytochromes. Myoglobin constitutes 50–80% of the meat pigments, depending on the species, age of the animal, and muscle type. Higher concentrations of myoglobin are found in beef than in pork, in older than in younger animals, and in muscles responsible for sustained activity than in muscles that are used sporadically, and less intensively. Hence, in bright, cherry-red beef, the myoglobin concentration ranges from 4 to 10 mg g^{-1} (wet matter basis) compared to 1–3 mg g^{-1} for grayish-pink pork. In dark-colored beef the range is from 16 to 20 mg g^{-1} compared to light-colored veal at 1–3 mg g^{-1}. The extensor carpi radialis, a locomotory muscle located in the forelimb, has twice the myoglobin concentration of the longissimus thoracis et lumborum, the major support muscle along the vertebrae (12 vs. 6 mg g^{-1}, wet matter basis). These differences in intensity of color are familiar to and, for the most part, accepted by consumers.

In the native deoxymyoglobin state (Mb), before exposure to oxygen, meat pigments are purplish-red (Figure 2). This can be seen when the interior of meat is first exposed during cutting or when fresh meats are vacuum packaged in oxygen-impermeable film. On exposure to atmospheric oxygen, the pigments are rapidly oxygenated, producing oxymyoglobin (MbO_2) and the bright red, 'bloomed' color of fresh meat. Brown metmyoglobin (MMb) is formed by oxidation of the pigment from its ferrous (Fe^{2+}) to its ferric (Fe^{3+}) iron state under conditions of low-oxygen partial pressure. Hence, in intact meat, the depth of oxygen penetration into the interior of the meat is marked by a layer of brown MMb.

In muscle, the three states of myoglobin exist simultaneously; *in vivo* MMb exists at a steady state of approximately 2–3%, whereas in meat color deterioration is not perceptible until greater than 30% of the pigments are in the oxidized

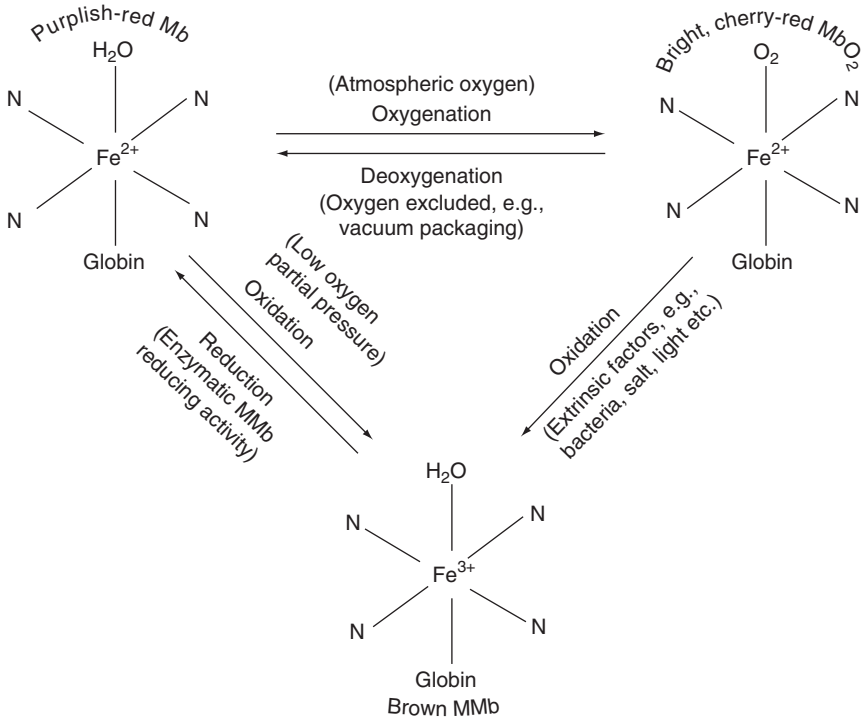

Figure 2 The normal cycle of myoglobin (Mb) pigment changes in fresh meat.

MMb state. Fresh beef is considered to have an unacceptable color when over 60% of the myoglobin is in the MMb form. Discoloration discounts at retail have a significant economic impact, calculated to be in excess of US$700 million per year in the US beef industry alone.

Though the conversion of MbO_2 to MMb is thermodynamically favored, reduction of MMb to ferrous myoglobin may also occur. The MMb is reduced nonenzymatically by reduced cytochrome b_5. In turn, cytochrome b_5 is regenerated to its active reducing form by cytochrome b_5 reductase (MMb reductase), which utilizes the reduced form of nicotinamide adenine dinucleotide (NADH). Once the reducing capacity of the meat is exhausted, complete MMb formation will occur. MMb reductase activity has been shown to differ among muscles, with, for example, the *longissimus* muscle having greater activity than the *psoas* major muscle.

In addition to loss of MMb reductase activity, color stability is reduced by any factors that cause denaturation of the globin (e.g., heat, salts, low pH, and ultraviolet light) and by low-oxygen tension. The formation of MMb is maximal at an oxygen pressure of approximately 4 mm Hg, and increasing oxygen pressure improves fresh meat color stability. The packaging environment can substantially affect the oxygen pressure to which meat is exposed, and can thus profoundly affect the color stability of fresh meat. Packaging systems with atmospheres of high-oxygen partial pressure extend fresh meat color shelf life. Vacuum-packaged meats retain the ability to oxygenate when exposed to oxygen. An anoxic atmosphere in master packs, with subsequent display in oxygen-permeable packaging at retail, has allowed the development of central packaging. However, failure to completely remove oxygen ($<1\%$) from these packages can result in pro-oxidative conditions and subsequent loss in color stability. The use of oxygen scavengers in which oxygen reacts with iron or low-molecular weight organic compounds such as ascorbate has proven efficacious for maintaining the color shelf life in meats that are stored under oxygen-depleted atmospheres during subsequent retail display.

Research into the biochemical mechanisms involved in heme pigment catalysis of lipid oxidation has shown that, during oxidation of MbO_2, both superoxide anion and hydrogen peroxide are produced, which can further react with iron to form hydroxyl radicals that facilitate lipid oxidation. Generally, MbO_2 shows higher prooxidant activity than MMb. However, MMb has been shown to react with hydrogen peroxide to form unstable hypervalent (Fe^{4+}) myoglobin species, perferrylmyoglobin and ferrylmyoglobin. Perferrylmyoglobin is a transient species that autoreduces rapidly to the more stable ferrylmyoglobin. Under conditions found in fresh meat of usual pH values (5.5–5.8) ferrlymyoglobin also autoreduces rapidly to MMb. Both of these hypervalent myoglobin species have been shown to initiate lipid oxidation through abstraction of a hydrogen atom from fatty acids. In turn, the aldehydes arising from lipid oxidation can induce myoglobin pigment oxidation to MMb. Hence, many of the lipid (α-tocopherol)- and water-soluble (ascorbic acid, dipeptides) antioxidants used to reduce rancidity will also be efficacious for preserving meat color. Many of these antioxidants, rather than having a direct effect on the myoglobin, act to delay the production of lipid peroxidation breakdown products such as peroxides, which can accelerate MbO_2 oxidation.

Oxidation of Proteins

Until recently, most research on oxidation and its effects in muscle foods concentrated on the lipid fraction, with limited study of protein oxidation (Pox). This may have been due to the greater susceptibility of lipids to oxidation, the generally later postmortem onset of Pox, and the challenges associated with measuring Pox in a complex meat matrix. However, the focus on Pox in relation to age-related disease in humans has provided methodologies and impetus to investigate Pox in the context of muscle foods.

Although the basic mechanisms underlying Pox are still under investigation, both the amino acid side chains and peptide linkages of the protein backbone have been shown to be susceptible to reaction with free radicals in the presence of oxygen. Hence, oxidative changes to proteins include protein cross-linking, amino acid side chain modification, and protein fragmentation. The chemical mechanisms are similar to those that occur in lipid oxidation but are less complex, and fewer oxidation products have been reported to date. In general, unsaturated double bonds susceptible to oxidation occur infrequently in proteins as they are found in only the aromatic amino acids tryptophan, tyrosine, and phenylalanine, and in the heterocyclic amino acid histidine. Amino acids most susceptible to metal-catalyzed oxidation include those with nitrogen-containing functional groups (arginine, lysine, proline) that yield carbonyl residues, and sulphur-containing amino acids (methionine and cysteine) that form cross-links and yield sulphur-containing residues. The extent of Pox has been generally estimated by measuring the appearance of protein carbonyl compounds in muscle foods using the dinitrophenylhydrazine method. The loss of thiol groups in proteins from muscle foods, which is also a good indicator of oxidation, has been determined spectrophotometrically using Ellman's reagent and, recently, with greater sensitivity, using a fluorescent reagent (ThioGlo®1).

Theoretically, amino acids with reactive side chains that include an imidazole ring, indole ring, suflhydryl, thioesther, or amino group (tryptophan, histidine, lysine, cysteine, and arginine) are most susceptible to reaction with lipid peroxidation products. However, the role of lipid peroxidation products in the initiation of Pox is still a matter of debate. In a complex matrix such as meat there seems to be little doubt these processes are not entirely independent; and due to the earlier onset of lipid oxidation, it would seem that lipid peroxide products must have a role in promoting Pox. In addition, the interaction between lipid and Pox must be highly dependent on the species, muscle type, type of muscle food, and a host of other environmental moderators. Of interest is the suggestion that proteins, particularly the myofibrillar proteins, may themselves act as an antioxidant within muscle foods. Their ability to scavenge free radicals and chelate metal ions may provide protection to other susceptible compounds, including lipids and proteins.

Research on the effect of Pox in muscle foods focused initially on changes to protein functionality during further

processing that results in alterations of the gel-forming ability, meat-binding ability, emulsification capacity, solubility, viscosity, or water-holding capacity of meat preparations. These changes in protein functionality can result in textural changes to meat products. However, the oxidative processes are complex and, depending on various intrinsic and extrinsic factors, may result in either improved or reduced functionalities. The intrinsic and extrinsic factors include the types of pro-oxidants or antioxidants, types of muscle or protein, specific protein side chains or amino acid residues located on the surfaces of protein molecules, the extent of oxidative modification, and the storage time. It has been suggested that mild-to-moderate protein modification results in improved protein functionality, whereas extensive alteration results in decreased functionality due to excessive aggregation and precipitation of the proteins.

In the past decade, attention has been directed toward the consequences of Pox in fresh, whole muscle during processing, fabrication, and storage. The findings indicate that Pox may have a negative effect on juiciness, tenderness, and nutritional quality. Pox-induced increases in cross-linking of the myofibrillar proteins is thought to contribute to reduced juiciness and tenderness, although Pox-based inactivation of μ-calpain may also be involved (see Section Enzymatic Factors). Increased cross-linking of the myosin tail region can stabilize the myofibrillar structure and constrain myofibrillar swelling, resulting in decreased tenderness and juiciness of fresh meat stored under high-oxygen packaging. Conversely, increased Pox during drying and salting may contribute to the unique and preferred textural characteristics of dry-cured meats and salted fish. Increased hardness of pâtés and cooked sausages during refrigerated storage has also been ascribed to Pox-induced cross-linking. Pox may decrease the nutritional value of muscle foods through both depletion of essential amino acids and decreased digestibility of oxidized muscle proteins, although the science is not yet conclusive. Through modification of the oxidation state of the heme iron, Pox can also contribute to negative effects on meat color. In addition, the formation of carbonyls during Pox may impact odor and flavor, and may contribute significantly to the unique flavor of dry-aged meats.

Enzymatic Factors

Enzymes, including lipoxygenases, peroxidases, and microsomal enzymes, which catalyze insertion of oxygen into polyunsaturated fatty acids with methylene interrupted dienes, have been identified in various animal tissues and contribute to enzyme-mediated oxidation of lipids. In contrast, phospholipases have been shown to inhibit lipid peroxidation by forming iron complexes with the free fatty acids liberated by hydrolysis of phospholipids in the membrane.

Several cytosolic enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, are active antioxidant enzymes in muscle. Superoxide dismutase controls the reactivity of superoxide anions and perhydroxyl radicals, both of which are involved in promotion of lipid oxidation. It also catalyzes the conversion of the superoxide anion to hydrogen peroxide. Hydrogen peroxide can be rapidly decomposed by transition metals to an extremely reactive hydroxyl-free radical, which can propagate oxidation. However, both catalase and glutathione peroxidase are active in converting peroxides into inactive derivatives, thereby preventing oxidative damage. In addition, glutathione peroxidase is capable of reacting directly with lipid peroxides, limiting oxidation.

Species differences in antioxidant enzymes have been reported with higher activities of superoxide dismutase and glutathione peroxidase in beef > turkey > pork, whereas activities of catalase are higher in pork > beef > turkey. Some evidence exists that the inactivation of antioxidant enzymes through heating contributes to the development of WOF. However, when catalase and glutathione peroxidase were added back into cooked muscle, only 15% of the lipid peroxidation was inhibited.

Because most enzymes are proteins, Pox may affect numerous enzyme-mediated processes in muscle foods. Perhaps the most attention to date has been given to the calpain enzyme system that plays a key role in postmortem tenderization. Irradiation of meat decreased tenderness and increased Pox in both the sarcoplasmic and myofibrillar fragments of meat, but the direct effect on μ-calpain was not measured. In contrast, storage under high oxygen also decreased tenderness but showed no inactivation of μ-calpain. Thus, although conclusive evidence that the μ-calpain enzyme undergoes Pox has not been obtained to date, this remains an active research area. There is also need for further understanding of the potential impact of Pox on other sarcoplasmic proteins.

See also: Chemical and Physical Characteristics of Meat: Adipose Tissue; Color and Pigment; Palatability. Conversion of Muscle to Meat: Color and Texture Deviations. Cooking of Meat: Flavor Development; Warmed-Over Flavor. Fish Inspection. On-Line Measurement of Meat Quality. Packaging: Modified and Controlled Atmosphere. Refrigeration and Freezing Technology: Applications; Thawing. Sensory Assessment of Meat. Spoilage, Factors Affecting: Microbiological

Further Reading

Baron, C.P., Andersen, H.J., 2002. Myoglobin-induced lipid oxidation. A review. Journal of Agricultural and Food Chemistry 50 (14), 3887–3897.

Brewer, S.M., 1998. What is "warmed-over flavour"? Pork quality factsheet. National Pork Board and the American Meat Science Association. Available at: http://www.porkboard.org (accessed 29.10.13).

Chan, K.M., Decker, E.A., 1994. Endogenous skeletal muscle antioxidants. Critical Reviews in Food Science and Nutrition 34 (4), 403–426.

Dave, D., Ghaly, A.E., 2011. Meat spoilage mechanisms and preservation techniques: A critical review. American Journal of Agricultural and Biological Science 6 (4), 486–510.

Faustman, C., Sun, Q., Mancini, R., Suman, S.P., 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science 86 (1), 86–94.

Fox Jr., J.B., 1987. The pigments of meat. In: Price, J.F., Schweigert, B.S. (Eds.), The Science of Meat and Meat Products, third ed. Westport, CT: Food & Nutrition Press, Inc., pp. 193–216 (Chapter 5).

Frankel, E.N., 1998. Lipid Oxidation. Dundee, Scotland: The Oily Press Ltd.

Howe, P., Buckley, J., Meyer, B., 2007. Long-chain omega-3 fatty acids in red meat. Nutrition and Dietetics 64 (4), S135–S139.

Huff-Lonergan, E., Zhang, W., Lonergan, S.M., 2010. Biochemistry of postmortem muscle – Lessons on mechanisms of meat tenderization. Meat Science 86 (1), 184–195.

Leray, C., 2003. Cyberlipid center: Resource site for lipid studies. Available at: http://www.cyberlipid.org (accessed 29.10.13).

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M., 2011. Protein oxidation in muscle foods: A review. Molecular Nutrition and Food Research 55, 83–95.

Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Science 71 (1), 100–121.

Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., Salvayre, R., 2008. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. British Journal of Pharmacology 153 (1), 6–20.

Pearson, A.M., 1986. Physical and biochemical changes occurring in muscle during storage and preservation. In: Betchel, P.J. (Ed.), Muscle as Food. London, UK: Academic Press Inc., pp. 103–134 (Chapter 3).

Schwimmer, S., 1981. Source Book of Food Enzymology. Westport, CT: AVI Publishing Co.

Younathan, M.T., 1985. Causes and prevention of warmed over flavor. Proceedings of the Annual Reciprocal Meat Conference, vol. 38, pp. 74–80. Chicago, IL: National Live Stock and Meat Board. Available at: http://www.meatscience.org (accessed 29.10.13).

STUNNING

Contents

CO_2 and Other Gases

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Glossary

Anoxia Severely reduced levels or complete lack of oxygen.
Breathlessness A sense or feeling of unable to breath.
Bronchoconstriction Narrowing of bronchi/respiratory tract.
Dyspnea Difficulty in breathing.
Hypercapnea Elevated levels of carbon dioxide.
Hyperventilation Increased rate and/or depth of breathing.
Hypoxia Reduced oxygen levels.

Introduction

In most of the developed countries, it is a statutory requirement that all animals, including poultry, slaughtered for human consumption are rendered immediately unconscious and that they remain so until death supervenes through loss of blood. Because the effect of a stunning method is momentary, the onus of preventing resumption of consciousness following stunning relies on the efficiency of the slaughter procedure, i.e., the prompt and accurate severance of blood vessels supplying oxygenated blood to the brain. By contrast, killing animals with gases can eliminate the chances of recovery of consciousness. Regulations governing the welfare of animals (during stunning or killing) in many countries, including the UK, prescribe certain gas mixtures for stunning/killing of pigs and domestic poultry. The relative merits of various gas mixtures used for stunning pigs and poultry are addressed in this article. Stunning of farmed fish using gas mixtures is also considered.

Reason for Use of Gas Stunning

Among the farm animals slaughtered for human consumption, pigs are arguably the most susceptible to stress during preslaughter handling and stunning. Electrical stunning is commonly used for rendering pigs unconscious before slaughter. With electrical stunning, some form of restraint (lifting or squeezing) is necessary to facilitate ideal placement of the stunning tongs and to achieve an effective stunning. Research has shown that isolation of individual animals from their pen mates and the application of any form of restraint can be distressing to pigs. By contrast, stunning of pigs with gases does not require a restraint and modern gas stunning devices involve killing in small groups. For example, pigs are loaded into a cage or cradle and lowered into a well, or passed through a purpose-built tunnel containing gas mixtures.

Multiple-bird electrical water bath stunning is the most common and cheapest method of rendering poultry unconscious before slaughter under commercial conditions, where high throughput rates (up to 220 birds per minute) are required. Under this system, conscious birds are hung upside down on a moving metal shackle line (process known as shackling) and passed through an electrified water bath, such that the current flows through the whole body toward the earthed shackle. There are many welfare concerns associated with the commercial electrical water bath stunning systems, such as unnecessary pain and suffering caused by uncrating, shackling, prestunning electric shocks, inadequate stunning, and recovery of consciousness leading to live birds entering the scald tanks. Owing to the complexity of multiple-bird electrical water bath stunning systems, it will be difficult to resolve the bird welfare problems. However, killing of poultry using gases, while the birds are still in their transport containers, will eliminate the problems associated with handling of live birds at the primary processing plant and electrical water bath

stunning systems. This concept was originally proposed in 1982 by the Farm Animal Welfare Council in the UK. All the gas stunning systems now operating in Great Britain involve killing in transport crates. Some gas stunning systems operating in Europe, however, involve tipping of live birds from transport modules on to a conveyor belt that passes through a gas stunning tunnel. There are gas stunning systems operating in North America that involve shackling of conscious poultry before stunning. Needless to say, the latter systems have failed to fully utilize the welfare benefits of gas stunning.

Gas Mixtures Evaluated for Stunning/Killing

Although a number of scientific and commercial establishments evaluated the commercial feasibility of stunning pigs and poultry with gases as early as 1950, the animal welfare and carcass and meat quality issues were addressed only during the latter part of the twentieth century. In this regard, most of the research and development was carried out in Scandinavian countries, Spain, and the UK.

Gas mixtures investigated so far have included:

- 40–90% by volume of carbon dioxide in air (hypercapnia).
- Mixtures containing a minimum of 30% by volume of carbon dioxide and 20–30% by volume of added oxygen in air (hypercapnic hyperoxia).
- Mixtures of argon and nitrogen with 2% by volume of residual oxygen in air (anoxia).
- A mixture of less than 30% by volume of carbon dioxide in argon or nitrogen or both with up to 5% by volume of residual oxygen (hypercapnic anoxia).

The normal atmospheric concentration of carbon dioxide is 0.003% by volume; however, the gas is cheaply and readily available as a by-product of the chemical/fertilizer industries. Argon and nitrogen occur naturally and can be separated from atmospheric air. The atmospheric concentration of argon is 0.94% by volume and that of nitrogen is 79% by volume, and thus nitrogen is cheaper than argon. Another advantage of using nitrogen is that it can be separated from atmospheric air in any part of the world with the minimum of cost and impact on the environment. Nitrous oxide has been used experimentally to stun pigs, but owing to toxicity on chronic exposure in humans, it is not used commercially.

Mechanisms of Induction of Unconsciousness

Carbon dioxide induces unconsciousness through inhibition of neurons. This mechanism is closely related to the fall in pH of the cerebrospinal fluid (CSF), which bathes the brain and spinal cord. It has been reported that unconsciousness begins when the CSF pH falls below 7.1 and reaches a maximum at pH 6.8. The level of γ-aminobutyric acid (GABA), which is the major inhibitory amino acid neurotransmitter, has been known to increase during distress and anxiety; it is not certain whether the increase in GABA level is due to the stress of induction of unconsciousness with this gas or a physiological mechanism involved in carbon dioxide-induced neuronal inhibition.

Inhalation of carbon dioxide does not lead to a reduction in the blood oxygen level and, therefore, anoxia does not accompany the inhalation of carbon dioxide at concentrations required for stunning animals. In addition, the anesthetic effect of carbon dioxide is independent of residual oxygen in the breathing mixture. For example, a mixture of 40% carbon dioxide and 30% oxygen will also render animals unconscious. The time to onset of unconsciousness in pigs is related to concentrations of carbon dioxide between 40% and 70% by volume in air. Increasing the concentration of carbon dioxide in an air mixture above 70% by volume does not reduce the time to loss of consciousness greatly. However, the times to loss of consciousness in terrestrial poultry species seem to be very similar during exposure to 40% by volume or more of carbon dioxide in air and are rather prolonged when 20% by volume or more of oxygen is added to the mixture. The time to onset of death in both species is related to the concentration of carbon dioxide and the duration of exposure to the gas. Gas mixtures containing carbon dioxide and 30% by volume or more of oxygen do not induce death and therefore require a killing procedure (e.g., further exposure to a high concentration of carbon dioxide in air).

In general, hypoxia or anoxia occurring as a result of the inhalation of argon or nitrogen induces unconsciousness by depriving the brain of oxygen. For example, it has been established that cerebral dysfunction occurs in mammals when the partial pressure of oxygen in cerebral venous blood falls below 19 mmHg. Brain oxygen deprivation leads to accumulation of extracellular potassium and a metabolic crisis as indicated by the depletion of energy substrates and accumulation of lactic acid in the neurons. These effects can occur within a matter of few seconds of inhalation of an anoxic agent. However, it is worthy of note that the survival times of various parts of the brain may differ according to the regional oxygen consumption rate. For example, the survival time of the cerebral cortex is considerably shorter than that of the medulla, in which the respiratory center is located. Normal brain activity may be restored in anoxia-stunned animals if oxygen is administered or they are allowed to breathe atmospheric air. Inevitably, the recovery of consciousness in these animals is rapid. It is therefore a statutory requirement in the UK that animals must be held within the gaseous atmosphere until they are dead.

Argon and nitrogen, along with xenon, are frequently referred to as inert gases. However, in contrast to argon or nitrogen having anesthetic properties under hyperbaric conditions, xenon is an anesthetic gas under normobaric conditions. It has been reported that inhalation of 80% xenon and 20% oxygen induced unconsciousness in humans via the inhibition of *N*-methyl-D-aspartate (NMDA) receptor channels, which are essential for maintaining neuronal excitation during the conscious state. Interestingly, induction of unconsciousness with xenon, argon, nitrogen, and nitrous oxide ('laughing gas') has frequently been described by humans as a euphoric or very pleasant way of losing consciousness, and this may be due to the effects of those gases on NMDA receptor channels. It is worth noting that the effects of a number of modern analgesics, sedatives, and anesthetics are also mediated via NMDA receptor channels in the brain.

Time to Onset of Unconsciousness during Exposure to Gas Mixtures

Although 'unconsciousness' has different interpretations, from the stunning and slaughter point of view it can be suggested to be 'a state in which the ability of the brain to process sensory stimuli, including pain, is lost.' In this regard, the time to loss of somatosensory-evoked potentials (SEPs) in the brain, induced by electrically stimulating a peripheral nerve, has been determined during exposure of pigs and poultry species to various gas mixtures. The time to loss of SEPs is found to be rapid with argon-induced anoxia. By contrast, the time to loss of brain responsiveness during exposure to carbon dioxide in air can be relatively long and highly variable. Inhalation of a mixture of 30% by volume of carbon dioxide in argon or nitrogen seems to have an additive effect on the brain in species that are known to be resilient to the effects of carbon dioxide or anoxia alone (e.g., waterfowl).

Welfare Concerns of Gas Stunning

The induction of unconsciousness with gas mixtures should be differentiated from asphyxia. The physiological definition of asphyxia implies a physical separation of the upper respiratory tract from the atmosphere. For example, drowning involves water as a separating medium, strangulation leads to constriction of the trachea, and choking is due to obstruction in the respiratory tract. Suffocation is frequently used as a synonym for asphyxia.

Unlike other established stunning methods, exposure of animals to gas mixtures does not produce an immediate loss of consciousness. It is therefore important to ensure that the induction of unconsciousness with gas mixtures does not compromise animal welfare.

It is known that breathlessness (dyspnea) can occur as a result of changes in the blood oxygen or carbon dioxide levels. For example, exercise induces breathlessness through the gradual increase in blood carbon dioxide concentration. However, inhalation of high concentrations of carbon dioxide results in rapid increases in blood carbon dioxide concentration, and this is more effective in producing dyspnea. It is also worth noting that the severity of breathlessness depends upon the rate at which the blood carbon dioxide increases. It has been reported that, in humans, an increase in blood carbon dioxide tension of 5 mmHg above normal will stimulate respiration, whereas the blood oxygen tension has to decrease by approximately 60 mmHg from the normal level before hypoxia stimulates the respiratory centers in the brain. It is therefore apparent that hypercapnia is a more potent respiratory stimulant than is hypoxia. Further evidence to support these concerns also emerges from studies involving pigs and poultry.

Aversion to the initial exposure to argon-induced anoxia or carbon dioxide has been used (e.g., passive avoidance tests in the presence of a reward) to determine the relative merits of gas mixtures. The results of this study clearly indicated that pigs do not show any aversion to 90% argon in air; the majority (75%) of pigs did not show aversion to 30% carbon dioxide in air, whereas the majority (88%) of pigs avoided an atmosphere containing a high (>80%) concentration of carbon dioxide in air. In this study, pigs that found carbon dioxide aversive withdrew their heads immediately on exposure and did not attempt again to obtain the reward offered. However, a minority of pigs made repeated efforts to obtain the reward.

It has been reported that human volunteers also find inhalation of 40% by volume of carbon dioxide extremely unpleasant. It is therefore not surprising to note that pigs, being phylogenetically close to humans, also experience the same aversion. Fasting pigs for up to 24 h before exposure to 90% carbon dioxide in air did not overcome the aversion. Similarly, a considerable proportion of chickens and turkeys have been reported to avoid atmospheres containing high concentrations (40% by volume or more) of carbon dioxide in air.

Passive avoidance tests also showed that pigs spent similar times feeding in air and under anoxia (Figure 1) and, as would be expected, several of them lost consciousness (as determined from the occurrence of loss of posture) while eating apples presented in the anoxic atmosphere.

Recent research carried out in an experimental slaughterhouse in Spain demonstrated that pigs show more aversion to gas mixtures containing nitrogen and either 15% or 30% carbon dioxide by volume than 90% argon by volume in air.

It is thus evident that the initial exposure to a high concentration of carbon dioxide is extremely aversive and, given a free choice, animals will avoid such an atmosphere. This is probably because carbon dioxide is an acidic gas and is pungent to inhale in high concentrations. Indeed, carbon dioxide gas has been used to stimulate the nasal mucous membrane in order to induce pain-evoked responses in the brain.

In addition, the severity of respiratory sounds occurring during the induction of unconsciousness in pigs (until they

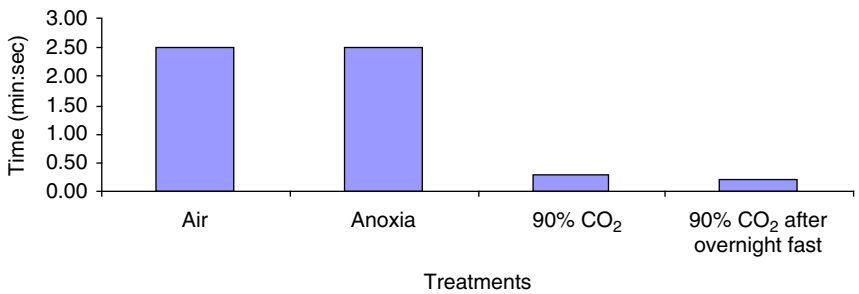

Figure 1 The average time (maximum 3 min) spent by pigs on feeding apples in various atmospheres.

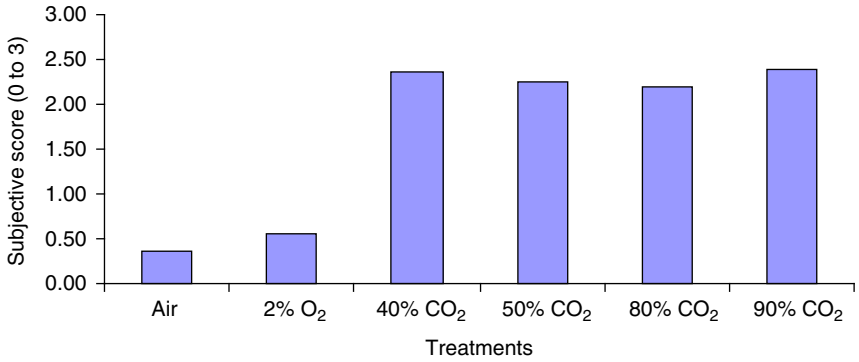

Figure 2 The severity of respiratory distress occurring in pigs during the induction of unconsciousness with gas mixtures.

lose posture) with gas mixtures has been subjectively rated and used to ascertain respiratory distress. It has been reported that exposure to 90% argon induced minimal respiratory distress, whereas, exposure to 40–90% by volume of carbon dioxide in air induced severe respiratory distress. Exposure to 30% by volume of carbon dioxide in an argon mixture induced moderate distress. Some pigs that were exposed to less than 70% carbon dioxide in air showed escape attempts. By contrast, during exposure to argon-induced anoxia, pigs lost posture without any evidence of behavioral arousal or escape attempts. See Figure 2.

Research carried out in an experimental slaughterhouse using a dip-lift system indicated that the incidence of pigs showing retreat and escape attempts was lower in 90% argon by volume in air than in the gas mixtures containing nitrogen and 15% or 30% by volume of carbon dioxide.

In poultry species, however, high concentrations of carbon dioxide induce severe head shaking, gasping, sneezing, and vocalizations, which can be considered as indicators of distress. This interpretation is based on the fact that these behaviors also occur during respiratory disease.

On the basis of the above evidence, it is suggested that anoxia is the best option on animal welfare grounds. The use of a mixture containing low concentrations (<30% by volume) of carbon dioxide in argon or nitrogen or both would appear to be better than using a high concentration of carbon dioxide in air.

However, those who wish to promote carbon dioxide for stunning argue that the cumulative stress associated with conventional electrical stunning methods can be greater than the stress of induction of unconsciousness with this gas. It is also claimed that the increased rate and depth of breathing occurring during carbon dioxide stunning of pigs enable them to breathe more of this gas and lose consciousness rapidly. It could also be argued that the cumulative stress caused to poultry during electrical water bath stunning is more than that would occur during stunning with carbon dioxide. On the basis of these arguments, carbon dioxide is widely used for stunning pigs and poultry.

Control of the temperature and humidity of the carbon dioxide in the stunning atmosphere could improve the welfare of animals. For example, in humans, nasal breathing of air increases the respiratory system's ability to warm and humidify the inspired air compared to oral breathing. By contrast, oral breathing, in particular during exercise-induced hyperventilation, results in drying and cooling of the upper respiratory tract, and this is one of the causes of exercise-induced asthma or bronchoconstriction. Under these circumstances, inhalation of warm and humidified air helps to alleviate distress, and this concept is widely used in human artificial respirators. Because animals exposed to carbon dioxide gas also show gasping (oral breathing), it is thought that administration of a warm and humidified gas mixture will help to reduce the severity of distress.

Commercial farming of several species of fish for human food has become popular in recent years. Traditionally, fish would be taken out of the water manually using nets or mechanically using pumps and placed in air or on ice, which is considered to be equivalent to asphyxiation in terrestrial vertebrates. Owing to the concern for their welfare at slaughter, the use of gas mixtures, especially carbon dioxide, for stunning salmon, trout, seabream, and seabass under commercial farming conditions has also been developed, at least in Europe. Carbon dioxide is highly soluble in water. Under commercial conditions, carbon dioxide is bubbled into water (sea or fresh water, depending upon the species of fish) contained in tanks until the water becomes saturated with the gas. Batches of fish are then placed in the CO_2-saturated water and held until they become completely sedated or motionless. However, research has shown that fish find immersion into CO_2-saturated water aversive, as indicated by rapid swimming and making escape attempts.

Commercial Implications

Experiments with the carbon dioxide stunning of pigs have shown that exposure to a minimum of 70% carbon dioxide for 90 s results in stunning; therefore sticking (bleeding or exsanguination) should be performed as soon as possible (e.g., ideally within 15 s of exiting the gas) to prevent resumption of consciousness. When the duration of exposure to this level of carbon dioxide is increased, the incidence of death also increases. Under high-throughput conditions, exposure of pigs to a minimum of 90% by volume of carbon dioxide in air for 3–5 min results in death in the majority of pigs, which can be recognized from the presence of dilated pupils and absence of gagging (rudimentary respiratory activity) at the exit from the gas.

In Denmark, where almost all pigs are stunned using carbon dioxide, a comprehensive automatic system for driving

large groups of pigs (15–16 pigs) from the lairage to the point of stunning, dividing them into small groups (e.g., 5–6 pigs) and loading them onto a lift, which is lowered into a carbon dioxide stunning unit, has been developed. In comparison with the conventional pig handling and loading systems and carbon dioxide stunning units, this group handling and stunning system is far better on animal welfare grounds.

It has been reported that exposure of pigs to either argon-induced anoxia or the carbon dioxide–argon mixture for 3 min resulted in satisfactory stunning; however, bleeding should commence within 15 s to avoid resumption of consciousness. A 5 min exposure to these gas mixtures followed by bleeding within 45 s prevented carcass convulsions during bleeding. The results also showed that exposure of pigs to argon-induced anoxia or the carbon dioxide–argon mixture for 7 min resulted in death in the majority of pigs. Owing to the prolonged exposure time required to kill pigs with anoxia, it is not used under commercial conditions. However, further research and development is needed to evaluate the feasibility of inducing unconsciousness with anoxia and then killing pigs by other means (e.g., induction of cardiac arrest in unconscious pigs using an electric current).

Chickens and turkeys can be killed with a minimum of a 2 min exposure to 50% by volume carbon dioxide in air, 90% by volume of argon or nitrogen in air, and a mixture containing less than 30% by volume of carbon dioxide in argon or nitrogen.

The Welfare of Animals (Slaughter or Killing) Regulations in the UK approved the use of a minimum of 70% by volume of carbon dioxide in air for killing pigs. However, on bird welfare grounds, this regulation does not allow the use of carbon dioxide for killing domestic poultry, except for disease-control purposes. Instead, two other gas mixtures have been approved for killing domestic poultry intended for human consumption:

- Argon, nitrogen, or other inert gases, or any mixture of these gases, in atmospheric air with a maximum of 2% oxygen by volume.
- Any mixture of argon, nitrogen, or other inert gases with atmospheric air and carbon dioxide provided that the carbon dioxide concentration does not exceed 30% by volume and the oxygen concentration does not exceed by 2% by volume.

However, the European Slaughter Regulation 1099/2009, which comes into force from January 2013, permits the use of:

1. Direct or progressive exposure of conscious pigs to a gas mixture containing more than 40% carbon dioxide for pigs.
2. Direct or progressive exposure of conscious pigs and poultry to an inert gas mixture such as argon or nitrogen leading to anoxia.
3. Direct or progressive exposure of conscious pigs and poultry to a gas mixture containing up to 40% of carbon dioxide associated with inert gases leading to anoxia.
4. Successive exposure of conscious birds to a gas mixture containing up to 40% of carbon dioxide, followed when they have lost consciousness, by a higher concentration of carbon dioxide.

In general, gas stunning/killing of pigs and poultry results in better carcass and meat quality than other established stunning methods. In comparison with electrical stunning, gas stunning or killing can reduce the incidence of broken bones in carcass and hemorrhaging in muscles. However, stunning with gas mixtures containing 40% by volume or more of carbon dioxide tends to retard the rate of rigor development and, hence, tenderness development. By contrast, stunning of pigs and poultry with argon and nitrogen mixtures or a mixture containing less than 30% by volume of carbon dioxide in argon or nitrogen mixture accelerates the rate of postmortem rigor development and tenderization of meat. This is found to be at least as effective as electrical stimulation of carcasses, especially in poultry. Therefore, these gas mixtures provide an opportunity for poultry processors to portion or separate breast muscles soon after chilling (in less than 2 h postmortem) without inducing toughness, provided the muscle temperature is also reduced rapidly by the use of an appropriate chilling method.

However, convulsions occurring as wing flapping after the loss of consciousness in poultry can increase the incidence of dislocated or broken wing bones. Owing to this and the cost of argon, the poultry industry would prefer to use gas mixtures causing less of this quality problem, especially methods involving exposure to low or gradually increasing concentrations of carbon dioxide in air as these methods have been known to cause very little or no wing damage in the carcasses.

Irrespective of the species of animals involved, the ever-decreasing competition in the fields of stunning equipment manufacturing and gas distillation and distribution are disconcerting on economic grounds.

Conclusions

In general, gas stunning of pigs in small groups and of poultry in transport crates can benefit animal welfare and improve carcass and meat quality. Anoxia induced with argon, nitrogen, and any mixtures of inert gases would appear to be the best option on animal welfare and carcass and meat quality grounds. By contrast, the induction of unconsciousness with carbon dioxide could be distressing to animals, and therefore the meat industry should be encouraged to seek potential alternatives.

See also: Carcass Chilling and Boning. Conversion of Muscle to Meat: Aging; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening; Slaughter-Line Operation and Pig Meat Quality. Environmental Impact of Meat Production: Primary Production/Meat and the Environment. Preslaughter Handling: Welfare Including Housing Conditions. Slaughter-Line Operation: Poultry. Stunning: Mechanical Stunning

Further Reading

Dalmau, A., Llonch, P., Rodreguez, P., *et al.*, 2010. Stunning pigs with different gas mixtures: Gas stability. Animal Welfare 19, 315–323.

Dalmau, A., Rodreguez, P., Llonch, P., Velarde, A., 2010. Stunning pigs with different gas mixtures: Gas stability. Animal Welfare 19, 325–333.

EFSA (European Food Safety Authority), 2012. Scientific Opinion on the electrical requirements for water bath stunning equipment applicable for poultry. EFSA Journal 10(6), 2757 (80 pp).

Gillies, J.A. (Ed.), 1965. A Text Book of Aviation Physiology. Oxford: Pergamon Press.
Gregory, N.G., 1998. Animal Welfare and Meat Science. Wallingford, Oxon: CABI Publishing.
Llonch, P., Dalmau, A., Rodreguez, P., Manteca, X., Velarde, A., 2012. Aversion to nitrogen and carbon dioxide mixtures for stunning pigs. Animal Welfare 21, 33–39.
Raj, A.B.M., 1999a. Behaviour of pigs exposed to mixtures of gases and the time required to stun and kill them: Welfare implications. The Veterinary Record 144, 165–168.
Raj, A.B.M., 1999b. Effects of stunning and slaughter methods on carcass and meat quality. In: Richardson, R.I., Mead, G.C. (Eds.), World Poultry Science Association (UK Branch) Symposium Series, Poultry Meat Science 25, 231–254.
Raj, A.B.M., 2006. Recent developments in stunning and slaughter of poultry. World's Poultry Science Journal 62, 467–484.
Raj, M., Tserveni-Gousi, A., 2000. Stunning methods for poultry. World's Poultry Science Journal 56, 291–304.
Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission, 2009a. Welfare aspect of the main systems of stunning and killing of farmed seabass and seabream. The EFSA Journal 1010, 1–52.
Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission, 2009b. Welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon. The EFSA Journal 1012, 1–77.
Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission, 2009c. Species-specific welfare aspects of the main systems of stunning and killing of farmed rainbow trout. The EFSA Journal 1013, 1–55.
Warriss, P.D. (Ed.), 2000. Meat Science: An Introductory Text. Wallingford, Oxon: CABI Publishing.

Electrical Stunning

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Glossary

Cardiac arrest A process that occurs when the heart ceases to function. It can occur, for example, through ventricular fibrillation.

Clonic muscle spasms A series of alternating muscular contractions and relaxations.

Consciousness A state of the mind that includes subjectivity, awareness, the ability to experience or to feel, wakefulness, and the executive control system of the mind.

Electroencephalogram (EEG) A measure of the electrical activity of the brain.

Electronarcosis or electroanesthesia A state of unconsciousness achieved by application of an electric current.

Generalized epileptiform activity The seizure of grand mal epilepsy, consisting of a loss of consciousness and generalized tonic convulsions followed by clonic convulsions.

Neurotransmitters A group of chemicals in the brain responsible for brain/nerve function. They include gamma amino-4-butyric acid (GABA), vasopressin, oxytocin, glutamate, and aspartate.

Slaughter The process of exsanguination or bleeding that involves sticking (severance of the brachiocephalic trunk at the thoracic inlet in cattle and pigs) and cutting both the carotid arteries in the upper neck (sheep, goat, and poultry).

Tonic muscle spasm A sudden, abnormal, involuntary muscular contraction consisting of a continued muscular contraction.

Introduction

Stunning Process

All animals, including poultry and fish, should be protected from anthropogenic excitement, and pain or suffering during transport, lairage, restraint, stunning, slaughter, or killing. Research in bird-, mammal-, and fish-slaughtering industries has linked improvements in animal welfare to improvements in meat quality.

Stunning of animals is applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover before exsanguination results in death. It is accepted that unconsciousness and insensibility should be induced immediately during stunning to minimize detrimental effects on animal welfare and meat quality.

Electrical stunning, also referred to as electronarcosis or electroanesthesia, is widely used all over the world in slaughterhouses on farmed animals such as cattle, sheep, pigs, poultry, and fish. Electrical stunning involves passing of an electric current of sufficient magnitude through the head of an animal such that a generalized epileptiform activity is induced in the brain (grand mal seizure-like state). In humans, generalized epileptiform activity involving both the cerebral hemispheres is always accompanied with unconsciousness, and therefore sentient animals are also considered to be unconscious and insensitive following electrical stunning.

The epileptic process is characterized by rapid and extreme depolarization of the resting membrane potential of neurons in the brain. Nevertheless, behavioral and clinical signs of recovery are not always sufficient for the assessment of the effectiveness of electrical stunning. Therefore, the use of electroencephalogram (EEG) recordings alongside responses to stimuli (visually evoked and somatosensory evoked responses) to assess unconsciousness and insensitivity are recommended.

History

Early in the nineteenth century scientists experimented with electricity in order to produce a state of anesthesia in animals and man, and later in the twentieth century, on animals to render them unconscious and insensible before slaughter. Most of the work in this connection was done in Germany, France, The Netherlands, and the USA. Leduc found that a constant current, which was rhythmically interrupted 90 times per second using a voltage of 5–20 V, was able to produce a comatose-like narcosis in rabbits. In other experiments on himself, his motor functions were paralyzed, whilst higher cerebral functions were not disrupted, but analgesia was present. Small animals showed tonic spasms before clonic contractions, which were often followed by convulsions when applying 300 mA on the head.

Many meat hygienists came to the conclusion that the animals were unconscious because of the absence of the corneal reflex. It is clear that the conjunctival reflex, i.e., shutting of the eyelids after the conjunctiva was touched, was only possible provided that the eyelids were free to react. Because the eyelids were brought into tonic spasms as soon as the circuit was closed, the reflex was prevented. In this condition, absence of the reflex did not mean anything as regards the condition of the central nervous system, i.e., the brain.

It was claimed that the 'electrolethaler,' which was designed for slaughter animals, was humane, instantaneous in action, economic in use, causing no blood splashing in the meat, safe, and ensured complete bleeding. The electrodes were placed behind the ears for a few seconds, and it was recommended to

place the electrodes as a second application on the head and back of the animal for 15–30 s. The voltage applied was 30–70 V. It was claimed that farm animals such as pigs, sheep, and calves were unconscious after the application of current. Fractures in the vertebrae of the carcasses were found and believed to be due to the sudden contraction of muscles.

Brain Stimulation

Anesthesia

Since the end of the past century, many investigations have been performed on electroanesthesia in animals and man. The anesthetic effect is brought about immediately with the onset of the flow of sufficient electric current through the brain, and the recovery is also very rapid after the end of electrical stimulation. In veterinary medicine, electroanesthesia might be used because it represents a simple, completely controllable, and low-cost method of anesthesia. During electroanesthesia, unconsciousness may be present but without a general epileptiform insult. It has been suggested that the ascending activating influence of the reticular formation of the brain stem suppress responses of the telencephalon or cortex (the seat of consciousness) during current delivery. Another suggestion is that the administered current interferes sufficiently with neuronal function at the thalamic (midbrain) level to cause anesthesia. It has also been observed that overt behavioral unconsciousness or loss of somatosensory potentials can occur without the development of polyspike activity in the EEG in sheep and poultry, respectively, when extremely high currents (almost 10 times than that is necessary to achieve effective stunning) are applied during stunning.

Stunning

Electroanesthesia is widely used for the stunning of slaughter animals. The amounts of current necessary to stun various species of farmed animals are presented in Table 1.

Effective electrical stunning can be ascertained from the occurrence of generalized epileptiform activity in the brain by using EEG. Generalized epileptiform EEG consists of relatively small waves increasing in amplitude in the tonic phase and decreasing in frequency in the clonic phase to result ultimately in a period of strong depression of electrical activity in pigs, sheep, calves, and poultry (Figure 1). Several studies involving sheep, in which neurotransmitters have been measured, coupled with pharmacological experiments, suggest the general epileptiform insult induced by an electrical stun is dependent on the release of vasopressin, oxytocin, glutamate, aspartate, and gamma amino-4-butyric acid (GABA). The first phase induced by the stun produces the tonic phase through the release of the excitatory neurotransmitter glutamate. This is followed by the release of GABA that provides a period of analgesia and also assists in the recovery if the animal is not slaughtered. The observed behavior of a general epileptiform insult is characterized by a phase of tonic muscle spasm followed by a phase of clonic muscle spasms and ultimately an exhaustion phase with muscle flaccidity. An eye reflex cannot be used as an indicator, because the reflex is blocked during the tonic phase and may occur spontaneously during the clonic phase. In sheep as well as in other mammals the extensors are stronger than the flexors that caused the extension. During head-only stunning, broilers may display wing flapping during and after stunning, which is sometimes intensive. Fish, which were able to move freely, initially showed limited tonic/clonic cramps, followed by heavy clonic contractions combined with uncoordinated movements or turning aside. The flexors and extensors in fish are considered to be equal in strength, which may explain the observation of limited tonic and clonic cramps.

Table 1 Recommended minimal current for electrical stunning of poultry, ruminants, pigs, and fish

Species	*Head-only*	*Water-bath/water tank*	*Head to body*
Broiler	240 mA	100 mA < 200 Hz^{-1}	
Turkey	400 mA	250 mA < 200 Hz^{-1}	
Ostrich		500 mA	
Duck and geese		130 mA < 200 Hz^{-1}	
Quails		45 mA < 45 Hz^{-1}	
Cow	1.28 A		
Calf	1.25 A		
Sheep and goat	1.0 A		1.0 A
Pigs	1.3 A		1.0 A
Eel	600 mA	0.64 A dm^{-2}	
Trout	500 mA		
African catfish	630 mA	1.6 A dm^{-2}	570 mA
Carp	240 mA	0.14 A dm^{-2}	
Salmon			670 mA
Cod		2.5 A dm^{-2}	
Turbot		3.2 A dm^{-2}	
Tilapia		1.0 A dm^{-2}	
Sea bass		4.3 A dm^{-2}	

The most common electrical stunning method for livestock uses a frequency of 50 Hz alternating current (AC) with sinusoidal waveform. The frequency can be as high as 1800 Hz, and the waveform can be square or rectangular. High-frequency electrical stunning can induce epileptiform activity in the brain; however, relatively higher amounts of current are necessary to induce epileptiform activity and the duration of unconsciousness also shorter than those with 50 Hz. A sufficiently prolonged period of unconsciousness and insensibility (e.g., 40 s) is necessary to facilitate exsanguination (bleeding out) and onset of death in unconscious animals. In this regard, a bipolar sine or square wave is found to be more effective than monopolar-pulsed direct currents. In 'head to body' stunning involving passage of a 50 Hz sine wave alternating current simultaneously through the brain and heart, the animal may die due to a heart failure, which is recordable on an electrocardiogram (ECG). The heart failure results in loss of blood pressure and lack of oxygen to the brain and affects the characteristics of general epileptiform insult.

Transcranial magnetic stimulation (TMS) is a recently developed noninvasive technique used in human psychiatry to treat depression with slowly repeated pulses to the frontal lobe or to induce seizures. A study was done to determine whether or not TMS with an adapted coil has potential for further development as a noninvasive stunning method for broilers and

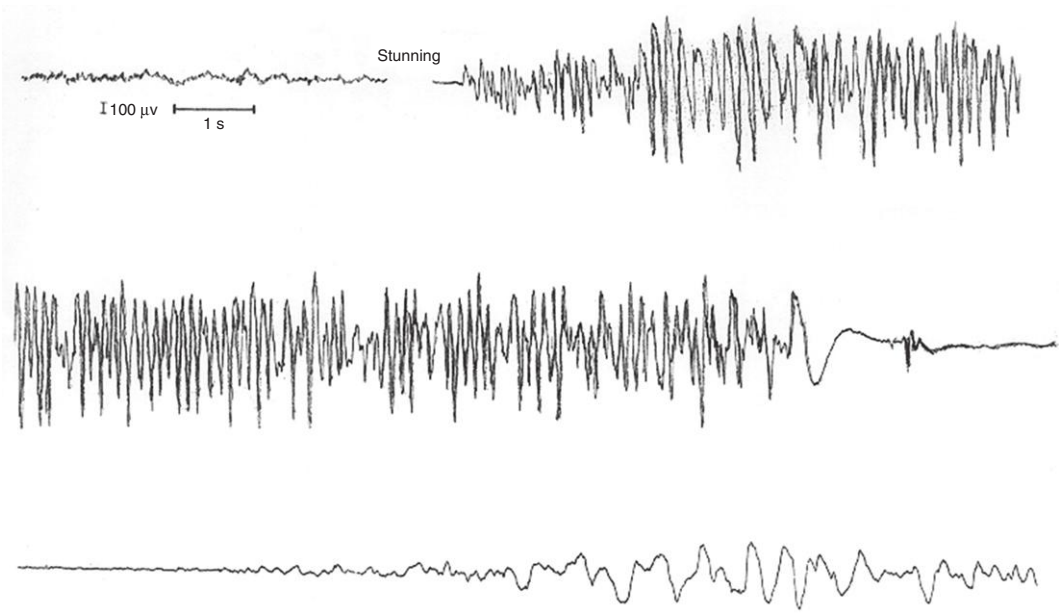

Figure 1 Trace of the EEG before and during a general epileptiform insult. The relatively small waves (initial phase) became larger (tonic phase) with an increase in amplitude and a decrease in frequency, followed by a period of strong depression of electrical activity (exhaustion phase) and recovery.

rabbits but further research and development is needed to optimize parameters.

Ethics

First, stunning of animals is applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover before death occurs via exsanguination. Second, stunning should produce sufficient immobility to facilitate safe shackling, hoisting, and exsanguination of animals. It is generally stated that unconsciousness should be induced, as soon as possible without imposing a detrimental effect on the welfare of the animal and the meat quality. For the application of stunning methods, it is necessary to confine or restrain the animal and to position it for stunning. The effectiveness of any method of preslaughter stunning can be seriously impaired by improper use of the restraining device on the animal and by preslaughter stress.

Stress before slaughter increases some neurotransmitters, which may affect the poststun reflexes and unconsciousness. Combining head-only stunning with exsanguination has a synergistic effect on the release of glutamate and aspartate, which increases the duration of unconsciousness and insensibility. Sticking (also referred to as slaughter, exsanguination, or bleeding) following a stun should be carried out as promptly as possible when using head-only stunning as it takes time depending on the species before brain responsiveness is lost following sticking. It is widely recognized that inducing a cardiac arrest at stunning has distinct welfare advantages: (1) it results in a rapid loss of brain function, (2) it ensures that the animal will not regain consciousness, and (3) it does not depend on the slaughter man performing an accurate stick. Sticking should involve severance of blood vessels supplying oxygenated blood to the brain. For example, sticking in cattle and pigs involves severance of the brachiocephalic trunk at the thoracic inlet and cutting both the carotid arteries in the upper neck of sheep, goat, and poultry.

Meat Quality

Various stunning methods and electrical parameters have been reported to have a different effect on postmortem rigor development and subsequent meat quality. The postmortem metabolism is largely a consequence of indirect stimulation through nervous pathways. Electrical stunning of lambs for 3–4 s can influence the pH measured in the loin and some other muscles. This is also the case in pork, and it may result in pale soft exudative meat, which is not necessarily related to genotype. In chickens, high-current electrical whole-body stunning at 100 mA and above resulted in higher initial muscle pH than nonelectrically stunned birds or birds stunned with 50 mA or less. Breast muscle shear values of birds whole-body stunned with currents lower than 100 mA were lower than or similar to, depending on the deboning time, stunning with currents higher than 100 mA.

Broken vertebrae can occur in pigs stunned with head-to-back electrode positioning if the voltage and the current are too high. A satisfactory stun with a minimum of fractures is obtainable when using 1.3 A with head-to-back stunning systems. Sinusoidal alternating currents of 50 Hz have a large stimulatory effect on skeletal muscles, which can be reduced by increasing the current frequency to an extent that prevents the occurrence of broken backs. The prevalence of broken vertebrae and pelvises could be reduced to zero by increasing the frequency from 50 to 1500 Hz when stunning head-to-back with 300 V for 3 s. The drawback of this approach is that

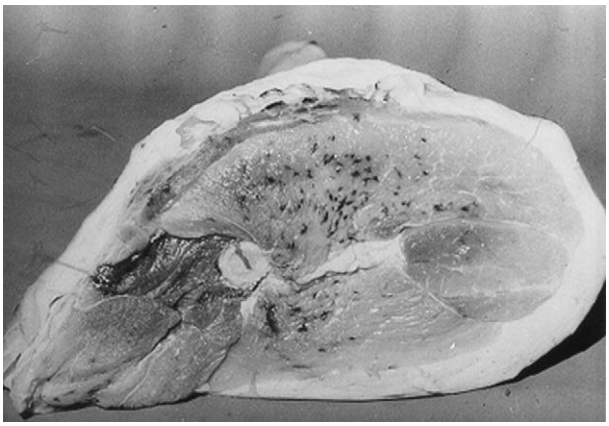

Figure 2 Hemorrhages in the muscles of the ham.

the effect on fibrillating the heart is also reduced. However, it might be possible to find an optimum combination of frequencies and current, and electrode position that stuns the pig and fibrillates the heart with minimum of damage to the carcass and meat.

Hemorrhaging in major muscles and surrounding tissues results in a decrease in the quantity (trimming) and quality of meat products, and hence causes economic losses to the meat industry. Hemorrhages can be induced by stunning; however, the underlying mechanism is considered to be multifactorial. The morphology of hemorrhages investigated was dependent on the tissue in which they occurred. In the pectoral muscles, extravasating blood was found to follow the direction of the muscle fibers. In fat tissue, the majority of the hemorrhages had a petechial appearance. More diffuse hemorrhages were found in loose connective tissue (Figure 2). The histological study of hemorrhages in different types of muscles showed that the morphological appearance of the blood extravasation is determined by the structure of the tissue as well as by the amount of blood leaving the circulation. Some hemorrhages were associated with hypercontracted and disrupted muscle fibers, indicating that they were caused by severe muscular strain. Many hemorrhages were found near venules or veins and were packed with erythrocytes, surrounded by intact adipocytes and connective tissue. Rupture was observed only in venous structures, such as postcapillary venules and small veins, not in arterial vessels. This strongly indicates that a local rise in venous blood pressure can cause rupture of venules and small veins.

The force experienced during electrical stunning probably depends on the posture or restraining method of the slaughter animals. For instance, shackling involves hanging live birds upside down, whilst suspended by their feet. The restrained legs carry the body weight of the birds. Electrical water bath stunning of broilers has the most detrimental effect with respect to muscle hemorrhaging. A poor bleed out can significantly increase hemorrhage conditions in broilers. Electrical stunning with currents that induce cardiac arrest in the majority of the broilers is associated with a high incidence of red wing tips. This is explained by inadequate bleeding of the birds after cardiac arrest. The wings of killed instead of stunned birds hang low resulting in stagnation of blood in the wing veins. The use of high frequency (500 and 1500 Hz) stunning currents resulted in a decrease in carcass downgrading and a marked reduction in the occurrence of breast muscle hemorrhages, which represent significant commercial benefits.

In lambs subjected to head-to-back stunning, higher currents and longer-stunning durations increased the severity and incidence of speckle in the legs but not in the loin. The reason for this is that its electrode placement ensures a maximal tetanic effect over the whole musculature of the loin. There are effects on speckle in the leg due to stimulation through the nervous system.

It is well known that ac currents between 50 and 100 Hz cause substantial injuries in salmon, but an older study indicates that dc currents although tending to improve the quality do not guarantee efficient stunning. The combined ac/dc supply used in the experiment was not only efficient for stunning but also did not provoke internal injuries such as spinal deformities or rupturing the aorta dorsalis in fish.

Practical Application

Ruminants

Sheep and calves are regularly stunned using head-only or head-to-back electrical methods. For head-only stunning, the current is either delivered via scissor-model stunning tongs with pointed steel electrodes placed on either side of the head or a pistol grip-like handpiece holding the electrodes. The head of animal may be wetted with a water jet in order to improve electrical conductivity between the head and stunning electrodes. The electrodes should be positioned on both sides of the head between the eye and ear such that they span the brain during head-only stunning. This type of stunning is termed 'head-only' stun that does not stop the heart. As the animal can potentially recover and the slaughterman cuts the throat before the animal recovers (i.e., effectively taking the life of the animal), this procedure is therefore consistent with halal slaughter.

During head-to-back or body stunning, one electrode should be positioned on the head and the other one on the withers or loin back such that the two electrodes span the brain and heart. The duration of insensibility associated with a head-only electrical stunning in sheep is 34–45 s, and recovery can be prevented by rapid exsanguination. Head-to-body stunning can cause cardiac arrest and as the spinal cord is also in the pathway, it additionally reduces the animal's reflexes with significant movement reduction, making it a good option when halal slaughter is not required.

In cattle, the head of the animal is usually restrained by two parallel bars, which also serve as stunning electrodes. After head-only stunning for 4 s with 1.5–2.5 A, the animal is rolled out of the stun box on to a conveyor and bled out by a throat cut within 10 s from the end of stun. While bleeding, a low voltage pulsed direct current is applied from nose to rump of the animal for a minimum of 30 s (80 V, 15 Hz). This results in a still carcass, enhancing worker safety and producing a degree of electrical stimulation that protects meat tenderness. Alternatively, head-only electrical stunning in cattle is swiftly followed by the application of a second current cycle from the

Figure 3 Correct placement of the stunning tongs on the head.

muzzle to brisket of the animal to induce cardiac ventricular fibrillation.

A double-rail conveyor restrainer for cattle or sheep in an upright position with the legs straddling and the body supported by the belly has been developed. The animals experienced less stress in this system. It is recommended to use a double-rail restrainer and stun the animal in an upright position.

Pigs

Most slaughter pigs are stunned electrically. The current is delivered via scissor-type stunning tongs positioned on both sides of the head between the eye and ear such that the electrodes span the brain for head-only electrical stunning (Figure 3). During head-to-back or -body stunning, one electrode is on the head and the other on withers, loin back, fore- or hindleg such that the current flows through the head as well as heart. In the case of the head-body position, a cardiac arrest may occur. It is considered that the pig skin has a high resistivity and penetrating this layer would improve current flow.

Several automatic electric stunning methods are available. One device consists of two V-type restrainers running at a different speed in order to separate the successive animals. At the end of the second restrainer, each pig touches the electrodes and the current is passed. In another method, pigs make automatic contact on the head with two electrodes at the end of one V-type restrainer. After stunning, the animals are turned out and fall onto a table. A third method is automatic electric stunning at a band restrainer. At the end of the restrainer, the nose of the pigs interrupts a beam of light that initiates the electrodes. The electrodes are positioned between the eye and ear. After 1 s of stunning a heart electrode is positioned behind the left shoulder for 1.5 s. As a result of the body current, the animals do not show muscle contractions.

Poultry

Water-bath electrical stunning is commonly used under commercial conditions where large throughput rates are required. In this system, birds are hung upside down on a moving, metal-shackle line (shackling), and passed through an electrified water bath, such that the current flows through the whole body toward the shackle, which serves as the earth. In water-bath systems, the electrical current is applied to the whole body, and the minimum current necessary to induce unconsciousness and insensibility depends upon the waveform and frequency of current used. However, the minimum currents recommended for broilers increase quality defects (hemorrhages, broken bones) of carcasses and meat. An alternative to whole-body electrical stunning is head-only stunning, where the stunning current only passes through the head of the birds. Head-only electrical stunning has been evaluated using a cone-shaped restrainer in which the broilers were suspended by their feet. Broilers may be insensible and unconscious after head-only electrical stunning with pin-electrodes using a current of 190±30 mA for 0.5 s. For practical implementation, a set current of 250 mA is recommended to overcome individual differences in resistance. To prevent recovery the stun should be followed by an immediate neck cut.

Fishes

Electronarcosis is used to immobilize fish for routine laboratory or farming practices. In general, 50–70 V depending on the length of the fish is used. Assessment of narcosis in fish by several researchers has been based on behavioral observations and clinical signs of recovery. It is assumed that narcosis lasts until the onset of opercular movement, the first response to a stimulus, and the commencement of swimming.

Electricity has been used in various studies to stun farmed fish species. It is known that the specifications for electrical stunning are not only dependent on fish species but also are partly determined by waveform, field strength of the current applied, and water conductivity. There are two approaches of electrical stunning applicable for use in practice. The fish species can be either stunned in water or after withdrawal from the water. Stunning in water involves exposing the fish to an electrical current administered via two plate electrodes in a tank. For stunning after dewatering, the fish is placed in a device that consists of rows of steel flaps as positive electrodes and conveyer belts or steel plates as negative electrodes. In principle, electrical stunning in water reduces stress in the fish, whereas applying stunning after dewatering the fish may result in exposing the fish to air longer.

Other Species

Ostriches are stunned by electrical means (head-only and 80/90 V) or by captive bolt and suspended by both legs by chains hanging from the ends of an upturned horizontal bar. The animal is then lifted and bled. Based on clinical parameters, it was recommended that the current to stun ostriches is 500 mA.

Preslaughter stunning of rabbits is now usually carried out by the preferred method of employing electrical currents. A wall mounted V-shaped metal electrode, with serrated edges for optimum contact, can be used as the stunning electrode. The head of the rabbit is placed into the V of the electrodes, which makes firm contact with the back of the eyes and the base of the ears to span the brain. Electrical stunning can be achieved using currents in excess of 140 mA.

See also: Exsanguination. Preslaughter Handling: Preslaughter Handling. Religious Slaughter. Slaughter, Ethics, and the Law. Slaughter-Line Operation: Sheep and Goats. Stunning: Slaughter: Immobilization

Further Reading

Anil, M.H., McKinstry, J.L., 1992. The effectiveness of high frequency electrical stunning in pigs. Meat Science 31, 481–491.

Barham, W.T., Schoonbee, H.J., Visser, J.G.J., 1987. The use of electronarcosis as anaesthetic in the cichlid, *Oreochromis mosambicus* (Peters). I. General experimental procedures and the role of fish length on the narcotizing effects of electric currents. Onderstepoort Journal Veterinary Research 54, 617–622.

Blackmore, D.K., Delaney, M.W., 1988. Slaughter of Stock. Palmerston North, New Zealand: Veterinary Continuing Education, Massey University Publ. No. 118.

Channon, H.A., Payne, A.M., Warner, R.D., 2003. Effect of stun duration and current level applied during head to back and head only electrical stunning of pigs on pork quality compared with pigs stunned with CO_2. Meat Science 65, 1325–1333.

Cook, C.J., Maasland, S.A., Devine, C.E., Gilbert, K.V., Blackmore, D.K., 1996. Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throatcutting. Research in Veterinary Science 60, 225–261.

Devine, C.E., Gibert, K.V., Ellery, S., 1982. Electrical stunning of lambs: The effect of stunning parameters and drugs affecting blood flow and behaviour on petechial haemorrhage incidence. Meat Science 9, 247–256.

Gregory, N.G., 2005. Recent concerns about stunning and slaughter. Meat Science 70, 481–491.

Hindle, V., Lambooij, E., Reimert, H.G.M., Workel, L.D., Gerritzen, M.A., 2010. Animal welfare concerns during the use of the water bath for stunning broilers, hens, and ducks. Poultry Science 89, 401–412.

Kranen, R.W., Lambooy, E., Veerkamp, C.H., Van Kuppevelt, T.H., Veerkamp, J.H., 2000. Histological characterization of hemorrhages in muscles of broiler chickens. Poultry Science 79, 110–116.

Lambooij, E., Anil, H., Butler, S.R., *et al*., 2011. Transcranial magnetic stunning of broilers: A preliminary trial to induce unconsciousness. Animal Welfare 20, 407–412.

Lambooij, E., Grimsbø, E., Van de Vis, J.W., *et al*., 2010. Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities. Aquaculture 300, 107–112.

Papinaho, P.A., Fletcher, D.L., 1996. The effects of stunning amperage and deboning time on early rigor development and breast meat quality of broilers. Poultry Science 75, 672–676.

Raj, A.B.M., 2006. Recents developments in stunning and slaughter of poultry. World's Poultry Science Journal 62, 467–484.

Sparrey, J.M., Wotton, S.B., 1997. The design of pig stunning tong electrodes – A review. Meat Science 47, 125–133.

Mechanical Stunning

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Glossary

Corneal reflex Blinking (fast or slow) in response to stimulus of the cornea.

Eye movements A correctly stunned animals will show fixed eyes: They are wide open and glassy, and no nystagmus (spontaneous rapid side to side (twitching) movements of the eyeballs). They will also not show eyeball rotation, in which the eyeball rolls so mostly pink sclera can be seen and little or no iris.

Spontaneous blinking Animal opens/closes eyelid on its own (fast or slow) without a stimulation.

Introduction

The Physiology of Mechanical Stunning

The main method for stunning cattle is the penetrative captive bolt.

It is intended to produce immediate unconsciousness and insensibility that lasts until death occurs from exsanguination. Well-serviced and -maintained weapons should consistently achieve proper stunning in cattle. The stun effectiveness (stun quality) can be influenced by variations in cattle breed, size and maturity, as well as differences in mechanical properties of commercially available guns.

There are two types of captive bolt guns: penetrating and nonpenetrating. In penetrating captive bolt guns, a metal rod is propelled from the muzzle of the gun by the discharge of a blank cartridge inserted in a chamber behind the proximal end of the bolt. In nonpenetrating captive bolt guns, a mushroom head-shaped bolt is propelled from the muzzle of the gun by the discharge of a blank cartridge inserted in a chamber behind the proximal end of the bolt. The use of the penetrating captive bolt is also referred to as concussion stunning and the nonpenetrating bolt as percussion stunning. The impact of the bolts on the head of an animal causes concussion of the brain and rupture of brain blood vessels, leading to unconsciousness.

The bolt velocity varies according to the gun powder content within a selected cartridge, which is usually color coded by the manufacturer. When a penetrating captive bolt gun is fired on the head, the sharp-rimmed bolt enters the skull and brain and then recoils automatically back into the barrel of the gun. The depth of penetration into the skull varies according to the length of the bolt and the power of the weapon. The captive bolt should create a large, deep, penetrating, and well-defined hemorrhagic track which traverses almost the full thickness of the brain. It should cause severe damage to the cerebellum, brainstem, and caudal aspect of the cerebral hemispheres with marked subarachnoid and intraventricular hemorrhages, especially adjacent to the entry wound and around the base of the brain. The rupture of arteries entering the brain ensures a lasting unconsciousness and insensibility during shackling, hoisting, and sticking (exsanguination or bleeding) procedures until death of the animal (Figure 1).

The bolt velocity and angle of firing determine the effectiveness of stunning, and the kinetic energy impacted on the skull travels to the basal area of the brain. Exsanguination should be carried out without undue delay following captive bolt stunning. In cattle, exsanguination is normally performed by inserting a knife at the base of the neck pointing toward the chest and severing the brachiocephalic trunk and anterior vena cava.

The effects of different bolt velocities at stunning on brain function may be investigated by looking at visual evoked responses (VERs) as an index of brain damage. VERs, induced by flashing a strobe light in front of the animal, are recorded along with the spontaneous brain activity by way of electroencephalograms (EEGs) recorded using electrodes attached to the head or electrocorticograms (ECoGs) recorded using electrodes implanted (under anesthesia) on the surface of the

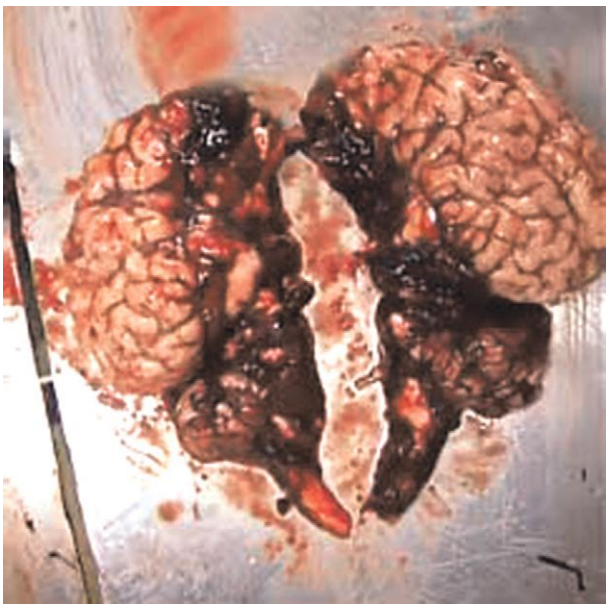

Figure 1 Typical brain hemorrhage in a well-stunned animal (notice heavy bleeding track down the central part of the brain).

brain area responsible for normal vision (known as visual cortex). The electrical activity evoked by the flashing light indicates the brain's ability to respond to external stimulus. VERs are present in conscious as well as unconscious animals, but their loss indicates an insult to the brain sufficient to cause failure of a primary sensory pathway, and are therefore used as an indicator of profound brain failure, and hence unconsciousness and insensibility.

The capability of penetrating captive bolt guns to obliterate VERs depends on bolt velocity and the shot accuracy. In an experiment conducted by Daly in 1987 on the effect of bolt velocity and diameter on abolition or retention of VERs, it was found that visual-evoked responses are abolished or significantly reduced in amplitude as energy transfer increases and that energy transfer increases with bolt diameter (**Table 1**).

The application of the shot should be at a point derived by the two lines between the ear base and the opposite eye (A, **Figure 2**).

Bolt velocity is reduced if (a) incorrect cartridges for the species and size of animal are used, (b) the cartridges are damp (kept in humid environments), and (c) the gun is dirty or has worn out or damaged parts. The impact of a captive bolt can also be seriously hindered if the operator applies the gun at an incorrect angle or too far away from the animal's forehead. In principle, the gun should be placed flat on the animal's forehead at a 90 degree angle to achieve the maximum impact. If animals are moving within the stun box, obtaining the correct shooting angle, location, and closeness on the forehead can be difficult. This can be especially a problem when shooting relatively small animals such as calves in a large stun box designed for adult cattle.

It is worth noting that sheep and cattle stunned in the poll position are less likely to lose VERs than those shot frontally. Also, increasing bolt velocity when stunning cattle increases the likelihood of them losing VERs, but even very high velocities are not invariably successful in this species. The impact of the bolt with the cranium is the principal determinant of effective stunning rather than the penetration of the bolt into the brain tissues. Furthermore, the tissue damage produced by the passage of the bolt through the brain tissue does not necessarily contribute to loss of VERs.

Table 1 Effect of bolt speed and bolt diameter of a captive bolt gun on energy transfer (joules), to the head of the animal and the prevalence of visual-evoked responses in the cortex of the brain

Bolt speed ($m\ sec^{-1}$)				
	47		*55*	
Bolt diameter (mm)	*Energy transfer*	*Evoked responses*	*Energy transfer*	*Evoked responses*
12	97 + 17	3/6	124 + 25	1/8
14	125 + 18	2/8	139 + 25	1/8
16	158 + 20	1/7	186 + 30	0/7

Source: Reproduced from Bouton, P.E., Fisher, A.L., Harris, P.V., Baxter, R.I., 1973. A comparison of the effects of some post-slaughter treatments on the tenderness of beef. Journal of Food Technology 8, 39–49.

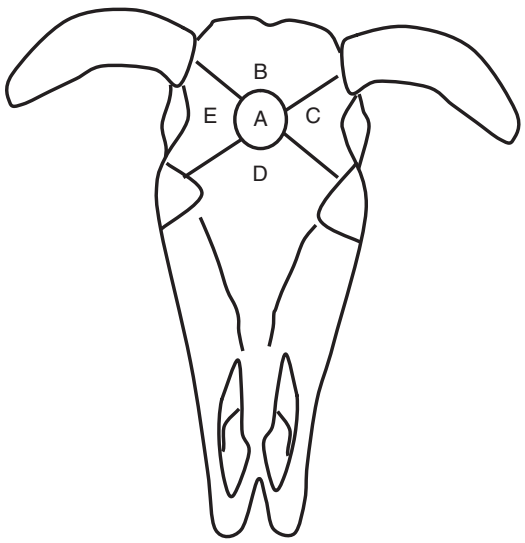

Figure 2 Accurate shot application.

Assessment of Stun Quality

The following criteria may be used to determine an effective stun:

1. Immediate collapse of the animal
2. Brief tetanic spasms that might be followed by uncoordinated hind limb movements
3. Immediate and sustained cessation of rhythmic respiration
4. Absence of coordinated attempts to rise
5. Absence of vocalization
6. Glazed "glassy" appearance of the eyes
7. Absence of eye movements and reflexes

Ineffective stunning is considered to occur if animals show any of the following symptoms: nystagmus, eyeball rotation, vocalization, rhythmic breathing, righting reflex, spontaneous blinking, or corneal reflex. Other symptoms may be failure to collapse properly, excessive ear or tail tonus, or excessive kicking.

Animals can show signs outside of these criteria due to neural reactivity occurring from various levels of physical injury to different parts of the brain. Despite the instantaneous brain trauma caused by penetrating captive bolt stunning, the physical manifestation of its effect can occur in stages. For example, kicking movements can occur at various times after the stun. The actual sticking process can also cause a further bout of kicking or front leg and head movements that are not necessarily associated with ineffective stunning. To be sure that there is no risk of recovery, eye reflexes must be absent. Bulls tend to display eye rotations and nystagmus more often than other cattle classes, and if seen in isolation, these symptoms represent a risk of recovery rather than a symptom of sensibility. Eye rotation can also sometimes occur for a short period immediately after stunning and persist for up to 15 s, then the eyeball centers in the socket and the glazed appearance of proper stunning occurs as the animal falls into a state of coma or death ensues. However, to prevent any risk of

recovery, and especially if seen with other symptoms, the animal should be restunned when these symptoms appear and remain for more than 15 s.

It is important when assessing stun quality that animals are inspected during the whole stun to stick period and during bleeding. A judgment should be based on the absence or presence of certain symptoms, and further inspection is warranted when symptoms outside of the deep stun criteria appear. For example, symptoms that indicate that the animal should be checked more closely for further signs include stiff ears usually held upright, gasping, stiffness in the jaw, absence of a protruding tongue while on the shackle or during sticking procedures, and excessive head or paddling movements that hinder the sticking process. An immobile tongue hanging out of the mouth is a valuable sign of an effective stun but is not a requirement for the diagnosis of effective stunning. If at any time animals show a positive corneal reflex or spontaneous blinking, the animal must be restunned immediately.

If an appropriate captive bolt gun is fired with the correct cartridge and positioned in the appropriate angle, captive bolt stunning should achieve a close to 100% success rate. The risk for inadequate stunning can be reduced in larger cattle (i.e., bulls) by using well-serviced, commercially available, pneumatically operated bolt guns because of the higher power and bolt velocity compared to conventional captive bolt guns. Brain damages seen in pneumatically operated bolt guns have been shown to be larger, with more severe hemorrhaging at the base of the brain, suggesting that the brain is shaken more vigorously within the cranium due to the impact of shooting cattle with these guns, contributing to higher stun quality. Problems with animal welfare can be minimized by designing handling facilities in abattoirs that consider natural species-specific behavioral principles. The effectiveness of the stun also depends on the skill of the abattoir staff, who should be adequately trained and certified. It is important to assess each situation separately when deciding whether standards at a given abattoir are satisfactory from an animal welfare perspective. However, by implementing quality assurance schemes in abattoirs including external stun quality auditing by appropriately trained personnel, animal welfare can be safeguarded.

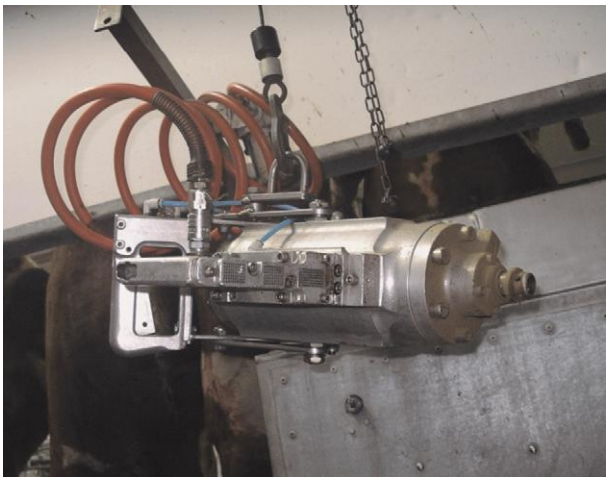

Figure 3 Pneumatic stunner.

Figure 4 A bull in a stun box with a neck restraint ready for stunning.

Practical Considerations

Bulls tend to be more difficult to stun than other cattle classes due to their thicker skull and greater hair mass on the forehead, which reduce bolt velocity as well as the transfer of energy to the brain. For consistent, effective stunning of bulls, it is therefore pertinent to use well-maintained, clean, and high-performance captive bolt guns. Bolt velocity (and stun effectiveness) can also be reduced in the event any part of the gun is damaged, worn, or dirty. In high-throughput abattoirs, the repeated use of the same captive bolt gun may cause overheating, reducing its efficacy. Changing guns frequently, e.g., after every 20 animals or so, may prevent this problem. It is also advisable to use a separate gun appropriate for the size of cattle, e.g., cows, steers and smaller cattle, and bulls. The use of a well-maintained pneumatically operated captive bolt gun and properly designed, constructed, and maintained neck restraints help to achieve effective stunning in bulls (Figures 3 and 4).

Free bullets can also be used in field conditions (e.g., during disease outbreaks) fired using either a conventional or modified rifle, with a high velocity bullet (9 mm) (Figure 5). In many abattoirs, however, due to the associated risk to human safety from possible ricochet, captive bolt guns are more often used.

Stun quality not only impacts on animal welfare but also greatly influences meat quality.

In small and older abattoirs, cattle are manually driven by a stock person from single line laneways into a final enclosed pen or 'stun box.' The stunning process involves a shooter leaning over the animal from an elevated platform and

Figure 5 Two modified weapons used for stunning bulls and cows. The original weapon is shown above and the two weapons below have been modified to activate the firing of the bullet only when the trigger is pressed and the rod under the muzzle is pushed in when placed on the animal's forehead.

Figure 6 A typical type of stunning system with captive bolt.

shooting the unrestrained animal in the forehead with a cartridge-fired captive bolt gun (Figure 6).

Recently, new automated designs have been developed to improve cattle handling and stunning. Cattle are loaded into the stun box with the help of a hydraulically operated moving gate, which pushes the animal forward into the stun box. The stun box is partly open in front, reducing the perception by the animal of 'dead end,' and facilitates voluntary forward movement of the animal. The animal can place its head through an opening where it is restrained by hydraulically closing metal bars on the side of the neck. A shelf can also be used to lift the head up by pushing under the animals chin (chin-lift) (Figure 7).

If cattle are stressed and frightened, they can attempt to escape the stun box or move the head out of reach of the operator making it difficult for the shooter to position the gun in the optimal area to achieve an appropriate stun. The purpose of restraint devices is therefore to hold the animal still and the chin-lift presents the forehead to make shooting easier and more accurate. It is important that push gates, neck

Figure 7 A restraint device for pneumatic stunning of cattle using both neck restraints and a chin-lift.

restraints and chin lifts, are operated slowly and smoothly to reduce fear reactions in the cattle. Operating these devices too quickly can startle the animals and set them into a panic response at the time of shooting.

Abattoir Audits

The guns and their maintenance records should be inspected and the type of cartridge should be appropriate for the type of cattle. A decent sample size needs to be inspected during stunning, and this probably requires spending a day making observations during the slaughter process. The system for loading the stun box, the restraint devices used, and observations of the animals' reactions in the stun box should be noted. If many animals ($>20\%$) are showing severe retreat or struggle and escape attempts during loading the stun box and at stunning, the facilities and the staff should be reviewed to identify the problem areas. A sample of the stun to stick times should also be noted (at least 10%). Prolonged sticking times can increase the recovery risk.

See also: Automation in the Meat Industry: Slaughter Line Operation. Equipment Cleaning. Exsanguination. Meat, Animal, Poultry and Fish Production and Management: Red Meat Animals. Microbiological Safety of Meat: Prions. Preslaughter Handling: Preslaughter Handling; Welfare of Animals. Quality Management: Abattoirs and Processing Plants. Religious Slaughter. Slaughter, Ethics, and the Law. Slaughter-Line Operation: Cattle; Pigs. Species of Meat Animals: Cattle; Sheep and Goats; Pigs. Stunning and Killing of Farmed Fish: How to put It into Practice?. Stunning: Slaughter: Immobilization

Further Reading

Algers, B., Anil, H., Blokhuis, H., *et al.*, 2009. Project to develop Animal Welfare Risk Assessment Guidelines on Stunning and Killing. Project developed on the proposal CFP/EFSA/AHAW/2007/01. Available at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902958022.htm (accessed 22.10.09).

Daly, C.C., 1987. Recent developments in captive bolt stunning. Humane slaughter of animals for food. In: Ewebank, R. (Ed.), Proceedings of a Symposium Organised by the Universities Federation for Animal Welfare. Potters Bar, England: Federation for Animal Welfare, pp. 15–20.

EFSA, 2004. Welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal 45, 1–29.

Relevant Websites

http://www.grandin.com/humane/restrain.slaughter.html
Dr. Temple Grandin's Web Page.

http://www.hsa.org.uk/
Humane Slaughter Association.

Slaughter: Immobilization

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Glossary

Avoidable pain and suffering Any additional pain and/or suffering of a food animal caused by how the animal is handled, restrained, slaughtered, or otherwise manipulated.
Head gate A mechanical restraining device placed in the path of an animal, commonly used for bovine animals, that, when closed, provides a narrow opening large enough to contain the neck of the animal, but too small for either the head or shoulders of the animal to pass through.
Immobilization Implicates measures that interfere with the animal's motor control, such as electric depolarization of the nervous system, to prevent directed movement, flight or attack.
Religious or ritual slaughter Slaughter of a food animal while observing religious rites, traditions, or requirements. Common examples are Halal and Kosher slaughter.
Restraint Restricting the space in which the animal can move about, usually by erecting physical barriers, such as a stun box, a cattle head gate, ropes, or manual force.

Humane slaughter involves methods that meet the dual objective of minimizing the risk of injury to the slaughter person and not inflicting avoidable pain and suffering onto the animal – critical for avoiding ethical (animal welfare) and meat quality problems.

Restricting an animal's movement such that the desired method of slaughter can be reliably employed requires much more than only the physical restraint. It is important to understand that the experience an animal had during an earlier part of the process, between being on a farm and being delivered to slaughter, which will affect later stages of the process.

At the time of slaughter, animals find themselves in unfamiliar environments, experiencing loud noises, strange odors, sometimes poorly designed facilities, are separated from their herd, and forced to interact with strangers – both animal and human. Most of our food animals are aware of their surroundings and are capable of directed movement to defend themselves, attack a threat, or get away from frightening situations.

The desired outcome of a functional and humane slaughter setup is restrained, nonstressed animals that are calmly and reliably slaughtered on a consistent basis. The animals' preslaughter and slaughter experience each play significant roles in achieving the desired outcome of safe and humane slaughter. It is imperative that the individuals involved have an understanding of animal behavior, handle animals appropriately and employ transport, preslaughter and slaughter facilities and equipment that are appropriate for the species, sex, age and number of animals being slaughtered.

The more rigid a method of restraint, the more risk it generally poses for the welfare of the animal and the more important it becomes to apply the restraint correctly and keep the time the animal has to cope with the restraint to a minimum.

The type and duration of preslaughter and slaughter handling and restraint vary greatly, depending on the economic circumstances, the part of the world in which it occurs, local customs and knowledge base, the species, size, and number of animals involved and the type of market that the end product is sold to and consumed in.

Western World

'Immobilization' is distinct from 'restraint.' Immobilization implicates measures that interfere with the animal's motor control, whereas 'restraint' refers to restricting the space in which the animal can move about. Immobilization before slaughter is generally not considered necessary where preslaughter stunning methods are used and not acceptable in the western world. Rather, the ability of the animal to move is greatly restricted by confining the animal in a small enclosure (e.g., 'knock box,' 'stun box') for the brief period of time it takes to stun the animal and/or initiate the bleeding process. A conveyor belt may be used that supports the animals' body but leaves the legs unsupported. Design features of the belt prevent struggling or escape. In bovine animals and pigs, such conveyor belt restrainers have a calming effect on the animals. The automated system presents an animal to the stunner at easily controllable intervals.

When a bovine animal moves to the slaughter area under its own power, a head gate that, when closed, creates a narrowing of the space to both sides of the animal's neck that, without applying painful pressure to the neck, is too narrow for the head to be pulled back or for the shoulders to push forward is most commonly used. Some forms of religious or ritual slaughter require even tighter restraint or a specific presentation of the animal. Examples are a head restraint that securely holds the head and extends the neck, or placing the animal into a rotating drum fixating the entire animal and intended to roll the animal on its back before the neck vessels being cut with a knife. For small ruminants, manual restraint during ritual slaughter is often sufficient.

Poultry are unable to see in low levels of light or areas that are lit with blue light. This can be used to ensure that they remain calm during slaughter, as well as employing handling practices that avoid sudden movements and loud noises, similar to what is recommended for other animal species.

When poultry are shackled (hung upside down on a conveyor in brackets that grasp the birds' legs) to restrain and

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transport them through the process of slaughter, the moving conveyor line brings the birds' breasts into contact with and moves them along a smooth breast bar to soothe or pacify the birds. This helps to ensure they remain calm as they approach and enter the automatic stunner and avoids wing flapping.

In gas stunning, the birds sometimes find immersion into the gas pit noxious and stressful. This is especially true if the gas concentration gradient is too steep or the descent into the gas pit is too rapid. Calm and relaxed birds that have been handled well before their entry into the gas stunning device are less likely to struggle to escape. Some gas stunning poultry plants attempt to restrain stressed birds and prevent them from escaping by placing a physical barrier over the crate as it descends into the pit.

In countries such as Australia, where night hunting of game animals is legal and common practice, bright spotlights trained onto the animals are used to temporarily immobilize animals while they are being shot with a firearm.

Fish

Crustaceans, such as lobsters, are restrained with an elastic band placed around their claws, minimizing the harm that they can do to each other and the people that remove them from the lobster tank and submerge them in a boiling pot of water (slaughter) for consumption.

Emerging Economies

In much of the world, slaughter, often of a single animal, provides for the family meal, a religious event or the local market. Slaughter may be carried out at home or at the farm. This is especially true for larger animals. Slaughter sometimes occurs at the local market only a short time before the meat being offered for purchase. In other circumstances – especially those involving fish and poultry – slaughter and evisceration may be carried out while the customer waits.

Immobilization and restraint in emerging economies are often different from methods seen in the western world. Some of these, often traditional, methods are not acceptable when assessed toward current animal welfare criteria and their inclusion in this article does not constitute an endorsement of their use.

Small ruminants, pigs and birds are held to the ground and their legs are then tied together so that they remain restrained up to and during slaughter.

Standing animals are sometimes tied (tethered) together in groups pending sale or slaughter. There is usually a rope attached to a post or building. However, much of the restriction to movement is supplied by the other animals in the group.

A method of restraining small ruminants is to have a person stand over the animal with their legs straddling the animal. The person's legs are pressed firmly against the shoulder and rib cage on each side of the animal's body. One hand (usually the left) is used to lift the animal's head and pull it firmly against the body of the person while the right hand holding a knife is used to cut the animal's throat.

A small ruminant may also be on its back and held to the ground by several helpers that also stretch the neck, allowing the slaughter person access to the neck with a knife.

Tying the small ruminant's head to a fixed object (e.g., pole) or having a helper hold the head while stretching the hind legs firmly allows rapid decapitation with a sharp saber or cutlass.

Confinement of animals up to the size of a pig in very small cages (metal, wooden, wicker, cardboard) is used during transport and sometimes even slaughter. For example, pigs may remain immobilized in a small form-fitting, basket-like cage for transport to the slaughter facility, while awaiting slaughter and may be bled out inside this cage by an operator using a knife on a long handle.

Akin to low levels of lighting used calm birds during catching, preslaughter handling and slaughter, in some parts of the world a fabric bag or hood is sometimes placed over the head and eyes of animals so that they cannot see. These animals remain quite calm, even if there are high levels of activity and noise around them.

Bags are sometimes used to hold and restrain poultry for transport and while they await slaughter. When this method of restraint is used, chickens are enclosed entirely in the bag. Larger birds, such as waterfowl, are often restrained with their body enclosed by the bag and their head and neck protruding.

In some places, poultry are picked up by their legs (restraint) and their breasts lie against a block of wood until the birds become quiet and do not move (similar to 'breast bars' in western-style poultry slaughter). Then the birds are decapitated with an axe. The birds are held (restrained) by their feet until the bleeding and involuntary movement have stopped.

A practice of immobilization of larger animals involving severing the spinal cord between the skull and the neck with a sharp, pointed knife ('puntilla') inserted into the foramen magnum is in use in some places. As soon as the knife severs the spinal cord, the animal collapses and can no longer move. The animal remains conscious throughout the bleeding process, until death through blood loss occurs, making this practice inhumane.

Immobilization of large animals by severing tendons on the extremities is used in some countries. The practice leads to the collapse of the animal and makes it impossible for the animal to move its extremities. Immobilizing animals by flexing the joints of the extremities and tying them in this position is used for smaller species.

Fish are held in tanks at markets pending slaughter. Fish placed in smaller containers are easier to grasp, especially if the water is warm and low in oxygen. Some individuals use their hands; others use small nets to catch the fish. The fish are held and immobilized by one hand while they are rendered (semi) unconscious with a blow to their head and eviscerated with the other hand.

Postslaughter Considerations

The slaughter process includes the transition from a live, conscious animal to a dead carcass. Inevitably, there is an intermediate state, where the animal is no longer aware of its

surroundings, but many organ systems are still functional. Voluntary, directed movement is replaced by involuntary kicking or paddling of extremities or flexing of the back. While this type of uncoordinated movement is a normal observation on a dying animal, it creates a hazard for workers, may slow the processing speed and can negatively affect meat quality. Immobilization of an irreversibly unconscious animal is acceptable and no animal welfare concerns exist at this point.

Where penetrating stunning devices are used, mechanical disruption of the brain stem and spinal cord ('pithing') is achieved by inserting a metal or plastic rod through the hole left by the stunning device and advancing it toward the foramen magnum. Vigorous manipulation of the rod destroys vital brain stem and spinal cord structures and will successfully prevent convulsions or kicking. The penetrating stunning device and the pithing rod must be matched in order to avoid a hole too small for the pithing rod, making enlargement of the hole necessary. There are downsides of the procedure associated with the potential for introducing pathogens into the carcass by means of a contaminated rod or brushing bacteria off the forehead onto the rod or into the skull cavity or causing proteins implicated in prion diseases, such as bovine spongiform encephalopathy (BSE), to cross from nervous tissue into the bloodstream resulting from the mechanical damage to brain tissue and blood vessels. Dissemination of pathogens throughout the carcass is possible at this stage, because the animal has not yet bled out and some blood circulation still occurs. These concerns led to pithing no longer being accepted practice in many countries. Some countries may allow pithing after the animal has been bled.

The use of electric current to immobilize animal carcasses is of increasing interest in slaughter practice, because it appears to avoid most of the pitfalls associated with mechanical methods of immobilization.

Electrical immobilization (EI) is the application of electric current to the carcass to temporarily disrupt the nervous system. In general the peak voltage is usually relatively low (80 V) with a pulsed waveform approximately 15 pulses per second and is not regarded as dangerous (higher frequencies have been used) and is widely used for cattle. The animals are not only immobilized while the current is applied (e.g., from head to legs), and workers are protected from movement, but the lack of movement continues after the current is switched off. There is an additional advantage of using these voltage waveforms in that immobilization effectively becomes low voltage electrical stimulation if the duration is long enough.

Nerve deactivation (ND) begins before carcass dressing will permanently disable nerve function and eliminates risk of injury to workers. If the voltage is higher than for EI, it can be applied for a shorter duration for a lasting effect. In the case of cattle that have been restrained and electrically stunned, they are already in an area where issues of electrical safety do not arise.

Electrical back stiffening (EBS) is used at the hide pulling stage to contract the carcass back muscles while the hide is being removed. This reduces the incidence of spinal fracture.

Electricity from the power grid must be modulated and transformed in order to have the desired effect. Technical applications exist that tailor the current to suit the characteristics of the particular carcass being dressed at the time, taking into account parameters such as carcass electrical response, carcass biochemical parameters, muscle glycogen levels, type of carcass, and carcass weight.

The EI, activation and deactivation methods listed above can be used individually or combined, at different stations in the process flow.

See also: Preslaughter Handling: Preslaughter Handling. Religious Slaughter. Slaughter, Ethics, and the Law. Stunning: CO_2 and Other Gases; Electrical Stunning; Mechanical Stunning

Further Reading

Blokhuis, H., 2004. Welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal 45, 1–29.

Chambers, P.G., Grandin, T., 2001. Guidelines for Humane Handling, Transport and Slaughter of Livestock. Food and Agriculture Organization of the United Nations. Bangkok, Thailand. Regional Office for Asia and the Pacific, RAP Publication.

Gregory, N., 2006. Anatomical and physiological principles relevant to handling, stunning and killing red meat species. Proceedings of the International Training Workshop Welfare Standards Concerning the Stunning and Killing of Animals in Slaughterhouses or for Disease Control, 26–29 September 2006 Bristol. Available from: Humane Slaughter Association, The Old School, Brewhouse Hill, Wheathampstead, Hertfordshire, AL4 8AN, UK.

Richards, I., 2006. Electrical treatment of carcasses, U.S. Patent Office, USPTO Full Text and Image Database, U.S. Patent 7,025,669. Available at: http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=7025669.PN.&OS=PN/7025669&RS=PN/7025669 (accessed 14.10.13).

Thiermann, A., World Organisation for Animal Health, 2013. Slaughter of animals. In: Terrestrial Animal Health Code. Paris: OIE, (Chapter 7.5), pp. 1–20. Available at: http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.5.htm (accessed 14.10.13).

Relevant Websites

http://www.inspection.gc.ca/english/anima/trans/transprace.shtml
Canadian Food Inspection Agency – humane handling and slaughter.

http://www.fao.org/DOCREP/003/X6909E/x6909e00.htm#Contents
Food and Agricultural Organisation.

http://www.hsa.org.uk/Humane%20Slaughter%20Information.htm
Humane Slaughter Association – preslaughter handling.

http://www.patentgenius.com/patent/7025669.html
Patent on carcass immobilization.

http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.5.htm
Slaughter for human consumption.

http://www.grandin.com
Temple Grandin livestock handling.

STUNNING AND KILLING OF FARMED FISH: HOW TO PUT IT INTO PRACTICE?

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Glossary

Electrical stunning The immediate induction of unconsciousness and insensibility in an animal or fish caused by the passage of an electrical current of sufficient strength through the brain.
Electrocardiogram Recording of the electrical activity of the heart muscles by using electrodes implanted or applied to the skin.
Electroencephalogram Recording of electrical activity of the brain, using implanted or surface electrodes.
Generalized epileptiform insult A state of brain characterized by abnormal, excessive or hypersynchronous neuronal activity throughout the brain, generally considered to lead to unconsciousness and insensibility.
Insensible Inability to perceive (and as a consequence respond to) stimuli.
Slaughter The killing of animals, especially farmed ones, for the production of food.
Stunning The process that renders an animal unconscious and insensible without causing avoidable stress and discomfort prior to death for a sufficient period of time to allow killing.
Stunning/killing The process that renders an animal unconscious and insensible without causing avoidable stress and discomfort that subsequently induces death.
Unconsciousness A state of unawareness (loss of consciousness) in which the brain is unable to process sensory input (e.g., during (deep) sleep, anesthesia or due to temporary or permanent damage to brain function).
Vestibulo-ocular reflex A reflex where eye movement occurs when a fish's body is moved/tilted along a longitudinal axis (also referred to as eye roll). The absence of this reflex is not an evidence of unconsciousness in fish.

Introduction

World aquaculture production of food from finfish comprised 36 million tons in 2010 and is increasing yearly up to 7–10%. The authors estimate that this production volume boils down to 7–120 billion of farmed fish slaughtered in 2010 (with an average weight of 5–0.3 kg, respectively). Asia accounted for 92% of world aquaculture finfish production by volume in 2010, whereas for Europe this was 5.2%.

Several types of aquaculture are used for the production of food fish: ponds, land-based intensive flow-through systems, cage farming and recirculation aquaculture systems. In Europe, the production of Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), and gilt-head seabream (*Sparus auratus*) rely mainly on cage culture at sea. For rainbow trout (*Oncorhynchus mykiss*), the ongrowing stage is done in different systems across Europe (for instance ponds and flow-through systems). In Asia, dominant fish are freshwater species such as various carp species and tilapia subspecies. Asian farmers perform ongrowing of these species predominantly in ponds.

Owing to increasing societal awareness, especially in Europe, Canada, Australia, and New Zealand, attention has been drawn to fish welfare in aquaculture, which is still growing. In view of the fact that mammals and birds should be spared of unnecessary stress and discomfort at slaughter, the question is raised whether this concern is also relevant for farmed fish. In humans, awareness of pain and fear apparently depends on proper functioning of specific regions of the cerebral cortex. Because fish lack a cerebral cortex, it might be argued that fish do not have a capacity to experience pain and fear (sentience). However, Braithwaite *et al.*'s recently reviewed studies showing that teleost fish species have the relevant functional areas in the telencephalon for cognition and emotion. The reported studies show that it is possible that teleost fish perceive pain and fear when they are not stunned before killing or slaughter. The number of fish species studied for behavioral and brain function with respect to cognition and emotion is, however, limited. Within the class of fish there is a diversity with respect to phylogeny, behaviors and habitats, consequently, a variability is found in brain structure and functions among fish. Hence, detailed studies on a wider range of fish species are needed to characterize further the taxonomic distribution of such capacities.

Commercially used methods, such as chilling of live fish by asphyxiation on ice or ice water slurry or killing by decapitation without prior stunning, might cause considerable pain and distress in these animals. These killing methods have been developed not to minimize stress but to achieve product quality control, efficiency, and processor safety. Therefore,

there is a need to develop methods for stunning or stunning/killing of farmed fish and implement them under commercial conditions and control the methods in that setting.

In this article, therefore, the focus is on:

Assessment of stunning and killing or stunning/killing of fish in a laboratory setting to establish ideal conditions for stunning or stunning/killing of fish without causing avoidable stress and discomfort and how the stunned fish can be killed without recovery of consciousness. Assessment of product quality parameters is also of interest as stunning or stunning/killing may affect its properties. A method such as percussion can be suitable for stunning/killing, whereas fish recover from an electrical stun.

In implementation of assessed stunning and killing or stunning/killing in a commercial setting, methods that are suitable for assessment are limited; it is likely that only observation of behavior and physical measurements to assess the equipment installed can be used.

Control of the process for stunning and killing or stunning/killing in a commercial setting can be achieved by using an effective management system. In a commercial setting, an approach that is process-oriented and focused on prevention rather than inspection only is needed, i.e., a quality assurance system.

Slaughter of Farmed Fish

Slaughter is the process for killing of animals intended for human consumption. The term slaughter is also used to depict killing of animals by bleeding. Most farm animals are killed by bleeding. In general, the following steps for slaughtering can be distinguished:

1. Transport from the rearing enclosure to the slaughterhouse or facility for slaughter at the farm.
2. Restraint (fixation of an animal for a proper application of a stunning method).
3. Stunning, i.e., rendering the animal unconscious and insensible so as to reduce avoidable stress and discomfort before killing. The application of a method like percussion can be suitable to achieve both stunning and killing; in this case, step 4 is not performed.
4. Killing of the stunned animals.

Figure 1 gives an impression of the four steps used in the slaughter process of Atlantic salmon, i.e., lairage, pumping, stunning, and killing.

The percussive stunner in Figure 1(d) is used, as this machine has the ability to bleed the electrically stunned salmon automatically.

Before transportation of live, farmed fish from a farm to harvest facilities for slaughter at the farm, the following process is normally carried out: fasting (withholding feed), crowding to facilitate capture of fish for loading into a transport vehicle, unloading of the fish and releasing them (e.g., in holding pen/tank for lairage) before commencing slaughter at the harvest facility or at the facilities for slaughter on the farm. The welfare of farmed fish could be at risk at each of these steps in the process. However, in this article the authors focus on the methods of stunning and killing of farmed fish. For assessment of welfare aspects of stunning methods, the general provision in the European Union (EU) legislation for warm-blooded slaughter animals can be used as a general term of reference. The general term of reference is met when stunning induces immediate loss of consciousness and sensibility in fish, which lasts until death or, when an instantaneous induction is not possible, the animal should be rendered unconscious and insensible, without causing avoidable pain and distress.

Stunning and Killing

For stunning and killing of farmed fish, a wide range of methods are used. Killing methods used at present for farmed fish include asphyxia by chilling on ice in air, live chilling in ice water slurry, exposure of live fish to a salt bath or ammonia, freezing, bleeding (exsanguination) by cutting blood vessels through the gills, and the transfer of fish to water saturated with carbon dioxide gas. Most of these methods do not induce unconsciousness and insensibility immediately, nor do they prevent avoidable stress and discomfort.

Only a limited number of methods have been shown to be able to result in immediate loss of consciousness and sensibility. The authors demonstrated that percussive stunning (a blow to the head) of Atlantic salmon resulted in an immediate onset of irreversible stunning, as judged from electroencephalogram (EEG) and electrocardiogram (ECG) recordings. However, this stunning/killing method, when delivered using a pneumatically operated bolt, required such a high pressure driving the percussive bolt that carcass damage of Atlantic salmon occurred, under the conditions used.

Electrical stunning can induce immediate loss of consciousness and sensibility in fish. However, reported data show that fish cannot be killed by the use of electricity, as the fibrillation of the heart is not permanent. This implies that electrical stunning should be followed by a killing method to avoid recovery of the stunned fish. Because stunning and killing are procedures that take some time, it is normally necessary to apply the electrical current not only at a certain voltage, but also for a certain duration of time, so as to allow subsequent killing before the fish have recovered. For example, Nile tilapia can be stunned using an electrical stun lasting for 5 s and the unconscious fish can be killed subsequently by chilling in ice water slurry.

A problem of electrical stunning, especially when fish are immersed in water during stunning, is that carcass damage might occur, such as muscle hemorrhages or a broken vertebral column. Roth *et al.* found that this problem could be overcome by exposing fish to the electricity after draining the water, so called 'dry stunning.' In this method, the fish are exposed to an electrical current via a series of rows of positive-plate electrodes and a conveyor belt acting as the negative electrode. Evidence shows a positive effect on the quality with a very low incidence of injuries in Atlantic salmon.

In Australia, Chili, Korea, and New Zealand, and some other countries outside of Europe, it is allowed to add the chemical compound Aqui-S^{TM} (with isoeugenol as the active ingredient) to the water in order to stun and kill fish. Using EEGs and ECGs isoeugenol can result in an effective and irrecoverable stun in cod. Isoeugenol is a food grade substance

Figure 1 (a) Stunning and killing of Atlantic salmon: lairage, (b) pumping of fish to the slaughter facilities, (c) electrical stunning after draining the water, and (d) killing the stunned fish by percussion and gill-cutting.

based on clove oil. Barriers to its use in the EU include the cost of overcoming the legislative requirements to introducing isoeugenol as anesthetic for food fish.

In Norway, live chilling of Atlantic salmon with controlled addition of low to moderate levels carbon dioxide (65–257 mg l^{-1}) and oxygen has been widely used to stun the fish. This method, however, has not yet been assessed with EEG and ECGs recordings.

How to Assess Stunning and Killing

Welfare Aspects

To establish whether the general term of reference is met after the application of a stunning method, the onset and duration of unconsciousness and insensibility in fish has to be assessed in a laboratory setting. Behavioral measures only are insufficient to assess the level of brain function of fish unequivocally. On the EEG, the electrical activity in the brain is monitored. In addition, nociceptive stimuli are administered to determine whether the stunned fish can be aroused, both on the EEG and behaviorally. When the fish do not respond, this implies that the fish remain unconscious and insensible until death occurs. The electrical activity of the heart (as determined using ECG) should also be recorded to assess stunning methods. The ECG can be used to determine, for example, whether fibrillation occurs or when the heart rate changes. In case of fibrillation, the circulation of blood in the body is reduced, including the supply of oxygen to the brain. Changes in heart rate might occur in fish that are subjected to live chilling on ice in air or using ice water slurry, carbon dioxide gas or chemical stunning. When these changes are observed before loss of consciousness, they can be signs of stress in fish.

Owing to the highly technical nature of recording the electrical activity in the brains and hearts of fish, conditions to achieve an effective stun in fish without causing avoidable stress and discomfort until death occurs need to be established in a laboratory.

For an instantaneous electrical stun, sufficient current should be passed through the brains of animals to induce a general epileptiform insult (where all brain parts are stimulated). The epileptic process is characterized by rapid and extreme depolarization of the membrane potential, but there is

heterogeneity of findings. It is generally assumed that animals are unconscious and insensible during a general epileptiform insult. Generalized epileptiform insults was observed in various fish species following electrical stunning. For percussive stunning, the patterns on the EEG, characteristic for an instantaneous stun, differ from those recorded in fish that are stunned using electricity. Percussive stunning of Atlantic salmon resulted in the appearance of theta and delta waves and spikes, which were followed by an isoelectric line. Exposure of cod to Aqui-S™ resulted in a slow increase in theta and delta waves and a decrease in the alpha and beta waves, which is indicative for loss of consciousness and sensibility.

For field observations (i.e., during slaughter on a commercial farm or in a harvest facility), registration of EEGs and ECGs might not be feasible. In this case, observation of behavior can be used. Spontaneous behavior (e.g., righting response and escape behavior), responses to stimuli, and physical reflexes (vestibulo-ocular reflex) can be used as preliminary behavioral observations to evaluate loss of consciousness and sensibility in fish. To scale the observations, a three-point scoring system can be used, where 0 designates no response, 1 refers to an attenuated or abnormal response or behavior, and 2 indicates a normal and clear response or behavior. Immediately after an effective electrical or percussive stun, rhythmic breathing as evidenced from the rhythmic gill movements should be absent, and the capacity of the fish to swim in a coordinated way lost. In an experiment with electrical stunning of Atlantic salmon followed by gill-cutting, we observed on the EEGs that one out of three fish recovered. The vestibulo-ocular reflex (eye roll) in the recovered fish was still absent. In properly stunned fish, the capacity to right themselves is also lost. When live chilling or gas stunning results in escape attempts, it is likely that the fish are conscious and stressed.

Fish can be motionless for a number of reasons, such as paralysis, exhaustion, chilling, or tonic immobility (feigning death). In such cases, motionless fish might well be conscious. Caution is therefore needed when using behavior and physical reflexes to determine the effectiveness of stunning methods in practice. Therefore, the use of EEG recordings, including evoked responses on the EEG by administering nociceptive stimuli to the fish, are necessary for an unequivocal assessment of the level of brain function in fish to determine whether or not the fish are effectively stunned.

Chemical stunning (i.e., the use of a gas and a combination of gases or a chemical such as Aqui-S™) or chilling of fish by exposing them to a drop in temperature do not result in an instantaneous stun. In such cases, the observation of behavior and the registration of EEGs and ECGs should be supplemented with stress-physiological measurements. Owing to their complexity, stress-physiological measurements might only be feasible in a laboratory setting.

Analysis of plasma cortisol, glucose and lactate, as indicators for stress in fish, might not be sufficient when Nile tilapia (*Oreochromis niloticus*) are exposed to a noxious stimulus as exposure of a fish to ice or ice water may be noxious. These stress parameters did not allow discrimination between a tailfin clip as noxious stimulus and the handling stress. However, the following parameters indicated a strong differential response in the clipped Nile tilapia: (1) a remarkable migration of chloride cells into the lamellar epithelium of the gills; the chloride cells in the gills are involved in the osmoregulatory performance of fish, (2) swimming activity of the Nile tilapia increased and the clipped fish spent more time in the light than in a dark region in the tank, and (3) the gill's mucus cells released their content.

Regarding exposing a fish's to rapid temperature drop, it is important to note that fish's homeostasis might be fine-tuned to a particular temperature. To understand the possibilities of analyzing the stress response in fish exposed to live chilling on ice or in ice water slurry, some information on the time course of physiological stress response is presented. It is known that changes in cortisol, glucose, and lactate levels in the blood of fish normally occur within a time frame of minutes. Hence, it is possible that due to rapid chilling of fish, no changes in these blood parameters can be detected, due to a fast decrease in metabolism in fish caused by the temperature drop in fish. Therefore, caution is needed to interpret stress-physiological parameters such as cortisol, glucose and lactate when analyzing fish exposed to rapid drop in temperature.

Physical Measurements

To assess whether equipment for stunning and killing of fish in practice meet the established criteria for stunning and killing or stunning/killing, physical measurements are needed. For electrical stunning, the strength of the electrical current (in water it is the height of the current density), its waveform, the applied voltage (in water it is the field strength) and duration of exposure of fish to the electricity need to be established, as well as the time interval between fish leaving the stunner and the application of a killing method. When percussion is applied, it should be measured whether the air pressure, which drives the bolt, is sufficiently high. For chemical stunning (gas and a combination of gases or in countries which allow Aqui-S™ for slaughter) the dosage and duration of exposure of the fish need to be assessed.

Product Quality

The assessment of product quality is relevant for the industry, as stunning and stunning/killing of fish can affect product quality parameters. In addition, a reduction of stress during slaughter might delay the onset of rigor mortis (i.e., the stiffening of the body after death), which is relevant for prerigor filleting of, for example, Atlantic salmon.

Analysis of product quality might not be feasible in a commercial setting and, therefore, these experiments should be performed in a laboratory. However, collection of stunned and killed fish in practice for analysis in a laboratory is doable.

To assess the effects of stunning and killing on product quality, a range of indicators can be used:

1. Appearance of the fish and fillet, for example, residual blood, fillet gaping, and color.
2. Technological properties of fish and fillet, such as texture, water holding capacity, drip loss, and fillet shrinkage (in relation to prerigor processing).

3. Freshness indicators: analysis of freshness, using K-value (calculated from adenosine triphosphate-degradation products).
4. Sensory properties and shelf-life; sensory traits of cooked fillets as texture, taste, flavor and odor, and microbial counts.

How to Control the Process of Stunning and Killing in Practice?

Current ethical concerns about aquaculture, which are broader than welfare of fish alone (for instance ecological aspects of aquaculture), drive the preparation of certification schemes that may include consideration of fish welfare, as for instance in the Freedom Food concept for Atlantic salmon. Increasing calls from nongovernmental organizations and supermarkets in Europe also promote optimization of fish welfare using a certification scheme with appropriate standards. Furthermore, embedding in auditing procedures for accreditation is required to ensure that labeled products comply with the established standards for optimized fish welfare.

Monitoring and auditing procedures, generally, do not focus on preventive measures and they might not control the entire process. To supplement existing procedures, a strategy is needed based on a thorough analysis of the conditions used during the whole process of stunning and killing, considering the specific requirements of a fish species. For this purpose, Quality Assurance appears to be a suitable approach, as it is process-oriented, efficient, focused on preventing hazards, and it involves establishing critical points/steps and standards for all steps in the production process. Previously, the authors developed a Quality Assurance system for safeguarding the welfare of fish at the fish farm – Fish Welfare Assurance System (FWAS). FWAS is based on the hazard analysis critical control points (HACCP) system. HACCP is an internationally acknowledged quality assurance system that is mandatory for the food industry in the EU and other countries outside of Europe. HACCP provides a management tool for food safety based on scientific principles while still being practicable, for example, small specialty shops that might be run by a single person, for example, a butcher.

Briefly, the FWAS consists of the following seven principles: (1) perform a hazard analysis and risk assessment, (2) determine Critical Control Points (CCPs), (3) establish target levels and critical limits for each CCP, (4) establish monitoring procedures at each CCP, (5) establish corrective actions, (6) establish verification procedures, and (7) establish a record keeping system.

The FWAS's structured approach is suitable to control proper stunning and killing of farmed fish in practice, as the basic premise is prevention rather than inspection.

Conclusion

In the authors' view, the following approach is needed to put stunning and killing of farmed fish into practice. The first step is to establish the specifications needed for stunning and killing by registration of EEG and ECGs, behavioral observations and, when a stunning method is not instantaneous, supplementary stress-physiological analysis. Owing to the complexity of these methods, they can best be performed in a laboratory setting. Product quality analysis, which may be only feasible in a laboratory setting, needs to be taken into account, as for example, electrical and percussive stunning can lead to carcass damage. This might have adverse economic consequences and these should be prevented or minimized. For the assessment of the welfare aspects of stunning and killing in a commercial setting, it is likely that behavourial measurements supplemented with physical measures (e.g., voltage, amperage, dosage, and percussion forces depending on equipment used) might suffice. To control the subsequent implementation of stunning and killing of farmed fish in practice, the authors foresee that development of a process-oriented assurance system is needed to safeguard and monitor fish welfare during stunning and killing.

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See also: Automation in the Meat Industry: Slaughter Line Operation. Species of Meat Animals: Finfish. Stunning: Electrical Stunning; Mechanical Stunning

Further Reading

Braithwaite, V., Huntingford, F., Van den Bos, R., 2013. Variation in emotion and cognition among fishes. Journal of Agricultural and Environmental Ethics 26, 7–23.

Broglio, C., Gómez, A., Durán, E., *et al.*, 2005. Hallmarks of a common forebrain vertebrate plan: Specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain Research Bulletin 66, 277–281.

Broglio, C., Rodríguez, F., Gómez, A., Arias, J.L., Salas, C., 2010. Selective involvement of the goldfish lateral pallium in spatial memory. Behaviorial Brain Research 210, 191–201.

Codex Committee on Food Hygiene, 2009. Recommended international code of practice – general principles of food hygiene. In: Food Hygiene Basic Texts, fourth ed., pp. 3–34. Rome: Food and Agriculture Organisation of the United Nations, World Health Organization and Food and Agriculture Organization of the United Nations.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Applied Animal Behaviour Science 86, 205–223.

Council Regulation (EC), 2009. On the protection of animals at the time of killing. No 1099/2009 Official Journal of the European Communities L303, 1–30.

Dalla Villa, P., Marahrens, M., Calvo, A.V., *et al.*, 2009. Final report on project to develop Animal Welfare Risk Assessment Guidelines on Transport-Project developed on the proposal CFP/EFSA/AHAW/2008/02, 143 pp. Available at: www.efsa.europa.eu/en/supporting/doc/21e.pdf (accessed 14.03.14).

Digre, H., 2011. Slaughter methods and processing of farmed Atlantic cod (*Gadus morhua*) – Welfare aspects and quality. Doctoral Thesis at NTNU 2011:79. ISBN 978-82-471-2687-5.

Durán, E., Ocaña, F.M., Broglio, C., Rodríguez, F., Salas, C., 2010. Lateral but not medial pallium ablation impairs the use of goldfish spatial allocentric strategies in a 'holeboard' task. Behaviorial Brain Research 214, 480–487.

EFSA, 2008a. Scientific report of EFSA on Animal welfare aspects of husbandry systems for farmed Atlantic salmon (Question No EFSA-Q-2006-033). EFSA Journal 736, 1–122. Annex I.

EFSA, 2008b. Scientific report of EFSA on Animal welfare aspects of husbandry systems for farmed European sea bass and Gilthead sea bream. (Question No EFSA-Q-2006-149). EFSA Journal 844, 1–89. Annex I.
EFSA, 2008c. Scientific report of EFSA on Animal welfare aspects of husbandry systems for farmed trout. (Question No EFSA-Q-2006-147). EFSA Journal 796, 1–97. Annex I.
EFSA, 2008d. Scientific report of EFSA on Animal welfare aspects of husbandry systems for farmed common carp. (Question No EFSA-Q-2006-148). EFSA Journal 843, 1–81. Annex I.
EFSA, 2009. Scientific Opinion of the Panel on Animal Health and Welfare on a request from European Commission on general approach to fish welfare and to the concept of sentience in fish. EFSA Journal 954, 1–26.
Erikson, U., Hultmann, L., Steen, J.E., 2006. Live chilling of Atlantic salmon (*Salmo salar*) combined with mild carbon dioxide anaesthesia I. Establishing a method for large-scale processing of farmed fish. Aquaculture 252, 183–198.
Erikson, U., Lambooij, B., Digre, H., *et al.*, 2012. Conditions for instant electrical stunning of farmed Atlantic cod after de-watering, maintenance of unconsciousness, effects of stress, and fillet quality – A comparison with AQUI-S™. Aquaculture 324–325, 135–144.
FAO, 2008. Glossary of Aquaculture Rome: Fisheries and Aquaculture Department, 424 pp. ISBN 978-92-5-005917-4.
FAO, 2012. The State of Word Fisheries and Aquaculture 2012, 230 pp. Rome: FAO Fisheries and Aquaculture Department. ISBN 978-92-5-107225-7.
Galhardo, L., Oliveira, R.F., 2009. Psychological stress and welfare in fish. Annual Review of Biomedical Sciences 11, 1–20.
Huntingford, F.A., Adams, C., Braithwaite, V.A., *et al.*, 2006. Current issues in fish welfare. Journal of Fish Biology 68, 332–372.
Ito, H., Yamamoto, N., 2009. Non-laminar cerebral cortex in teleost fishes? Biology Letters 5, 117–121.
Iversen, M.H., Økland, F., Thorstad, E.B., Finstad, B., 2013. The efficacy of Aqui-S vet. (iso-eugenol) and metomidate as anaesthetics in European eel (*Anguilla anguilla* L.), and their effects on animal welfare and primary and secondary stress responses. Aquaculture Research 44, 1307–1316.
Kestin, S.C., van de Vis, J.W., Robb, D.H.F., 2002. A simple protocol for assessing brain function in fish and the effectiveness of stunning and killing methods used on fish. Vet Record 150, 302–307.
Kooi, K.A., Tucker, R.P., Marshal, R.R., 1978. Fundamentals of Electro Encephalography, second ed. New York, NY: Harper and Row, pp. 125–145.
Lambooij, B., Digre, H., Erikson, U., *et al.*, 2013. Evaluation of electrical stunning of Atlantic cod (*Gadus morhua*) and turbot (*Psetta maxima*) in seawater. Journal of Aquatic Food Product Technology 22, 371–379.
Lambooij, E., Digre, H., Reimert, H.G.M., *et al.*, 2012. Effects of on-board storage and electrical stunning of wild cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) on brain and heart activity. Fisheries Research 127–128, 1–8.
Lambooij, E., Gerritzen, M.A., Reimert, H., *et al.*, 2008. A humane protocol for electro-stunning and killing of Nile tilapia in fresh water. Aquaculture 275, 88–95.
Lambooij, E., Grimsbø, E., van de Vis, J.W., *et al.*, 2010. Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities. Aquaculture 300, 107–112.
Lambooij, E., Van de Vis, J.W., Kloosterboer, R.J., Pieterse, C., 2002. Welfare aspects of live chilling and freezing of farmed eel (*Anguilla anguilla*, L.): Neural and behavioural assessment. Aquaculture 210, 159–169.
Lines, J.A., Robb, D.H., Kestin, S.C., Crook, S.C., Benson, T., 2003. Electric stunning: a humane slaughter method for trout. Aquacultural Engineering 28, 141–154.
Lines, J.A., Spence, J., 2012. Safeguarding the welfare of farmed fish at harvest. Fish Physiology and Biochemistry 38, 153–162.
Lopes da Silva, H.F., 1983. The assessment of consciousness: general principles and practical aspects. In: Eikelenboom, G. (Ed.), Stunning of Animals for Slaughter. The Hague: Martinus Nijhoff, pp. 3–12.
Morzel, M., Sohier, S., van de Vis, J.W., 2002. Evaluation of slaughtering methods of turbots with respect to animal protection and flesh quality. Journal of the Science of Food and Agriculture 82, 19–28.
Müller-Graf, C., Berthe, F., Grudnik, T., Peeler, E., Afonso, A., 2012. Risk assessment in fish welfare, applications and limitations. Fish Physiology and Biochemistry 38, 231–241.
Nieuwenhuys, R., Ten Donkelaar, H.J., Nicholson, C., 1998. The Central Nervous System of Vertebrates, vol. 2, 1524 pp. Heidelberg: Springer-Verlag.
Robb, D.F.H., Kestin, S.C., 2002. Methods used to kill fish: field observations and literature reviewed. Animal Welfare 11, 269–282.
Roth, B., Birkeland, S., Oyarzun, F., 2009. Stunning, pre slaughter and filleting conditions of Atlantic salmon and subsequent effect on flesh quality on fresh and smoked fillets. Aquaculture 289, 350–356.
Roth, B., Slinde, E., Imsland, A., Moeller, D., 2003. Effect of electric field strength and current duration on stunning and injuries in market-sized Atlantic salmon held in seawater. North American Journal of Aquaculture 65, 8–13.
Roques, J., Abbink, W., Geurds, W., Van de Vis, H., Flik, G., 2010. Tailfin clipping, a painful procedure: Studies on Nile tilapia and common carp. Physiology and Behavior 101, 533–540.
Roques, J.A.C., Abbink, W., Chereau, G., *et al.*, 2012. Physiological and behavioral responses to an electrical stimulus in Mozambique tilapia (*Oreochromis mossambicus*). Fish Physiology and Biochemistry 38, 1019–1028.
RSPCA, 2010. RSPCA welfare standards for farmed salmon. RSPCA, Horsham, UK. Available at: http://content.www.rspca.org.uk/cmsprd/Satelliteblobcol=urldata&blobheader=application%2Fpdf&blobkey=id&blobnocache=false&blobtable=MungoBlobs&blobwhere=1232991903901&ssbinary=true (accessed 25.09.13).
Salas, C., Broglio, C., Durán, E., *et al.*, 2006. Neuropsychology of learning and memory in teleost fish. Zebrafish 3, 157–171.
Terlouw, C., Auperin, C.A.B., Berri, C., *et al.*, 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. Animal 2, 1501–1517.
Van de Vis, H., Kestin, S.C., Robb, D., *et al.*, 2003. Is humane slaughter of fish possible for industry? Aquaculture Research 34, 211–220.
Van de Vis, H., Kiessling, A., Flik, G., Mackenzie, S. (Eds.), 2012a. Welfare of Farmed Fish in Present and Future Production Systems. Heidelberg, Germany: Springer.
Van de Vis, J.W., Poelman, M., Lambooij, E., Bégout, M.-L., Pilarczyk, M., 2012b. Fish welfare assurance system: Initial steps to set up an effective tool to safeguard and monitor farmed fish welfare at a company level. Fish Physiology and Biochemistry 38, 243–257.
Van den Burg, E.H., Peeters, R.R., Verhoye, M., *et al.*, 2005. Brain responses to ambient temperature fluctuations in fish: reduction of blood volume and initiation of a whole-body stress response. Journal of Neurophysiology 93, 2849–2855.
Vargas, J.P., López, J.C., Portavella, M., 2009. What are the functions of fish brain pallium? Brain Research Bulletin 79, 436–440.
Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiological Reviews 77, 591–625.

Relevant Websites

http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/htmfile=chapitre_1.7.4.htm
Killing of farmed fish for disease control purposes. The World Organisation for Animal Welfare (OIE).

http://www.oie.int/index.phpid=171&L=0&htmfile=chapitre_1.7.3.htm
Welfare aspects of stunning and killing of farmed fish for human consumption. The World Organisation for Animal Welfare (OIE).

http://www.oie.int/index.phpid=171&L=0&htmfile=chapitre_1.7.2.htm
Welfare of farmed fish during transport. The World Organisation for Animal Welfare (OIE).

SUSTAINABLE MUSCLE FOODS INDUSTRY

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Glossary

Distiller's Grains with Solubles (DGS) These are cereal by-products of the distillation process used for biofuel production.

Feed Conversion Rate (FCR) It is a measure of an animal's efficiency in converting feed mass into increased body mass.

Life cycle analysis (LCA) It is a technique to assess environmental impacts associated with all the stages of a product's life from conception to consumption.

Polylactic acid (PLA) Biobased material from corn or sugarcane by-products that can be used in biorenewable packaging.

Sustainability Defined as the effective use of agricultural crops with reduced use of energy and water, and a minimization of losses within the food chain and is still profitable.

Background

Food production in general puts a lot of pressure on the environment. A growing global population to more than 9 billion people by 2050 will require approximately twice as much food, whereas high-quality proteins will become the limiting factor in a healthy diet according to the Food and Agriculture Organization (FAO). The demand for animal protein will continue to grow, especially in developing countries as they become more affluent. Production of meat requires substantial amounts of feed grains, which, in turn, uses vast amounts of arable land. Currently, 40% of the land on the earth is used for food production. On the one hand, it is important to produce enough food of high quality, but on the other hand, food production has not been optimized in the past with respect to sustainability issues. For every 1 kg of high-quality animal protein produced, livestock are fed between 3 and 10 kg of feed grain.

Sustainability can be defined in many different ways, but in this article, it is interpreted as making more effective use of crops with reduced use of energy, reduced use of water, and minimization of losses through better chain management and logistics and optimal valorization of by-products and residues within the whole of the food chain. The standard methodology that is used to assess sustainability of any product or process is life cycle analysis (LCA). The total impact is often expressed in terms of the 'carbon footprint,' which gives an overall indication of the environmental impact of the product and its use. Although LCA is useful for the analysis of complete chains, it does not give insight into where inefficiencies are located and how these can be reduced. Profitability must be a component of 'sustainability' because no enterprise can continue unless it is profitable.

LCA studies on meat production seldom extend beyond the agricultural production stage. Food production, preservation, and distribution consume a considerable amount of energy, which contributes to the total CO_2 emission. LCA studies that cover more of the life cycle indicate that the agricultural production part is the main source for greenhouse gas emission in the life cycle of meat products. A recent study from the National Pork Board in the USA revealed that crop production, manure management, and retail distribution and consumption had the most impact on CO_2 production in the pork production chain. Pig production from nursery to finishing accounted for 60% of emissions, whereas retail and consumer parts of the chain accounted for less than 10% each. This paper will focus on the entire production chain for meat and the opportunities that are available to optimize the efficiency and sustainability of meat production chains.

Livestock Production

It is estimated that more than 60% of the arable land is used for the production of animal feeds. One of the approaches to reduce the environmental impacts of food is to optimize both the livestock and systems in which meat is produced. Animals need feed to grow and Table 1 shows that different types of animals require different energy input levels to produce 1 kcal of protein. Lamb and beef are the least efficient forms of animal protein production, but one has to keep in mind that these can also be grown on land that is less suitable for other types of agricultural production. Furthermore, both species can also be a by-product of wool or dairy production. The biological differences will remain as pigs will never be able to eat grass and the higher yields of broilers are due to their relative small size in comparison with pigs and beef cattle. In organic agricultural systems, livestock have more freedom of activity resulting in a higher feed-to-gain ratio.

Other important traits in livestock production that can be optimized are growth rate and feed conversion of animals. The animal breeding industry has been focusing on increasing lean

Table 1 Ratio of fossil energy input required to produce 1 kcal of animal protein

Livestock and animal products	*Ratio of energy input to protein output (kcal)*
Lamb	57:1
Beef cattle	40:1
Eggs	39:1
Swine	14:1
Dairy (milk)	14:1
Turkey	10:1
Broilers	4:1

Source: Reproduced from Pimentel, D., Pimentel, M., 2003. Sustainability of meat-based and plant-based diets and the environment. American Journal of Clinical Nutrition 78 (Suppl.), 660S–663S.

growth rate and improving feed conversion rates (FCR) in the past decades. This has reduced the total feed intake and age at slaughter in most species. Improvements in broiler genetics, nutrition, and other management changes in the period of 1957–2001 have resulted in 2001 broilers that reached processing time in one-third of the time required for a 1957 bird with more than threefold decrease in the amount of feed consumed. Similar improvements in productivity over the past 40 to 50 years can be shown for pigs. Genetic improvement has led to a reduction in greenhouse gas emission for broilers and pigs and global warming potential per kg of animal product in the past 20 years of up to 30%.

Optimization of feed composition is a major tool toward more sustainable meat production systems. Livestock feed is produced out of different feed ingredients. These feed ingredients can be whole crops (e.g., grains, rapeseed, peas, and soybeans) or by-products (e.g., pulp and milling products). Animals are very well able to digest low-quality vegetable by-product sources to produce high-quality meat proteins. Future research should focus on increased utilization of these types of lower quality by-products. Examples of alternative proteins for animal feed would not only be insect or algae proteins but also safe reintroduction of animal by-products. Concerns about food safety and the BSE crisis have understandably inhibited previous procedures of recycling animal and food waste, such as supplementing animal feed with rendered animal material. Beyond changes in behavior of the food industry, retailers, and the general public to reduce food wastage, imaginative yet safe systems are required to recycle biological material discarded throughout the meat production chain. Traditional protein sources for animal feed as soybean and rapeseed in the future will be replaced by distiller's grains with solubles (DGS). DGS are by-products of the production of bioethanol. Different developments, like the responsible soy initiative, aim at global solutions to improve the sustainability of agricultural food production.

Processing Optimization

The meat industry is continuously optimizing carcass and meat product processing conditions by using operational excellence projects to reduce costs of production and make better use of all the by-product streams. Water is primarily used to ensure food safety and hygiene during operation. Overall water consumption has been reduced by recycling and reuse under stringent food safety restrictions. Refrigeration and production of hot water are the major energy-consuming activities in meat processing with lesser amounts used for lighting, motors, and the like. The Australian red meat industry recently published an industry environmental sustainability review in which they monitored the changes in water and energy usage between 2003 and 2008–09. Overall, there was a reduction in raw water usage by 11% and a reduction in waste water generation by 13% compared with 2003. Energy usage levels had increased by approximately 18% since 2003 because additional energy sources were included compared with 2003 and some of the companies had started value-added processes, which are more energy intensive. Similar results were found by Ramirez *et al*. for a 10-year period in which they analyzed energy use and energy efficiency developments for the meat industry in four different European countries. They concluded that energy consumption increased between 14% and 32%, partly due to a shift from beef to broiler and pork processing and due to an increased demand for value-added meat products. However, strong hygiene regulations could explain between one- and two-thirds of the increase, whereas the role of increasing shares of frozen and cut fresh meat was found not to be of significance.

Energy-related issues will become even more important in food processing plants as increasing sales of ready-to-eat meals and a greater demand for different and flexible range of products by consumers will lead to a larger energy demand. These changes in consumer behavior, together with raised energy prices, hardened price competition, and potential policy instruments such as CO_2 taxation will stimulate the meat industry to continue their focus on reduction in water and energy usage. Reduction in water and energy usage currently is an integrated part of the corporate social responsibility agenda of all global meat companies. In a case study, it was shown that, even in a modern meat plant where many energy-saving measures have taken place, there is still a technical potential for saving 30% of the external heat demand and more than 10% of the mechanical shaft work used in the plant.

It should be kept in mind, depending on the species, that between 40% and 60% of the processed animal is directly suitable for human consumption, whereas the rest are useful by-products. Head, bones, skin, heart, lungs, intestines, and blood are removed in the slaughter process. None of these by-products are wasted and are used for a wide variety of food, feed, and nonfood applications like gelatin, different protein hydrolysates, fats, bone powder, etc. A comprehensive overview of all the products that can be made from pigs include medicine, heart valves, brakes, chewing gum, porcelain, soap, toothpaste, cosmetics, conditioner, and biofuel.

New Product Development

Meat fulfils some key important nutritional requirements and is part of a healthy diet. Both a meat-based and a lacto-ovo vegetarian diet require significant quantities of

nonrenewable fossil energy to produce and are not sustainable in the long term according to Pimentel and Pimental. However, approximately 1.3 billion people are employed, either directly or indirectly, in livestock production and processing and provide economic livelihood for societies in addition to the dietary contributions they provide. However, the meat-based diet requires more energy, land, and water resources than the lacto-ovo vegetarian diet. Except for Japan (with its high fish intake), meat is now the single largest source of animal protein in all affluent nations, and it remains among the most desirable, high-status foodstuffs in all countries. In some of the developed countries, there have been recommendations to reduce the average meat consumption without negatively affecting the healthy diet requirements. Consumers do not easily reduce or replace meat for more sustainable alternatives. Nonvegetarian households are reluctant to decrease meat consumption just for environmental reasons, specifically reduced carbon footprint. Furthermore, current meat substitutes are not comparable in sensory perception to real meat products, and there is a low level of repetitive purchase by nonvegetarian consumers. Meat was judged more positively overall, which explains the choice for meat. Substantial voluntary reductions of meat consumption are not very likely. Because a large part of the meat production is sold as minced or further processed products, it should be possible to incorporate varying shares of plant-derived proteins and fibers into meat products and thus increase their sustainability. Development of hybrid products with a similar sensory profile would be an option to reduce current meat consumption levels. A commercial example of such a hybrid product development can be found where minced meat has been mixed with 30% plant protein and fibers and reducing the fat content of the product as well.

Another area of product development for the meat industry will be packaging. Even though packaging material represents a relatively small part of the carbon footprint from the meat production chain, it is perceived by consumers as having a negative impact on sustainability and being environmentally unfriendly. An increasing part of total meat production is sold as case-ready products using modified atmosphere conditions to increase shelf life of the products. The need for convenience and ease of preparation will increase the demand for different types of meat-packaging options. The purpose of food packaging is to preserve the quality and safety of the food it contains from time of production to time of consumption. The majority of the current meat-packaging materials are based on petroleum derivates. As petroleum costs are increasing, renewable packaging for meat, such as materials based on Polylactic acid (PLA) from corn or sugar cane by-products, will become more feasible in the future. Different bio-based materials have become available made from a variety of renewable and sustainable agricultural commodities that can be applied for meat products and that have been tested for retention of product quality characteristics, handling properties, and microbiological stability of muscle foods. With consumer demanding more environmentally friendly packaging, new and novel food grade packaging materials or technologies have been and continue to be developed.

Conclusions

The challenge for the next 50 years in meat production is to increase the productivity of major livestock species in order to address the food needs of the world, but at the same time minimizing the environmental impact. A number of technologies and techniques are available to continuously improve feed conversion, reproduction, and overall production efficiency in beef and dairy cattle, pigs, and poultry. New processing technologies will reduce the water and energy usage in animal processing and further processed meat production. Although a voluntary reduction in meat consumption is not very likely, it is possible to develop new minced-based meat products that are enhanced with plant and/or by-product proteins and that taste and present nutritional value that is as good as found in the original products.

Further Reading

Cutter, C.N., 2006. Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. Meat Science 74, 131–142.

Eilert, S., 2005. New packaging technologies for the 21st century. Meat Science 71, 122–127.

Elferink, E.V., Nonhebel, S., 2007. Variations in land requirements for meat production. Journal of Cleaner Production 15, 1778–1786.

FAO, 2009. Declaration of the World Summit on Food Security, 16–18 November 2009. Rome, Italy: Food and Agricultural Organization of the United Nations.

Foster, C., Green, K., Bleda, M., Dewik, P., 2006. Environmental impacts of food production and consumption. A Final Report to the Department of Environment, Food and Rural Affairs. London: Manchester Business School, Defra.

Fritzson, A., Berntsson, T., 2006. Efficient energy use in a slaughter and meat processing plant – Opportunities for process integration. Journal of Food Engineering 76, 594–604.

Gilland, B., 2002. World population and food supply. Can food production keep pace with population growth in the next half century? Food Policy 27, 47–63.

Havenstein, G.B., Ferkel, P.R., Qureshi, M.A., 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed diets representative 1957 and 2001 broiler diets. Poultry Science 82, 1500–1508.

Hoek, A.C., 2010. Will novel protein foods beat meat? Consumer acceptance of meat substitutes – A multidisciplinary research approach The Netherlands: Thesis Wageningen University. ISBN 978-90-8585-536-1.

Hume, D.A., Whitelaw, B.A., Archibald, A.L., 2011. The future of animal production: Improving productivity and sustainability. Journal of Agricultural Science 1–8.

Meindertsma, C., 2008. Pig 05049, second ed. Flocks Uitgeverij. ISBN 978-90-812413-1-1.

M.L.A., Meat & Livestock Australia, 2010. Industry environmental sustainability review 2010. Published June 2011. Sydney, NSW: Meat and Livestock Australia. ISBN 978174915693.

Nonhebel, S., 2005. Renewable energy and food supply: Will there be enough land? Renewable and Sustainable Energy Reviews 9 (2), 191–201.

Nonhebel, S., Moll, H.C., 2001. Evaluation of options for reduction of greenhouse gas emissions by changes in household consumption patterns. IVEM Research Report (OS) 106. The Netherlands: University of Groningen.

Pimentel, D., Pimentel, M., 2003. Sustainability of meat-based and plant-based diets and the environment. American Journal of Clinical Nutrition 78 (Suppl.), 660S–663S.

Ramirez, C.A., Patel, M., Blok, K., 2006. How much energy to process one pound of meat? A comparison of energy use and specific energy consumption in the meat industry of four European countries. Energy 31, 2047–2063.

Roy, P., Nei, D., Orikasa, T., *et al.*, 2009. A review of life cycle assessment (LCA) on some food products. Journal of Food Engineering 90, 1–10.

Smil, V., 2002. Worldwide transformation of diets, burdens of meat production and opportunities for novel proteins. Enzyme and Microbial Technology 30, 305–311.

Van Der Steen, H.A.M., Prall, G.F.W., Plastow, G.S., 2005. Application of genomics to the pork industry. Journal of Animal Science 83 (E Suppl.), E1–E8.

Relevant Websites

ftp://ftp.fao.org/docrep/fao/Meeting/018/k6050e.pdf
Food and Agricultural Organization of the United Nations.

www.pork.org/sustainability
Pork Checkoff from the National Pork Board in Des Moines USA.

www.responsiblesoy.org
Round Table on Responsible Soy Association.

www.smithfieldcommitments.com/csr-reports
Smithfield.

www.hackplus.de
VION Food Group.

www.vionfoodgroup.com/flipbook/annual-report-2010
VION Food Group.

TENDERIZING MECHANISMS

Contents

Chemical

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Glossary

Ageing It is the process of meat tenderization that occurs over time – it commences after rigor mortis.
Contractile proteins It includes actin and myosin, which form the thin and thick filaments of skeletal muscle. These two proteins interact chemically (to form actomyosin), which gives muscle the ability to contract and relax. Associated with actin are the proteins troponin and tropomyosin.
Costameres It connects Z-disks to the sarcolemma and are made up of proteins such as talin, vinculin, desmin, and dystrophin.
Cytoskeletal proteins These are a set of filamentous structural proteins (includes actin, titin, nebulin, and desmin).
Electrical stimulation Application of an electric current through a carcass postmortem that accelerates the rigor process.
Myofibril It is comprised of contractile, structural, and regulatory proteins. The contractile protein is composed of myofilaments, which are in turn made up of thin and thick filaments. Structural proteins include titin and nebulin. Titin is the largest protein in skeletal muscle (approximately 3700 kDa) and it provides elasticity to the sarcomere. The regulatory proteins include troponin and tropomyosin.
Proteolysis It is the degradation of proteins into smaller subunits that occurs with ageing and also during turnover of living muscle.
Rigor It is a term for individual muscle fibres that have been depleted of adenosine triphosphate and the actomyosin bond has formed.
Rigor mortis It is a term used when muscles stiffen after all muscle fibres enter rigor.
Sarcomere The basic unit of skeletal muscle defined by the distance between two Z-disks. Z-disks are dense protein structures into which the contractile protein actin is attached along with proteins such as titin and nebulin. Z-disks are the anchor points for the contractile proteins that allow contraction and relaxation.
Shear force It is the force (N) applied to a standardized piece of cooked meat to shear it.
Shortening It is a process that occurs when prerigor muscle is cooled below 10 °C when the pH is still above 6.0. Additionally it also occurs as muscles enter rigor at high temperatures (rigor shortening).
Tenderization It is the enzymatic process that takes place after rigor mortis that makes meat tender.
Ultimate pH It is the pH attained when muscles reach rigor mortis.

Introduction

The process that leads to an improvement in tenderness can be termed 'tenderization' and this is driven by proteolysis of myofibrillar, cytoskeletal, and costameric proteins. Proteolysis of some of the myofibrillar proteins can precede tenderization (i.e. before rigor mortis) and the degree of disruption of these proteins (such as titin and nebulin) will influence the final stage of meat tenderness. Other proteins, like actin and myosin, are not generally degraded under normal storage temperatures. The introduction of exogenous solutions containing ions such as Ca^{2+} or Na^{+} has been shown to impact the tenderization of meat. The action of Ca^{2+} is strongly linked to the activation of the calpains as opposed to altering protein structure *per se*, whereas Na^{+} based solutions can lead to solublisation of proteins and thus improve tenderness. Connective tissue provides support in muscle at a number of levels via the endomysium, perimysium, or epimysium and maintains the integrity of the contractile apparatus. This connective tissue is not significantly degraded during 'normal' tenderization. However chemical mechanisms have been implicated in the solubility of connective tissue, particularly based on the use of organic acids. Commercial application of systems to introduce chemical-based solutions to meat have been restricted to vascular infusion, but the efficacy of this approach for improving tenderness is debatable.

Rigor Bonding

After death, muscle filaments are in a continual state of contraction and relaxation. As adenosine triphosphate (ATP) is depleted, the filaments enter rigor and a contracted state, but this process does not occur uniformly throughout a muscle fiber. As a consequence of the interaction between the contractile proteins actin and myosin, and other associated filament proteins, the overlap between thick and thin filaments increases, leading to an increase in toughness and a decrease in the width of the A band within the sarcomere. The degree of overlap will be influenced by temperature, with extreme contraction (increased overlap) occurring under low temperatures, known as 'cold shortening.'

Based on theoretical studies of muscle biochemistry, several workers have proposed that the binding 'state' of actin and myosin can be manipulated as fibres enter rigor. These binding states are proposed to arise through a change in contact between myosin heads (S1) and specific regions of actin monomers reflected by the sequences of amino acids in different regions. The relative proportion of different states of actin–myosin interactions is proposed to vary in relative proportions as muscle enters rigor. The hypothesis is that this would affect subsequent toughness, and so changes in conformational states of actomyosin at rigor could explain the toughening of muscle at this stage.

Several studies have linked changes in the ease with which actomyosin can be dissociated (as an indicator of binding strength) and tenderization, but these studies did not provide definite evidence to support the hypothesis. Indeed, when the potential confounding effects of proteolysis (due to endogenous enzymes) have been taken into account, the hypothesis is not supported.

Infusion/injection of meat with ionic compounds in solution termed 'meat enhancement' can manipulate several biochemical processes, depending on the postmortem time of injection. For example, prerigor infusion can have a dramatic effect on the rate of glycolysis, the rate and state of contraction, the oxidative processes and rate of the proteolysis whereas postrigor injection will affect mainly proteolysis and oxidative processes. Early prerigor enhancement can result in dramatic toughening. The impact of the infused compounds on meat quality will be greatly dependent on the postmortem time of the treatment (reflecting the pH and the temperature of the meat), the concentration of the infused compounds (level of activation or modification) and the method of infusion (the distribution of the compounds in the meat). Each combination of the above factors can lead to unique outcomes for different species and within these combinations a set of factors can be optimized for the best outcome.

Injection of Metal Ions and Ionic Strength

Metal ions such as Ca^{2+} and Mg^{2+} have many functions in regulating muscle contraction and enzyme activity, and for this reason extensive research has been conducted to examine the effect on tenderization when such ions are injected into muscle. There is a significant rise in ionic strength as muscle enters rigor, without any exogenous introduction of ions, and this has been proposed to contribute to tenderization. The rise in ionic strength mirrors the decline in pH (Figure 1) and is attributed to an alteration of protein structure and the release of bound ions and metabolites. A rise in osmolality has been shown to increase the solubility of proteins, which could make them more liable to enzymatic degradation and thus be part of a synergistic mechanism. The extent of the increase in ionic strength is muscle specific, with higher osmolalities in fast-twitch glycolytic muscles.

The introduction of metal ions into muscle has been studied extensively as a means of understanding the potential synergy between a rise in ionic strength and enzyme activity. Prerigor injection of high ionic solutions creates an atypical

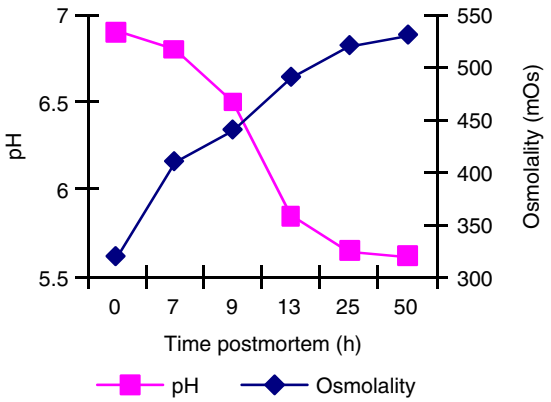

Figure 1 Postmortem decrease in muscle pH (—■—) and increase in osmolality (—◆—) in bovine Musculus longissimus. Adapted from Ouali, A., Vigon, X., Bonnet, M., 1991. Osmotic pressure changes in postmortem bovine muscles: Factors of variation and possible causative agents. Proceedings 37th International Congress of Meat Science and Technology, Kulmbach, pp. 452–455.

environment within the muscle, and this may by itself result in premature membrane rupturing and the release of endogenous enzymes. Isolating the mode of action of ion injection on tenderization has been a major challenge since ions such as Ca^{2+} can cause shortening when injected prerigor as well as activate endogenous enzymes. One approach that has been used to speed up the onset of rigor is electrical stimulation, so that earlier postmortem injection of ions could occur. Infusion with 0.3 mol l^{-1} $CaCl_2$ has been shown to accelerate tenderization and lower shear force values compared with control samples and the reduction in shear force has been attributed to an increased level of activity of μ-calpain and also m-calpain at the highest concentration of $CaCl_2$. The requirement for such high levels of $CaCl_2$ may reflect the barriers that exist to the translocation of ions into the cell; a high level could ensure that some of the ions reach the target enzymes, by effectively swamping the cells. Injection of hot-boned muscle (prerigor) with 0.3 mol l^{-1} $CaCl_2$ does elicit significant shortening, but the muscle is still significantly more tender than control muscle, or muscle injected with 0.15 mol l^{-1} NaCl. In this case the effective Ca^{2+} concentration would be much higher than would occur intrinsically because it has been clearly shown that maximum Ca^{2+} concentration is not reached until the postrigor period. A summary of the reported effects of Ca^{2+} on tenderness is given in Table 1. Injection of sodium chloride (NaCl), combined with phosphates, will enhance the ability of muscle proteins to bind with water and could reflect increased solubility of these proteins and this is the principle applied for the brine treatment of pork. Sodium chloride and polyphosphates accelerate the degradation rates of titin and troponin-T as well as the appearance of 95 and 30 kDa degradation products, which leads to higher tenderization rates and these effects have been attributed to an increased pH due to the high buffering capacity of polyphosphates.

In some instances, sodium pyrophosphate has been injected into muscle in combination with NaCl. Although this has been observed to cause increased calpain activity leading to a reduction in shear force, the ability of pyrophosphate to compete with ATP binding sites on myosin, and thus reduce the extent of muscle contraction, is also a possible explanation for the reduction in toughness. When myofibrils that have been treated to remove endogenous proteolytic enzymes (i.e. calpains), are incubated with 0.3 mol l^{-1} $CaCl_2$, some key myofibrillar proteins show no degradation with time, indicating that protein solubility alone cannot explain tenderization. Equally, this is consistent with activation of the calpains as the major contender for the mechanism that drives tenderization. A major drawback of injection with $CaCl_2$ is that the meat tends to be regarded as having a bitter flavour and to be less acceptable to consumers, due to the fact that Ca^{2+} can stimulate lipoxygenase activity and the mitochondrial respiration process leading to higher rate of oxidative processes. For this reason, commercial adoption of $CaCl_2$ infusion into meat has been limited. The extent of the synergy between enzyme activity and changes in ionic strength is yet to be fully clarified.

Injection of Acids and Other Compounds

A number of organic acids such as acetic, citric, and lactic have been used to tenderize meat. Administration is often by marination, but this requires extended storage periods for penetration into the meat. A number of studies have investigated the efficacy of these acids after injection into meat. These acids cause a significant decrease in pH, creating an environment that is optimal for cathepsin activity (these are lysosomal proteases), which is confirmed by the degradation of proteins such as myosin, a protein that is not degraded under normal (control) postmortem conditions. A drop in pH leads to swelling of the meat. Another effect of this treatment is the weakening/solubilization of perimysial collagen and lowering of the temperatures required for denaturation of connective tissue. On the whole, organic acids lead to an increase in meat tenderness (Table 2). Since the effect of acid injection is largely dependent on the buffering capacity and overall pH reduction in meat, the type of acid used is very important to achieve desired quality attributes. Lactic acid lowers the pH compared with citric and acetic acids, and illicits more dramatic changes in the meat. Combinations of ions and organic acids have also been used to enhance tenderization.

Vascular Infusion

Another approach that has been developed commercially is the vascular infusion of a chemical mix into carcasses via the arteries immediately after death at a rate of 10% of live weight. The chemicals include sugars, phosphates, and sometimes salts. A reduction in toughness has been reported in a couple of studies using this methodology (Figure 2), although other studies have reported no difference in either objective tenderness (shear force) or consumer assessments of tenderness. One of the reports of increased tenderization was associated with increased fragmentation of myofibrils and increased degradation of myofibrillar proteins such as troponin. Unfortunately, the methodology does cause paler meat colour, with higher weep, and thus tends to be less acceptable to retailers, wholesalers and consumers. The main advantage is the increase in carcass weight resulting in increased returns per kilogram of carcass weight. The mode of action that occurs through use of the chemicals has not been confirmed, but could include disruption of muscle cells and increased proteolysis or changes in osmolality. The rate of glycolysis increases with infusion and this may in turn accelerate the activity of the calpains. More recently, this same approach has been investigated, but with the infusion of an ice-slurry throughout the carcase, which has been shown to reduce shear force, but the mechanism was not quantified. Infusion of plant extracted enzymes has also been effective at reducing shear force.

Related to this concept is the prerigor injection or infusion of carcases to manipulate the rate of glycolysis and thus pH fall. It has been reported that prerigor injection of either sodium fluoride or sodium citrate can increase the level of muscle glycogen and thus reduce the absolute decline in pH. Given the link between pH and the activity of the calpains, this could presumably enhance the rate of tenderization. These compounds have resulted in a higher ultimate pH, a reduction in sarcomere length, and in case of sodium citrate a reduction in shear force (Figure 3). For the immediate postmortem period when the citric acid cycle is still operative, citrate is produced, which has an inhibitory effect on

Table 1 Summary of effects of various meat calcium ($CaCl_2$) infusion tenderization treatments applied during the post mortem period

Dose level and concentration of added solution	*Muscle/Species*	*Ageing time (days)*	*Postmortem time of injection (h)*	*Ageing Temperature (°C)*	*% Shear force*[a]	*Sensory tenderness*[b]	*References*
0.075 mol l^{-1} (10% of animal liveweight)	LL (ovine)	1	0.5	1–2	–1.7	–	Koohmaraie *et al.* (1989)
		6			11.7		
0.15 mol l^{-1} (10%)		1			–28.1		
0.15 mol l^{-1} (10%)		6			–14.3		
0.3 mol l^{-1} (10%)		1			–60.0		
0.3 mol l^{-1} (10%)		6			–45.5		
0.3 mol l^{-1} (10%)		1			–50.3		
0.175 mol l^{-1} (10% of cut weight)	LL (bovine)	7	0.5	2	–21.9	10.2%	Wheeler *et al.* (1993)
0.175 mol l^{-1} (10% of cut weight)	SM				–2.9	–	
0.175 mol l^{-1} (10% of cut weight)	TB				–12.6	–	
Water	LL				2.9	–26.5%	
Water	SM				–7.9	–	
Water	TB				–	–	
0.175 mol l^{-1} (10%)	LL	6	24		–29.2	19.6%	
0.175 mol l^{-1} (10%)	SM				–25.6	–	
0.175 mol l^{-1} (10%)	TB				–15.0	–	
Water	LL				–22.4	9.8%	
Water	SM				–13.1	–	
Water	TB				–	–	
0.2 mol l^{-1} (5%)	LL	6	24	2	–21.7	9.8%	
0.2 mol l^{-1} (5%)	SM				–25.6	6.7%	
0.2 mol l^{-1} (10%)	LL				–23.7	11.8%	
0.2 mol l^{-1} (10%)	SM				–10.9	6.4%	
0.25 mol l^{-1} (5%)	LL	6	24		–31.4	13.7%	
0.25 mol l^{-1} (5%)	SM				–17.1	8.9%	
0.25 mol l^{-1} (10%)	TB				–15.6	3.7%	
0.25 mol l^{-1} (10%)	LL				–25.0	15.7%	
0.25 mol l^{-1} (10%)	SM				–8.5	–4.3%	
0.25 mol l^{-1} (10%)	TB				–3.4	1.8%	
0.3 mol l^{-1} (10% of cut weight)	SM (bovine)	0	1	2	–24.8	–	Boleman *et al.* (1995)
			12		–1.8		
			24		–8.5		
		10	1		–33.1		
			12		–20.3		
			24		–13.5		
0.1 mol l^{-1} (10% of cut weight)	LL (bovine)	2	1	4	–70.6[c]	16.9%	Rousset-Akrim *et al.* (1996)
		6			–50.0	4.4%	

		14			–43.9	–9.6%	
		2	24		–53.6	40.8%	
		6			–44.2	40.0%	
		14			–39.3	13.5%	
0.2 mol l^{-1} (5% of cutweight)	LL (bovine)	7	48	1	–23.6	17.3%	Wheeler *et al.* (1997)
		35			–23.3	12.1%	
		7	36		–19.4	12.7%	
		35			–4.3	13.1%	
0.3 mol l^{-1} (10% of animal liveweight)	LL (bovine) SM	14	0.1	2–4	65.7	–19.7%	Dikeman *et al.* (2003)
					–2.1	1.8%	

[a]Difference from control (negative means tender, positive means tougher).
[b]Tenderness rating (difference from control positive means more tender).
[c]Myofibrillar resistance by compression method.
Abbreviations: LL, *Longissimus lumborum*; SM, *Semimembranosus*; TB, *Triceps brachii*.

Table 2 Sensory assessment (ease of first bite and chewiness; 1 = extremely tough to 10 = extremely tender) for beef Musculus pectoralis profundus treated with 0.5 mol l^{-1} lactic acid at either 1 or 24 h postmortem and aged for 2 or 14 days

	Lactic acid (1 h)	*Lactic acid (24 h)*	*Control*	*Ageing period (days)*
Ease of first bite	6.7a	7.4a	4.3b	2
Chewiness	6.0a	6.3a	5.3a	2
Ease of first bite	6.0a	6.4a	4.2b	14
Chewiness	5.7a	6.1a	4.4b	14

Note: Means followed by a different letter (a, b) within a row are significantly different ($P < 0.05$).

Source: Adapted from Berge, P., Ertbjerg, P., Larsen, L.M., *et al.*, 2001. Tenderization of beef by lactic acid injected at different times post mortem. Meat Science 57, 347–357.

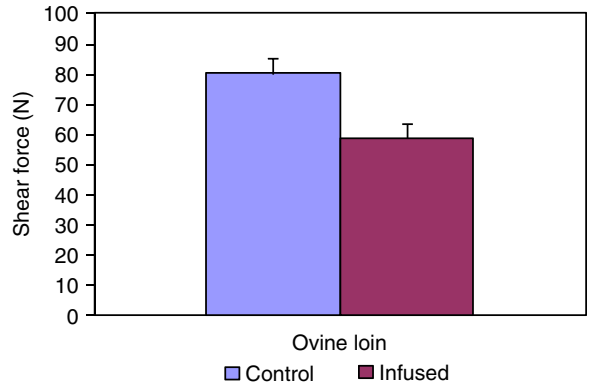

Figure 2 Effect on the shear force of ovine loin (24 h postmortem) after infusing carcasses with a solution of 0.1% maltose, 0.21% glycerine, 0.23% dextrose, and 0.14% blend of sodium and potassium tripolyphosphate in water. Adapted from Farouk, M.M., Price, J.F., Salih, A.M., 1992. Post-exsanguination infusing of ovine carcasses: Effect on tenderness indicators and muscle microstructure. Journal of Food Science 57, 1311–1315.

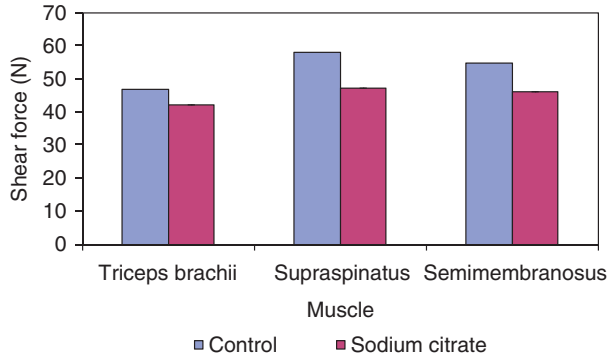

Figure 3 Effect on the shear force of bovine muscles (72 h post mortem) after prerigor injection with a solution of 200 mmol l^{-1} sodium citrate compared with noninjected muscle. A significant difference ($P < 0.05$) in shear force between treatments was found for the supraspinatus muscle. Adapted from Jerez, N.C., Calkins, C.R., Velazco, J., 2003. Prerigor injection using glycolytic inhibitors in low-quality beef muscles. Journal of Animal Science 81, 997–1003.

phosphofructokinase. Because this enzyme is an important regulatory enzyme in glycolysis, this may partially explain the apparent reduction in glycolysis reported by the injection of sodium citrate.

Similarly, injection of sodium bicarbonate has been found to slow the rate of pH decline in porcine muscle, decrease drip loss and darken meat, but again no mode of action has been proposed. It is probable that the response was mediated through a buffering effect whereby the free hydrogen ions produced as a result of glycolysis become complexed with the bicarbonate.

Selection of compounds that can inhibit critical steps in the glycolytic pathway will control the decline in pH. Another approach would be to increase the concentration of creatine phosphate to buffer ATP levels and thus prolong the postmortem time period before ATP is utilised and, therefore, prevent an immediate fall in pH. The inhibition of AMP-activated protein kinase (AMPK) would also presumably lead to a reduction in the rate of pH fall postmortem, as would regulation of the activity of glycogen debranching enzyme (GDE), by a reduction in glycogenolysis. A delay in activity of GDE until the carcase temperature dropped below 39 °C would result in a reduction in the rate of pH decline. Whichever mechanism was applied, the critical concentration of the inhibitor would have to be determined, for high concentrations would potentially lead to a reduction in glycolysis to the extent that the final pH was too high creating other quality issues.

Nonenzymatic Tenderization Meditated by Calcium Ions

There is a hypothesis that Ca^{2+} ions cause nonenzymatic weakening of the myofibrillar structure, and thus tenderization. It is suggested that the significant rise in the concentration of free calcium as rigor develops leads to fragmentation of proteins such as nebulin, desmin, and titin and the weakening of Z-disk proteins through the liberation of phospholipids. Several facts bring into question the validity of this theory: (1) when myofibrils, which have been treated to remove endogenous proteolytic enzymes (i.e., calpains), are incubated with 0.3 mol l^{-1} $CaCl_2$, some key myofibrillar proteins show no degradation with time; (2) troponin T is readily degraded in postmortem muscle, but there is no claim that it is directly affected by calcium; (3) when Triton X-100, which causes a release of Ca^{2+} ions, is combined with a calpain inhibitor, toughness is not reduced; (4) inhibition of specific proteases has been found to prevent tenderization in the presence of an increasing concentration of free calcium; and (5) there is clear evidence for protein degradation/deposition in living muscle as a result of calpain activity, so there is no reason to suggest activity to cease in postmortem muscle, particularly in the early period of rigor development.

It has been shown using autoradiography that Ca^{2+} ions bind to titin, and more specifically by using fluorescence detection they bind to the major sub fragment of this degraded protein. Since calpains bind to Ca^{2+} ions for activation and titin has been shown to be a good substrate for these enzymes, these results are not inconsistent with the proposed role of the calpains in tenderization. Also, this partially reconciles some of the results to emerge from the theory that Ca^{2+} ions cause nonenzymatic weakening of the myofibrillar structure.

See also: Carcass Composition, Muscle Structure, and Contraction. Conversion of Muscle to Meat: Aging. Tenderizing Mechanisms: Enzymatic; Mechanical

Further Reading

Brown, T., Richardson, I.R., Wilkin, C.-A., Evans, J.A., 2009. Vascular perfusion chilling of red meat carcasses – a feasibility study. Meat Science 83, 666–671.

Boleman, S.J., Boleman, S.L., Bidner, T.D., McMillin, K.W., Monlezun, C.J., 1995. Effects of postmortem time of calcium chloride injection on beef tenderness and drip, cooking and total loss. Meat Science 39, 35–41.

Dikeman, M.E., Hunt, M.C., Schoenbeck, J., *et al.*, 2003. Effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on meat quality and sensory traits of steaks and ground beef. Journal of Animal Science 81, 156–166.

Hopkins, D.L., Thompson, J.M., 2001. The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. Meat Science 57, 1–12.

Hopkins, D.L., Thompson, J.M., 2002. Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact of tenderisation in beef and sheep meat. Australian Journal of Agricultural Research 53, 149–166.

Kauffman, R.G., van Laack, R.L.J.M., Russell, R.L., *et al.*, 1998. Can pale, soft exudative pork be prevented by post-mortem sodium bicarbonate injection? Journal of Animal Science 76, 3010–3015.

Ke, S., Huang, Y., Decker, E.A., Hultin, H.O., 2009. Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. Meat Science 82, 113–118.

Koohmaraie, M., Crouse, J.D., Mersmann, H.J., 1989. Acceleration of post-mortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. Journal of Animal Science 67, 934–942.

Kylä-Puhju, M., Ruusunen, M., Puolanne, E., 2005. Activity of porcine muscle glycogen debranching enzyme in relation to pH and temperature. Meat Science 69, 143–149.

Lee, S., Stevenson-Barry, J.M., Kauffman, R.G., Kim, B.C., 2000. Effect of ion fluid injection on beef tenderness in association with calpain activity. Meat Science 56, 301–310.

Ouali, A., 1992. Proteolytic and physiochemical mechanisms involved in meat texture development. Biochimie 74, 251–265.

Rousset-Akrim, S., Got, F., Bayle, M.C., Culioli, J., 1996. Influence of $CaCl_2$ and NaCl injections on the texture and flavour of beef. International Journal of Food Science and Technology 31, 333–343.

Stephens, J.W., Dikeman, M.E., Unruh, J.A., Haub, M.D., Tokach, M.D., 2006. Effects of pre-rigor injection of sodium citrate or acetate, or post-rigor injection of phosphate plus salt on post-mortem glycolysis, pH, and pork quality attributes. Meat Science 74, 727–737.

Takahashi, K., 1996. Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: The non-enzymatic mechanism of meat tenderization. Meat Science 43, s67–s80.

Wheeler, T.L., Koohmaraie, M., Lansdell, J.L., Siragusa, G.R., Miller, M.F., 1993. Effects of post-mortem injection time, injection level, and concentration of calcium chloride on beef quality traits. Journal of Animal Science 71, 2965–2974.

Wheeler, T.L., Koohmaraie, M., Shackelford, S.D., 1997. Effect of post-mortem injection time and post injection aging time on the calcium-activated tenderization process in beef. Journal of Animal Science 75, 2652–2660.

Enzymatic

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Glossary

Calpains A group of enzymes, including μ-calpain and m-calpain, that when activated by calcium degrade the cytoskeletal proteins.
Calpastatins Endogenous inhibitor of μ- and m-calpains.
Cathepsins Cysteine proteases that degrade many different types of proteins.
Contractile proteins Muscle proteins, such as actin and myosin, that are directly involved in the contractile process and minimally involved in tenderization.
Cytoskeletal proteins A set of structural proteins (includes titin, nebulin, and desmin) are denatured by calpains during tenderization.
Endogenous proteases Proteases involved in muscle remodeling in life that take on the role of degrading cytoskeletal proteins postmortem.
Exogenous enzymes Protease enzymes usually of plant material that when applied to meat degrade myofibrillar and connective tissue proteins.

Introduction

The process of meat tenderization is a complex phenomenon. It is accomplished naturally by the presence of endogenous enzymes and it can be augmented by the application of exogenous enzymes, typically purified from plant sources. In postmortem muscle, natural tenderization begins at slaughter and continues to occur while the meat is held at refrigerated temperatures over the next 2–3 weeks. This natural tenderizing process is often referred to as 'aging' the product.

Tenderization via natural aging is done by the actions of enzymes in the muscle that function to regulate the growth and repair of living muscle, often by serving a role in the initiation of the removal of damaged proteins so that new proteins can be inserted into the appropriate muscle structure. The majority of these endogenous enzymes act on the structures of the myofibril, the main contractile organelle of the muscle cell. The tenderization that occurs via these endogenous enzymes starts at death and is essentially slowed or has ceased by 7 days postmortem in most species.

Tenderization via the application of exogenous enzymes is generally less specific than endogenous enzymes. These enzymes target not only myofibrillar proteins but also the connective tissue proteins of the muscle. Most plant enzymes have the capacity to tenderize the product to a greater degree than is possible with endogenous enzymes, leading to the need for the processor to carefully monitor the process to avoid 'over-tenderizing' the product and creating an overly soft, or even 'mushy' texture to the meat.

Both types of enzymes are important in the production of tender meat products. An appropriate understanding of the enzyme systems being relied on for tenderization is important to allow maximal tenderness development in a given product.

Enzymatic Tenderization

Role of Endogenous Proteases

During aging of meat, major structural changes occur in the muscle tissue. Many of these changes are associated with myofibrils, the contractile elements of muscle cells, and their linkages to the cell membrane (sarcolemma) through the cytoskeletal network. As myofibrils make up approximately 80% of the volume of the muscle cell, disruption of myofibrillar structure and, in particular, the cytoskeletal network has the greatest influence on meat tenderness during aging. Changes in the connective tissue are minimal during aging, although the amount of connective tissue that varies between different cuts influences the basic tenderness. Degradation of some proteins linking myofibrils to the sarcolemma and to each other has been observed during the early postmortem period. Other changes that are correlated with increased tenderness include breakages within the myofibrils themselves, particularly within the I-band. These breakages lead to increased fragility and fragmentation of the myofibrils.

The histochemical and biochemical evidence indicates that much of the tenderization associated with postmortem aging is due to the action of the enzymes, which are known to be endogenous to the muscle. Some of the major myofibrillar and cytoskeletal proteins that are known to be degraded early during postmortem aging include (but are not limited to) titin, nebulin, desmin, and troponin-T. Interestingly, the most abundant proteins of the myofibril, actin and myosin, are not significantly degraded during postmortem aging.

Cathepsins

Early research on the mechanism responsible for the development of meat tenderness during aging focused on the cathepsins. The cathepsins are endogenous proteases found in

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lysosomes in living muscles. The most frequently studied cathepsins with respect to meat tenderness include cathepsins B, D, L, and H. The majority of the cathepsins are active at acidic pH values (usually between pH 5 and 6). These proteases were originally of interest because in living tissue lysosomes are one of the major sites of protein degradation. Additionally, these enzymes are active at acidic pH values, near the pH values found in postrigor meat.

Characteristics of the cathepsins

Cathepsin B (EC 3.4.22.1) is a glycoprotein that has a molecular weight of approximately 25 000. It is a cysteine protease and has been shown to have activity over the pH range of 4–6.5. Cathepsin B degrades many proteins in the muscle, including myosin and actin. Cathepsin D (EC 3.4.23.5) is an aspartyl protease with an approximate molecular weight of 42 000. This glycoprotein has been shown to have activity over the pH range 2.5–5.0. Like cathepsin B, cathepsins D and L (EC 3.4.22.15) will degrade myosin and actin. Cathepsin L has also been shown to have activity against α-actinin, troponin-T, and troponin-I and is active over the pH range 3.0–6.5. Cathepsin H (EC 3.4.22.16) is a cysteine protease with a molecular weight of approximately 25 000 and is active from pH 5.5 to 6.5. Like all of the cathepsins mentioned in this article, cathepsin H has a high specific activity against myosin.

In addition to degrading many myofibrillar proteins, several of the cathepsins have the ability to hydrolyze connective tissues, especially cathepsins B and L. Cathepsin B has been shown to have activity against collagen and proteoglycans. Cathepsin L has been shown to have activity against collagen, proteoglycans, and elastin. Some studies have indicated that limited proteolytic alteration of collagen occurs especially after long periods of aging.

Cystatin

Muscle also contains a family of potent cysteine-type protease inhibitors collectively known as cystatins. These cystatins are found distributed throughout the muscle cell and in the living muscle are thought to modulate the activity of cysteine proteases. In postmortem muscle, the pH should favor the activity of many of the cathepsins and because of this it might be expected that myosin and possibly actin would be among the proteins degraded. However, there is little evidence of either myosin or actin degradation in postmortem muscle. The presence of cystatins may help explain why there is little evidence of cathepsin activity in postmortem muscle.

Although there is some evidence that proteins like myosin and actin can be degraded when meat is stored at relatively high temperatures or for extremely long periods of time, myosin and actin are not degraded in the first week after slaughter under normal storage conditions. Therefore, the current evidence implicating the cathepsins in the tenderization of meat during the early stages of normal postmortem aging (when most tenderization occurs) is somewhat limited. Owing to this, in recent years, a much larger effort has been focused on the calpain enzyme system, a system that seems to degrade the same proteins that are degraded in postmortem muscle under normal meat storage conditions. Unlike the cathepsins, calpains degrade the majority of proteins into relatively few fragments, which is similar to what is seen in meat. It should be stressed that other enzyme systems like the serine proteases and the proteasomes may have a role in postmortem proteolysis. Recent studies on the proteasome endopeptidase complex (EC 3.4.25.1) in bovine skeletal muscle have suggested that it could play a role in protein degradation late in the aging process. At this stage, however, there is insufficient evidence to substantiate involvement of these other enzyme systems.

Calpain System

The endogenous calpain system has been implicated as playing a major role in the proteolysis of muscle proteins under postmortem conditions. Some of the proteins that have been shown to be substrates of calpains include titin, nebulin, troponin-T, desmin, synemin, talin, and vinculin. Most of these proteins have structural roles within the muscle cell. Degradation of these proteins has been associated with a weakening of the muscle cell and the myofibrillar structure and with tenderness.

Calpain enzymes

The calpain system is composed of several isoforms of tissue-specific and ubiquitous calcium-dependent cysteine proteases (calpains, EC 3.4.22.17), and their specific competitive inhibitor, calpastatin. The two best-characterized isoforms of calpains are the so-called ubiquitous forms μ-calpain and m-calpain. They are referred to as ubiquitous because they are found in most tissues. These proteases are named μ-calpain and m-calpain in reference to the amount of calcium they require for activation *in vitro*. μ-Calpain requires between 3 and 50-μM calcium for half-maximal activity, whereas m-calpain requires between 0.4 and 0.8-mM calcium for half-maximal activity. Both μ- and m-calpain are heterodimers composed of an 80- and a 28-kDa subunit. The 28-kDa subunit is identical in both μ-calpain and m-calpain. The *C*-terminus of this subunit has four sets of amino acid sequences that predict calcium-binding EF hand structures; however, the exact function of this subunit is not known. The 80-kDa subunits of μ- and m-calpains are similar, but are encoded for by different genes. The 80-kDa subunit is composed of four domains. Domain I, the *N*-terminal domain, has no sequence homology to any known polypeptide. Domain II is the catalytic domain and contains a cysteine residue as well as a histidine and asparagine residue that form a catalytic triad similar to that seen in other cysteine proteinases (including papain). Recent determination of the crystal structure of m-calpain has shown that in the absence of calcium, critical regions of the catalytic domain, domain II may be misaligned. The region of domain II (referred to as domain IIa) that contains the cysteine residue and the region of domain II that contains the critical histidine and aspargine residues (domain IIb) appear to be held slightly apart and rotated, potentially rendering the protease inactive. Release of specific structural constraints, possibly triggered by calcium, may play an important role in conferring activity to the enzyme. It has been speculated that conformational changes in domain I and III may play critical roles in calpain activation by their potential influence on the active site conformation in domain II. The

amino acid sequence of domain III is not homologous to any other known protein, but has two sets of sequences that predict EF hand Ca^{2+}-binding sites. The crystal structure of m-calpain suggests that this domain resembles the C2-domain found in several Ca^{2+}-regulated proteins like protein kinase C. Domain IV is a calmodulin-like domain that has four sets of sequences that predict EF hand Ca^{2+}-binding sites.

The protease μ-calpain is active under *in vitro* conditions mimicking postmortem muscle pH, ionic strength, and temperature. Although the calpain enzymes are active under postmortem conditions, their level of activity is somewhat compromised by the low pH and high ionic strength conditions that develop within the meat during storage. During postmortem storage in beef and pork, μ-calpain has been shown to become increasingly associated with the myofibril. It has been suggested that this myofibril-associated μ-calpain may indeed be active.

Although calcium is necessary for their activation, both μ- and m-calpain will also autolyze (selfdegrade) when incubated with calcium. Autolysis reduces the mass of the 80-kDa subunit of μ-calpain to 76 kDa, and the mass of the 80-kDa subunit of m-calpain is reduced to 78 kDa. The 28-kDa subunit of both enzymes is reduced to 18 kDa. Brief autolysis also reduces the Ca^{2+} concentration required for half-maximal activity of either enzyme. Extended autolysis leads to inactivation of the enzyme. Autolysis occurs under situations that allow activity, both in living cells and in postmortem muscle, but the significance of this is not clear. Both autolyzed and unautolyzed forms of the enzymes have been shown to have activity. However, the autolyzed form of μ-calpain appears to be more hydrophobic and binds tightly to subcellular organelles, including myofibrils. The presence of the autolyzed form of μ-calpain in postmortem tissue has been suggested to indicate that activity has occurred.

One of the tissue-specific forms of calpain, often referred to as p94 or novel calpain-1, deserves mention. This muscle-specific calpain isoform was the first tissue-specific calpain identified. The messenger ribonucleic acid for p94 in muscle has been reported to be as much as 10 times that of either μ- or m-calpain. The p94 peptide appears to be a single polypeptide that has a structure similar to the large catalytic subunit of μ- and m-calpain. It has a predicted molecular weight of 94 000 – slightly larger than the catalytic subunit of the ubiquitous calpains. This larger size is due to three unique regions: one in the *N*-terminus, one in the catalytic domain, and one at the interface of domains III and IV. Unlike μ- and m-calpain, which are sarcoplasmic proteins, p94 is associated with the myofibrillar fraction. More specifically, p94 appears to be closely localized if not bound to the large myofibrillar protein titin. This calpain has proven to be very difficult to study as it autolyzes rapidly during conventional extraction procedures and so it has been very difficult to ascertain its role, if any, in proteolysis/tenderization.

Calpastatin

Calpastatin, the endogenous inhibitor of μ- and m-calpain, has been found in all the tissues that contain calpains. Calpastatin in the skeletal muscle is a single polypeptide that contains within its structure four repeating domains that each has calpain inhibitory activity. Theoretically, then one calpastatin molecule may inhibit at least four calpain molecules. Calpastatin plays a major role in the regulation of the expression of calpain proteolytic activity. The amount of calcium required to allow half-maximal binding of calpastatin to calpains is generally lower than that required for half-maximal activity of the unautolyzed and autolyzed forms of m-calpain and for half-maximal activity of autolyzed μ-calpain. Calpastatin binding is reversible as calcium chelators can cause calpastatin to dissociate from calpain.

The level of inhibitory activity of calpastatin declines during postmortem aging (Figure 1). The level of inhibitory activity of calpastatin that remains at approximately 24 h after slaughter is associated with tenderness. Calpastatin is actually degraded in postmortem muscle and this rate of degradation is related to the rate at which it loses its ability to inhibit calpain. Both degradation of calpastatin and its loss of inhibitory activity are related to the rate of proteolysis and tenderization observed in meat. There is good evidence that calpains are at least partially responsible for the degradation of calpastatin. Currently, the conditions that promote calpain degradation of calpastatin in postmortem muscle have not been defined.

Even though there has been much research done on the calpain system over the years, relatively little is known about its regulation. Certainly, the endogenous inhibitor of the calpains, calpastatin, is involved, but there is evidence to suggest that other mechanisms may also be important, particularly in meat. Environmental factors in the early postmortem muscle cell can influence calpain activity and inhibition of calpain by calpastatin. These can include factors like pH and ionic strength. Therefore, it is important to examine other mechanisms that may affect calpain activity to fully explain how calpain activity is regulated in meat. Alterations in pH or ionic strengths in early postmortem meat have the potential to cause conformational changes allowing for increased hydrophobicity. This increased hydrophobicity has been hypothesized to lead to aggregation of the enzyme. Likewise, pH/ionic

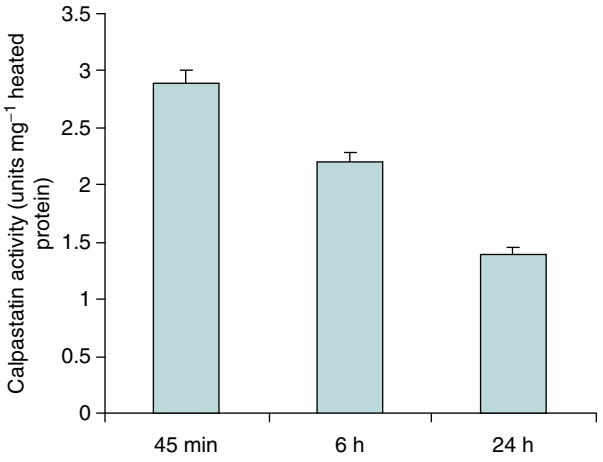

Figure 1 Effect of aging time on calpastatin activity from porcine longissimus muscle. Data taken from Melody, J.L., Lonergan, S.M., Rowe, L.J., *et al.*, 2004. Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science 82, 1195–1205.

strength changes may alter the conformation of substrate proteins and render them less susceptible to cleavage by μ-calpain. A slightly accelerated pH decline has been shown to be associated with more rapid attainment of ultimate tenderness and more rapid proteolysis. However, a greatly exaggerated rate of pH decline, like the rapid pH decline that results in pale, soft, and exudative pork, seems to result in very limited aging potential. Hypothetically, a rapid pH decline would lead to an increased level of activity of catheptic enzymes and increased proteolysis; however, in most cases, this does not seem to occur. The product that has an exceptionally rapid pH decline has often been shown to also exhibit limited proteolysis of muscle proteins associated with tenderization. Low pH values have been shown to destabilize μ-calpain and to promote more rapid autolysis and/or activation and subsequent inactivation in *in vitro* studies and may do the same in muscle tissue. Therefore, the rate of pH decline may play a very pivotal role in the attainment of ultimate tenderness.

Caspase Enzyme System

Caspases are a family of enzymes that are involved in apoptosis, or programmed cells death. Cell death by apoptosis is characterized by a systematic and organized dismantling of a cell. Common hallmarks of apoptosis include shrinkage of the cell, cell membrane blebbing, chromatin condensation, damage to deoxyribonucleic acid, and the formation of apoptotic bodies without causing a generalized inflammatory response. Apoptosis is adenosine triphosphate (ATP) dependent, which may seem to be incongruous with postmortem tissue; however, in most postmortem muscle ATP can be produced for a period of time via anaerobic glycolysis, which may be different from the classical necrotic state. Necrosis is typically caused by a catastrophic loss of energy and is a passive process. It is accompanied by a loss of membrane integrity and swelling of organelles. Thus, in reality, as the loss of energy in the early postmortem cell is a gradual process, the argument could be made that early postmortem tissue resides in a 'nether region' between the two states of apoptosis and necrosis.

The apoptotic process is choreographed by the caspases. Caspases are cysteine proteases that require their substrates to have aspartate residues. There are more than a 1000 substrates that have been identified for the caspases, and they include myofibrillar and cytoskeltetal proteins. Activation of caspases can be initiated by pathological events including ischemic/hypoxic conditions.

There are two general classes of caspases, initiatior caspases (caspases 8, 9, 10, and 12) and effector or executioner caspases (caspases 3, 6, and 7). Initiator caspases are activated when a stimulus for apoptotic events is received. Once they have been stimulated, these initiator caspases activate the executioner caspases by cleaving a linker that separates the small and large subunits of the catalytic domain. Once activated, the executioner caspases are responsible for the enzymatic cleavage of substrates that are ascribed to the caspase system.

Since the early 2000s, caspases have been suggested to play a role in postomortem proteolysis related to tenderness. Many of the caspase substrates are proteins that have been shown to be at least partially degraded during the early postmortem period. These include (but are not limited to) actin, troponin-T, desmin, and myosin light chains.

The question of whether or not caspase enzymes or calpain enzymes are the predominant systems involved in early postmortem proteolysis has been hotly debated. It has proven difficult to rule out either system. Indeed, there is evidence that the two systems work together in the cell. For example, it has been shown that the calpain inhibitor, calpastatin, can be cleaved by caspases 1, 3, and 7, thereby directly influencing the activity of the calpain system. Thus, it may not be a question of which enzyme system is responsible for postmortem proteolysis, but rather, how do the two systems work together. For a more detailed discussion of relevant research on this topic, the reader is referred to the Further Reading section of this article.

Exogenous Enzymes

In addition to allowing the endogenous enzymes to tenderize meat, exogenous enzymes, mostly of plant origin, have been used to augment the tenderization process. The most commonly used plant enzymes are papain (from papaya), bromelain (from pineapple), and ficin (from figs/ficus). More recently, actinidin (from kiwi) has been investigated, as has been zingibain (from ginger).

Papain, bromelain, and ficin are all cysteine proteases and have a broad spectrum activity, cleaving a wide variety of bonds, thus degrading a large number of muscle proteins. These proteases are active in the pH range found in meat (papain, pH range 5.8–7; bromelian, pH range 5–7; and ficin, pH range 5–8). The ideal temperature range for these proteins is approximately 50–60 °C, making them maximally active on heating. Actinidin has a higher pH range than the aforementioned enzymes (ideal range is 7–10, but can have activity at pH 5–7) but the temperature range is similar. Zingibain is obtained from a crude extract of ginger. It has a maximum activity at pH 6–7 and a temperature of 60 °C.

These plant-derived enzymes are very effective tenderizers. In addition to acting on myofibrillar proteins, most will also act very effectively on connective tissue proteins as well. In fact, one of the major challenges of using these enzymes is countering the effect of overtenderizing. However, continued research in the application of these enzymes is yielding better ways to utilize them. For further detailed information, the reader is referred to a review by Bekhit *et al.* (2013).

Conclusions

On the basis of available data the major candidate to explain proteolysis of myofibrillar proteins and thus tenderization postrigor is the calpain protease system. The mode of action of the calpains is not yet fully defined and questions remain as to the role of m-calpain given the *in vitro* requirement for a Ca^{2+} ion concentration exceeding that observed in postmortem muscle. The existence of the calpains in living muscle and other tissues is consistent with the involvement of these enzymes in tenderization (which reflects protein degradation) but suggests a mode of action more intricate than previously thought.

An increase in ionic strength postmortem may assist degradation of proteins by enzymes and also lead to solubilization of proteins, but in itself is not the sole mechanism causing tenderization. Equally, changes in the binding of actomyosin (the complex of contractile proteins formed at rigor) or cleavage of myofibrillar proteins due to Ca^{2+} ions do not offer plausible explanations for the mechanism that results in tenderization. It also appears that the cathepsin proteases are unlikely to have a role in early postmortem cleavage of proteins (proteolysis) and thus tenderization and this also applies to other enzyme groups such as the serine proteases, proteasomes and matrix metallopeptidases. Recent work on caspases has indicated that they may be worthy of further investigation, especially with respect to their interaction with the calpain system.

The use of exogenous plant enzymes is a useful method to tenderize meat cuts beyond what can be achieved via postmortem aging alone and is a viable method to use particularly for cuts that have high amounts of connnective tissue.

See also: Carcass Composition, Muscle Structure, and Contraction. Conversion of Muscle to Meat: Aging; Glycogen; Glycolysis; Rigor Mortis, Cold, and Rigor Shortening. Tenderizing Mechanisms: Chemical; Mechanical

Further Reading

Bekhit, A., Hopkins, D., Geesink, G., Bekhit, A., 2013. Exogenous proteases for meat tenderization. Critical Reviews in Food Science and Nutrition. doi:10.1080/10408398.2011.623247.

Goll, D.E., Boehm, M.L., Geesink, G.H., Thompson V.F., 1997. What causes postmortem tenderization? In: Proceedings 50th Reciprocal Meat Conference, Iowa, pp. 60–67. Ames, IA: American Meat Science Association.

Goll, D.E., Thompson, V.F., Li, H., Wei, W., Cong, J., 2003. The calpain system. Physiological Reviews 83, 731–801.

Goll, D.E., Thompson, V.F., Taylor, R.G., Ouali, A., Chou, R.G., 1999. The calpain system in skeletal muscle. In: Wang, K.K., Yuen, R.W. (Eds.), Calpain: Pharmacology and Toxicology of Calcium-Dependent Protease. Philadelphia, PA: Taylor and Francis, Publishers, pp. 127–160.

Hopkins, D.L., Thompson, J.M., 2001. Inhibition of protease activity 2. Degradation of myofibrillar proteins, myofibril examination and determination of free calcium levels. Meat Science 59, 99–209.

Hopkins, D.L., Thompson, J.M., 2001. The relationship between tenderness, proteolysis, muscle contraction and dissociation of actomyosin. Meat Science 57, 1–12.

Hopkins, D.L., Thompson, J.M., 2002. Factors contributing to proteolysis and disruption of myofibrillar proteins and the impact of tenderisation in beef and sheep meat. Australian Journal of Agricultural Research 53, 149–166.

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D.D., Parrish, Jr., F.C., Olson, D.G., 1996. Proteolysis of specific muscle proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem muscle. Journal of Animal Science 74, 993–1008.

Huff-Lonergan, E.J., Lonergan, S.M., 1999. Postmortem mechanisms of meat tenderization: The roles of the structural proteins and the calpain system. In: Xiong, Y.L., Ho, C.-T., Shahidi, F. (Eds.), Quality Attributes of Muscle Foods. New York, NY: Kluwer Academic/Plenum Publishers, pp. 229–251.

Kemp, C.M., Parr, T., 2012. Advances in apoptotic mediated proteolysis in meat tenderisation. Meat Science 92, 252–259. doi:10.1016/j.meatsci.2012.03.013.

Kemp, C.M., Sensky, P.L., Bardsley, R.G., Buttery, P.J., Parr, T., 2010. Tenderness – An enzymatic view. Meat Science 84, 248–256.

Lamare, M., Taylor, R.G., Farout, L., Briand, Y., Briand, M., 2002. Changes in proteasome activity during postmortem aging of bovine muscle. Meat Science 61, 199–204.

Moldovenu, T., Hosfield, C., Lim, D., *et al*., 2002. Ca^{2+} switch aligns the active site of calpain. Cell 108, 649–660.

Monin, G., Ouali, A., 1991. Muscle differentiation and meat quality. In: Lawrie, R. (Ed.) Developments in Meat Science, vol. 5. London: Elsevier Applied Science, pp. 89–157.

Ouali, A., 1992. Proteolytic and physiochemical mechanisms involved in meat texture development. Biochimie 74, 251–265.

Strobl, S., Fernandez-Catalan, C., Braun, M., *et al*., 2000. The crystal structure of calcium free human m-calpain suggests an electrostatic switch mechanism for activation by calcium. Proceedings of the National Academy of Sciences USA 97, 588–592.

Takahashi, K., 1996. Structural weakening of skeletal muscle tissue during postmortem ageing of meat: The non-enzymatic mechanism of meat tenderization. Meat Science 43, s67–s80.

Takahashi, K., 1999. Mechanism of meat tenderization during post-mortem ageing: Calcium theory. In: Proceedings 45th International Congress of Meat Science and Technology, pp. 230–235. Yokohama: Wageningen Academic Publishers.

Uytterhaegen, L., Claeys, E., Demeyer, D., 1994. Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. Journal of Animal Science 72, 1209–1223.

Relevant Website

www.calpain.net
Calpain Research Portal.

Mechanical

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Glossary

Aging The process of meat tenderization that occurs over time – it commences after rigor mortis.
Contractile proteins Actin and myosin, which form the thin and thick filaments of skeletal muscle. These two proteins interact chemically (to form actomyosin), which gives muscle the ability to contract and relax. Associated with actin are the proteins troponin and tropomyosin.
Costameres Connect Z-disks to the sarcolemma and are made up of proteins such as talin, vinculin, desmin, and dystrophin.
Cytoskeletal proteins A set of filamentous structural proteins (includes actin, titin, nebulin, and desmin).
Electrical stimulation The application of an electric current through a carcass postmortem that accelerates the rigor process.
Myofibril Comprises contractile structural and regulatory proteins. The contractile protein is composed of myofilaments that are in turn made up of thin and thick filaments. Structural proteins include titin and nebulin. Titin is the largest protein in skeletal muscle (up to 3700 kDa) and provides the elasticity to the sarcomere. The regulatory proteins include troponin and tropomyosin.
Proteolysis The degradation of proteins into smaller subunits that occurs with aging, but also in turnover of living muscle.
Rigor A term for individual muscle fibers that have been depleted of adenosine triphosphate and in which the actomyosin bond has formed.
Rigor mortis A term describing muscle stiffening after all muscle fibers enter rigor.
Sarcomere The basic unit of skeletal muscle defined by the distance between two Z-disks. Z-disks are dense protein structures to which the contractile protein actin is attached along with proteins such as titin and nebulin. Z-disks are the anchor points for the contractile proteins that allow contraction and relaxation.
Shear force The force (N) applied to a standardized piece of cooked meat to shear it.
Shortening A process that occurs when prerigor muscle is cooled below 10 °C. It also occurs as muscles enter rigor at high temperatures (rigor shortening).

Introduction

Methods to reduce meat toughness by mechanical or physical means can be employed during either the prerigor or postrigor phase as muscle is converted to meat. The methods fall into two broad categories: those that prevent shortening during rigor or those that disrupt the meat structure either by physical or enzymatic means when applied some time after slaughter. In the former category, carcass-hanging methods, or excision of cuts combined with devices to restrict shortening, have been found to impact on tenderness levels. The mechanism behind these methods is essentially a restriction of the degree of actin and myosin overlap as muscle enters rigor and a reduction in fiber cross-sectional area. For the latter category of meat disruption, electrical stimulation, ultrasonic waves, blade tenderization, pressure treatment, and freeze–thawing have been investigated. These methods depend on one or more mechanisms: decreased actin and myosin overlap, physical damage to sarcomere and connective tissue structure, or altered rates of proteolysis.

Although carcass-hanging methods have been found very effective for reducing the toughness of loin and hindquarter cuts and are generally cost-effective, they provide no benefit to forequarter cuts. The other methods provide the opportunity for more cuts to be improved, but the practicality and effectiveness is in some cases questionable. Inevitably, any method that reduces the density of muscle fibers in a unit area or causes disruption of sarcomere structure will lead to a reduction in the toughness of cooked meat.

Shear force is an objective instrumental measure of tenderness, where high shear values indicate increasingly unacceptable meat (tougher). Tenderness can also be assessed by sensory evaluation, where high numbers indicate greater levels of satisfaction (less tough).

Carcass Methods

Tenderstretching

The decline postrigor in shear force from a peak attained during rigor is illustrated in Figure 1. Numerous studies have shown that temperature conditions prerigor dramatically influence the level which the peak reaches. Prerigor methods that physically prevent a large rise in the peak shear force (indicated by arrows) will confer an immediate postrigor advantage in tenderness.

Suspension of carcasses by the obturator foramen or aitchbone so that the back leg falls into the walking position (Figure 2) was given the name tenderstretch. Researchers in the US first investigated this method and Australian researchers conducted extensive follow-up research. The technique places tension on the hind leg and loin muscles and physically prevents them shortening (i.e., reduces the overlap of actin and myosin).

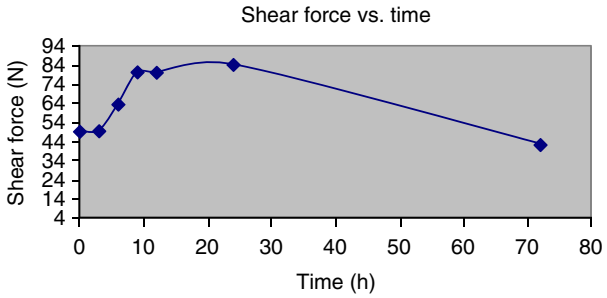

Figure 1 Time course changes in shear force of ovine longissimus during postmortem storage. Maximum contraction occurs somewhere between the arrows as the muscle enters rigor. Data from Wheeler, T.L., Koohmaraie, M., 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. Journal of Animal Science 72, 1232–1238.

Table 1 Warner–Bratzler shear values (N) for muscles measured at 2–3 days postmortem obtained from sides of beef hung by either the Achilles tendon or aitchbone

Muscle	*Method of suspension*	
	Achilles tendon	*Aitchbone*
Semimembranosus	82.4	50.0
Gluteus medius	78.5	39.2
Longissimus	107.9	55.9
Vastus lateralis	86.3	53.0
Biceps femoris	63.7	65.7
Semitendinosus	59.8	58.8
Infraspinatus	62.3	58.8
Psoas major	35.3	49.0

Source: Adapted with permission from Bouton, P.E., Fisher, A.L., Harris, P.V., Baxter, R.I., 1973. A comparison of the effects of some post-slaughter treatments on the tenderness of beef. Journal of Food Technology 8, 39–49.

Figure 2 The carcass is suspended by the aitchbone so that the back leg drops and the backbone straightens and maximum tension is placed on these muscles. Photograph courtesy of J.M. Thompson.

The extent of the improvement across a range of muscles is clearly demonstrated in Table 1 for cuts taken from beef carcasses. Shear force was significantly reduced by tenderstretch in the majority of hindquarter muscles. The notable exceptions to this trend were the m. biceps femoris and m. semitendinosus. This latter muscle is stretched in Achilles-hung sides and in the case of the m. psoas major it actually shortens in tenderstretched carcasses.

By weighting the hind legs, a further reduction in shear force (20%) can be achieved, particularly in the loin muscle; it has recently been shown that this technique causes significant disruption of the sarcomere, with distortion of the Z-disk and actual tearing of the filaments (Figure 3). It is this disruption and a decreased overlap of actin and myosin that results in the dramatic reduction in shear force.

The improvement in tenderness is so dramatic that the need for prolonged aging is virtually eliminated; in addition, the variation in tenderness along the loin muscle is reduced. Commercial adoption of this technique has seen resurgence as processors have developed ways to handle and store tenderstretched carcasses, such as adopting methods to rehang carcasses from the Achilles tendon after attainment of rigor and streamlining the processing of carcass movement and boning. Pelvic suspension (or tenderstretch) alone has not been patented. Cargill Incorporated in the US have patented pelvic suspension as part of a wider meat tenderization system incorporating separating vertebrae (a variant of the tendercut technique below), pelvic suspension, electrical stimulation, and immediate subsequent Achilles suspension. In this methodology pelvic suspension is used for less than 10 min, preferably less than 2 min, restricting muscle contraction only as the muscles approach rigor. No claims are made as to the efficacy of this process.

Tendercut

An alternative method to tenderstretch has been developed called 'tendercut.' This method offers an advantage in carcass handling because the leg is still hung by the Achilles tendon. The tendercut process was initiated by Claus and Marriott in 1991 (Virginia Polytechnic Institute and State University, USA). The tendercut process applies tension on muscles by breaking the vertebrae and pelvic bones in the hot carcass. This process involves sawing the vertebral column at the 12th/13th rib junction and/or the ischium at the rump/butt junction (Figure 4). In addition to breaking the vertebrae at the 12th/13th rib junction, all tissues surrounding the loin are cut, such that only a dorsal component is holding the forequarter to the hindquarter. The adipose tissue dorsal to the longissimus muscle is also cut to expose the epimysium and this cut is then continued around the medial side of the loin muscle and the m. multifidus dorsi is completely severed. Intercostal connective tissue and muscle are then cut between the 12th/13th costal bones. This latter cut is extended approximately

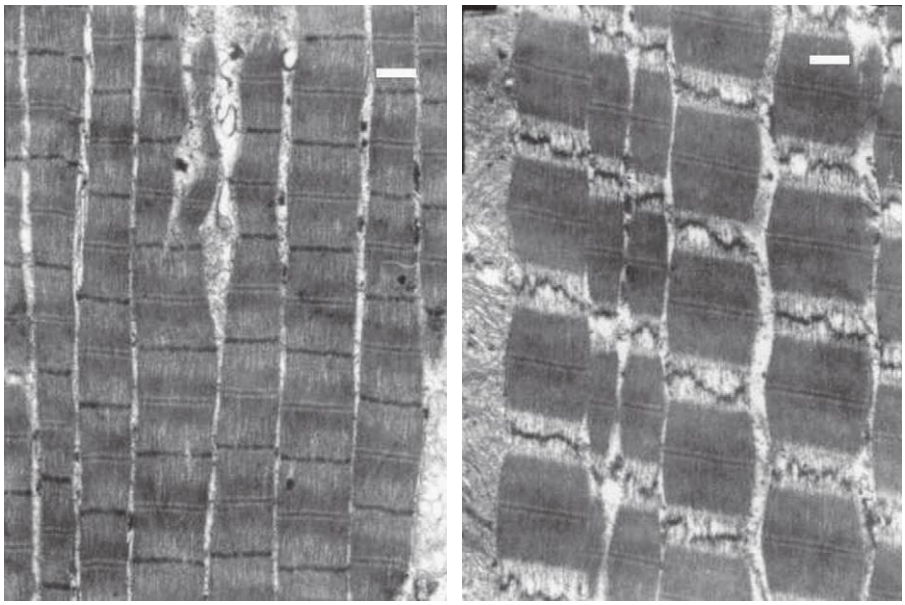

Figure 3 Images of ovine longissimus captured with an electron microscope (original magnification×10 000) from (a) a carcass tenderstretched and weighted with 2 kg weights and (b) a sample from a normally hung (by Achilles tendon) carcass shown for comparison. White bars = 500 nm. Reproduced from Hopkins, D.L., Garlick, P.R., Thompson, J.M., 2000. The effect on the sarcomere structure of super tenderstretching. Asian-Australasian Journal of Animal Science 13 (Supplement C), 233, with permission of the Asian-Australasian Journal of Animal Science.

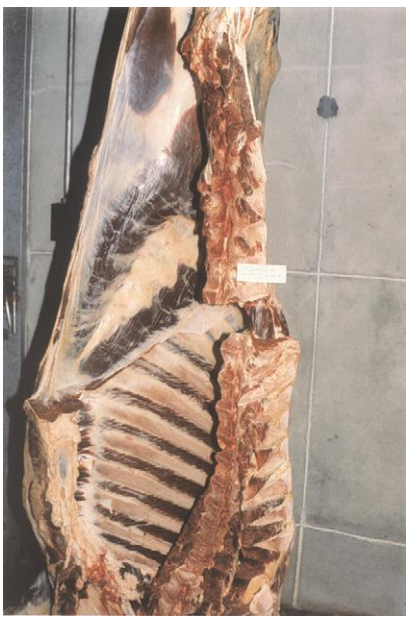

Figure 4 A photograph showing a severed vertebral column as used as part of the tendercut method. Photograph courtesy of J.R. Claus.

12 cm from the lateral edge of the loin muscle. The second cut severs the ischium at the site used to separate the butt/rump joints, the junction between the 4th/5th sacral vertebrae and connective tissues. The fillet muscle must be freed from its attachment and deflected forward during sawing. In addition, care must be taken while sawing the ischium to minimize damage to the rump cut. The technique can be applied by use of either cuts or a single cut, in which case fewer muscles will be affected.

Compared to tenderstretching, the tendercut process overcomes the need for additional chiller space and avoids the need to train boners on new cutting lines, but is much more difficult to carry out on a processing line. Based on published evidence it also appears that this method does not reduce the shear force of loin and leg muscles to the same extent as tenderstretching. However, like the former method, tendercut does reduce the proportion of unacceptable loin steaks when tested by consumers, and in one study the proportion of unacceptable scores for overall tenderness was reduced from 19% to 2.5%. The magnitude of the gain achieved within either of these hanging methods will be influenced by the chilling regime, with a lower improvement expected under slow chilling conditions.

Electrical Stimulation

Accelerated fall in postmortem pH is one of the main outcomes of electrical stimulation; however, there is some evidence that stimulation does cause physical disruption of muscle. Histological images of stimulated muscle at times show the appearance of contractile bands containing predominantly stretched, ill-defined, and disrupted sarcomeres.

Contracture bands are not a direct consequence of electrical current passing through the muscle, but are rather due to the supercontracture caused through localized excessive release of calcium ions from the sarcoplasmic reticulum. This suggests that they are a consequence of abnormal, perhaps localized, calcium release from the sarcoplasmic reticulum through a

tetanic contracture. This extra calcium could lead to tenderization through activation of enzymes such as the calpains, but any reduction in toughness could alternatively be due to a purely physical effect via a reduction in fiber density in a unit area. It has been shown quantitatively in beef m. longissimus that sarcomeres adjacent to contracture bands have a higher frequency of I-band fracture.

Ultrastructural changes are fiber type-specific and depend on the duration and effectiveness of the applied stimulation, the current frequency, and the interaction between current frequency and voltage. If the time interval between successive stimuli is more than approximately 0.25 s, the muscle tetanic shortening is reversible. However, when a higher frequency of current is applied, muscle may not have enough time for relaxation between succeeding twitches, and irreversible contracture bands are formed. At present, it is not possible to determine how much improvement in tenderness as a result of electrical stimulation stems from a reduction in fiber density in unit area as a consequence of contracture as opposed to increased activity of enzymes due to spikes in the concentration of free cytoplasmic Ca^{2+}. Quantitative studies examining ultrastructural alteration, shortening, and proteolysis are necessary under the same experimental conditions to clarify the contribution of these variables to improvements in tenderness. A combination of stimulation and tenderstretching has not been found to confer a significant additive advantage over separate treatment of muscles with either method.

Cut Methods

It has been demonstrated that if prerigor muscle can be excised from the carcass and held to prevent shortening (e.g., by wrapping), then this can provide a potentially economical way to speed up processing and at the same time minimize toughening. This does require chilling at a temperature that minimizes rigor shortening. Several different approaches have been developed in recent years.

Pi-Vac Elasto-Pack System®

The Pi-Vac Elasto-Pack System® is a method of tightly wrapping hot-boned muscles in an elastic wrapping material prerigor to prevent shortening and toughening of the meat. The system uses a highly flexible packaging sleeve, which is expanded using a partial vacuum to allow the meat to be inserted. Once the vacuum is turned off the flexible packaging retracts to its normal dimensions. This exerts longitudinal forces on the meat, preventing the contraction of the muscle. Almost all of the oxygen is also forced out of the packaging. The subsequent bound meat product has been labeled TenderBound. This technology can come in three different sizes (Figure 5) and some commercialization of this approach has occurred in Europe. By adopting the concept of super tenderstretching by using weights and applying it to hot-boned beef muscle it has been shown that tenderness could be achieved equivalent to that realized with the Pi-Vac Elasto Pack System®. Additionally, the Pi-Vac Elasto Pack System® produced meat with the lowest variation, indicating that this method does something different to meat structure, but there have been few published studies outlining the benefits or otherwise of the technology.

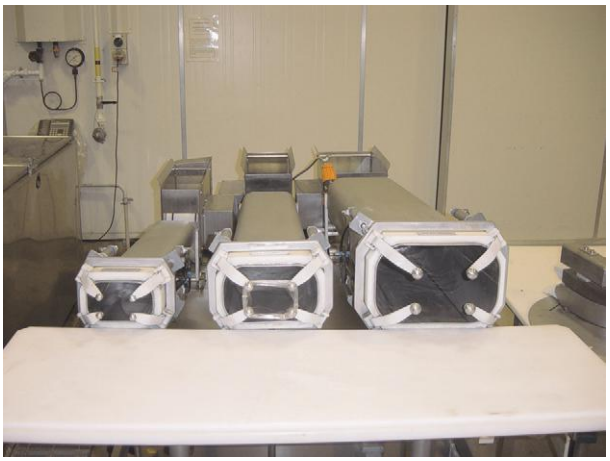

Figure 5 The Pi-Vac Elasto-Pack System showing three different sizes. The middle size shows the plastic film that is used inside the machine. Photograph courtesy of Hans-Werner Meixner.

SmartStretch™

This technology was designed to apply air pressure/vacuum to excised individual prerigor muscles to stretch the muscle into an even form and package it so as to retain the form. The technology was patented by Meat and Wool New Zealand Limited and Meat and Livestock Australia Limited as the 'Boa' and was subsequently registered as SmartStretch™. As with all stretching systems the aim was to either stretch sarcomeres or prevent the contraction of sarcomeres during rigor, with some resultant tenderness benefits. The machine's operation is based on an externally ribbed flexible sleeve surrounded by inflatable bladders that are housed within an airtight chamber that air can be pumped into or out of. Air is pumped out of the chamber to create negative pressure, which causes the sleeve to expand, allowing the meat to be inserted. Air is then pumped into the inflatable bladders causing the meat to be compressed by force perpendicular to the direction of the muscle fibers. This also applies peristaltic action, moving the meat toward the same end of the sleeve that it was inserted into. Positive pressure is then applied to the exterior of the sleeve by pumping air into the chamber, forcing the meat upwards and into packaging as shown in Figure 6.

As with the Pi-Vac system the application of SmartStretch™ is for hot-boned muscles and work has been conducted in Australia, mostly with sheep and beef meat from old animals. Initial experiments with sheep meat were promising. A 24% increase in m. semimembranosus length resulted in shear force reductions of 46% at 0 days aging and 38% at 5 days aging and was matched with a significant increase in sarcomere length. A further study examined the effect when the muscles were stretched as part of a whole sheep meat hind leg. A 14% increase in leg length resulted in a shear force reduction in the m. semimembranosus of just 16% and of 18.4% in the m. biceps femoris at 0 days aging and no significant difference at 5 days aging. Significant increases were found in sarcomere length

Figure 6 Stretched beef being ejected from the flexible sleeve into the packaging. Photograph courtesy of D.L. Hopkins.

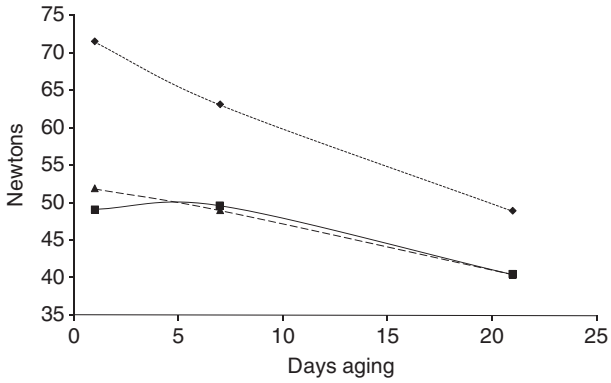

Figure 7 Effect of hanging/stretching method and aging on tenderness. ◆–◆, Achilles-hung carcasses; ■–■, Tenderstretch-hung carcasses; and ▲– –▲, SmartStretch™-treated meat. Adapted from Geesink, G., Thompson, J., 2008. Utilising the 'Boa' stretching technology to improve the quality of hot boned striploins. Report No. RE-221941, Armidale, NSW, Australia: University of New England, School of Environmental and Rural Science. Available at: www.redmeatinnovation.com.au/innovation-areas/eating-quality/products/smartstretch/validation-trials (accessed 06.09.12).

following stretching. However, a number of the studies conducted on beef m. semimembranosus, m. longissimus lumborum, and rostbiff (mainly m. gluteus medius) were inconclusive as to the SmartStretch™ system's impact on beef tenderness. Increasing stretch in the m. semimembranosus from 34% to 52% had no impact on shear force, but this was proposed to reflect the fact that once a basal level of stretch was achieved further stretching would not have an effect. A 21% increase in length of the m. semimembranosus and rostbiff also had no significant impact on shear force or on sensory results, although the shear force values were so high that tasters could not discriminate between the tough and the extremely tough product. A reduction in the variability in shear force was found.

By contrast, when younger cattle (maximum dentition score of 2) were used the results showed a significant improvement in hot-boned meat tenderness by the use of SmartStretch™. Initial work in beef comparing SmartStretch™ to Tenderstretch, 'Superstretch' (Tenderstretch plus a pulley system to pull the hindlimb toward the forequarter), and Achilles suspension showed similar tenderness improvements in the m. longissimus lumborum resulting from all three stretching treatments in prime cattle. The results for two stretching treatments are shown in Figure 7. Follow-up work in young cattle showed that tenderness of the rostbiff (m. gluteus medius), as reflected in reduced shear force measurement, was significantly improved in 0 day aged stretched samples over the unstretched hot-boned control. After 8 days aging there was no longer a difference in the tenderness between stretch treatments. Sarcomere length was significantly increased by stretching in both studies.

Blade Tenderization

The physical disruption of muscle structure must reduce the density of cooked meat fibers in unit area and therefore impart an improvement in tenderness. Blade tenderization is used for this purpose commercially. Commonly, cuts of meat are placed on a conveyor and pass through a machine that consists of spear-shaped blades arranged on a mounted head. Blade density can be greater than one blade per 1 cm^2 of head and the pattern of cut can also be varied. Other types of devices, which combine a large screw and pressure to extrude the meat through a slit, also cause significant disruption and reduce toughness (by fracturing fibers). Repeated treatment of cuts by blade tenderization may confer some additional benefit, but the gain is marginal and good sanitation is required to avoid bacterial contamination between cuts.

Commercial blade tenderization devices are often used for infusing solutions into meat and these solutions may contribute to tenderization. Histological examination of treated meat samples shows that the muscle fibers are torn and fragmented by the blade, as is connective tissue, but areas between points of blade penetration will be unaffected. Despite this localized effect, the density of blades compensates and this technique confers significant improvement to a range of cuts (see Figure 8), but particularly for some of the toughest cuts of the hind leg such as the topside (m. semimembranosus). In contrast, there is much less benefit for inherently tender cuts such as the rib-eye (m. longissimus thoracis).

Ultrasonic Waves

Sound waves travel through material at different speeds and impart energy to the transmission material, which has the potential to cause physical disruption, particularly when the wavelength is similar to the size of structural units of the material. Both frequency and wavelength can be varied, but few studies using meat have focused on high-frequency (>1 MHz) ultrasonic waves, with more attention on low-frequency waves. Theoretically, disruption of muscle cell integrity could also lead to a leakage of Ca^{2+} from the sarcoplasmic reticulum and release of cathepsins from the lysosomes, hastening the onset of enzyme activity.

Indeed, there is a suggestion that prerigor treatment of m. semimembranosus from beef with ultrasound at 2.6 MHz

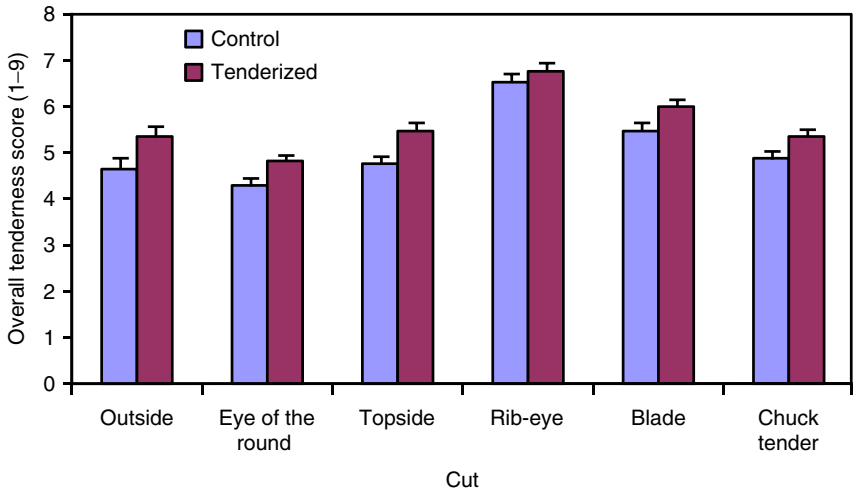

Figure 8 Impact of mechanical tenderization on the tenderness of cuts of meat from beef carcasses. Modified with permission from Jeremiah, L.E., Gibson, L.L., Cunningham, B., 1999. The influence of mechanical tenderization on the palatability of certain bovine muscles. Food Research International 32, 585–591.

(intensity 10 W cm^{-2}) can elevate the levels of free cytosolic Ca^{2+} and does lead to an increase in sarcomere length (~10%). Despite this effect, no clear advantage in reducing toughness has been reported and this applies equally to low-frequency, low-intensity ultrasound.

The size of meat sections and the use of muscles with high connective tissue content (e.g., m. semitendinosus) could be some of the reasons for the apparent lack of effect of ultrasound on toughness. From a practical perspective, the transmission of sound waves causes a dissipation of energy and this results in a rise in material temperature. As a consequence, a number of studies have submerged the meat in cold water, which would potentially limit commercial adoption. It would appear that the use of ultrasound to increase tenderization rate currently has limited applicability, although it may be feasible to integrate its use with hot-boning operations in which meat is passed through a water-cooling submersion system, at which time the meat could be exposed to a barrage of ultrasonic waves.

Hydrodynamic Pressure

One of the most novel techniques that have been developed to reduce toughness is the Hydrodyne process. In this process a small amount of explosive is used to generate a shock wave that travels through water in fractions of a millisecond. The idea was proposed by John Long, a mechanical engineer, who developed the concept further with the help of Morse Solomon (USDA, Beltsville, MD, USA), and Eric Staton who had experience in the use of explosives.

The process requires that the meat be held within a container and surrounded by water; because meat has a high content of water, the shock waves travel through the meat. When the meat is held inside a metal or plastic container, the shock waves are reflected from the walls and intersect; this increases the pressure, leading to physical disruption of the encapsulated meat. Electron micrographs of treated meat show I-band proteins totally separated from the Z-disk and fractures at the A-band/I-band junction, and thus the increase in tenderness would appear to be mediated through physical degradation of muscle structure in particular myofibrillar proteins. As for any method that results in such a degree of disruption, it is also feasible that cellular structure is damaged, leading to the release of protein-degrading enzymes (i.e., calpains or cathepsins) or activators of these enzymes such as Ca^{2+}, hastening proteolysis. A somewhat related method is to apply hydrostatic pressure to meat; in this case it is the pressure of a liquid on immersed meat that results in a reduction in toughness (see next section).

Early development of the Hydrodyne process showed that the reduction in toughness was influenced by the amount of explosive that was used, the number of detonations, and whether the meat was fresh or frozen. The process was more effective for fresh meat and two detonations using 50 g of explosive were as effective as one detonation using 100 g of explosive. The effect on toughness, as measured by shear force, is dramatic (Figure 9), with improvements of up to 70%. The magnitude of improvement is sufficient to make even cuts high in connective tissue acceptable for table meat (e.g., topside, Figure 9). In the case of the data shown in Figure 9, the cuts were excised from the carcass 1.5 h after death and stored for 1 day at 2–4 °C, explaining the high shear values for control cuts, indicative of cold-induced shortening, and suggesting that the Hydrodyne process was able to overcome this toughening effect. As for many techniques, the magnitude of the effect is influenced by the toughness of control samples and when it was applied to relatively tender pork loins the improvement was only 17%, but the evidence indicates that this technique will also reduce the variation in shear force.

The process has not yet been successfully commercialized and the concept is still under development. A current prototype (Figure 10) can tenderize 270 kg of meat at once. The unit consists of a 3.2-ton steel tank filled with water, which is covered with a 2.3-ton steel dome (see photograph below), 2.7 m in diameter. Cuts of meat, encased in water and pressure-resistant wrapping, are placed into the tank, which is 3 m

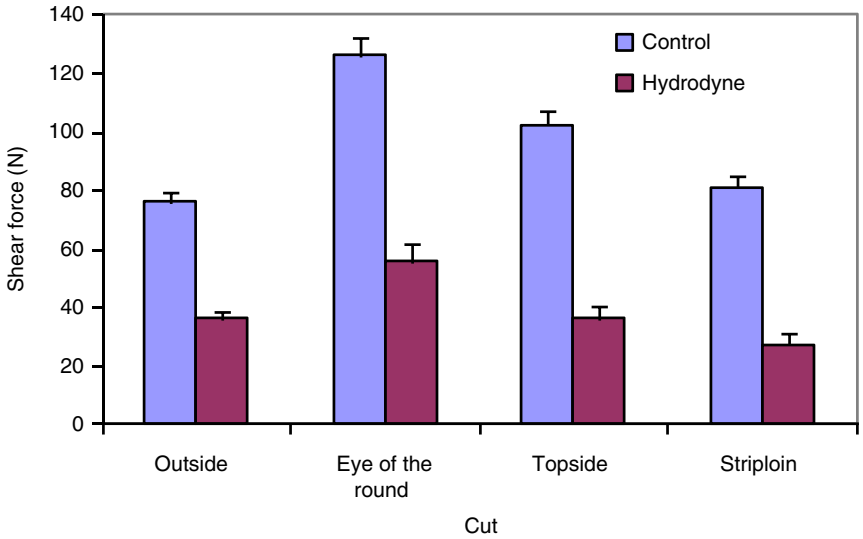

Figure 9 Impact of Hydrodyne treatment on the shear force (N) of beef cuts (8 samples per muscle) after 1 day of aging, and treatment of the cuts after freezing and subsequent thawing. Modified from Solomon, M.B., Long, J.B., Eastridge, J.S., 1997. The Hydrodyne: A new process to improve beef tenderness. Journal of Animal Science 75, 1534–1537.

Figure 10 A large-scale Hydrodyne unit (1060 l) showing the inner section where the encased meat and surrounding water are held, with the large dome in the background. Photograph courtesy of M.B. Solomon.

in the ground, and an explosive charge is detonated in the water less than a meter from the meat. The tank's dome holds in water that is forced upwards. Refinement of the process continues with a focus on variables such as container type (metal vs. plastic), the material used to pack the meat, location of the explosive (distance from meat), and the type of explosive. Future models may be based on generation of shock waves with an alternative to the use of explosives.

Hydrostatic Pressure

Subjecting meat to high pressure via a surrounding liquid has been adopted in countries such as Japan, Australia, and the US in order to extend shelf-life and reduce bacterial counts. The technique is combined with heat treatment (e.g., to 60 °C) while the muscle is under pressure. The method has a high capital cost, limiting adoption, but can also be used to decrease the toughness of meat. First reports on the technique were published in the 1970 s after research in Australia. The technique can be applied to both prerigor and postrigor muscle and, similar to hydrodynamic shock waves, will cause a disruption of muscle structure that is confirmed in electron microscopy studies. Prerigor meat exhibits accelerated glycolysis under pressure/heat treatment and this treatment leads to a significant reduction in shear force (Figure 11). In this case, the shear force values of the m. longissimus from control carcasses indicate cold-induced shortening, yet the negative effects of prerigor excision of muscles through shortening can be overcome by pressure treatment.

Hydrostatic pressure can cause the degradation of specific myofibrillar proteins such as titin and depolymerization of actin, but also appears to decrease the contribution that connective tissue makes to overall toughness. At 100 MPa, structures such as lysosomes are observed to alter shape and, as the pressure rises, disruption of membranes occurs. This disruption leads to the release of catheptic enzymes into the cytoplasm and absorption onto myofibrils. However, given the lack of evidence that these enzymes play a significant role in tenderization, these seem unlikely to contribute to the

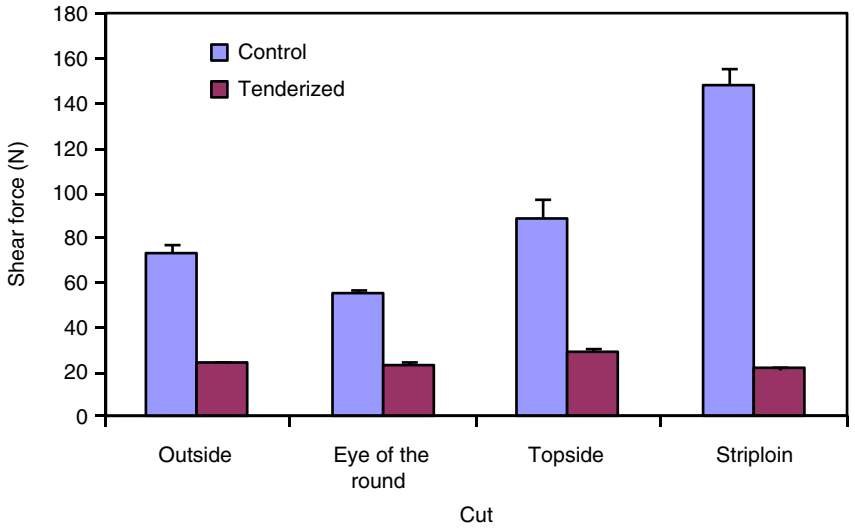

Figure 11 Effect of prerigor pressurization (~100 MPa) at 35 °C for 2 min on the shear force of beef cuts. Modified from MacFarlane, J.J., 1973. Prerigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. Journal of Food Science 38, 294–298.

beneficial effect that arises from this technique. A greater reduction in calpastatin activity than in μ-calpain at pressures above 100 MPa suggests that this enzyme could contribute to the degradation of muscle structure mediated through a rise in the level of free Ca^{2+}, although at pressures above 200 MPa μ-calpain appears to be inactivated. Detailed research examining the effect of this type of pressure on enzyme activity, physical changes in muscle structure, and the impact on tenderness (i.e., shear force) remains to be conducted before the exact mechanism is established. A variation on this approach is to combine pressure treatment with freezing. This involves cooling samples under pressure (200 MPa) to −20 °C and releasing pressure to reach equivalence with atmospheric pressure, causing supercooling of the sample.

Freeze–Thaw

Freezing muscle (i.e., at −20 °C) leads to shrinkage of muscle fibers mediated through a dehydration of cells and significantly increases the fragmentation of myofibrils. The extent of these effects will be influenced by the size of samples and the freezing rate, which impact on thermal gradients within the sample. Reductions of more than 20% in shear force values have been observed in muscle after freezing and thawing during the early stages of aging compared to muscle tested from the fresh state after the same period of aging. The effect will not be observed in 'aged' meat. There are likely to be several explanations for this reduction, which may well interact in a synergistic manner: proteolysis during the thawing phase, physical damage due to the freeze–thaw cycle, or diminished activity of enzyme inhibitors such as calpastatin.

See also: Chemical and Physical Characteristics of Meat: Palatability; Protein Functionality. Connective Tissue: Structure, Function, and Influence on Meat Quality. Conversion of Muscle to Meat: Aging; Color and Texture Deviations; Glycolysis. Cutting and Boning: Hot Boning of Meat. Electrical Stimulation. Muscle Fiber Types and Meat Quality. Tenderizing Mechanisms: Enzymatic

Further Reading

Bouton, P.E., Fisher, A.L., Harris, P.V., Baxter, R.I., 1973. A comparison of the effects of some post-slaughter treatments on the tenderness of beef. Journal of Food Technology 8, 39–49.

Claus, J.R., Wang, H.J., Marriott, N.G., 1997. Prerigor carcass muscle stretching effects on tenderness of grain-fed beef under commercial conditions. Journal of Food Science 62, 1231–1234.

Devine, C.E., Wahlgren, N.M., Tornberg, E., 1999. Effect of rigor temperature on muscle shortening and tenderisation of restrained and unrestrained beef m longissimus thoracics et lumborum. Meat Science 51, 61–72.

Duckett, S.K., Klein, T.A., Leckie, R.K., *et al.*, 1998. Effect of freezing on calpastatin activity and tenderness of callipyge lamb. Journal of Animal Science 76, 1869–1874.

Ferrier, G.R., Hopkins, D.L., 1997. Tenderness of meat cooked from fresh, frozen and thawed states. Proceedings 43rd International Congress of Meat Science and Technology, Auckland, pp. 560–561.

Geesink, G., Thompson, J., 2008. Utilising the 'Boa' stretching technology to improve the quality of hot boned striploins. Report No. RE-221941, Armidale, NSW, Australia: University of New England, School of Environmental and Rural Science. Available at: www.redmeatinnovation.com.au/innovation-areas/eating-quality/products/smartstretch/validation-trials (accessed 06.09.12).

Got, F., Culioli, J., Berge, P., *et al.*, 1999. Effects of high-intensity high-frequency ultrasound on ageing rate, ultrastructure and some physico-chemical properties of beef. Meat Science 51, 35–42.

Homma, N., Ikeuchi, Y., Suzuki, A., 1995. Levels of calpain and calpastatin in meat subjected to high pressure. Meat Science 41, 251–260.

Hopkins, D.L., Garlick, P.R., Thompson, J.M., 2000. The effect on the sarcomere structure of super tenderstretching. Asian-Australasian Journal of Animal Science 13 (Supplement C), 233.

Hwang, I.H., Devine, C.E., Hopkins, D.L., 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness – a review. Meat Science 65, 677–691.

Jeremiah, L.E., Gibson, L.L., Cunningham, B., 1999. The influence of mechanical tenderization on the palatability of certain bovine muscles. Food Research International 32, 585–591.
MacFarlane, J.J., 1973. Pre-rigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. Journal of Food Science 38, 294–298.
O'Sullivan, A., Korzeniowska, M., White, A., Troy, D.J., 2003. Using a novel intervention technique to reduce the variability and improve tenderness of beef longissimus dorsi. Proceedings 49th International Congress of Meat Science and Technology, Campinas, Brazil, pp. 513–514.
Petersohn, R.A., Topel, D.G., Walker, H.W., Draft, A.A., Rust, R.E., 1979. Storage stability, palatability and histological characteristics of mechanically tenderized beef steaks. Journal of Food Science 44, 1606–1611.
Sakata, R., Oshida, T., Morita, H., Nagata, Y., 1995. Physico-chemical and processing quality of porcine m. longissimus dorsi frozen at different temperatures. Meat Science 39, 277–284.
Solomon, M.B., Long, J.B., Eastridge, J.S., 1997. The Hydrodyne: A new process to improve beef tenderness. Journal of Animal Science 75, 1534–1537.
Toohey, E.S., van de Ven, R., Thompson, J.M., Geesink, G.H., Hopkins, D.L., 2012. SmartStretch™ Technology. V. The impact of SmartStretch™ technology on beef topsides (m. semimembranosus) meat quality traits under commercial processing conditions. Meat Science 92, 24–29.
Taylor, J.M., Hopkins, D.L., 2011. Patents for stretching and shaping meats. Recent Patents on Food, Nutrition & Agriculture 3, 91–101.
Taylor, J.M., Toohey, E.S., van de Ven, R., Hopkins, D.L., 2012. SmartStretch™ technology. IV. The impact on the meat quality of hot-boned beef rostbiff (m gluteus medius). Meat Science 91, 527–532.
Wheeler, T.L., Koohmaraie, M., 1994. Prerigor and postrigor changes in tenderness of ovine longissimus muscle. Journal of Animal Science 72, 1232–1238.

Relevant Website

www.mla.com.au/off-farm
Meat & Livestock Australia.

TENDERNESS MEASUREMENT

RW Purchas, Massey University, Palmerston North, New Zealand

Glossary

Intrinsic determinant A feature within the meat that plays a role in determining the level of specific meat quality characteristic such as tenderness.

Proteolysis The chemical reaction that results in breakdown of proteins into smaller parts as peptides or individual amino acids.

Sarcomere length The distance between adjacent Z discs in a muscle fiber or fibril.

Shear force The force required to shear or cut through a piece of meat, that is used as an index of meat tenderness.

Tenderometer An instrument designed to measure the tenderness of meat.

Introduction

Meat tenderness appears at first glance to be simply a measure of the biting effort required. Unfortunately, however, this is an oversimplification as careful mechanical measures of such forces seldom account for more than approximately 60% of the variation in tenderness as assessed by trained panels of people. This is despite the fact that many mechanical devices have been developed, and a number of these are widely used in studies of factors affecting meat tenderness. The inconsistencies between mechanical and sensory assessments of tenderness are at least partly because a consumer's sensory perception of meat tenderness is often influenced by components other than the dominant one of the simple biting force required.

In this article, approaches to the measurement of meat tenderness are outlined, and some terms used to describe tenderness are described first. The main characteristics within meat that play a role in determining its tenderness, the so-called 'intrinsic determinants' of tenderness, are discussed because they influence the extent to which a particular measurement method will be appropriate for a specific situation. Approaches to the measurement of meat tenderness outlined here include primarily the use of mechanical devices such as the Warner–Bratzler shear device, but also the use of descriptive/trained and consumer sensory panels, and some other laboratory methods.

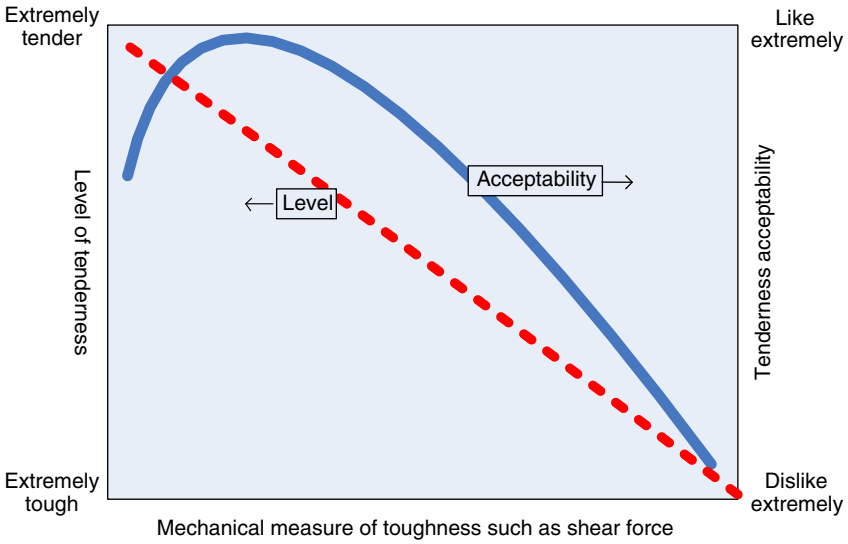

Figure 1 A diagrammatic illustration of how the relationship between sensory tenderness and mechanical measures of tenderness differ depending on whether the sensory panelists score on an acceptability scale (blue solid line), as for a consumer panel, in which case extremely tender meat may be less acceptable, or a level-of-tenderness scale (red dashed line) as used by a trained panel. Note that the closeness of the relationship between mechanical measures and sensory assessments of tenderness is not generally as close as implied by these lines.

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Table 1 Some intrinsic determinants of meat tenderness. An intrinsic determinant is a structural or metabolic characteristic within the meat that can be responsible for differences in meat tenderness. The suitability of methods for measuring differences in tenderness for any group of meat samples will depend on which of these or other determinants are responsible for the differences

Intrinsic determinant	*Relationship with tenderness*	*Relative importance and situations where it might be particularly important*
1. Concentration of connective tissue. This tissue consists primarily of the fibrous protein collagen but will also include some elastin and other substances.	Other things being equal, meat containing more connective tissue will be less tender, but this will depend on the nature of the connective tissue and the cooking conditions.	Of medium importance. More important for comparisons (1) between different muscles, (2) between meat samples from older animals or between samples from animals varying widely in age, and (3) when cooking conditions have been mild (i.e., most collagen will not be not dissolved when final internal temperatures are less than approximately 60 °C).
2. The extent of cross-linking between peptide chains within collagen molecules in meat.	Other things being equal, meat containing collagen with less cross-links will be more tender because such collagen will dissolve to form gelatin faster and at lower temperatures.	This is an important source of variation in tenderness if samples vary widely in the level of cross-linking, as might be expected if they are from animals varying widely in age (cross-linking increases with increasing age). It is also more important for fast cooking methods such as frying because the solubilization of collagen is to some extent time dependent. The type of cross-links may influence the extent to which tenderness is affected.
3. The ultimate pH (pH_{ult}) of the meat, as determined primarily by the amount of lactic acid present, which, in turn, is a function of the glycogen levels at the time of slaughter.	Other things being equal, an increase in pH_{ult} from approximately 5.5 (the normal pH_{ult} for meat from a well-fed and unstressed animal) to 6.1 (the peak of toughness, 5.8–6.1) will often lead to tougher meat. With further increases from approximately 6.2 to 7.0, however, the meat becomes tenderer with other deficiencies.	An important determinant of tenderness in some situations where the variability of pH_{ult} is high. In many situations it is of low importance because there is little variation in pH_{ult} between animals.
4. The extent to which muscle is contracted when it sets in rigor mortis, as commonly assessed by the average sarcomere length.	Other things being equal, a greater degree of contraction (shorter sarcomere lengths) will be associated with tougher meat. This relationship is not linear and muscle shortened by more than approximately 40% of its resting length will actually be tenderer due to structural damage.	Very important as a determinant of tenderness under cold shortening conditions when low temperatures prerigor induce muscle contraction. Thaw shortening of meat frozen before the onset of rigor mortis can also lead to very tough meat. If shortening is prevented in some way, this is not an important determinant.
5. The extent to which certain proteins in meat are broken down postmortem through the action of proteolytic enzymes such as the calpains and cathepsins.	Other things being equal, a greater degree of protein breakdown is associated with tenderer meat, but the extent of this effect will depend on the specific proteins that are cleaved.	An important determinant of the extent to which tenderness improves with aging of meat at temperatures above freezing. It also accounts for some genetic differences in tenderness through varying levels of either proteolytic enzymes (e.g., the calpains) and/or their inhibitors (e.g., calpastatin).
6. The concentration of intramuscular fat (marbling) in muscle. Levels vary from less than 2% in many lean meat products through approximately 3–4% when the marbling first becomes clearly visible, up to levels of more than 30% in very heavily marbled products.	Other things being equal, more highly marbled meat will be somewhat tenderer. The reasons for this are unclear but probably include the fact that the meat will tend to be more juicy, muscle fibers and connective tissue are diluted by fat, and a reduced likelihood of prerigor shortening.	This determinant seldom accounts for more than 10% of the variation in tenderness but is more likely to be more important when there is a wide variation in marbling levels.

Descriptive Terms and Scales

When meat tenderness is measured by a sensory panel, higher values usually indicate more tender meat either on an acceptability (hedonic) scale or on a level of tenderness scale. However, these two scales do not necessarily correspond, as, although a lower acceptability of tenderness is usually due to a greater toughness, this is not always the case as meat can also be less acceptable because it is too tender to the point of being mushy and textureless, as illustrated in Figure 1. When measured mechanically, tenderness is usually expressed in terms of some measure of resistance such as shear force in kilogram (kgF) or newtons (N), so that, in contrast to sensory scores, low values indicate more tender meat. Being aware of these distinctions is crucial when interpreting published data on meat tenderness. Unfortunately, there is no harmonization of the shear force values between the various measurement devices. The Warner–Bratzler values are

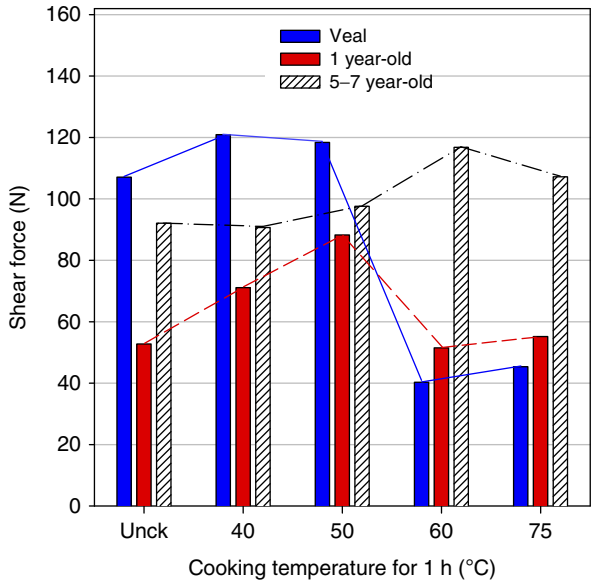

Figure 2 Toughness (as assessed by Warner–Bratzler shear force) of uncooked meat and meat cooked to temperatures from 40 to 75 °C for samples of deep pectoral muscle from veal calves, yearling steers, and 5–7 year old cows. Veal meat was tougher at temperatures <50 °C because of higher collagen concentrations but was more tender at higher temperatures because the collagen present was more readily solubilized on heating. Adapted with permission from Bouton, P.E., Harris, P.V., 1972. The effects of cooking temperature and time on some mechanical properties of meat. Journal of Food Science 37, 140–144.

approximately 0.7 times those of the MIRINZ tenderometer, but the relationships with other devices is not standardized. This is a limiting factor in international usage and comparisons of processing.

Meat texture is a term that is sometimes used as a synonym for tenderness, which can be confusing as it is also used to denote the appearance of fineness or coarseness of meat. When used as a descriptor of palatability rather than appearance, the term will often have a broader meaning than tenderness and may include assessments of features such as mealiness, coarseness, cohesiveness, juiciness, chewiness, and fattiness as well as the force required to bite through a sample.

Intrinsic Determinants of Meat Tenderness

Muscle is a complex and sophisticated biological tissue. It is, therefore, not surprising that there are many structural and metabolic characteristics of meat that can affect its tenderness at the time of consumption or measurement following cooking. Some of these features, which are termed the intrinsic determinants of tenderness, are listed in Table 1. For two similar meat samples any difference in tenderness will sometimes be totally attributable to only one intrinsic determinant, but usually several of those listed in Table 1, and possibly others as well, are involved. This background on intrinsic determinants is relevant to the measurement of tenderness because the suitability of some methods will depend on the extent to which the various determinants are responsible for variation in tenderness.

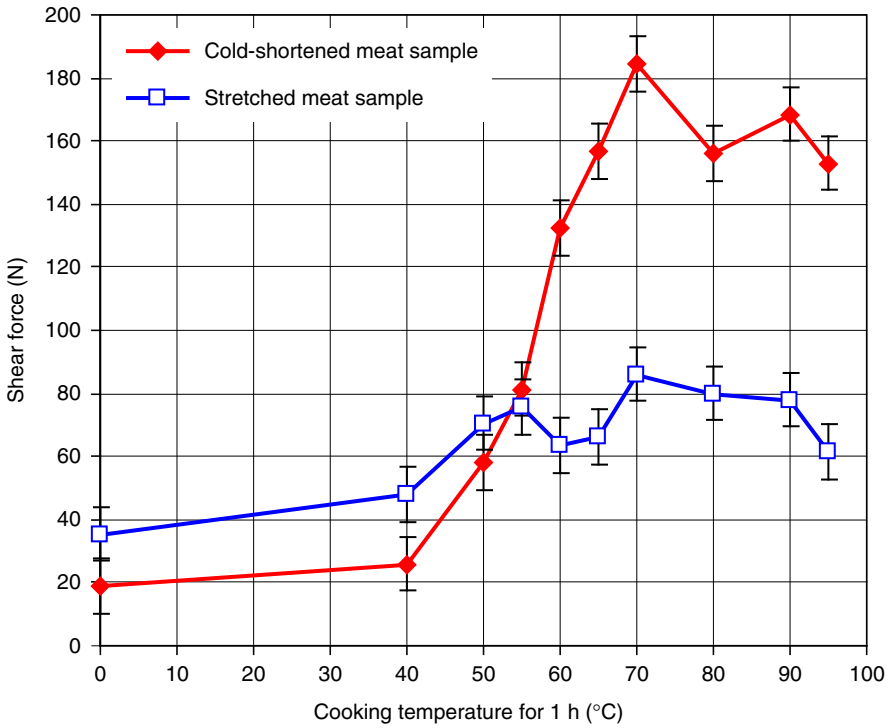

Figure 3 Results showing a large effect of cold shortening on changes in Warner–Bratzler shear force with increasing cooking temperature such that the toughening effect of cold shortening was only apparent after cooking to 60 °C or more. Samples were of beef semitendinosus muscle. Data adapted from Bouton, P.E., Harris, P.V., Shorthose, W.R., 1976. Dimensional changes in meat during cooking. Journal of Textural Studies 7, 179–192.

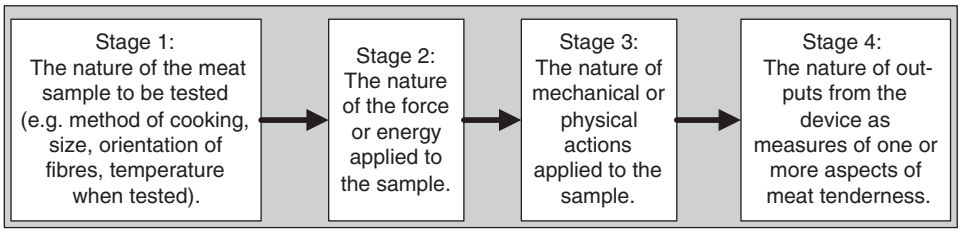

Figure 4 Four stages during any mechanical method of meat tenderness measurement when the nature of the procedure followed may influence the measurements obtained.

Table 2 Examples of mechanical actions used to obtain measures of meat tenderness. Because of variation in tenderness within a muscle, it is usual practice to make from 6 to 12 measurements on cores (1 or 2 per core) from the same steak to obtain a satisfactory overall measurement

Mechanical action	*Examples of devices and the aspects of tenderness measured*
1. A shearing action where the force required to cut through a meat sample is measured by the movement of metal blades relative to each other in a scissors-type action. Measurements made by the so-called shear devices often encompass tensile and compression stresses as well as shear stresses.	The most widely used device in this category is the Warner–Bratzler (WB) shear, which measures the force required to pull a cutting blade with rounded edges between two metal plates when a cylindrical core of meat is placed within a vee-shaped opening in the blade (**Figure 5(a)**). Alternative forms of this basic system involve the replacement of the vee-shaped shearing edges with a straight horizontal edge, and the use of samples that are cuboidal rather than cylindrical. The use of cuboidal samples and a straight edge produces force-deformation curves with higher peak forces. A good example is the slice shear force device that uses a 50 mm square blade to shear through a slab of cooked meat that is 10 mm thick and 50 mm wide and that has been carefully prepared so that the shear is at right angles to the fiber direction. Although WB shear forces primarily reflect the myofibrillar contribution to tenderness, the difference between peak force and initial yield also provides an indication of the connective tissue contribution.
2. A biting action where the force required to bite through a sample is recorded. The biting part is usually in the form of one or two blunt metal that are vee-shaped 'teeth' of the same width as the sample that they bite through (**Figure 5(b)**).	The Volodkevich and MIRINZ tenderometers are good examples of instruments based on this principle, although a number of others have been produced. Results obtained are usually highly correlated with WB shear values and reflect primarily the myofibrillar contribution to tenderness, but differences in tenderness due to other determinants (e.g., connective tissue or marbling fat) also contribute. The 'slice shear force' system and the Meullenet–Owens razor shear are essentially biting-type actions, with a sharper 'tooth' in the latter case.
3. A compressing action where a plunger is pushed into the meat sample which may or may not be constrained on two sides (**Figure 4(c)**). The maximum compression force is usually measured when the sample is compressed to a specified proportion (commonly 80%) of its initial thickness.	A number of devices based on measures of compression have been developed with variation in the nature of the plunger and the way the meat sample is held. Compression tests are well suited to provide information on rheological parameters. Compression tests on uncooked meat have provided useful information on subsequent cooked-meat tenderness in some studies. These tests appear to reflect the connective tissue contribution to tenderness to a greater extent than shear or biting tests.
4. A penetrating action where the force required to penetrate the meat samples is measured.	This approach has been widely tested, usually with a bank of pins of some sort, but the results have been variable and this approach has not been widely used.
5. Measures of tensile strength or the force required to stretch and break a meat sample by pulling in a direction either parallel to the fibers or perpendicular to the fibers.	The force required to separate muscle fibers perpendicular to their length is indicative of the connective tissue contribution to tenderness and is referred to as 'adhesion.' Other tensile strength measures are not commonly used.
6. Measures of the energy (usually electrical energy) required to mince a sample of meat under standard conditions.	Moderately good correlations with sensory tenderness have been shown, but the approach is rarely used.
7. Measures of the effects of a standard homogenizing treatment in terms of the size of the fragments produced. Commonly termed the myofibrillar fragmentation index (MFI).	This is primarily a measure of the fragility of the muscle fibers and is used as an index of the past proteolytic activity within the meat. The fragment size may be measured by levels of turbidity, by filtration, or by direct measures of fragment size using a microscope and often with the aid of an image analysis program.

Measurement of Tenderness of Cooked versus Uncooked Meat

Almost all meat is cooked before consumption, so tenderness measurements are best made on samples that have been cooked in the way the meat is normally eaten. This is the usual practice, but it would also be very useful if meat in an intact carcass could be assessed for tenderness (preferably in a non-intrusive manner) so that the information could be used as a grading criterion and premiums be given for carcasses yielding more tender meat. Unfortunately, the accurate measurement or prediction of cooked-meat tenderness using uncooked meat is difficult for two main reasons. First, changes in tenderness with cooking vary depending on the temperature and time involved and on characteristics such as collagen solubility and the ratio of collagen to muscle fibers in the meat. This is because the collagen contribution to tenderness decreases with increasing temperature as an increasing proportion is converted into insoluble gelatin, whereas the contribution of muscle fibers increases as the proteins within the fibers denature (see Figure 2). Results in Figure 2 illustrate this effect with the shear value for veal being highest at lower temperatures before collagen dissolves, but lowest at higher temperatures because collagen in veal is more soluble. Shear force can potentially be predicted by using noninvasive near-infrared spectroscopy (NIR) on uncooked meat where it has been shown to perform as well as by the reference method – it measures chemical changes as the meat tenderizes. However, NIR tenderness evaluations will be most accurately close to consumption and any prediction will, therefore, need to take account of both the time of measurement and cooking procedures.

Second, some factors, such as cold shortening, have a major effect on the tenderness of cooked meat but no effect or even the opposite effect on uncooked meat. This is illustrated in Figure 3, where the toughening effect of cold shortening only became apparent when samples of beef semitendinosus were cooked to temperatures greater than 55 °C.

Thus, it is only when tenderness differences are due to intrinsic determinants that have similar effects on cooked and uncooked meat that direct measures of tenderness on uncooked meat will be particularly useful. Because of variable cooking effects, it is important in tenderness research experiments that cooking methods be standardized with respect to sample size to be cooked, cooking temperature, final internal temperature, and cooking time. Cooking methods should also be similar to those commonly used for the type of meat involved. Cooking methods differ to some extent between meat research laboratories, which means that the direct comparison of results need to be made with care, but the fact that a range of methods are used increases the chance of important interactions between cooking parameters and other factors affecting tenderness being revealed.

Mechanical Methods of Tenderness Measurement

Many devices have been developed to mechanically or physically measure the forces required to disrupt meat in some way. They can be categorized according to the way the force is applied, the type of action (biting, shearing, compressing, etc.), the way the meat is prepared and orientated within the device, and the way the measurements are expressed (Figure 4).

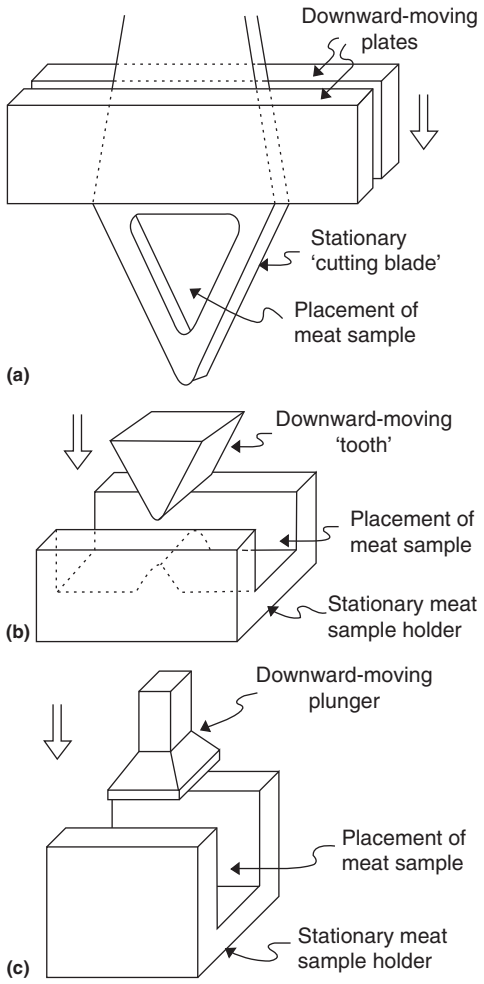

Figure 5 Diagrams showing three examples of types of mechanical action used to measure meat tenderness: (a) shearing; (b) biting; and (c) compressing. For some devices, the part shown as moving will be stationary and vice versa.

Most devices have a constant rate of movement (the crosshead speed) with changes in the force required being measured with movement through the sample to produce a force-deformation curve. For some devices, a sinusoidal change in rate of movement is employed. Alternatively, the force is increased at a constant rate, so that a curve of distance through the sample against force or time is produced. In both cases, the output most commonly reported is the maximum or peak force required to complete the test. Outputs in addition to the peak force that are sometimes reported include:

1. The area under the curve as a measure of the total work done.
2. An initial yield force represented by a shoulder or peak on the rising side of the force-deformation curve. This has been shown to be a useful indicator of the myofibrillar contribution to tenderness.

Table 3 Important aspects of the measurement of meat tenderness by sensory panels are illustrated here by comparing features of a consumer panel and a 'descriptive,' 'analytical,' or 'trained' panel

Feature	*Consumer panel*	*Descriptive or analytical panel*
1. Broad aim	To assess the acceptability of the tenderness of the samples for a specified population of meat consumers.	To determine whether differences in tenderness or certain components of tenderness differ between groups of samples.
2. Number of panelists	Relatively large numbers are required in order to obtain a representative sample of the population. A minimum of 6 would be needed for a trained panel, and absolute minimum of 50 for a consumer panel, with 100 more preferable.	Relatively low numbers may be used, especially if panelists are highly selected and trained.
3. Selection of panelists	Selection should be such that a representative sample of the population of interest is obtained.	Panelists are commonly selected on their ability to detect small differences in the parameters of interest in a repeatable and consistent manner.
4. Training of panelists	Training is minimal or nonexistent as it is important that panelists represent typical consumers.	Training with meat samples of the type to be assessed is important so that all panelists are conversant with the terms used and the ranges that are more likely to be encountered.
5. Site where samples are assessed	The site should be similar to the environment where meat is normally consumed such as in the home or at a commercial eating place.	Tasting is carried out under controlled conditions so that effects of extraneous variables (lighting, smells, other people, etc.) are minimized.
6. Methods of sample preparation	Methods should match closely those normally used by consumers, although detailed instructions on preparation methods will normally be given.	Methods are closely controlled and designed to facilitate the acquisition of answers to the questions being addressed.
7. Number of samples and complexity of the questions asked	A small number of samples and of questions per sample. Questions should be expressed in terms of tenderness acceptability or desirability rather than the level of tenderness or related characteristics. Acceptability scales are referred to as hedonic scales.	The number of samples and the number and complexity of the questions can be greater because the panelists are trained. Panelists should not be expected to provide useful acceptability assessments. An example of characteristics covering a range of tenderness components is given in **Table 4**.

3. The difference between the peak force and initial yield force as an indication of the connective tissue contribution to tenderness.
4. The peak force or work required to pass a certain proportion of the way through or into a meat sample (used mainly with compression tests where compression by 20% or 80% is often used).
5. Rheological parameters such as stress and elasticity can be calculated when more than one cycle of actions are carried out for some forms of measurement such as by compression.
6. A measure of the electrical power required can be used when a meat sample is minced under standard conditions.

Examples of types of mechanical action that have been used to obtain measures of meat tenderness are outlined in **Table 2**. This is not an exhaustive list and some devices involve more than one action, three examples of which are shown in **Figure 5**.

For most mechanical measures of tenderness, the sample is arranged so that the force to cut or shear across muscle fibers is measured, but additional, and sometimes quite distinctly different, information is obtained when the sample is turned by 90° either horizontally or vertically from this usual orientation.

Sensory Methods of Tenderness Measurement

The use of groups of people (sensory panels or taste panels) to assess the tenderness of meat samples is the ultimate test because tenderness has to be defined in terms of people's perceptions. Despite this, sensory methods are not used as widely as mechanical methods because of variation between people (even after a period of training), and because the approach is slower, more expensive and requires larger amounts of meat. Many types of sensory panel have been used, but their main features are summarized in **Table 3** by comparing a consumer panel with a descriptive (also known as an analytical, trained, or laboratory) panel. The items listed in **Table 4** illustrate the multidimensional nature of meat tenderness and hence the limitations of simple mechanical measures, although it should be noted that in that example the closely related characteristics of both tenderness and texture were being assessed.

Within a sensory panel, there are several alternative ways in which panelists may record their assessments, including:

1. On a category scale of 5–9 steps, with each step given a specific description (e.g., a scale from extremely tender to extremely tough). A simplification is where only some of the steps are described.
2. A line scale, usually 100 or 150 mm in length, with no steps but with descriptors at the extremes and sometimes at the midpoint. Panelists mark a point on the line for each sample.
3. An open-ended line scale with a single anchor point that is described. This approach is referred to as 'magnitude estimation' and is a form of ratio scaling where the tenderness of one control sample is assigned a specific value and all others are compared with the control.

Table 4 The complexity of tenderness as a characteristic of meat is illustrated by the examples in this table of characteristics of lamb texture that panelists were asked to assess in a study investigating the texture and tenderness of lamb meat

Aspect of tenderness or texture	*Subcategories within the aspect*
1. Elasticity on partial compression	
2. First bite properties	a. Compressibility
	b. Moisture release
	c. Fat amount
	d. Fattiness
	e. Cohesiveness
3. Mastication properties	a. Number of chews
	b. Chewiness
	c. Rate of breakdown
	d. Fibrousness
	e. Coarseness of fiber
	f. Moisture release
	g. Moisture absorption
	h. Cohesiveness
	i. Fattiness
	j. Fat amount
	k. Uniformity
	l. Density
	m. Connective tissue (6 options)
	n. Connective tissue amount
4. After-feeling properties	a. Ease of swallowing
	b. Mouthcoating type (2 options)
	c. Mouthcoating amount
	d. Fat amount
	e. Particle type (15 options)
	f. Particle amount
	g. Tooth packing
5. Amplitude (an overall impression)	

Source: Adapted from Jeremiah, L.E., 1988. A comparison of flavour and texture profiles for lamb leg roasts from three different geographical sources. Canadian Institute of Food Science and Technology Journal 21, 471–476.

4. Two or more samples are ranked with respect to specific characteristics in a difference test. Panelists may also record the size of the difference between adjacent samples in a ranked list. The triangle test is a special form of difference test where panelists identify the different sample in a set of three, two of which are the same.

Examples of ways in which sensory panels can be made more objective include:

1. Having the panelists count the number of chews required before meat is swallowed.
2. Using electromyography to record the action of the masseter (cheek) muscles during chewing.
3. Using time-intensity methodology whereby the panelist records (with the aid of a computer mouse) the change in tenderness as the meat is consumed.

Other Methods of Tenderness Measurement or Prediction

Generally, methods other than those employing sensory panels or mechanical methods involve measuring characteristics associated with only one or two intrinsic determinants of tenderness (Table 1) and hence will only be useful when only those determinants are responsible for the tenderness differences of interest. Thus, the value of these methods tends to be limited to particular situations. Examples are as follows:

1. Intramuscular fat content: Relationships with tenderness are not close but are almost always positive as explained in Table 1 (item 6). Although intramuscular fat levels are most often measured by solvent extraction, they can also be assessed reasonably accurately by NIR spectroscopy or by visual appraisal or VIA below.
2. Sarcomere length: Shorter sarcomere lengths, which can be measured by microscopy or laser diffraction, tend to be associated with less tender meat as explained in Table 1 (item 4).
3. Collagen content: Higher concentrations of collagen, as assessed by the hydroxyproline content in meat, will often mean lower levels of tenderness (Table 1, item 1).
4. Collagen solubility: High collagen solubility, as assessed by the extent to which it is dissolved by a standard heating treatment, tends to be associated with more tender meat (Table 1, item 2).
5. Ultimate muscle pH: Ultimate pH is either measured by a pH meter and probe or can be predicted from glycogen levels at slaughter. Its relationship with tenderness is outlined in Table 1 (item 3).
6. Video image analysis (VIA): Several characteristics can be measured by VIA including color, marbling fat, and connective tissue content, so the possibility of predicting tenderness exists.
7. Meat color: The relationship with tenderness is not close and probably arises at least in part from the link between color and pH. Meat color may be assessed subjectively with the aid of standard colors, with a reflectance spectrophotometer to give L^*, a^*, and b^* values or by VIA.
8. NIR spectroscopy: This technique will also provide a measure of a number of characteristics of meat when a cut surface of uncooked meat is scanned, including fat content and color as well as meat tenderness. The measurements can be made very quickly and preliminary results have been promising when it is used as a means of sorting carcasses or cuts of meat into two categories. For example, the terms 'predicted tender' and 'not predicted tender' have been used for the two categories in some studies.
9. SDS-PAGE and Western blotting: This technique can be used to quantitate the proteolysis of muscle proteins, which is usually associated with more tender meat. The proteins which are usually targeted are troponin-T, which breaks down to a 30 kDa protein, titin, which breaks down from a singlet to a doublet or triplet, and nebulin. Other muscle proteins have also been used as indicators of proteolysis.
10. Myofibrillar fragmentation index (MFI): This technique can also be used to quantitate the proteolysis of muscle proteins, which is usually associated with more tender meat. The procedure involves homogenizing the meat in a

standard way in buffers and measuring the size of the fragmented myofibrils to get an index.

See also: Carcass Composition, Muscle Structure, and Contraction. Chemical and Physical Characteristics of Meat: Chemical Composition; Palatability. Conversion of Muscle to Meat: Aging; Rigor Mortis, Cold, and Rigor Shortening. Cooking of Meat: Cooking of Meat. Measurement of Meat Quality: Measurements of Water-holding Capacity and Color: Objective and Subjective. On-Line Measurement of Meat Quality. Prediction of Meat Attributes from Intact Muscle Using Near-Infrared Spectroscopy. Sensory and Meat Quality, Optimization of. Tenderizing Mechanisms: Chemical; Enzymatic; Mechanical

Further Reading

AMSA, 1995. Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat. Chicago: American Meat Science Association.

Bouton, P.E., Harris, P.V., 1972. The effects of cooking temperature and time on some mechanical properties of meat. Journal of Food Science 37, 140–144.

Bouton, P.E., Harris, P.V., Shorthose, W.R., 1976. Dimensional changes in meat during cooking. Journal of Textural Studies 7, 179–192.

Chrystall, B., 1994. Meat texture measurement. In: Pearson, A.M., Dutson, T.R. (Eds.), Quality Attributes and Their Measurement in Meat, Poultry and Fish Products. Advances in Meat Research, vol. 9. London: Blackie Academic, pp. 316–336.

Harris, P.V., 1976. Structural and other aspects of meat tenderness. Journal of Texture Studies 7, 49–63.

Jeremiah, L.E., 1988. A comparison of flavour and texture profiles for lamb leg roasts from three different geographical sources. Canadian Institute of Food Science and Technology Journal 21, 471–476.

Lawless, H.T., Heymann, H., 1998. Sensory Evaluation of Food. Principles and Practice. New York: Chapman & Hall.

Lepetit, J., Culioli, J., 1994. Mechanical properties of meat. Meat Science 36, 203–237.

Prieto, N., Roehe, R., Lavin, P., Battern, G., Andres, S., 2009. Application of near infrared spectroscopy to predict meat and meat products quality: A review. Meat Science 83, 175–186.

Stephens, J.W., Unruh, J.A., Dikeman, M.E., *et al.*, 2004. Mechanical probes can predict tenderness of cooked beef longissimus using uncooked measurements. Journal of Animal Science 82, 2077–2086.

Tornberg, E., 1996. Biophysical aspects of meat tenderness. Meat Science 43, S175–S191.

Wheeler, T.L., Vote, D., Leheska, J.M., *et al.*, 2002. The efficacy of three objective systems for identifying beef cuts that can be guaranteed tender. Journal of Animal Science 80, 3315–3327.

THERMOPHYSICAL PROPERTIES

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Glossary

Density The ratio of an object's mass to its volume.
Enthalpy The total heat content of an object, often expressed per unit mass.
Freezing temperature The temperature at which a material starts to freeze.
Heat capacity The derivative of enthalpy with temperature, under either constant pressure or constant volume conditions.
Latent heat The amount of heat that is released or absorbed when a material changes phase between solid and liquid (melting/freezing), liquid and gas (boiling/condensing), or solid and gas (subliming/deposition).
Moisture diffusion The movement of water through a solid.

Introduction

The thermophysical properties of meat products are relevant for many purposes, including:

- calculating chilling, freezing and cooking times, and heat loads;
- designing drying, salting, and other value-adding processes;
- modeling meat processing operations;
- calculating yields and mass balances in meat processing; and
- calculating behavior during transportation.

The quality and microbial status of a meat product are usually strongly dependent on the thermal and physical processes to which the meat is subjected, so the ability to model thermal and physical processes accurately is important when one needs to calculate the meat quality attributes and the microbial growth that may result from these processes. In turn, accurate knowledge of the thermophysical properties of meat products and the materials typically found in meat processing operations is essential to the accurate modeling of the thermal and physical processes.

Typical values and simple methods of estimating these properties are provided in this article but when accurate values are required for process design (for instance), reference should be made to the books and articles that report data for the specific materials to be used.

The SI unit for temperature is Kelvin, K, but the degree Celsius, °C, is the same size and is often more convenient for the temperatures of importance to meat science, so both temperature units are used in this article.

Density

The density of a substance is the ratio of its mass to its volume, as shown in eqn [1].

$$\rho = \frac{m}{V} \quad [1]$$

In this equation, ρ is the density in kg m^{-3}, m is the mass of the substance in kg, and V is the volume of the substance in m^3.

The specific volume of the substance, v, in m^3 kg^{-1}, is defined as the reciprocal of the density, as shown in eqn [2].

$$v = \frac{1}{\rho} \quad [2]$$

The concept of density is not as straightforward as one might imagine at first, because it depends on both the mass and volume of the substance. Mass is fairly simple and it can be measured with considerable accuracy but the meaning of a substance's volume is more complex.

The most important issue in measuring volume is whether one should or should not include any void space that may be around or within the substance in which one is interested. A cut of fresh meat would not be expected to contain much void space within itself. Its volume could, therefore, be measured by (for instance) measuring the amount of liquid that it displaces when it is completely submerged. Even then, meat will typically absorb some moisture over time and so the amount of liquid displaced will reduce as this absorption takes place. This effect is particularly important for pieces of meat with high ratios of surface area to volume, such as poultry or offal.

A package containing meat could contain a substantial amount of void space, often air, and one must then take considerable care. The appropriate volume to use when calculating density will depend on the purpose for which it is required. For example, if one was interested in the mass of packaged meat that one could fit into a shipping container, one would use the volume of the whole package and therefore calculate the figure known as the 'bulk density.' Alternatively, when one is calculating the chilling time for the package, one often needs to convert the thermal properties from a

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volumetric to a mass basis. In this case, one should use the volume of the meat contained in the package.

Finally, some meat products such as free flow frozen meat pieces can contain significant amount of air within their overall dimensions, so the relevant density will depend on whether the product is being heated or cooled as individual pieces or as a collection of pieces in a package.

Fresh meat comprises mostly water, protein, fat, and, sometimes, bone. Dry protein has a density somewhat greater than that of water and dry fat has a density somewhat less than that of water. Many products that are predominantly made of lean meat, therefore, have a density close to that of water, i.e., between approximately 950 and 1080 kg m^{-3} in the range 0–100 °C. If it is necessary to estimate the density of a meat product with greater accuracy, the volume of the whole product is usually equal to the sum of the volumes of the components that make up that product. Exceptions to this rule apply when the components dissolve in each other (as with alcohol and water, for instance), but this does not usually apply for the components making up meat products. Thus, if one knows the composition of a meat product, one can estimate its density as shown in eqn [3].

$$\frac{1}{\rho} = \sum_{i=1}^{n} \frac{x_i}{\rho_i} \qquad [3]$$

In eqn [3], x_i is the mass fraction of the ith component, ρ_i is the density of the ith component in kg m^{-3}, and n is the number of component materials in the meat product.

Table 1 shows density values for some common components of meat products at typical processing temperatures.

The densities of most materials vary with temperature, generally decreasing as the material's temperature increases. For water-based materials, such as meat, the density of the water component is affected by its hydrogen-bonding behavior, with the result that the density of liquid water reaches a peak at approximately 4 °C. Similarly, although the densities of most materials increase when they freeze, water is again unusual and ice is less dense than liquid water under most conditions.

The density of a meat product below its freezing temperature can be estimated using eqn [3], but it is necessary to include both water and ice as components and also to estimate the fraction of water that is still liquid and the fraction that has become ice from the temperature, T, of the meat. An approximate value of the ice fraction at a given temperature, below the initial freezing temperature, is given by eqn [4], where T_f is the initial freezing temperature of the meat in °C, x_{ice} is the mass fraction of ice, and x_{water} is the total mass fraction of water (frozen and unfrozen).

Table 1 Approximate density values for some meat components at typical food processing temperatures

Component	*Density (kg m^{-3})*
Water	1000
Ice	920
Protein	1400
Fat	900–950
Air	1.29 (at 0 °C)

$$\frac{x_{ice}}{x_{water}} = 1 - \frac{T_f}{T} \qquad [4]$$

Equation [4] overestimates the ice fraction when the temperature T is low (typically −20 °C and below) because it does not consider the bound water in the food, which never freezes no matter how low the temperature becomes.

Freezing Temperature

The water content of meat is in the form of a complex solution with many solutes present in different concentrations. The presence of these solutes depresses the initial freezing temperature of the solution (and, hence, of the meat) below the freezing temperature of pure water (0 °C).

The freezing temperature of any solution is depressed from the freezing temperature of the pure solvent. If one assumes that the solute is insoluble in the solid solvent, the freezing point of the solution is determined by the equilibrium of chemical potentials between the pure solid and the liquid solution. The details of the mechanism are described in physical chemistry texts. In simple terms, when the first ice crystal forms in the meat, it comprises almost pure ice, thereby causing the diffusion of the solutes into the remaining solution, increasing the concentration of that solution, and further depressing the solution's freezing temperature. As additional ice crystals form, the concentration of the remaining liquid increases further and its freezing temperature is depressed still further. This results in meat freezing progressively over a range of temperatures.

Meat has a freezing temperature range, depending on the concentration of the water solution at any point in time, so the expression 'freezing temperature' usually refers to the initial freezing temperature at which ice crystals first begin to form. Even this temperature can be difficult to determine, because the formation and growth of ice crystals requires that there be sufficient 'undercooling' or 'supercooling' below the initial freezing temperature to initiate nucleation and drive crystal growth. At high freezing rates (as may be found in cryogenic freezing) or in some special circumstances, the amount of undercooling can be many degrees Celsius. At typical meat industry freezing rates, however, undercooling amounts are typically no more than a few tenths of a degree Celsius, and can, therefore, be neglected. The need for undercooling means, among other things, that it is possible to store meat in an environment that is a little colder than its freezing temperature without ice crystals beginning to form, in order to maximize its storage life while maintaining a chilled state.

The initial freezing temperature of meat depends on its composition, but −1 °C is often assumed to be representative for lean meat with a high (approximately 70–80%) moisture content. Meat products with higher fat content, added components, such as salt, and dried meats can have freezing temperatures several degrees lower than this.

Compositional approaches based on the average molecular weight of solutes have been found to estimate the initial freezing temperature of meat with an average absolute error of approximately 0.25 °C. If a more precise estimate of the initial

freezing temperature is required, the data must be obtained by measurement.

Glass Transition

A glass is a metastable noncrystalline solid with a very high viscosity. This state can be formed when a liquid is cooled very quickly and its molecules slow their movement to the point where they cannot reach the preferred crystallized state. The characteristic temperature, below which a glass is formed, is known as the 'glass transition temperature,' or T_g'. Because the rate of deterioration of a food product is often determined by the diffusion of solutes through the product, the ability to store products near or below T_g', where this diffusion rate is very slow, could be expected to greatly extend the storage life of a product. The absence of ice crystals in a glassy product is also advantageous because the growth of ice crystals can damage cell walls and cause solute diffusion that can degrade the quality of the product over time, or when it is thawed.

T_g' is typically measured using a differential scanning calorimeter, but it is difficult to measure accurately. Values have been reported by some researchers to be in the range $-11\,^{\circ}\mathrm{C}$ to $-13\,^{\circ}\mathrm{C}$ for mackerel, cod, trout, mutton, and beef, and $-13\,^{\circ}\mathrm{C}$ to $-17\,^{\circ}\mathrm{C}$ for chicken.

Enthalpy, Latent Heat, and Specific Heat Capacity

Enthalpy (for which the symbol is usually H) is the heat content of a substance per unit mass of that substance, measured in $\mathrm{J\,kg^{-1}}$. Thus, to change the temperature of a kilogram of meat from an initial temperature, T_1, to a higher temperature, T_2, it would be necessary to add an amount of heat equal to the difference between the enthalpy of that meat at T_1 and its enthalpy at T_2.

Enthalpy has no physically defined zero point, so zero points can be defined arbitrarily. This has the unfortunate consequence that different authorities have defined the zero point differently, and so it is important to ensure that the zero point is defined consistently (or that an appropriate adjustment is made) when comparing enthalpy data from different sources. For foods, the zero point is often defined to be $-40\,^{\circ}\mathrm{C}$ (so that enthalpies in normal ranges of temperature are always positive), or sometimes $0\,^{\circ}\mathrm{C}$, both at a pressure of 1 atm. For other materials that are commonly used in food processing (refrigerants or steam, for instance), the zero point can be defined quite differently – for instance, enthalpy may be set equal to zero for saturated liquid at the material's triple point. None of this should be important as calculations should only be done with enthalpy differences, but it can be a source of confusion. Similarly, there is no significance in an enthalpy value being less than zero because this is just a result of where the zero point has been defined.

The specific heat capacity, C, is the amount of heat required to raise the temperature of a unit mass of material by a unit temperature change, measured in $\mathrm{J\,kg^{-1}\,K^{-1}}$. Specific heat is usually defined under conditions either at constant volume (written as C_v) or constant pressure (written as C_p). This can make a significant difference for the specific heat of a gas but, because the pressure dependence of specific heat for food materials is small except at extremely high pressures, specific heat values for foods are usually reported at constant pressure and these values can be applied with good accuracy for all conventional food processing operations.

Enthalpy and specific heat are related by eqn [5].

$$C = \frac{\mathrm{d}H}{\mathrm{d}T} \qquad [5]$$

When freezing, a substance will release a considerable amount of heat without any temperature change as its liquid content becomes solid. This is known as the latent heat of freezing, H_f, measured in $\mathrm{J\,kg^{-1}}$. For a pure substance, such as water, this results in a discontinuity in eqn [5] at the freezing temperature, T_f, because the enthalpy changes substantially with no change in temperature. Even for meat and other food products, C can become quite large near the initial freezing temperature and this can make some calculations relatively difficult. As a result, it is often better, near the freezing point, to calculate in terms of enthalpy instead of specific heat, if possible.

H_f for water is $334\,\mathrm{kJ\,kg^{-1}}$, but the water content of meat becomes ice over a range of temperatures, as estimated by eqn [4]. As a consequence, some of the water content of a meat product will remain unfrozen even well below the initial freezing temperature and the latent heat content of meat is released during freezing, based on the change in ice fraction with temperature.

For the temperature ranges of practical interest in meat processing, the heat capacity of meat can be estimated from the heat capacities of its components, weighted by their mass fractions, as shown in eqn [6], where C_i is the heat capacity of the ith component in $\mathrm{J\,kg^{-1}\,K^{-1}}$.

$$C = \sum_{i=1}^{n} C_i x_i \qquad [6]$$

When no phase change, such as freezing, takes place, the heat capacities of most materials change only relatively slowly with temperature and so can be assumed to be constant. Some approximate heat capacities for meat components are shown in Table 2.

The heat capacity of meat fat can be strongly dependent on the composition of that fat so, for accurate work, it is necessary to understand that composition in detail or to measure the heat capacity. This can be particularly important for cooking processes, where each fat fraction will melt at a different temperature. The latent heat required to melt each fat fraction can be accounted for either by assuming a higher effective heat capacity

Table 2 Approximate heat capacity values for some meat components at typical food processing temperatures

Component	*Heat capacity ($\mathrm{kJ\,kg^{-1}\,K^{-1}}$)*
Water	4.18
Ice	2.09
Protein	2.01
Fat	1.98
Air	1.005

for the overall fat in that temperature range, or by dealing separately with the thermal properties of each fat fraction.

If eqn [6] is used to calculate a specific heat capacity for frozen meat of a specific composition, C_S, and for unfrozen meat of the same composition, C_L, the change in enthalpy, ΔH, between a frozen temperature, T_1, and an unfrozen temperature, T_2, can be estimated from eqn [7], where x_{ice} is the mass fraction of ice at the temperature T_1.

$$\Delta H = C_S(T_f - T_1) + H_{f,water}x_{ice} + C_L(T_2 - T_f) \qquad [7]$$

It is sometimes useful, particularly when modeling food processing operations, to express enthalpy, latent heat, and specific heat capacity in volumetric terms (i.e., J m^{-3}, J m^{-3} and J m^{-3} K^{-1}, respectively). This can be achieved by multiplying the mass-based property values by the density of the substance.

Thermal Conductivity

The thermal conductivity, k, of a material defines the ease with which heat passes through the material, measured in W m^{-1} K^{-1}. For a slab of material (e.g., meat) where heat passes directly from one side to the other (i.e., the edges are perfectly insulated), thermal conductivity is defined by eqn [8].

$$Q = \frac{kA}{x}(T_a - T_b) \qquad [8]$$

In eqn [8], Q is the rate of heat flow through the slab in W, A is the cross-sectional area of the slab perpendicular to the direction of heat flow in m^2, x is the thickness of the slab in the direction of heat flow in m, and T_a and T_b are the temperatures on each surface of the slab in °C. Although thermal conductivity is defined in eqn [8] as applying to a slab, the concept applies equally to any physical geometry.

The thermal conductivity of a substance depends on the composition of the substance, as with heat capacity, but it is also strongly dependent on the structure of the substance. As a consequence, it is considerably more difficult to predict the thermal conductivity of a material than to predict heat capacity, for instance. Literature reviews have listed dozens of generic theory-based prediction methods. Including empirical curve-fits just for food products would increase this number considerably. At the same time, thermal conductivity is relatively difficult to measure with high levels of accuracy and so even the measured data reported in the literature are often subject to uncertainties of perhaps ±5–10%.

The accuracies of thermal conductivity prediction models are strongly dependent on the ratios of the component thermal conductivities. For small component conductivity ratios, different models typically produce similar, accurate predictions. Thus, referring to Table 3, for fresh meat comprised only of water, protein, and fat, the largest ratio of thermal conductivities is approximately 3 and many models are good enough for most purposes, including an average of the component thermal conductivities weighted by the component mass fractions, as was used above for specific heat capacity. For frozen meat containing ice, unfrozen water, protein, and fat, the thermal conductivity ratio is approximately 12 and it is important to take care over which model is used. For meat packages that include frozen meat and air, the thermal conductivity ratio is nearly 100 and substantial errors could be made by using the wrong predictive model. Owing to the substantial difference between the thermal conductivity of water and that of ice, the thermal conductivity of meat also changes substantially as it freezes or thaws.

Table 3 Approximate thermal conductivity values for some meat components at typical food processing temperatures

Component	*Thermal conductivity (W m^{-1} K^{-1})*
Water	0.57
Ice	2.2
Protein	0.18
Fat	0.18
Air	0.025

Accurate data have been reported in the literature for a range of meat products. A review of the literature shows that for fibrous materials, such as meat, it can even be necessary to determine whether the heat flow is across or along the length of the meat fibers, because the thermal conductivity can be significantly different for these two cases. For rough calculations, however, it is sometimes sufficient to assume typical thermal conductivity values of approximately 0.5 W m^{-1} K^{-1} for unfrozen and 1.5 W m^{-1} K^{-1} for frozen lean meat.

Moisture Diffusivity

The diffusivity of moisture through a material can be defined by analogy with thermal conductivity, following eqn [8], but replacing heat flow with moisture flow and temperature with moisture content. Just as knowledge of the thermal conductivity of a meat product is important when designing or analyzing thermal processes, knowledge of the moisture diffusivity is important when designing or analyzing processes that involve drying. Drying can occur deliberately, such as when manufacturing jerky or biltong, or as a side effect of another process, such as when chilling unwrapped meat cuts or carcasses in air.

Moisture diffusion is arguably even more sensitive to the attributes of the material through which the moisture is to pass than is heat diffusion. As a result, there have been relatively few reliable measurements of moisture diffusivity in meat products and even these measurements differ considerably in their reported values, apparently due to subtle differences in attributes between the different meat products studied.

In addition, for meat and many other materials, moisture diffusivity is strongly dependent on the moisture content of the material. Many methods for measuring moisture diffusivity rely on drying some or all of the sample, so the diffusivity value measured by those methods can change substantially during the measurement process.

The most reliable moisture diffusivity values reported for meat to date have probably been those for raw minced beef, where measurements have ranged from 0.3×10^{-10} to

$5 \times 10^{-10}\ m^2\ s^{-1}$, with dependencies reported based on moisture content and temperature. The accurate measurement and estimation of moisture diffusivity for whole muscle meat remains an active area of research.

See also: Canning. Cooking of Meat: Heat Processing Methods. Modeling in Meat Science: Refrigeration. Refrigeration and Freezing Technology: Applications

Further Reading

Amos, N.D., Willix, J., Chadderton, T., North, M.F., 2008. A compilation of correlation parameters for predicting the enthalpy and thermal conductivity of solid foods within the temperature range of $-40\ ^\circ C$ to $+40\ ^\circ C$. International Journal of Refrigeration 31, 1293–1298.

ASHRAE, 2010. ASHRAE Handbook – Refrigeration, SI ed. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers.

Atkins, P., De Paula, J., 2009. Atkins' Physical Chemistry, ninth ed. Oxford: Oxford University Press.

Boonsupthip, W., Sajjaanantakul, T., Heldman, D.R., 2009. Use of average molecular weights for product categories to predict freezing characteristics of foods. Journal of Food Science 74 (8), E417–E425.

Brake, N.C., Fennema, O.R., 1999. Glass transition values of muscle tissue. Journal of Food Science 64 (1), 10–15.

Carson, J.K., 2006. Review of effective thermal conductivity models for foods. International Journal of Refrigeration 29, 958–967.

Green, D.W., Perry, R.H. (Eds.), 2007. Perry's Chemical Engineers' Handbook, eighth ed. New York, NY: McGraw-Hill.

McCabe, W.L., Smith, J.C., Harriott, P., 2004. Unit Operations of Chemical Engineering, seventh ed. New York, NY: McGraw-Hill.

Motarjemi, Y., Hallstrom, B., 1987. A study of moisture transport in minced beef. In: Jowitt, R., Escher, F., Kent, M., McKenna, B., Roques, M. (Eds.), Physical Properties of Foods, vol. 2. London: Elsevier, pp. 61–64.

Rahman, M.S., 2009. Food Properties Handbook, second ed. Boca Raton, FL: CRC Press.

Sahagian, M.E., Goff, H.D., 1995. Chapter 1: Fundamental aspects of the freezing process. In: Jeremiah, L.E. (Ed.), Freezing Effects on Food Quality. New York, NY: Marcel Dekker, pp. 1–50.

Sunooj, K.V., Radhakrishna, K., George, J., Bawa, J.S., 2009. Factors influencing the calorimetric determination of glass transition temperature in foods: A case study using chicken and mutton. Journal of Food Engineering 91, 347–352.

Relevant Websites

https://www.ashrae.org/
American Society of Heating, Refrigerating and Air-Conditioning Engineers.

http://www.iifiir.org/
International Institute of Refrigeration.

Index

Notes
Cross-reference terms in italics are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references – not all of these cross-references have been included in the index cross-references.

The index is arranged in set-out style with a maximum of three levels of subheading. Major discussion of a subject is indicated by bold page numbers. Page numbers suffixed by *T*, *F*, and *B* refer to Tables, Figures, and Boxes respectively. *vs*. indicates a comparison.

This index is in letter-by-letter order, whereby hyphens and spaces within index headings are ignored in the alphabetization. For example, acid meat is alphabetized after acidification, not after acid(s) or *F*-value is after *Fusarium*, and not at the start of the F section. Prefixes and terms in parentheses are excluded from the initial alphabetization.

Where index subentries and sub-subentries pertaining to a subject have the same page number, they have been listed to indicate the comprehensiveness of the text.

A

B

C

D

E

F

G

H

I

J

K

L

M

N

P

Q

R

S

T

U

V

X

Y

Z