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Lawrie's Meat Science

Ninth Edition

Edited by

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Preface

Lawrie's Meat Science constitutes a classical reference as a textbook in the meat world because it has been used by numerous generations of meat professionals since its first edition in 1966. The former eighth edition dates back to 2017 and the contents were spread throughout 22 chapters. The knowledge on meat science has progressed rapidly in the recent years; therefore, an updated compilation of the recent developments was needed by meat scientists. This new ninth edition of the book is arranged as an edited book as was the previous eighth edition and also keeping the textbook format, with a compilation of 23 chapters with new approaches, combining updated revised chapters with new ones dealing with the sustainability of animal production and meat processing, and the future meat market, especially regarding the trends in consumption of processed meats partly replaced by plant- or insect-based proteins, cultured meat, organic meat, and pandemic planning for the meat industry.

The main goal of this book is to provide the reader with a comprehensive resource, covering the wide field of meat science and including leading-edge technologies (i.e., nanotechnology and novel preservation technologies) and techniques (i.e., proteomics, genomics, and metabolomics). The book brings together all the advances in the production of animals, the structure and composition of the muscle, its conversion into meat, the different technologies adopted for preservation and storage, the eating and nutritional quality, meat safety, traceability and authenticity, sustainability, and future trends through the production systems, processing industry, and distribution until reaching the consumer.

I sincerely hope that readers will find this book interesting as the intention is to provide them with useful information. The book is written by a select group of the most experienced and distinguished international contributors, and I wish to thank all of them for their dedication and good job because without them this book would not have been possible. I also thank the production team at Woodhead Publishing, especially Mrs. Judith Clarisse Punzalan (Editorial Production Manager) and Mr. Surya Jayachandran (Production Manager) for their dedication during the preparation and elaboration of the chapters and during the publication of the book.

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Chapter 1

Introduction

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Meat science is a discipline that requires a complete understanding of the complexities of antemortem and postmortem factors that impact the final product for the consumer. Subsequent chapters in this book will delve into these factors more deeply, but an overview of some of the background and current issues related to meat production is an important starting point for this journey.

1.1 Meat and muscle

The basic definition of meat is the flesh of animals used for food. For the most part and for most societies, meat comes from domesticated livestock with the primary species being cattle, hogs, and sheep. Although skeletal muscle makes up the greatest proportion of the products produced and consumed, various organs and other offal items are important food components for many nations and often contribute greatly to the export markets for those countries that produce more than what can be consumed domestically.

One example of a technical definition of meat can be found at U.S. Department of Agriculture (2016a):

> *Meat. (1) The part of the muscle of any cattle, sheep, swine, or goats which is skeletal or which is found in the tongue, diaphragm, heart, or esophagus, with or without the accompanying and overlying fat, and the portions of bone (in bone-in product such as T-bone or porterhouse steak), skin, sinew, nerve, and blood vessels which normally accompany the muscle tissue and that are not separated from it in the process of dressing. As applied to products of equines, this term has a comparable meaning.*

1. Meat does not include the muscle found in the lips, snout, or ears.
2. Meat may not include significant portions of bone, including hard bone and related components, such as bone marrow, or any amount of brain, trigeminal ganglia, spinal cord, or dorsal root ganglia.

Regulatory authorities within governments must define what constitutes "meat" for its citizens to ensure proper labeling and prevention of adulteration, and it is

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expected that this definition will vary from country to country. This definition from the United States has been updated since the occurrence of bovine spongiform encephalopathy in the mid-1980s as reflected by the reference to the absence of items now considered as "specified risk material" (U.S. Department of Agriculture, 2016b).

1.2 Meat from other animals

Throughout the world, there are many other animals used for primary or secondary sources of meat for consumption. The buffalo (*Bubalus bubalis*) is an important source of draft power, milk, meat, and hides in many Asian countries, with the greatest numbers present in India, China, Pakistan, and Nepal (Nanda and Nakao, 2003). Desert camels (*Camelus dromedarius*), in addition to their historic use as a transporter, their drought tolerance, and their ability to adapt to harsh arid and semiarid zones, provide food for parts of Africa (Kurtu, 2004; Yousif and Babiker, 1989) and the Middle East (Elgasim and Alkanhal, 1992; Kadim et al., 2006).

The goat (*Capra aegagrus hircus*) is a great contributor to the development of rural zones and people (Dubeuf et al., 2004) and historically has been a great source of meat, milk, fiber, and skin. Dubeuf et al. (2004) stated that goats are found on all continents, with the greatest numbers being in Asia (especially China and India), Africa (especially Nigeria and Ethiopia), Europe (especially Greece and Spain), and the Americas (especially Mexico and Brazil). For species such as goats, sometimes meat production is secondary to that of milk or fiber, which often diminishes the value of meat in the marketplace.

The horse (*Equus ferus caballus*) is used as a source of human food in some cultures, with the majority of horse meat production/importation occurring in Asia and Western Europe (Gill, 2005). Gill (2005) also stated that the Western European countries with the greatest amounts of horse meat produced, exported, and/or imported were Italy, Belgium, France, and the Netherlands. Gade (1976) stated that the acceptance of horse meat in France as a food item for humans would be one of the few documented cases of a change in attitude from aversion to that of acceptance and was probably driven by food-shortage crises of the past.

For many years, the United States slaughtered horses with most of the more-valued cuts destined for Western Europe and the less-valued cuts remaining for use in pet food manufacturing or use in zoos. In 2005, the first successful attempt by the US Congress to find a way to stop horse slaughter was through an act that prevented federal monies from being used to pay the salaries or expenses of inspectors. Even though this bill expired several years later, the US budget passed in early 2014 reinstated the ban on the use of federal monies for inspection of horse meat. Nonetheless, there are EU-approved horse slaughter facilities in Canada and Mexico that handle much of the volume of North American horses that are destined for slaughter.

Horse meat production and consumption were brought to international headlines when in 2013, in parts of Ireland and the United Kingdom, processed beef products were found to have been contaminated/adulterated with horse meat (Abbots and Coles, 2013). Regan et al. (2015), in a survey of the aftermath of this incident, found three factors that were related to how consumers assigned responsibility and blame for the adulteration: (1) the deliberately deceitful practices of the food industry, (2) the complexity of the food supply chain, and (3) the demand from (other) consumers for cheap food. Mislabeling/misbranding products, especially related to substituting lower-priced for higher-priced meats, can and do have serious regulatory consequences, but may, most importantly, erode consumer confidence and trust for the meat industry.

The domestic rabbit (*Oryctolagus cuniculus*) meat consumption is centered in the Mediterranean countries and is impacted by historical, economical, and social evolution (Dalle Zotte, 2002). Dalle Zotte and Szendro (2011) observed that rabbit meat could be used as a functional food (providing multiple health benefits including nutrition, well-being, and reduction of disease) because of how diet could be used to influence the fatty acid composition and vitamin content of the meat.

Exotic or game meat is one for which there are certain countries that have abundant wildlife where animals can be hunted in the traditional form or where animals can be farmed using the latest reproductive technologies, advanced nutrition schemes, and sanitary slaughter and cutting operations to provide meat through commerce. Hoffman and Cawthorn (2013) compared several species of wildlife to show the proximate composition of meat (principally from the *M. longissimus thoracis et lumborum*). As one would expect based on the overall leanness of these animals, Hoffman and Cawthorn (2013) found that the ungulates, African species, including the springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus dorcas phillipsi*), kudu (*Tragelaphus strepsiceros*), and impala (*Aepyceros melampus*), and ungulates, cervidae, including red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), and reindeer (*Rangifer tarandus*) had protein contents from 19.3% to 23.6% and fat contents from 1.7% to 4.6% based on a raw weight basis. Hoffman and Wiklund (2006) stated that game meat and venison from southern Africa are increasingly being exported into Europe and the United States, and that how they are produced (wild, free range, or intensive production), harvested, the nutritional quality, and traceability are all factors that play a role into the consumer acceptance of this meat.

1.3 Domestication of livestock

There are exciting technologies, such as mitochondrial and nuclear DNA, available to better understand how, when, and where livestock domestication occurred (Bruford et al., 2003). Bruford et al. (2003) stated that there are three principal areas of livestock domestication: (1) southwest Asia also known as the Fertile Crescent and toward the Indus Valley, (2) East Asia (China and countries

south of China), and (3) the Andean chain of South America. Species such as cattle, sheep, goats, pigs, and buffalo were domesticated in the two Asian regions, whereas the South American region is where llamas and alpacas were domesticated (Bruford et al., 2003). Most studies point to domestication of livestock to have occurred around 10,000 years ago.

Evidence to point to when domestication occurred most often focused on when a reduction in size of the animal was observed. Zeder (2008) stated that this reduction in size most likely was the difference in the strategies between hunters, who would have targeted large animals to maximize their hunt, and herders, who would have slaughtered the females (smaller than their male counterparts) at the end of the reproductive life and the younger males not needed for herd propagation. Zeder (2008) also revealed that archeological evidence related to the sequence and timing of long bone growth and the determination of sex-specific subpopulations can be used to generate harvest profiles for male and female animals that are capable of distinguishing between the prey strategies of hunters from the harvest strategies of herders.

1.3.1 Cattle

The wild aurochsen (*Bos primigenius*) were the ancestors of modern-day cattle with two possible domestication events occurring in southwest Asia, which gave rise to the taurine (*Bos taurus*) and zebuine (*Bos indicus*) cattle (Loftus et al., 1994). Ajmone-Marsan et al. (2010) stated that the maternal lineages of taurine cattle originated in the Fertile Crescent with a possible contribution of South European wild-cattle populations, and that the zebu cattle originated from the Indus Valley. Domestication of these two different types of cattle has allowed them to be used in a wide variety of environments throughout the world, providing meat, milk, hides, and labor to promote the development of the human population over the millennia (Ajmone-Marsan et al., 2010).

Initial migration of cattle from their domestication sites to Africa and Europe allowed for more development over time and is the subject of many studies following mitochondria DNA haplotype distributions to evaluate where the subsequent development occurred (Achilli et al., 2008; Ajmone-Marsan et al., 2010; Beja-Pereira et al., 2006). Achilli et al. (2008) showed that the aurochsen in Northern or Central Europe may have contributed to additional gene flow to the T haplogroups (*B. taurus*), and that the haplogroup Q may have been acquired from a different population of aurochsen that ranged only south of the Alps (Achilli et al., 2009).

The development of cattle throughout Europe, Africa, and Asia occurred for thousands of years, but with the discovery and conquest of the Americas, cattle accompanied the humans to the New World (Ajmone-Marsan et al., 2010) and set in place the development of the cattle industries in North and South America that have grown into major beef-producing regions over the past 500 years.

B. taurus cattle from Europe and *B. indicus* cattle from Southwest Asia made their way to these lands at different times and for different reasons, and even new breeds of cattle (e.g., Santa Gertrudis, Brangus, Beefmaster) were developed based on planned breeding programs between these two species. Hundreds of breeds of cattle are found around the world, each known for some growth, quality, and/or composition feature with some used in pure breeding or as part of planned crossbreeding operations to produce beef for a varied marketplace.

1.3.2 Swine

Larson et al. (2010) have shown, using both genetic and archeological pieces of evidence, that pigs were domesticated in East Asia. That said, the authors also believe that the most common modern domestic haplotypes found in Central China also are the most common Asian haplotypes found across East Asia, in Australian feral pigs, and in modern European and American breeds, which occurred most likely during the 18th century when Asian pigs were used to improve the European breeds. Larson et al. (2010) further stated that pigs were disseminated throughout these regions through human migration as well as the natural migration across land bridges into various countries.

Of great interest in the domestication of swine (*Sus scrofa*) is the role played by the wild boar where at least two European wild boar lineages have been found and that the possibility that other wild boar lineages also may have been domesticated (Larson et al., 2005). Larson et al. (2005) stated that even though some of the wild progenitors of many of the Eurasian domesticates are either extinct or have little or no phylogeographic structure, the distribution of the surviving wild boar gives researchers the opportunity to determine the origins of the current domestic lineages.

Another important issue related to how swine entered Europe from Asian was that it appeared that at least two paths were followed, one a northern route—the Danubian Corridor—which followed the Danube and Rhine River Valleys, and the other more along with northern Mediterranean region (Larson et al., 2005). Larson et al. (2007) evaluated ancient DNA related to the Neolithic expansion in island Southeast Asia and found that there were two separate, human-mediated dispersals of *Sus* from Asia into the Pacific and a third within Wallacea (islands between Borneo, New Guinea, and Australia). These pigs likely originated in East Asia and were introduced to these areas as humans migrated to them.

Pork production today features many different breeds or genetic lines designed for specific markets. Large commercial farms, many of which are farrow-to-finish operations, ensure that enough market-ready hogs are available for processing into finished goods. From a minor contribution standpoint, there are some heritage breeds (e.g., Mangalitsa, Red Wattle, Gloucestershire Old Spot) that have gained in popularity from those who wish to preserve and promote these animals.

1.3.3 Sheep

It may be that the domestication of sheep (*Ovis aries*) was the easier of the three major species because of their relatively small size and ease of herding. Chessa et al. (2009) citing others that sheep were the first species to be domesticated also stated that although sheep were reared primarily for meat, during the fifth millennium before present (B.P.) in Southwest Asia and the fourth millennium B.P. in Europe, specialization for products such as wool may have caused a replacement of primitive domestic populations with those more suited for wool production.

Hiendleder et al. (1998) evaluated the mitochondrial DNA from several sources of sheep from European, African, and Asian breeds along with mouflon (*Ovis musimon*). The authors identified two major domestic sheep mitochondrial DNA lineages, which they termed European and Asian lineages, and within branches that contained European mouflon (*O. musimon*). It is of interest that there were two different lineages in cattle (*B. taurus* and *B. indicus*) and swine (*Sus vittatus* and *S. scrofa*) that go along with the theory of two different lineages in sheep (Hiendleder et al., 1998). Finally, the authors hypothesized that some modern domestic sheep and European mouflon derive from a common ancestor that is not from the urial and argali groups and has not yet been identified.

Chessa et al. (2009) used retrovirus integrations to study the history of sheep domestication. The authors found that there was a secondary population expansion of improved domestic sheep, which were most likely out of Southwest Asia. This finding provided valuable insights into the history of pastoralist societies that involved sheep husbandry.

Not all agree about the number of domestication events for sheep. Pedrosa et al. (2005) found evidence of an additional maternal lineage in sheep, which would then mean that there were at least three domestication events for sheep rather than the previous theory of just two (Hiendleder et al., 1998, 2002).

Once sheep were exported throughout the world, they played an important role in the economic development of so many countries as important sources of meat, fiber, and milk. Some sheep are raised primarily for their meat and some primarily for their wool with different breeds developed to fit different niches. Development of synthetic fibers and the unique flavor aspects of lamb meat have somewhat dampened the demand for sheep over the past half century, but the sheep industry continues to be an important component for much of the world.

1.4 Trends and developments

Six specific areas are included so that a quick overview can be provided related to trends and developments in meat production, animal welfare, sustainability, kosher and halal, cultured meat, and plant-based meat alternatives.

1.4.1 Meat production

Meat production varies around the world with respect to countries that produce the most meat. For beef and veal (Table 1.1), the top five producing countries in 2020 are the United States, Brazil, European Union, China, and India. For pork (Table 1.2), the top three producing countries in 2020 are China, European Union, and the United States. Because of an outbreak of African Swine Fever in China (Zhou et al., 2018), China's pork production has dropped by about 33% from 2017 to 2020 (from 54,518 to 36,340 thousand metric tons, carcass weight equivalent), but the country is still the leading pork-producing country in the world.

Imports and exports play a major role in the economic viability of each country. Imports may provide a way to be sure that there is enough meat of a particular kind for a country, whereas exports may be a way to improve the balance of trade and increase the revenue livestock producers receive. For beef and veal (Table 1.3), the top four importing countries are the China, United States, Japan, and South Korea, whereas the top four exporting countries are Brazil,

TABLE 1.1 Beef and veal production, summary of selected countries for the year 2020.

Country	1000 metric tons (carcass weight equivalent)
United States	12,379
Brazil	10,100
European Union	7810
China	6670
India	4270
Argentina	3230
Australia	2123
Mexico	2079
Pakistan	1820
Russia	1378
Canada	1310
Others	7863

From U.S. Department of Agriculture, Foreign Agricultural Service, Livestock and Poultry: World Markets and Trade. Available from: https://downloads.usda.library.cornell.edu/usda-esmis/files/73666448x/zs25z463c/np194471v/livestock_poultry.pdf.

TABLE 1.2 Pork production, summary of selected countries for the year 2020.

Country	1000 metric tons (carcass weight equivalent)
China	36,340
European Union	24,150
United States	12,843
Brazil	4125
Russia	3611
Vietnam	2467
Canada	2130
Mexico	1451
South Korea	1403
Philippines	1115
Others	5765

From U.S. Department of Agriculture, Foreign Agricultural Service, Livestock and Poultry: World Markets and Trade. Available from: https://downloads.usda.library.cornell.edu/usda-esmis/files/73666448x/zs25z463c/np194471v/livestock_poultry.pdf.

TABLE 1.3 Beef and veal imports and exports, summary of selected countries for the year 2020.

Country	1000 metric tons (carcass weight equivalent)
Total imports	
China	2782
United States	1516
Japan	832
South Korea	549
Hong Kong	513
Russia	363
European Union	285
Chile	313
Egypt	230

TABLE 1.3 Beef and veal imports and exports, summary of selected countries for the year 2020.—cont'd

Country	1000 metric tons (carcass weight equivalent)
Canada	249
Malaysia	206
Others	1515
Total exports	
Brazil	2539
Australia	1476
United States	1341
India	1284
Argentina	819
New Zealand	638
Canada	513
Uruguay	412
European Union	350
Paraguay	371
Mexico	343
Others	719

From U.S. Department of Agriculture, Foreign Agricultural Service, Livestock and Poultry: World Markets and Trade. Available from: https://downloads.usda.library.cornell.edu/usda-esmis/files/73666448x/zs25z463c/np194471v/livestock_poultry.pdf.

Australia, United States, and India. For pork (Table 1.4), the top three importing countries are China, Japan, and Mexico, whereas the top three exporting countries are the European Union, the United States, and Canada.

For sheep meat, Colby (2015) and Ramírez-López et al. (2020) published information related to the global sheep meat production, consumption, and export based on the United Nations Food and Agriculture Organization information. The top sheep-meat-producing countries in 2013 were China (24%), Australia (8%), New Zealand (6%), United Kingdom (3%), and Iran (2%), whereas the top sheep-meat-consuming countries were China (27%), Sudan (4%), and the United Kingdom, Turkey, Algeria, Australia, and India with 3% each. The top sheep-meat-exporting countries in 2013 were Australia (36%), New Zealand (35%), and the United Kingdom (9%).

TABLE 1.4 Pork imports and exports, summary of selected countries for the year 2020.

Country	1000 metric tons (carcass weight equivalent)
Total imports	
China	5281
Japan	1412
Mexico	945
South Korea	554
United States	410
Hong Kong	378
Philippines	167
Canada	273
Australia	201
Vietnam	225
Chile	135
Others	956
Total exports	
European Union	4546
United States	3303
Canada	1543
Brazil	1178
Mexico	344
Chile	295
Russia	156
China	100
Argentina	34
South Africa	17
Others	59

From U.S. Department of Agriculture, Foreign Agricultural Service, Livestock and Poultry: World Markets and Trade. Available from: https://downloads.usda.library.cornell.edu/usda-esmis/files/73666448x/zs25z463c/np194471v/livestock_poultry.pdf.

1.4.2 Animal welfare

Political and social attention to animal welfare has increased during the first two decades of the 21st century. Much has originated from the increasing numbers of livestock that are raised in confinement and the distances many animals are shipped to processing facilities. Many activist groups have targeted livestock production, in general, and intensified operations, in particular, as nothing more than cruel enterprises that exploit animals for profit without regard for their welfare or the environment. Undercover videos showing animal abuse, whether staged or not, have been used to place pressure on retailers and foodservice establishments and to seek publicity for the causes of the activist groups. Social media has allowed for wide distribution of negative images and stories of animal welfare to audiences throughout the world. Most of the major retail and foodservice operations now require extensive animal welfare audits on at least a yearly basis to ensure that at the processing plant level, appropriate handling, and stunning programs are in place.

Farm animal welfare is a complex issue that addresses scientific, ethical, and economic factors (Webster, 2001). One approach to developing an adequate plan to ensure that appropriate animal welfare was the creation of the "Five Freedoms" (Farm Animal Welfare Council, 2009):

1. Freedom from hunger and thirst—by ready access to fresh water and a diet designed to maintain full health and vigor;
2. Freedom from discomfort—by the provision of an appropriate environment including shelter and a comfortable resting area;
3. Freedom from pain, injury, or disease—by prevention or through rapid diagnosis and treatment;
4. Freedom to express normal behavior—by the provision of sufficient space, proper facilities, and company of the animal's own kind; and
5. Freedom from fear and distress—by the assurance of conditions that avoid mental suffering.

Research in animal handling and welfare continues to increase in importance for the livestock and meat industries. Understanding animal behavior is key to designing effective vehicles and facilities to transport and handle livestock. This is especially important in species such as swine, where animals are often raised together before being marketed. Støier et al. (2016) found that using a "group-based principle," where pigs were kept in the same groups during transport, lairage, and stunning, reduced aggression and fighting by keeping unfamiliar pigs away. They also found that pigs in groups of 15, rather than groups of 45, were easier to move at unloading, to pens in lairage, and to the stunner, and many quality factors were improved. Best practices designed around animal handling benefit both animal welfare and meat quality.

TABLE 1.5 Criteria and subcriteria used in WelfareQuality to develop an overall animal welfare assessment plan.

Criteria	Subcriteria
Good feeding	1. Absence of prolonged hunger 2. Absence of prolonged thirst
Good housing	3. Comfort around resting 4. Thermal comfort 5. Ease of movement
Good health	6. Absence of injuries 7. Absence of disease 8. Absence of pain induced by management procedures
Appropriate behavior	9. Expression of social behaviors 10. Expression of other behaviors 11. Good human-animal relationship 12. Absence of general fear

From Botreau, R., Veissier, I., Butterworth, A., Bracke, M.B.M., Keeling, L.J., 2007. Definition of criteria for overall assessment of animal welfare. Animal Welfare 16 (2), 225–228.

Botreau et al. (2007) reported the criteria and subcriteria used in WelfareQuality to develop an overall welfare assessment based on the "Five Freedoms" (Table 1.5). It is important to provide criteria that are as objective as possible so that successful assessments of animal welfare can be made by third-party auditors or others tasked with these evaluations.

In North America, the Professional Animal Auditor Certification Organization or PAACO (https://animalauditor.org) was developed to create auditor-training programs for the livestock and poultry industry. This organization provides uniform minimum standards for auditors and audits through education and training, developing procedures based on best practices and providing animal science and veterinary professionals an assessment of specific criteria for the auditing process. It is common for employees who handle livestock at large meat-processing facilities in the United States to have to be PAACO-certified before they can perform their duties.

Adequate animal welfare is an important component of producing high-quality meat, but the public pressure on livestock producers to ensure that the highest standards of production will be driven primarily by those outside of agriculture. Understanding the importance of adequate animal welfare in the livestock production chain will only continue to be more important in the future.

1.4.3 Sustainability

Producing meat using sustainable systems has been a topic for some time and a one for which there are no good answers as to how to define, measure, or

much less improve sustainability. Much of the discussion on sustainability is now focused on how to feed the inhabitants of the world by the year 2050 when the population reaches around 9 billion people and what will be the role of the livestock industry in helping meet the food needs of this many people without resulting in a significant negative impact to the environment (see Chapter 22).

Most measures of sustainability focus on three metrics (Capper, 2013): (1) economic viability, (2) environmental stewardship, and (3) social responsibility. National and multinational corporations address these metrics in a variety of annual reports hoping to satisfy those who question the sustainable practices of each.

One of the most publicized and critical assessments of livestock production and the environment was found in the United Nations Food and Agriculture Organization report entitled "Livestock's Long Shadow: Environmental Issues and Options" by Steinfeld et al. (2006). Here is a paragraph from the Executive Summary of that report:

> *The livestock sector emerges as one of the top two or three most significant contributors to the most serious environmental problems, at every scale from local to global. The findings of this report suggest that it should be a major policy focus when dealing with problems of land degradation, climate change and air pollution, water shortage and water pollution and loss of bioavailability.*

Not all is bad news when it comes to changes at least in the beef industry and how it impacts the environment. Capper (2011) compared beef production practices in the United States in 1977 with those in 2007. Her findings were that the 2007 system required considerably fewer resources than the 1977 system with 69.9% of animals, 81.4% of feedstuffs, 87.9% of water, and only 67% of the land required to produce 1 billion kg of beef (Capper, 2011). In addition, the beef production system of 2007 achieved these reductions in input while producing only 81.9% of the manure, 82.3% of the CH_4, and 88.0% of the N_2O compared with 1977. Finally, Capper (2011) stated that the carbon footprint per billion kilograms of beef produced was reduced by 16.3% over this 40-year comparison. The world livestock sector must work tirelessly to document the increased efficiencies and decreased waste associated with the production of livestock for food or face possible scrutiny from those who believe that raising livestock for meat is a wasteful endeavor.

1.4.4 Kosher and halal

The dietary laws of Jews and Muslims directly impact the slaughter, processing, and consumption of meat products for these two groups. According to Regenstein et al. (2003), the kosher or kashrus dietary laws determine which foods are "fit and proper" for consumption for the Jewish consumer who observes these laws, are Biblical in origin, and which can be found in the original five books of the Holy Scripture, the Torah. The halal dietary laws determine

which foods are "lawful" or permitted for Muslims and are found in the Quran and in the Sunna, the practice of the Prophet Muhammad, as recorded in the books of Hadith, The Traditions (Regenstein et al., 2003).

Kosher laws focus on three primary issues: (1) allowed animals, (2) the prohibition of blood, and (3) the prohibition of the mixing of milk and meat (Regenstein et al., 2003). Provisions for allowed animals center primarily on ruminants with split hooves that chew their cud, traditional domestic birds, and fish with fins and removable scales. Of equal importance is the list of those animals that are prohibited, which includes the pig, wild birds, sharks, dogfish, catfish, monkfish (and similar fish), and all crustacean and molluscan shellfish. Prohibition of blood also focuses on how the animal is slaughtered to ensure that there is complete removal of blood during this process. A specially trained rabbit or "shochet" uses an extremely sharp knife or "chalaf" designed for this purpose to cut the animal's throat. There are multiple steps in the later process, where soaking and salting (thus the need for kosher salt) are used to draw as much blood from the meat as possible. Finally, the prohibition on the mixing of meat and milk prevents the two from being consumed together or benefit from owing a business where the two are mixed. Careful reviews of manufacturing processes by various rabbinical certifications organizations ensure that the products are either a meat product, or a dairy product, or a neutral product (neither meat nor dairy or "pareve") so that Jewish consumers can avoid mixing meat and dairy. As you can see, the kosher dietary laws begin with what kind of animals can be eaten, how they are slaughtered, and how the products can be manufactured and/or consumed.

The halal dietary laws define food products as "halal" (permitted) or "haram" (prohibited) and define several key areas of concern including (1) prohibited animals, (2) prohibition of blood, (3) method of slaughtering/blessing, (4) prohibition of carrion, and (5) prohibition of intoxicants (Regenstein et al., 2003). In addition to the prohibition of pork, carnivorous animals such as lions, tigers, cheetahs, cats, dogs, and wolves, and birds of prey such as eagles, falcons, osprey, kites, and vultures also are prohibited. Those animals that are permitted include meat from domesticated animals, mostly ruminants with split hooves, such as cattle, sheep, and goats in addition to camels and buffaloes. Birds that do not use their claws to hold down food such a chickens, turkeys, ducks, geese, pigeons, doves, partridges, quails, sparrows, emus, and ostriches are permitted. Blood that is "poured forth" is prohibited from consumption. Slaughtering requirements include (1) using only halal species, (2) must be slaughtered by an adult Muslim, (3) Allah's name must be invoked at the time of slaughter, and (4) slaughtering must be done by cutting the throat of the animal to induce rapid and complete bleeding and a quick death.

Farouk et al. (2016) stated that Islam teaches that there should be zero tolerance to animal abuse throughout halal meat production, and that slaughtering practices must follow those put forth by the Prophet Muhammad. Four viewpoints were stated as it relates to the balance between halal meat production and

animal welfare: (1) the scientific approach to animal welfare, (2) the ethic-based approach to animal welfare, (3) the Islamic dietary laws, and (4) the Islamic ethic about the role of animals in the world. Farouk et al. (2016) also summarized the importance of animal welfare in halal meat production focusing on (1) staff training regarding religious and regulatory aspects of slaughter, (2) empathy and compassion assessment of applications before employment, (3) installation of closed-circuit television cameras around lairage and slaughter sites to monitor worker activity, (4) follow-up training to minimize "compassion fatigue," (5) incorporating animal welfare requirements in halal certification, (6) using Imams to give mosque-based sermons related to animal welfare issues, and (7) incorporating mobile-based humane slaughter units to assist with local/neighborhood and small-scale halal slaughter.

Performing either kosher or halal slaughter in high-speed commercial meat-processing plants can be a challenge because of the line speeds and throughput of these operations. There is no stunning allowed for kosher slaughter although there are a few rabbinical certification programs that will allow the application of mechanical stunning for cattle shortly after the animal's throat is cut and the bleeding process has begun. This postslaughter stunning is used as a way for the animals to have fewer involuntary reflexes such as kicking to reduce the likelihood of worker injury during the initial shackling and landing steps of slaughter. For halal slaughter, the preference is for animals to not be stunned, but reversible forms of stunning (e.g., electrical) are widely accepted and practiced (Farouk et al., 2014). Farouk et al. (2014) further stated that the use of preslaughter head-only stun and neck cut can cause blood splash or ecchymosis in some halal systems as one example of some of the meat quality issues that may be found when kosher and halal slaughter is performed commercially.

1.4.5 Cultured meat

One novel approach to providing meat for the future is through the development of cultured meat. Cultured meat, where meat would be grown from cells, has been hailed as a way to address various animal welfare, food safety, and sustainability issues often associated with traditional livestock and meat production systems (Bekker et al., 2017; Bryant and Barnett, 2018; Goodwin and Shoulders, 2013).

It should not be surprising that much of the work in this area has been dedicated to finding what consumers think about the process and whether they will be willing to purchase cultured meat in the future. Interesting work by Mancini and Antonioli (2020) reveals that for certain groups such as nonmeat eaters, they were not willing to try, buy, or pay a premium price for cultured meats when provided additional positive information regarding this product. In addition, Mancini and Antonioli (2020) found that vegetarians still felt that cultured meat still came from animals, and they would prefer plant-based meat substitutes as an alternative. Bryant and Barnett (2020) reported that there could be a large

potential market for culture meats around the world, and that this product was more appealing than food technologies such as genetically modified organisms (GMOs) and alternative proteins such as insects.

There will be continued further work on the production and marketing of cultured meat (see Chapter 23). Although cultured meat may never overtake conventional meat production, its role in feeding people who have an interest in this novel product will be an important component of the study of meat science.

1.4.6 Plant-based meat alternatives

Using plant-based materials as extensions in or replacements for meat products is not new, which have been used for decades as ways to reduce costs or to provide replacements for those who did not want to consume meat because of health, religious, or other reasons. In recent years, the emphasis has shifted to manufacturing plant-based meat alternatives as ways to provide consumers with meat-like products that are perceived to be better for the environment, human health, and animal welfare concerns (He et al., 2020). Hu et al. (2019) even suggested that plant-based meat alternatives can be a part of a healthy and sustainable diet and that technological innovations are needed to increase their production as recommendations for decreases in meat consumption are being made.

The challenge with manufacturing plant-based meat alternatives is to give the consumer the same taste and texture characteristics they expect when they eat meat products. Technology has accomplished so much in improving these characteristics, but Michel et al. (2021) recommended that manufactures of plant-based meat alternatives concentrate on replicating processed meat products instead of whole muscle cuts. Estell et al. (2021) also noted that consumers were less interested in products that mimicked chicken and fish, but products such as burger patties, sausages, and mince were more frequently chosen, and that taste was a driving issue in the participants of their study.

It is unfortunate that many of the studies on plant-based meat alternatives focus on their use, not so much for their benefits, but because of the negative issues stated about livestock and meat production. Manufacturing plant-based meat alternatives is well funded globally (Choudhury et al., 2020), and it likely will become a greater part of the protein market in years to come (see Chapter 23).

1.5 Conclusions and future trends

Meat production is a vital part of the world economy making important contributions to local, national, and international trade. There may be multiple paths to the future of meat production. The first may be as consumers in developing countries gain more purchasing power, the demand for meat will increase substantially, providing a valuable source of high-quality protein in

the diets of many people. The existing market in developed countries may face another challenge as consumers who have significant purchasing power may limit their consumption of meat for a variety of reasons or may demand that there be more options in the marketplace such as organic, free range, humanely raised, sustainably raised, or whatever may be a marketing niche where the perceived benefits of how and where the animals are born, raised, and harvested are thought to be better than traditional schemes. The popularity of meat as a nutritious and delicious food will stay high for some time to come, but challenges to how it is produced and its impact on the world's environment will be under increasing pressure for the immediate and the foreseeable future.

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Chapter 2

Factors influencing the growth of meat animals

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2.1 Introduction

Growth in animals is an increase in total tissue mass or the combined accretion of protein, fat and bone over time that reflects changes in the size, development and structure of various organs and tissues (Black, 1988; Owens et al., 1995). Growth is continuous during the lifetime of an animal, starting from zygote formation until the animal reaches its mature weight at the adult stage (Arango and Van Vleck, 2002). Most fetal growth occurs during the final third of pregnancy in cattle and sheep and from about day 70 onwards in pigs, although genesis and growth of vital organs and body tissues commence during early gestation (see Greenwood and Dunshea, 2009). Birth and early postnatal development are characterized by maturation of organs essential for survival and subsequent growth. Growth of bone continues until closure of the growth plates postpuberty, at which time mature skeletal size is attained. Although bone and muscle continue to grow in an essentially linear manner during postnatal growth to mature size, adipose tissue grows at an increasing rate. Hence, during postnatal growth, the percentage of bone and muscle in the body declines while that of adipose tissue increases.

Muscle growth continues as a result of hypertrophic (cell enlargement) accretion of muscle protein coupled with the incorporation of nuclei from muscle satellite cells until mature lean body mass is attained. Growth of fatty tissues during postnatal life is supported by postnatal hyperplasia (cell multiplication), particularly at younger ages, and by hypertrophy of adipocytes until a maximal size is reached, beyond which they do not grow. Adipose depots continue to grow when the supply of energy in excess of requirements for other bodily functions is exceeded and involve both hypertrophy and hyperplasia. Fat is deposited in the body in visceral (around vital organs), subcutaneous, intermuscular or

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intramuscular locations. The time lines of growth of muscle and adipose tissue in pigs and cattle are shown diagrammatically in Du et al. (2017).

When reared under "ideal" dietary and environmental conditions, the pattern of growth or growth curve, i.e., the mass or cumulative weight plotted against age, is sigmoidal or "S-shaped" (Black, 1988). In most species, the point of inflection from an accelerating phase to a decelerating phase of growth occurs at about the age of puberty, and relative growth rate (gain per day per unit of body weight on that day) normally decreases from conception to mature weight. Growth curves or functions have been a standard approach to evaluating growth in animals (e.g., Arango and Van Vleck, 2002 (cattle); Brunner and Kühleitner, 2020 (sheep); Kebreab et al., 2010 (pigs)). Among the most commonly used are logistic, Richards, Gompertz, von Bertalanffy and Brody curves. These curves include three to four parameters that are often regarded as new traits to be studied to better understand the genetic architecture of growth patterns. Predictive models of growth have now evolved into more mechanistic and dynamic models of growth and body composition (e.g., Hoch and Agabriel, 2004).

2.2 Measurement of growth and body/carcass composition

With appropriate calibrated equipment and operator care, animal weight can be measured accurately. However, measured live weight can vary substantially over short periods without any change in the gross energy content of the animal mainly due to changes in the quantities of food and water present in the gastrointestinal tract. In particular, with grazing ruminants, substantial within- and between-day variation in live weight ("gut-fill effects") may be associated with the daily pattern of grazing, drinking, urinating and defecating, as well as fluctuations in food supply associated with herbage defoliation, especially under rotational grazing.

The ability to accurately measure body or carcass composition is important for performance testing, grading/assigning a value, (genetic) selection or payment for meat-producing animals. Estimation of body composition of the live animal can be assessed visually, but this can be very operator-subjective. "Fleshiness" (McGee et al., 2007) and body condition scoring (McGee et al., 2005a), which also involves physical palpation of the animal, are subjective assessments of subcutaneous body fatness, although the latter is more widely used on mature animals, cows and ewes. Body or linear measurements, such as back length, height at withers, height at and width of pelvis and chest width, girth and depth which are physically measured using a tape or calipers, as appropriate, are used to characterize size and shape (e.g., McGee et al., 2007). Carcass shape is considered important commercially and is depicted as "muscularity" in the live animal and conformation score in the carcass. Carcass measurements, such as carcass length and depth and, leg length width and thickness, have been used to estimate carcass shape. Visual muscularity scores taken preslaughter were

shown to have significant positive correlations with carcass meat proportion ($r = 0.5–0.7$) and negative correlations with carcass bone ($r = -0.7$ to -0.9) and fat ($r = -0.1$ to -0.4) proportions in beef cattle (Conroy et al., 2010).

Advances in imaging technology coupled with the decreasing cost of imaging equipment will facilitate its application in animal production. Applications of two-dimensional imaging to date have included estimating live weight (gain) of pigs and cattle. Miller et al. (2019) examined three-dimensional imaging coupled with machine learning in beef cattle production. The performance of prediction models for live weight ($R^2 = 0.7$), carcass weight ($R^2 = 0.88$) and saleable lean meat yield ($R^2 = 0.72$) demonstrates the potential of this approach.

Carcass evaluation for beef, sheep and pigs differs worldwide (Delgado-Pando et al., 2021). In Europe, beef and sheep carcasses are classified according to the official EU beef carcass classification scheme for conformation (E,U,R,O,P scale with E best and with an additional score S for superior double-muscled carcasses—(S)EUROP) and fatness (1–5 with 5 being fattest). This classification is based on visual assessment. In some countries, each class is now divided into three subclasses giving a 15-point continuous scale for conformation and fat score. Kempster et al. (1982) reported that carcass conformation score had little practical value as a predictor of carcass composition or lean meat proportion and distribution within breed. However, in a mixed breed population, the value of carcass conformation depended on its ability to identify breed (type) differences in carcass characteristics. In agreement, Conroy et al. (2010) using genotypes encompassing Holstein-Friesian, beef × Holstein-Friesian and > 0.75 late-maturing continental breed crosses showed significant correlations of 0.66–0.78 and 0.29–0.50 between carcass conformation score (15-point scale) with meat proportion and proportion of high-value meat cuts, respectively, indicating its usefulness as a predictor of lean meat yield and carcass value in a mixed breed population. In beef cattle, the proportion of variation in carcass lean meat yield explained by the 15-point EUROP grid (conformation and fat scores) was 0.55–0.75, whereas the proportion of variation in high-value cuts—cube roll, striploin and filet—was lower ($R^2 = 0.28–0.57$) (Craigie et al., 2012).

The most direct method for measuring composition is to completely dissect the whole body into viscera, skin, bones, muscle and fatty tissue. As this is very labor-intensive; a more common, less detailed, method of dissection is separation of the carcass into primal meat cuts to estimate lean yield. In beef cattle, for example, complete-body dissection (McGee et al., 2008), whole-side carcass dissection (Conroy et al., 2010) and, partial—e.g., hind-quarter (McGee et al., 2005b) or 6–10 ribs joint carcass dissection—which are used to "predict" whole-carcass composition, are often used for research purposes. Advances in noninvasive techniques for determination of body and carcass composition are mainly based on the development of electronic and computer-driven methods to provide objective phenotypic data (Scholz et al., 2015). Each method has its advantages and disadvantages vis-à-vis accuracy, reliability, financial cost,

portability, speed, ease of use under field conditions, safety and, for in vivo measurements, the need for animal fixation or sedation (Simeonova et al., 2012; Scholz et al., 2015). Objective carcass measurement technologies include X-ray-based technologies, nuclear magnetic resonance–based technologies, bioelectromagnetic methods, ultrasound, video image analysis and optical and spectroscopic probes (Delgado-Pando et al., 2021). Simeonova et al. (2012) concluded from their review which focused on methods for determining body protein content in vivo, especially in pigs, that ultrasonic analysis (two-dimensional B-mode scans; $R^2=0.36–0.38$), bioelectrical impedance ($R^2=0.81$) and the D_2O-dilution technique are the most economical and acceptably accurate methods that can be applied under field conditions, whereas magnetic resonance imaging (MRI) (indirect, $R^2=0.89–0.97$; direct, $R^2=0.62$), X-ray computed tomography (CT) ($R^2=0.92–0.98$), total body electromagnetic conductance (TOBEC) ($R^2=0.94$) and the measurement of isotope K^{40}, are precise, but too expensive under most conditions. Scholz et al. (2015) concluded from their review, which encompassed cattle, sheep, and pigs, that CT was most accurate, followed by MRI and dual-energy X-ray absorptiometry (DXA), whereas ultrasound can be used for all sizes of farm animals, even under field conditions. In beef cattle, for example, ultrasonic measures of muscle and fat depth of the *M. longissimus dorsi* are correlated with carcass conformation ($r=0.80–0.83$) and fat ($r=0.54–0.63$) scores and carcass meat ($r=0.52–0.63$) and fat proportions ($r=0.53–0.59$), respectively (Conroy et al., 2010). Video image analysis technology can replace visual carcass assessment (as practiced in the EU) and is capable of improving the precision and accuracy of saleable meat yield % prediction, even for specific carcass joints (Craigie et al., 2012; Miller et al., 2019). Other methodologies for estimating body composition include adipose cell size (McGee et al., 2005a), nitrogen balance (Hristov et al., 2019) and metabolic profiling (Fitzsimons et al., 2014).

2.3 Animal influences on growth of farm animals

There are large variations both within and among animal species in size, growth rate, body composition and metabolism (Black, 1988). Growth and mature size are determined by genetic and nongenetic factors. The genetic make-up of an individual includes additive and nonadditive genetic combinations that determine growth (Arango and Van Vleck, 2002). These combinations interact with intrinsic factors such as gender, age, physiological status and extrinsic factors such as the environment and climate, nutrition, management and maternal effects to determine the ultimate phenotype expression of growth (Arango and Van Vleck, 2002).

2.3.1 Physiological age/stage of maturity

All animals within a species or breed do not grow, develop or mature at the same chronological age. The increase in weight is associated with different growth

patterns for the organs that make up the body; most internal organs are early-maturing, i.e., they approach their mature weight before final body weight is reached (Black, 1988). Among other organs, the order of maturation is skeleton, muscle and fat, with only fat approaching maximum weight at a faster rate than whole body during the final stages of growth (Black, 1988). The species, breed and sex of an animal affect its pattern of organ development (Black, 1988) as well as extrinsic factors. Relative organ and tissue growth patterns are often examined using the allometric growth equation $Y = a \times x^b$, i.e., the ratio of the rate of change of the part to the whole (McGee et al., 2005b). With increasing slaughter weight in cattle, the proportions of noncarcass parts, hindquarter, bone, total muscle and higher-value muscle decrease, while the proportions of noncarcass and carcass fats, fore-quarter and marbling fat all increase. Therefore, at younger ages and lighter body weights, bone is present at greater proportions than muscle and fat, respectively, than at older ages and heavier weights. However, the rates of these changes differ among breed types. Protein accretion declines to zero when cattle reach their mature body size even though mature animals can continue to accrete fat (Owens et al., 1995). Likewise, as sheep genotypes grow toward maturity, in absolute terms, body, carcass, bone and muscle weights all increase but, comparatively relative to carcass weight, the proportion of fat weight (fatness) increases, whereas the proportions of muscle weight (leanness) and especially bone, decrease (Nsoso et al., 1999). Similarly in the pig, the ratio of daily carcass lean gain to fat gain declines as live weight increases.

2.3.2 Genetics

2.3.2.1 Breeds

Growth and carcass composition differ between breeds of farm animal species. In beef cattle, there is relatively large variation between *Bos taurus* breeds (Cundiff et al., 2004; Alberti et al., 2008; Berry, 2021) and *Bos indicus* breeds (Thrift et al., 2010; Retallick et al., 2017) for growth and carcass traits. Of the prominent European *Bos taurus* breeds, large late-maturing breeds (e.g., Charolais, Belgian Blue), in general, have higher live weight gain than smaller late-maturing breeds (e.g., Limousin, Piedmontese), early-maturing breeds (e.g., Hereford, Angus) and dairy breeds (e.g., Holstein-Friesian). On a constant age basis, differences between dairy and early-maturing beef breeds in growth and slaughter traits are small, but the latter have lower feed intake and better carcass conformation (Keane, 2011, Table 2.1). Late-maturing beef breeds also have lower feed intake and better carcass conformation and in addition, have a higher growth rate, kill-out proportion and carcass muscle proportion (Keane, 2011). However, due to the genetic selection, when comparing breed types, cognizance is required of the year the study was undertaken and the relevance of that population to the modern-day population, and when assessing results across country borders, caution is necessary given the dissimilarities in animal strains,

TABLE 2.1 Ranking of Holstein-Friesian (HF = 100) and beef × HF steers for intake, growth, slaughter traits, muscle weight and *M. longissimus* area at a similar age.

Sire breed	HF[a]	HE	LM	PM	RO	BL	SM	BB	CH
Feed intake (g/kg live weight)	*18.2*	98	96	94	92	96	98	97	97
Slaughter weight/day (g)	*803*	103	98	95	101	102	106	104	107
Kill-out proportion (g/kg)	*527*	102	105	105	104	105	104	105	104
Carcass weight/day (g)	*425*	105	103	100	104	107	109	109	111
Carcass conformation score[b]	*2.19*	133	136	139	139	132	136	138	143
Carcass fat score[c]	*3.52*	125	103	86	97	91	103	95	90
Muscle weight (g/day)	*256*	102	109	113	115	116	116	119	117
M. longissimus area[d]	*22.3*	103	117	118	117	110	108	112	114
Muscle: bone ratio	*3.22*	105	117	115	114	115	109	117	116
Higher value muscle (g/kg muscle)	*446*	100	102	103	103	101	102	102	102

[a]Actual values for HF, values for other genotypes expressed relative to HF value = 100; EU Beef Carcass Classification Scheme Scales: [b]1 (P = poorest) to 5 (E = best); [c]1 (leanest) to 5 (fattest). [d]cm^2/100 kg carcass.
BB, Belgian Blue; *BL*, Blonde d'Aquitaine; *CH*, Charolais; *HE*, Hereford; *LM*, Limousin; *PM*, Piedmontese; *RO*, Romagnola; *SM*, Simmental.
Modified from Keane, M.G., 2011. Ranking of Sire Breeds and Beef Cross Breeding of Dairy and Beef Cows. Teagasc, Occasional Series No. 9, Grange Beef Research Centre March 2011. http://hdl.handle.net/11019/1327.

especially dairy cattle, in different countries (Berry, 2021). For example, in the germplasm evaluation program in the United States, the magnitude of differences in postweaning growth between British and European Continental cattle breeds has decreased considerably over time (Cundiff et al., 2004; Retallick et al., 2017).

As breeds differ in their rate of maturation and average mature weight, breed comparisons must be interpreted in the context of the slaughter endpoint. In breed comparisons where animals are slaughtered at a constant age, later-maturing genotypes are leaner, whereas slaughtering on a fat-constant basis means later-maturing genotypes are heavier. Therefore, standardizing measurements of body composition to the same stage of maturity of body weight generally results in less variation in carcass composition than standardizing to the same age or weight.

There are breed differences in the distribution of body fat with dairy breeds of cattle depositing a higher proportion of their total fat internally and a lower proportion subcutaneously than beef breeds (McGee et al., 2005b). Differences between breed types in kill-out proportion can be explained by differences in gut contents—consequent on differences in feed intake—differences in the proportions of gastrointestinal tract and metabolic organs, differences in hide proportion and differences in offal fats (McGee et al., 2007).

Selection to improve carcass value is frequently based on growth and muscularity of young males. Extremes in muscling are evident in double-muscled cattle such as Belgian Blue, Piedmontese, (Culard) Charolais and double-muscled sheep such as Texel and Beltex. This is due to mutations which make the myostatin gene inactive resulting in muscle hypertrophy. In cattle, this results in better carcass conformation, increased kill-out (dressing) proportion, higher carcass lean meat content and a lower content of bone and fat. However, also associated with the extremely high carcass yields is a reduction in size of most vital organs and possibly lower animal robustness.

Similar to cattle, there are differences in growth and carcass traits among sheep breeds. Lambs sired by rams (breeds) of low mature weight reach a fixed weight at a later age than those sired by rams of high mature weight and reach a similar degree of fat cover at an earlier age and lighter weight than the larger breeds. When slaughtered at a constant fatness, however, differences between prominent terminal sire breeds are relatively small (Hanrahan, 1999).

Correspondingly, modern pig breeds which have been genetically improved to achieve fast growth and a lean meat deposition differ from “local” pig breeds with respect to fat deposition, whereby the latter have higher adipogenic potential and low muscle mass deposition (Poklukar et al., 2020).

While many breed comparison studies have focused on the sire, there is also a dam breed (maternal) effect, especially on preweaning growth as the (suckled) offspring are partially reared by the mother’s milk (e.g., Sapkota et al., 2020). It should be noted that although many of the common breed types are productive in a wide range of environmental and management conditions, their ranking for

various production traits may vary with extremes in nutrition or environment, i.e., genotype × environment interactions.

2.3.2.2 Crossbreeding

Breed diversity can be exploited via breed substitution, systematic crossbreeding systems or composite (synthetic) breed development. Crossbreeding exploits available heterosis or hybrid vigor and complementarity between different breeds or population lines. For example, in temperate climates, the heterosis of postweaning gain among *Bos taurus* breeds is c. 5%, carcass fatness is 3%–4% and leanness is −2%; these differences are much greater when *Bos taurus* and *Bos indicus* breeds are crossed in tropical climates (Renand et al., 1992). Individual heterosis effects for carcass weight in British × British, British × Continental, Continental × Continental, British × Zebu and Continental × Zebu cattle breed types equated to 10.3, 13.1, 16.4, 42.0 and 24.6 kg, respectively (Williams et al., 2010). Similarly, composite populations (breeds) of cattle and sheep offer an alternative breeding system that is generally competitive with crossbreeding for using heterosis and can be easier to manage on a continuing basis.

2.3.2.3 Within breed differences

In addition to breed differences, there is a large amount of genetic variability for growth and carcass traits within breed and line. The efficiency of genetic gain under selection depends on the intensity of selection, the accuracy with which the genetic merit (breeding value) of each animal is known, the genetic variance among animals and the generation interval. In particular, the generation interval varies between farm animal species, being highest for cattle and decreasing for sheep and pigs, in that order.

Heritability is the proportion of total phenotypic variation in a trait that is explained by genetic variation between individuals. Mean-weighted heritability estimates in beef cattle for growth traits (birth, weaning, postweaning and mature weight) ranged from 0.13 to 0.50, generally increasing with age (Koots et al., 1994). Mean heritability estimates for carcass weight, backfat thickness, *longissimus* muscle area and marbling score (measured at, or adjusted to age, weight or fat depth slaughter endpoints) in beef cattle were similar and moderate—0.40, 0.36, 0.40 and 0.37, respectively—although they varied greatly across studies (Rios-Utrera and Van Vleck, 2004). For sheep, weighted mean heritability estimates for growth traits ranged from 0.12 to 0.57—also increasing with age—and values for carcass weight, fat depth and eye muscle measurements were 0.20, 0.30–0.32 and 0.29–0.41, respectively (Safari et al., 2005). Similarly, heritability estimates are published for growth and carcass traits in pigs (Akanno et al., 2013).

Although genetic selection has increased productivity of livestock species considerably, there is evidence that animals in a population selected for high production efficiency seem to be at risk for behavioral, physiological and im-

munological problems, which can result in undesirable metabolic, reproduction and health traits. Such selection can also be associated with meat quality issues. An important consideration in comprehensive genetic improvement programs is whether genetic effects among traits and trait systems are correlated, and especially so if genetic correlations are antagonistic. Consequently, it is not recommended that any genetic improvement program focus on any single trait. In practice, economically weighted, multiple-trait genetic selection indices are used to identify superior animals (e.g., Clarke et al., 2009; Berry, 2021), usually based on the expected profitability of their progeny. Genetic indices are extending to include more animal robustness-related traits (Friggens et al., 2017). Application of modern reproduction techniques, availability of sufficient reliable phenotypic data, the integration of DNA technologies and marker-assisted selection, such as genomics, coupled with the employment of state-of-the-art precision agricultural technologies to measure animal phenotypes (phenomics) to further increase the accuracy of breeding values, should result in enhanced genetic selection and progress in meat animals through structured animal breeding programs (Rexroad et al., 2019; Mohammadabadi et al., 2021).

2.3.2.4 Efficiency of growth

Efficiency of resource utilization is an essential contributor to the continued sustainability of the production of food of animal origin. Consequently, there is considerable interest in enhancing the efficiency of feed utilization within the production of ruminants (Kenny et al., 2018) and pigs (Patience et al., 2015), but also with an increasing emphasis on reducing the environmental "hoof-print" of livestock production. Growth rate and feed efficiency (feed conversion ratio (FCR) or feed conversion efficiency (FCE)) have been the standard metrics for evaluating the growth in meat animals in both genetic and nutritional studies. Because feed efficiency is correlated with both growth rate and body composition, improvements in growth and body composition (more lean, less fat) resulting from improved nutrition or genetic selection have resulted in improvements in feed efficiency in growing animals. Breed differences in feed efficiency must be interpreted in the context of the (slaughter) endpoint as ranking of breeds can vary with changes in the slaughter endpoint. For example, ranking of cattle breeds for efficiency of live weight gain per unit of energy consumed was inversely correlated with the number of days of maintenance required to reach the endpoint (Cundiff et al., 2004). On a constant age basis, beef breeds and beef crossbreds are generally more feed-efficient than beef × dairy breeds, which in turn are more efficient than dairy breeds (Friesian and Holstein); within the beef breeds, late-maturing breeds are more feed-efficient than early-maturing breeds, especially in terms of muscle production (Keane, 2011). At a common fatness endpoint, steers with British breed sires were more efficient than those with Continental European breed sires because they reached the endpoint in fewer days; however, the opposite was evident when expressed in terms of retail product (edible meat) endpoint (Cundiff et al., 2004). Differences in the rates

of water, protein and fat deposition influence efficiency and rate of body weight gain primarily because fat has higher energy density than either protein or water (Owens et al., 1995).

More recently, there is increased interest in residual feed intake (RFI) as an alternative measure of feed efficiency in cattle (Kenny et al., 2018), sheep (Tortereau et al., 2020) and pigs (Gilbert et al., 2017). RFI is defined as the difference between actual feed intake (dry matter or energy) and that predicted on the basis of maintenance (weight) and production (growth) and is a moderately heritable trait. Unlike FCR and FCE, it is phenotypically independent, although not necessarily genetically independent, of the production traits from which it is derived. This means that antagonistic responses, such as increased mature size of animals, can be reduced. There is significant interanimal variation in feed efficiency in animals offered feed to appetite (Kenny et al., 2018). For example, phenotypic differences in dry matter intake between the most feed-efficient and -inefficient terciles of up to 15% in young growing cattle have been reported. Concomitant with this, there is also significant genetic variance in the RFI trait. Research to date has clearly shown that feed efficiency in ruminants and pigs is a complex multifaceted trait, under the control of many biological processes (Gilbert et al., 2017; Cantalapiedra-Hijar et al., 2018).

2.3.3 Gender

The term gender as discussed here refers to intact males, castrated males and females. Growth rate and the composition of gain of animals are significantly affected by differences in gender, arising mainly from effects of sex steroids. In general, with cattle, sheep and pigs, intact males grow more rapidly, utilize feed more efficiently and produce a carcass with a higher yield of retail product with less fat and more red meat than castrates (or females). In cattle, the superiority of bulls over comparable steers for live weight gain, carcass weight and lean meat yield was 0.08–0.20, 0.09–0.14 and 0.20, respectively, and they produce carcasses with better conformation and 0.27–0.35 less fat (O'Riordan et al., 2011). Ram lambs grow more rapidly (0.10–0.15) and produce leaner (0.07–0.12) carcasses than castrated lambs (wethers) and also grow faster than females pre- (0.08) and postweaning (0.17) (e.g., Hanrahan, 1999). Boars have superior growth, up to 0.09, and leaner carcasses, up to 0.20, than castrates (Lundström et al., 2009). In ruminants and pigs, the differences in favor of intact males over castrates can vary across studies, due to factors such as breed, feeding system, diet and slaughter weight. In terms of converting feed to live weight gain, the feed conversion efficiency of bulls is 0.13–0.17 better than steers (O'Riordan et al., 2011) and that of boars is up to 0.14 better than castrates (Lundström et al., 2009).

2.3.4 Genetic modification

Traditionally, animals were genetically modified using conventional breeding and selection practices to produce improved livestock, e.g., crossbreeding as

described in Section 2.3.2. Although the capacity of transgenic technology to produce livestock with enhanced characteristics has been available since 1985, the few high-profile reports are typically proof-of-principle studies, and commercial application of genetically modified animals is still limited and under development. However, the recently developed nuclease-mediated genome editing technology has stimulated interest in the generation of genome-edited livestock. Precision editing of the endogenous genome without introducing foreign DNA could become a new breeding technology to produce genetically modified organisms for human consumption. Several nucleases have been successfully used for gene editing, including zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and the clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) protein 9 system. The natural mutation in the myostatin gene which results in muscle hypertrophy was mentioned earlier. In an early example of gene editing, a ZFN-mediated mysostatin mutation in Chinese Meishan pigs led to developmentally normal animals that exhibited an increase in muscle mass by 100% and a decrease in fat accumulation compared with wild-type animals. Of the gene editing tools presently available, CRISPR is the most accessible, is relatively easy to use and is highly specific and programable (Brandt and Barrangou, 2019). Using CRISPR/Cas9, goats were "edited" to have increased body mass, while pigs were "edited" to be leaner. These examples demonstrate the potential of this technology, but societal and regulatory challenges remain before commercial application in the livestock industry. Van Eenennaam et al. (2021) have summarized the transgenic food animals developed to data and discussed the opportunity cost of the regulatory delay in approving their commercial use.

2.4 Nonanimal influences on growth of farm animals

2.4.1 Nutrient supply

In meat animal production systems, optimum economic efficiency is greatly influenced by supplying the appropriate amount and balance of nutrients to achieve a commercially desirable endpoint, usually carcass weight and saleable lean meat yield. Relationships between the amount of nutrients required by an animal to achieve such endpoints are under continued investigation and have resulted in "rationing systems" to guide producers. As energy and protein are the most important initial considerations, currently available systems are briefly reviewed below. Since the ruminant digestive tract presents particular challenges to estimating the nutrients available to the animal after ruminal fermentation and subsequent digestion in the gastrointestinal tract, the focus is on ruminants.

2.4.1.1 Energy feeding systems

Six of the major feeding systems used around the world for ruminants—namely the metabolizable energy (ME) system in the United Kingdom (AFRC) and

Australia (CSIRO), and the net energy (NE) systems in France (INRA) and North America (NASEM and RNS) and Brazil (BR-Corte) were reviewed by Tedeschi et al. (2017) and Cabezas-Garcia et al. (2021). There is no difference in principle between these systems, all of which consist of two components:

a. feed evaluation for energy concentration in feeds, i.e., the energy supply from consumed feeds, and

b. calculation of energy requirements for the various functions of animal production.

For feed evaluation, all systems use ME concentration as the basic energy term for feed ingredients. Metabolizable energy concentration in feeds can be accurately measured using digestibility trials and calorimeter chambers to measure gross energy intake and energy outputs in feces and urine and as methane in breath, although this is time-consuming and costly. Net energy concentration in feeds cannot be measured, but estimated using its ME concentration multiplied by energetic efficiencies. Energetic efficiencies vary with ME used for different functions of animal production, e.g., efficiency of utilization of ME for maintenance (k_m), for lactation (k_l), for live weight gain (k_g) and for pregnancy (k_f). In NE systems, energy supply from feeds is based on an NE term, and as a result, a single feed can have different NE concentrations depending on the functions of animal production. In the ME system, only one ME value is assigned to a feedstuff, because energetic efficiencies are only used in calculating ME requirements for various functions of animal production.

In calculating energy requirements, all systems recognize that NE requirement (NE_r) of beef cattle and sheep is the sum of their requirements for maintenance (NE_m), milk production (NE_l), live weight gain (NE_g) and fleece growth (NE_w) and fetal growth (NE_f). However, there are differences between systems in calculating these components and the respective efficiencies of utilization of ME.

2.4.1.2 Protein feeding systems

The major protein feeding systems used worldwide for ruminants—including the United Kingdom, North American, French, Australian, Dutch, German and Nordic systems—were reviewed by Tedeschi et al. (2017). In the past, protein rationing for ruminants was based on digestible crude protein (DCP). This system simply measured feed CP intake and CP output in feces, and the remaining protein was assumed to be available for animal production. The major deficiency of DCP was that the effect of rumen fermentation on the availability of feed protein, or the contribution of microbial protein produced in the rumen to the overall protein economy of the animal, was not recognized. In the early 1990s, most models adopted the metabolizable protein (MP) system. All systems share the same principle, i.e., available dietary protein for animal production includes digestible protein from both feeds (digestible undegradable dietary protein) and rumen microorganisms (digestible microbial protein). The sum of digestible undegradable dietary protein and digestible microbial protein is referred to as MP.

In calculating protein requirement, all systems recognize that total net protein (NP) requirements (NP_r) of beef cattle and sheep is a sum of requirements for maintenance (NP_m), milk production (NP_l), live weight gain (NP_g) and fleece growth (NP_w) and fetal growth (NP_f). For feed evaluation, degradable dietary protein is calculated from total CP multiplied by protein degradability. Protein degradability is usually measured in vivo using the dacron/nylon bag technique with rumen fistulated cattle and sheep or in vitro using proteolytic enzymes and adjusted for variations in the outflow of digesta from the rumen. In all systems, undegradable dietary protein is calculated as the difference between total CP intake and the protein degraded in the rumen. The latter is degraded into ammonia in the rumen as a result of enzymes secreted by rumen microorganisms, and the ammonia can then be used by rumen microorganisms as a nitrogen source for microbial growth. Production of microbial protein depends on the availability of degradable CP and also the availability of energy. Theoretically, the synchronous supply of degradable CP and fermentable ME/degradable organic matter promotes the optimum microbial growth in the rumen. However, nonincorporated ammonia is excreted, and therefore dietary protein may be wasted on high-protein diets and also contribute to environmental pollution. On the other hand, nonprotein nitrogen (e.g., urea) can be converted into protein when low-protein diets are fed. All systems present two approaches to estimate the microbial protein production. If degradable protein is insufficient, microbial protein production is calculated from rumen degradable protein supply. If degradable protein is oversupplied, microbial protein production is calculated from fermentable ME/degradable organic matter. Current protein rationing systems for ruminants do not directly consider the profile of animal acids available for absorption from the small intestine. In contrast, for pigs, the requirement for specific amino acids is included in dietary recommendations. An example is given in Table 2.2 (van der Peet-Schwering and Bikker, 2018). Research will likely result in future protein feeding systems for ruminants that are based on individual amino acid supply rather than protein per se.

For whichever feeding system used, the producer can match the requirements of the animal for a particular rate of growth/carcass weight with the most economically available feedstuffs.

2.4.1.3 Other nutrients

Minerals and vitamins are required for the normal functioning of almost all metabolic processes in animals. Dietary deficiencies or excesses of certain minerals and vitamins can result in ill-health and loss of productivity in meat animals. Mineral and vitamin requirements for growing ruminants have been published as part of many of the aforementioned feeding systems. Recent findings with regard to phosphorous, chromium, cobalt, copper, manganese, zinc, vitamins A, D, E and B_{12} and biotin, choline, niacin and thiamine have been summarized by Spears and Weiss (2014). As for ruminants, nutrient requirement guidelines have been compiled for pigs, e.g., “Nutrient Requirements of Swine” (NRC, 2012).

TABLE 2.2 Recommendations for standardized ileal digestible (SID) essential amino acids in starter, grower and finisher pig diets (expressed as % of SID lysine).

Sire breed	Starter diet (25–50 kg)	Grower diet (50–80 kg)	Finisher diet (80–120 kg)
Lysine	100	100	100
Methionine + cystine	60	61	62
Threonine	66	67	68
Tryptophan	20	20	20
Isoleucine	53	53	53
Valine	67	67	67
Leucine	100	100	100
Histidine	32	32	32
Phenylalanine + tyrosine	95	95	95

Modified from van der Peet-Schwering, C.M.C, Bikker, P., 2018. Amino Acid Requirement of Growing and Finishing Pigs. Wageningen Livestock Research Report 1101, 35 pp.

2.4.2 Exogenous agents

These agents include steroid hormones, β2 agonists, somatotropin, immunological approaches and gut-active compounds. Many of these products require regulatory approval and, depending on the country and animal species or category, may or may not be permitted for use. For example, growth-promoting hormones and selected β2 agonists are permitted for use in beef production in the United States, Canada, Australia, South Africa and some South American countries. However, the use of hormones and β2 agonists in beef production is not permitted in the European Union (EU), and consequently, the EU does not permit the importation of meat produced using these technologies.

2.4.2.1 Steroid hormones

The inferior growth of castrated animals was discussed in Section 2.3.3. The effect of the removal of the gonads can be restored by the administration of either male hormones (androgens) or female hormones (oestrogens). These include naturally occurring steroids such as oestradiol-17β, progesterone and testosterone and synthetic steroids such as zeranol, melengestrol acetate and trenbolone acetate. Administration as removable implants is the preferred mode of usage. Estrogenic products are effective in steers, androgenic products are effective in heifers, and combination products are also effective. These anabolic

agents increase rates of muscle protein synthesis and deposition and/or decrease protein degradation, and decrease the amount of fat at a particular live weight. Although hormonal growth promoters increase feed intake by 5%–10%, they decrease the amount of energy required for maintenance, increasing the amount available for growth, thereby improving feed efficiency by 5%–15%. Daily gain can be improved by up to 25% when aggressive implant strategies are used in cattle fed high-concentrate diets (see Greenwood and Dunshea, 2009). The use and efficacy of hormonal growth promoters in the Australian (Hunter, 2010) and US (Johnson and Beckett, 2014) beef industries were comprehensively reviewed. Unlike for ruminant animals, anabolic implants appear to have little effect upon growth performance and carcass quality in pigs and so are not used in pork production.

2.4.2.2 β2 agonists

The catecholamine receptors are classified as α, β1 and β2 adrenergic, on the basis of their binding pattern and on the response they elicit. β2 agonists bind to β2 receptors and promote metabolism and are of particular interest in relation to meat animals. When β2 agonists (such as cimaterol, clenbuterol, ractopamine and zilpaterol) are included in the diet of growing cattle and pigs, they cause a marked repartitioning between fat and protein whereby animals become leaner. While there is general agreement that protein deposition is increased during β-agonist treatment, effects on fat deposition have been equivocal. β2 agonists act by increasing lipolysis and decreasing lipogenesis and by reducing protein breakdown. The magnitude of these changes is influenced by dose and duration of treatment with the β-agonist, the type of β-agonist and the animal species. For example, β-agonists do not appear to decrease fat deposition in pigs, whereas they have pronounced effects on fat deposition in ruminants. Treatment of pigs with β-agonists, particularly ractopamine, generally increased average daily gain (10%), decreased daily feed intake (3%–5%) and increased carcass lean content (50%, approx.). In finishing cattle, β-agonists increased growth rate by 0.15–0.19 kg/day, decreased daily feed intake by 0–0.12 kg/day, increased carcass weight by 6.2–15 kg, decreased marbling score by 5–23 units and increased the cross-sectional area of the *longissimus* muscle by 2–8 cm^2 (Lean et al., 2014). Hormones and β2 agonists currently used in cattle and pig production were comprehensively reviewed by Aroeira et al. (2021).

2.4.2.3 Somatotropin

Somatotropin (ST) is a naturally occurring protein hormone produced by the anterior pituitary gland and secreted into the circulation. ST has an integral role in the coordination of lipid, protein and mineral metabolism in mammalian species. Elevation of plasma ST redirects nutrients toward increased muscle and bone growth and decreased adipose tissue growth in meat animals (Dunshea et al., 2016). Exogenous porcine ST (*p*ST) treatment (regular injection) consistently

improves average daily gain, feed conversion efficiency and protein deposition and reduces fat deposition and the effects appear to be dose-dependent. In all animal species, nutrient intake needs to be maximized for the full benefits of ST to be realized (Dunshea et al., 2016).

Other growth-promoting compounds include, but are not limited to, cysteamine, chromium, betaine and dietary neurolepetics (e.g., tryptophan and potassium bromide) (Dunshea et al., 2016).

2.4.2.4 Gut-active compounds

There are some additives aimed at altering the nature of the fermentation in the rumen of ruminants, but the majority are aimed at monogastric species or at young ruminants prior to rumen development.

Ruminants

Ionophores and nonionophores are antibiotic growth promoters (AGPs) used as feed additives for beef cattle. Of these compounds, most information is available for the ionophore antibiotics, monensin and lasalocid. Ionophores typically selectively inhibit Gram-positive bacteria in the rumen with a consequent increase in propionic acid production and an improvement in ruminal nitrogen metabolism. Inclusion of monensin in cattle diets increased growth rate by 29 g/day, decreased feed consumption by 268 g/day and improved the feed-to-gain ratio by 0.53 kg/kg (Duffield et al., 2012). Corresponding values for lasalocid were 40 g/day, 0 g/day and 0.41 kg/kg (Golder and Lean, 2016).

Concerns about the use of antibiotics in livestock feeding possibly contributing to antibiotic resistance in bacteria that might represent a risk to human health led the EU to prohibit the use of AGP in 2006. This prohibition together with increasing consumer preference for products of production systems that did not use antibiotics has led to research and development of "natural" alternatives such as live/dead yeast, yeast culture media, bacteria, essential oils and fibrolytic enzymes. Of these materials, the majority investigated to date have been bacteria. However, there have also been numerous studies using molds and yeast, the majority of which were the yeast *Saccharomyces cerevisiae* and the molds *Aspergillus niger* and *Aspergillus oryzae*. Feeding combinations of live bacteria to feedlot cattle would result in a 2.5%–5.0% increase in growth, a 2.0% improvement in feed efficiency, although the dry matter intake may be inconsistent (Buntyn et al., 2016). Research on alternatives to antibiotics in ruminant nutrition is ongoing, and more recently, the use of essential oils and fibrolytic enzymes shows promise. Currently, there is also much interest in the development of additives to decrease methane production in the rumen as ruminants are now considered to make a substantial contribution to global greenhouse gas emissions. It remains to be seen if there is a growth enhancement benefit from such additives.

Monogastrics

In monogastric production, antibiotics are used firstly to improve overall animal health with an ancillary benefit of an increase in body weight and increased feed efficiency. As with rumen modifiers above, recent research has resulted in the development of an array of possible alternatives (Table 2.3). Some of these include direct-fed microbials (Buntyn et al., 2016) but also probiotics, prebiotics,

TABLE 2.3 Potential alternatives to antibiotics.

Gut flora modifiers and stabilizers	**Nutrient modifiers**
Antibacterials	*Enzymes*
Single organic or inorganic chemicals, e.g., Emtryl (dimetridazole), copper sulfate, zinc oxide nitrates	Usually targeted at complex polysaccharides to break down gels and antinutritive factors to deactivate them, e.g., β-glucanase; may also be targeted at organic dietary phosphate complexes (phytates), e.g., phytase
Probiotics	*Zeolites and clay minerals*
Often cultures of favorable bacteria that have been freeze-dried, e.g., specific strains of *Lactobacillus*; sometimes yeasts or yeast extracts	Said to absorb (sequester) toxic molecules such as ammonia and amides
Chemical probiotics	*Surfactants*
Specific sugars, such as mannose or mannans, which interfere with the attachment of pathogens to the gut wall	Lecithins and saponins
Organic acids	**Physiological regulators**
Usually added with the intention of providing a favorable environment for lactobacilli, e.g., lactic acid, fumaric acid	*Stimulants*
Nutraceuticals	e.g., caffeine
Usually regarded as natural products of plants that have antiseptic, antiyeast or antifungal properties; often a mixture of essential oils, e.g., oregano oil	*Tranquillizers*
Prebiotics (nutrabiotics)	e.g., aspirin
A substrate acting as a fermentable substrate for favorable bacteria, e.g., fructans, mannans, hemicellulose	

Modified from Lawrence, L.J., Fowler, V.R., Novakofski, J.E., 2012. Growth of Farm Animals, third ed. CABI Wallingford UK, Cambridge, MA, USA, 352 p.

enzymes, acidifiers, plant extracts, essential oils, zeolites, clay minerals and neutraceuticals such as copper and zinc. The reader is directed to Thacker (2013) for a comprehensive discussion on alternatives to antibiotics in pig production.

2.4.3 Immunological approaches to increasing growth

Consumer resistance to physical or surgical castration in farm animals has encouraged the development of other methods to suppress the gonads. More graded reduction of male hormone production could permit control of aggression and fertility (and of boar taint in pigs) while retaining sufficient circulating androgens and oestrogens to produce desired anabolic effects. One alternative method is immunocastration. This involves actively immunizing the animal against gonadotrophin-releasing hormone (GnRH). The antibodies raised against GnRH prevent the release of gonadotrophins from the pituitary gland, and as a consequence, testosterone production in the testes ceases temporarily. Improvac is a commercial vaccine which was primarily developed to decrease "boar taint" in male pigs by reducing the concentrations of skatole and androstenone below threshold levels but also increased growth rate and feed intake (Dunshea et al., 2001). Although there are fewer studies in cattle, immunocastration can increase average daily gain over that of "control" bulls (Amatayakul-Chantler et al., 2012) or physically castrated steers (Marti et al., 2015), although results are more variable than for swine.

Importantly, the effects of immunocastration appear to be additive with other technologies used in the livestock industries, thereby allowing for utilization of the increased feed intake observed with immunocastration to be incorporated into lean tissue. For example, the growth-promoting effects of porcine somatotropin can be further enhanced when used in conjunction with immunocastration. Similarly, there are additive effects of ractopamine and immunocastration in pigs (see Dunshea et al., 2016). In cattle, the increase in daily gain in response to hormone growth promotants is also augmented by immunocastration (Amatayakul-Chantler et al., 2012).

2.4.4 Environmental influences on animal growth

Other important factors that affect livestock growth and productivity are health/disease and climate. Meta-analyses have been conducted on of the effects of infection/disease on growth of pigs Kippera et al., 2011; Pastorelli et al., 2012) and cattle (Larson, 2005). With regard to temperature, animals generally attempt to maintain their body temperature at a constant value which is optimum for biological activity. In an environment of low or high temperature, development may be retarded. For example, exposure to high temperature (and humidity) can overcome the capacity of cattle to dissipate heat and lead to an increase in body temperature that exceeds the physiological limits resulting in "heat stress." In this condition, dry matter intake is reduced and growth decreases (Greenwood

and Dunshea, 2009). Animals may show signs of adaptation to the prevailing climate. Thus, cattle of more temperate regions have a somewhat less compact frame, while tropical cattle have an angular frame, larger extremities, a large dewlap (i.e., the fold of skin hanging between the throat and brisket of certain cattle) and tend to have a lighter coat color which has very short hair. Some of the greater heat tolerance of tropical cattle can be attributed to a relatively thinner layer of the subcutaneous fat, i.e., a thinner "insulation" layer of tissue. Fatter animals and/or with a heavier hair coat (i.e., higher insulation) and/or darker-coated animals (e.g., Angus cattle) are more sensitive to heat (Summer et al., 2019). The zone of thermal neutrality in an animal is that in which metabolic heat production is independent of air temperature. The zone of thermal neutrality for cattle is broad (c. 20°C), whereas this zone is much narrower in the pig. The effects of high ambient temperature on growth performance of sheep (Marai et al., 2007) and pigs (Renaudeau et al., 2011) have also been reviewed. Given the increasingly severe weather fluctuations associated with climate change, adapting to cold and heat stress is likely to become a bigger issue in animal production in the future.

2.5 Interactions between animal and nonanimal influences on growth of farm animals

The general pattern of growth from conception to maturity has been briefly summarized in Section 2.1 (see also Lawrence et al., 2012). Emerging data indicate that while prenatal growth can be influenced greatly by maternal nutrient supply, subsequent postnatal growth can be permanently affected by the uterine experience, and there may be interactions between the two phases. The focus here will be on nutritional effects on growth.

2.5.1 Growth in utero

2.5.1.1 Fetal developmental programing

The embryonic and early fetal periods, encompassing early-to-mid gestation, represent phases of maximal development as genesis of most organs and tissues commences and is completed during this time. Factors that regulate fetal growth, development and birth weight include placental size and capacity for nutrient transfer; parity, age and size of the dam; maternal, paternal and fetal genotypes and litter size. Epidemiological studies on humans and experiments on animals (mostly rodents) have demonstrated adverse consequences for adult health and well-being due to stressors imposed during fetal life, resulting in the "Fetal Origins" and "Thrifty Phenotype" hypotheses and the concept of developmental plasticity (Robinson et al., 2013). Fetal developmental programing is the response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development qualitatively and/or quantitatively (Du et al., 2017).

Fetal programing/maternal nutrition effects on productivity have recently been reviewed in cattle (Greenwood et al., 2017; Robinson et al., 2013), sheep (Kenyon and Blair, 2014) and pigs (Oksbjerg et al., 2013). In general, studies with cattle and sheep indicate that the level of nutrition in early pregnancy is unlikely to influence fetal growth and offspring birth weight unless nutritional restriction is severe and prolonged. In addition, it seems that maternal nutritional excess has limited impact on birth weight. Results from large-scale beef cattle studies carried out in Australia were recently summarized by Greenwood and Dunshea (2009) and by Robinson et al. (2013). Within a pasture-based production system, severe, chronic maternal nutritional restriction resulted in fetal growth retardation and reduced birth weight. Growth-retarded offspring continued to have reduced live weight until slaughter at 30 months of age with little evidence of compensatory growth after weaning. At feedlot exit at 30 months of age, for every 1 kg difference in birth weight, there was a difference of 4.4 kg in live weight. Fetal growth retardation, resulting in low birth weight, may limit skeletal and muscle growth potential and result in a predisposition toward reduced mature lean body mass and increased fatness at any given postnatal live weight (reported for sheep (Kenyon and Blair, 2014), cattle (Moloney and Drennan, 2006) and pigs (Oksbjerg et al., 2013; Gatford et al., 2018)). Maintenance energy requirements are likely to be less due to smaller size, and animals that are smaller at any given age during growth are likely to be at an earlier stage of the allometric growth patterns for specific body components contributing to increased requirements for protein relative to energy. These factors, which are not specific effects of fetal programing, may interact with factors that are thought to be programed, such as a thrifty phenotype due to prenatal growth restriction (Robinson et al., 2013). In pigs, characterized by multiple fetuses, pigs of light birth weight tend to have a lower growth to slaughter when compared with their larger littermates and similarly tend not to display compensatory growth (Oksbjerg et al., 2013). Increasingly, studies are being conducted that seek to limit intrauterine growth retardation and variability in growth between fetuses within porcine litters and/or enhance fetal growth and development.

Most of the studies on fetal programing have focused on the maternal influence. Recently, Khatib (2021) examined the impact of prepubertal diet in rams on production and reproduction traits, DNA methylation and transmission to offspring. From weaning until puberty, 12 rams were fed a control diet (basal concentrate diet of the farm) and 12 rams (of twin pairs) were fed a treatment diet (basal concentrate diet plus rumen-protected methionine). Methionine-treated rams reached puberty earlier than the control rams. The offspring of the treated rams showed differences in overall growth and scrotal size, thus demonstrating that the paternal diet led to altered phenotypes in the offspring.

2.5.1.2 Epigenetics

Epigenetics may be defined as heritable changes in gene expression resulting from alterations in chromatin structure but not in DNA sequence. Epigenetic

changes to the genome are caused by DNA methylation, histone modification and/or noncoding microRNAs. Epigenetic modifications can result from internal as well as external stimuli, thus allowing gene expression in the fetus to best fit with environmental stimulation. A prominent example of epigenetics comes from human epidemiological studies. Severe food restriction in Western Holland during World War II resulted in women from that area being undernourished during the last trimester of pregnancy and giving birth to babies with reduced weight. In the absence of any further dietary restriction, these babies also gave birth to babies of lower birth weight in the subsequent generation (Susser and Stein, 1994).

There is now accumulating evidence that maternal diet-induced "programing" can be transmitted across generations in meat animals. For example, when groups of ewes were fed to 100% or 150% of their nutrient requirement, resulting in obesity in the latter animals, there was no difference in lamb birth weight between the groups. Lambs born to obese ewes, however, had greater visceral adiposity at birth when compared with lambs born to nonobese ewes (Long et al., 2010). Female offspring were returned to similar body weights and adiposities, bred to a single ram and fed only to requirements from conception throughout gestation (Shasa et al., 2015). As with the first-generation offspring, no differences were observed in birth weight in lambs from obese grandmothers or nonobese grandmothers. However, second-generation lambs born to first-generation offspring of obese mothers had significantly more visceral adiposity when compared with second-generation lambs born to first-generation offspring of nonobese mothers, indicating a clear intergenerational effect.

Khatib (2021) defined intergenerational epigenetic inheritance as "the transmission of epigenetic marks between two generations" and transgenerational epigenetic inheritance as "transmission across multiple generations" and concluded that "at present, there is no definitive evidence of transgenerational epigenetic inheritance in farm animals or mammalian species in general." In the study of Khatib (2021) above, the paternal diet influenced epigenetic modifications in the sperm, which in turn altered DNA methylation and gene expression and contributed to the altered phenotypes in the offspring. The magnitude, parental origin and persistence of prenatal effects, in particular those mediated by epigenetic modifications of the genome, need to be better quantified.

2.5.2 Postnatal growth

2.5.2.1 Plane of nutrition

Animals on different planes of nutrition, even if they are of the same breed and weight, will differ greatly in form and composition (see Lawrence et al., 2012). Factors that influence the partition of absorbed nutrients and hence affect body composition include: maturity of the animal; level of feeding relative to maintenance; protein absorption relative to requirements; balance of protein to energy in the diet and available for absorption by the animal; prior nutrition that may

result in compensatory growth and affect the composition of gain (below); and climatic conditions that may limit growth rate and fat synthesis. Animals of the same genotype when fed to grow rapidly to a given age or weight will generally have a lower proportion of lean tissues and a higher proportion of fat than those fed to grow more slowly, i.e., growth rate per se will increase fat deposition relative to protein deposition (Owens et al., 1995). Although fast rates of growth caused by a high plane of nutrition can lead to an earlier onset of the fattening phase of growth, the nature of the diet is also an important growth-regulating factor as fat deposition can be influenced by the energy and protein concentration in the diet. In pigs, restricting energy intake by feeding a low-energy (low fat and/or high fiber) diet while supplying adequate protein will reduce carcass fat deposition. Feeding excess protein, i.e., excess essential amino acids, to pigs will result in a higher proportion of lean in the carcass, but the effect is primarily a result of energy restriction relative to protein. The converse, restricting protein supply while supplying adequate energy will increase fat deposition.

2.5.2.2 Compensatory growth

In animal production systems which evolve to optimize economic efficiency, it may not be appropriate for growth be at a maximum throughout postnatal life. This is frequently the case in beef production systems in temperate climates whereby cattle are programed to grow relatively slowly over the expensive indoor winter period prior to grazing cheaper pasture or in more extreme climates are "backgrounded" on poor pastures prior to finishing on a high energy ration in a feedlot (Australia and United States) (e.g., Greenwood and Dunshea, 2009). In both scenarios, producers seek to exploit the phenomenon of compensatory growth. Compensatory growth is the ability of an animal to undergo accelerated growth when offered unrestricted access to high-quality feed after a period of restricted feeding or undernutrition. This phenomenon has also been reported for pigs (Menegat et al., 2020). Compensatory growth is greatest when animals are relatively mature at the end of the period of dietary restriction, the period of restriction is short (3 months for cattle) and not overly severe, and the diet during the restricted period is different than that during the period of realimentation. The influence of the type of diet during recovery was examined by Tudor et al. (1980). Calves were fed a high or low plane of nutrition immediately postpartum until weaning at 200 days of age. With each group, calves were then offered a concentrate feed or pasture until slaughtered at ~400 kg live weight. There was an interaction between postweaning nutritional restriction and the nature of the recovery ration. Thus, nutritionally restriction resulted in animals that were fatter than their nonnutritionally restricted counterparts when they recovered on a concentrate ration. In contrast, there were no differences in body composition between the restricted and well-nourished calves when they recovered on pasture (Table 2.4).

TABLE 2.4 Effects of recovery from severe nutritional restriction during artificial rearing from birth to weaning at 200 days of age on carcass composition when cattle are recovered on pasture or a concentrate diet (intensive) to the same carcass weight.

	Preweaning nutrition	
Trait	**Low**	**High**
Weaning BW, kg	39.3*	165.7**
Age at slaughter, months		
Intensive recovery	21.1*	15.2**
Pasture recovery	30.1*	25.3**
Carcass weight, kg		
Intensive recovery	212.9	213.7
Pasture recovery	213.6	214.4
Fat, % carcass weight		
Intensive recovery	34.1*	22.9**
Pasture recovery	23.6	23.8
Protein, % carcass weight		
Intensive recovery	14.0*	15.3**
Pasture recovery	16.5	16.4*

*$p<0.05$
**$p<0.01$
Modified from Tudor G.D., Utting D.W., O'Rourke P.K., 1980. The effect of pre- and post-natal nutrition on the growth of beef cattle. III. The effect of severe restriction in early postnatal life on the development of the body components and chemical composition. Aust. J. Agric. Res. 31, 191–204.

2.5.2.3 Interaction between prenatal and postnatal growth

The literature is unclear as to whether animals that were growth retarded in utero can exhibit compensatory body weight growth during postnatal life. Where maternal nutrient restriction has resulted in a decrease in birth weight, the subsequent effect on growth and carcass fatness is likely to be influenced by the duration of the recovery period, the quality of the ration and the slaughter weight. Nutrition during the preweaning/early postpartum period may ameliorate or exacerbate longer-term outcomes for productive characteristics affected by growth and nutrition during fetal life. Under a pasture-based Australian beef production system, there were few interactions between effects of maternal nutrition during pregnancy and maternal nutrition during lactation (7 months) prior to backgrounding on a common ration (19 months) and finishing on a high-energy

feedlot ration (4 months). Growth-retarded offspring achieved target slaughter body weights older than their nongrowth-restricted counterparts (Robinson et al., 2013). In the study of Tudor et al. (1980) above, cows were fed either a submaintenance or above-maintenance diet during the last trimester of pregnancy, but the maternal dietary restriction did not decease birthweight, and there was no interaction between prenatal and postnatal nutrition. Similarly, interactions between prenatal and preweaning nutrition for postweaning growth, feed intake, feed efficiency and carcass characteristics were not evident in the study of Stalker et al. (2006). Nissen and Oksbjerg (2011) found no interaction between birth weight and the protein concentration of the postweaning ration in the growth of pigs. In production systems where the interval between weaning and slaughter is short, e.g., in young (>16 months) bull production, the impact of maternal nutrition and postweaning ration composition on growth and development merits study.

2.6 Future developments

The principles of animal and tissue growth enunciated in the mid-20th century remain intact today. Much of the succeeding research summarized, in brief, in this chapter has been concerned with understanding how the patterns of tissue growth are influenced by the type of animal and nonanimal factors. The ultimate focus in meat animal production is to produce, in a cost-efficient and sustainable manner, products that meet consumer requirements with respect to composition and quality. These endpoints are addressed elsewhere in this book. There has been rapid progress in the development of techniques that are allowing investigation, at a cellular and tissue level of the fundamental processes underpinning animal growth and development. In addition, advances such as functional genomics, rapid and large-scale phenotypic data capture, bioinformatics, etc. will accelerate the understanding of growth and efficiency in meat animals. This is rapidly leading to genomic selection and editing tools such as CRISPR whereby selection of animals with defined characteristics for particular production systems will be possible. Personalized nutrition or nutrigenomics is receiving much attention in human nutrition. This will likely be possible in animal nutrition leading to "precision/smart" animal production. This will also lead to a decrease in the variation currently observed between animals of the same species and breed when fed similarly.

Societal issues such as antibiotic resistance (mentioned above), the consumer-driven prohibition of exogenous growth-promoting agents in EU, concerns about animal welfare, the use of foods in animal production that are suitable for human consumption, resource limitations and climate change provide challenges to meat animal production globally and to the research community. The latter concern has stimulated considerable interest in reducing the environmental hoofprint, particularly greenhouse gas emissions, from livestock. In this regard, sustainable efficient livestock production must be looked at more holistically. The influence of the novel production systems required to achieve this goal on the growth of meat animals will no doubt be subject of future research.

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Chapter 3

The structure and growth of muscle

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3.1 Introduction

This chapter summarizes the main points of the structure, development and growth of the tissues that make up meat. Meat is the flesh of animals that is consumed as food; technically organs such as liver and kidneys and glands such as the thymus come under that description. Practically speaking, deboned meat sold for human consumption consists of muscle, connective tissues associated with muscle and fat. Other organs and tissues are termed offal. There are three types of muscle tissue: cardiac muscle (heart), smooth muscle and skeletal (or striated) muscle. Here we shall deal only with skeletal muscle, intramuscular connective tissue (IMCT) and adipose tissue (fat). Such is the depth of current knowledge concerning all aspects of muscle structure and of the genetic factors controlling them, that only a condensed description can be given here, with emphasis mainly focused on the aspects most relevant to meat quality. The sources cited mostly give exhaustive coverage of the topics referenced and should be consulted for in-depth discussion of detailed information and the investigations that underpin current knowledge.

Both muscle tissue and connective tissue are hierarchical structures, i.e., there are several nested layers of structures, with molecules being assembled into fibrillar structures that are then assembled into larger entities and so on. In describing these, we have the choice of going from macroscopic to molecular organization, or vice versa. This chapter will take the macroscopic-to-microscopic approach, describing the distribution of muscles in the carcase in terms of meat cuts and the various architectures of individual muscles before going on to talk about the microscopic and molecular structure of muscle fibers, adipose tissue and IMCT.

Before describing this pyramid of hierarchical structures, we must recognize that the molecules comprising all structures within an animal's body, including the bones, muscles, tendons and nerves that make up the musculo-skeletal

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system, are ultimately the products of gene expression. The bovine genome contains approximately 22,000 genes and 3 billion base pairs of nucleotides. In 2009, the bovine genome project identified 91% of the genome of a Hereford bull (Zimin et al., 2009). Humphray et al. (2007) mapped 98% of the pig genome, and Jiang et al. (2014) have sequenced the genome of Texel sheep. Whole genome sequences (91%) of chicken (Wallis et al., 2004) and goat (Dong et al., 2013) are also available.

An additional genome mapping exercise (Gibbs et al., 2009) revealed 37,470 single-nucleotide polymorphisms (SNPs) in 497 cattle from 19 geographically and biologically diverse breeds. A range of different, commercially available, SNP genotyping arrays (SNP chips) are available—to study genetic diversity between breeds and subpopulations of animals and in gene association studies for production and functional traits. Increasing data availability on a large number of animals and the use of high-density SNP panels on large numbers of multibreed sample (Rowan et al., 2019) are extending the accuracy of genetic imputation, and there are now attempts to apply genome-wide fine mapping to wide ranges of cattle populations around the world (Xiang et al., 2021). RNA-sequencing (RNA-seq), also called whole-transcriptome shotgun sequencing (WTSS), looks set to supersede microarrays as the next generation of methodologies to investigate gene expression profiles (Singh et al., 2019). As with cattle, sheep and pigs also have gene polymorphisms which are associated with phenotypic differences between breeds and subpopulations. Thus, our understanding of the effects of genotype on animal growth and development has undergone rapid development in the last 10–15 years.

The expression of the genome of a given meat animal is variable, depending on nutrition, hormones, growth factors, the presence of infections and other physiological stimuli, including exercise and psychological stress. In embryogenesis and fetal development of vertebrates, there are at least 7000 genes whose patterns of variation with time recapitulate developmental timing (Bozinovic et al., 2011). Nutrition (especially maternal nutrition in mammals) influences the outcome of many of the developmental processes for muscle tissue.

In addition, we must realize that, in the embryogenesis and development of an individual animal, its genome is expressed differently in different parts of the musculoskeletal system, i.e., the hundreds of different muscles in an individual animal vary in structure and composition as a result of different expression of the animal's genome. It is estimated that there are up to 800 separate muscles in mammalian bodies. In terms of the major muscles of obvious commercial value in meat species, estimates of the number of muscles in cattle, sheep, goats and pigs are in the range of 300. Each of these has a different size, shape, structure and composition, because each one is tuned to a different function in the body—but they are all differential expressions of a single genome. In postnatal animals, there is strong evidence that the cells making up different muscles and their associated connective tissue and adipose tissue respond differently to a range of stimuli applied to the whole animal, and this complicates gene association

studies, as the phenotypic or functional traits being studied in mature animals may not only vary from muscle to muscle, but from animal to animal due to nutritional and environmental inputs. Biological variability is therefore inherent in muscle structure, but the general structure of muscles and their associated connective tissue and adipose tissue follows a common pattern, which is summarized here.

3.2 The hierarchy of structures relevant to meat: From macroscopic to molecular

3.2.1 Carcass level: Meat cuts

The empty carcass produced as a result of the slaughter process is usually cut in half longitudinally for chilling, and the half carcasses are divided into wholesale cuts (primal cuts). From these primal cuts, retail cuts (subprimals) are prepared for sale. The division of half carcasses into primal cuts varies a little from country to country, and the division of primals into retail cuts varies much more so between countries. A detailed description of the different cuts and their names in various countries, and the muscles they contain, is given by Swatland (2004). The United Nations Economic Commission for Europe (UNECE) has published a guide, based on the inputs from over 30 countries, which attempts to provide a common description for meat cuts suitable for international trade (UNECE, 2016). Standard bovine cuts described by UNECE (2016) are shown in Fig. 3.1, and the major muscles of a beef carcass are shown schematically in Fig. 3.2. The Food and Agriculture Organization of the United Nations (FAO) also has a generic meat-cutting guide for beef, pork and lamb carcasses (FAO, 2016).

3.2.1.1 Distribution and percentage of fat and lean muscle in the growing animal

The increase in live weight of an animal with time (i.e., live weight gain) is an easily measured parameter of growth. However, in the growth of animals for meat, the increase in those components saleable as meat is clearly the most important parameter. The empty carcass weight (or dressed weight) of an animal post mortem (with intestines, skin, blood and head removed) has a good correlation with live weight. The dressing-out percentage of a carcase is simply the (hot) carcass weight divided the live weight × 100. Dressing-out percentages are highest for pigs (70%–74%) because the skin is left on the carcass as part of the saleable yield. Cattle yield dressing percentages in the range of 58%–63%, while sheep (fleece removed) are in the range of 50%–54%, with goats a shade lower.

The composition of the chilled carcass is defined as the proportion of bone, muscle tissue and fat. There is a consensus that, at birth, the ratio of muscle to bone is about 2:1, and there is little fat.

As the animal grows, muscle grows faster than bone, and so the muscle-to-bone ratio increases marginally. Fat growth starts later, but then proceeds at a

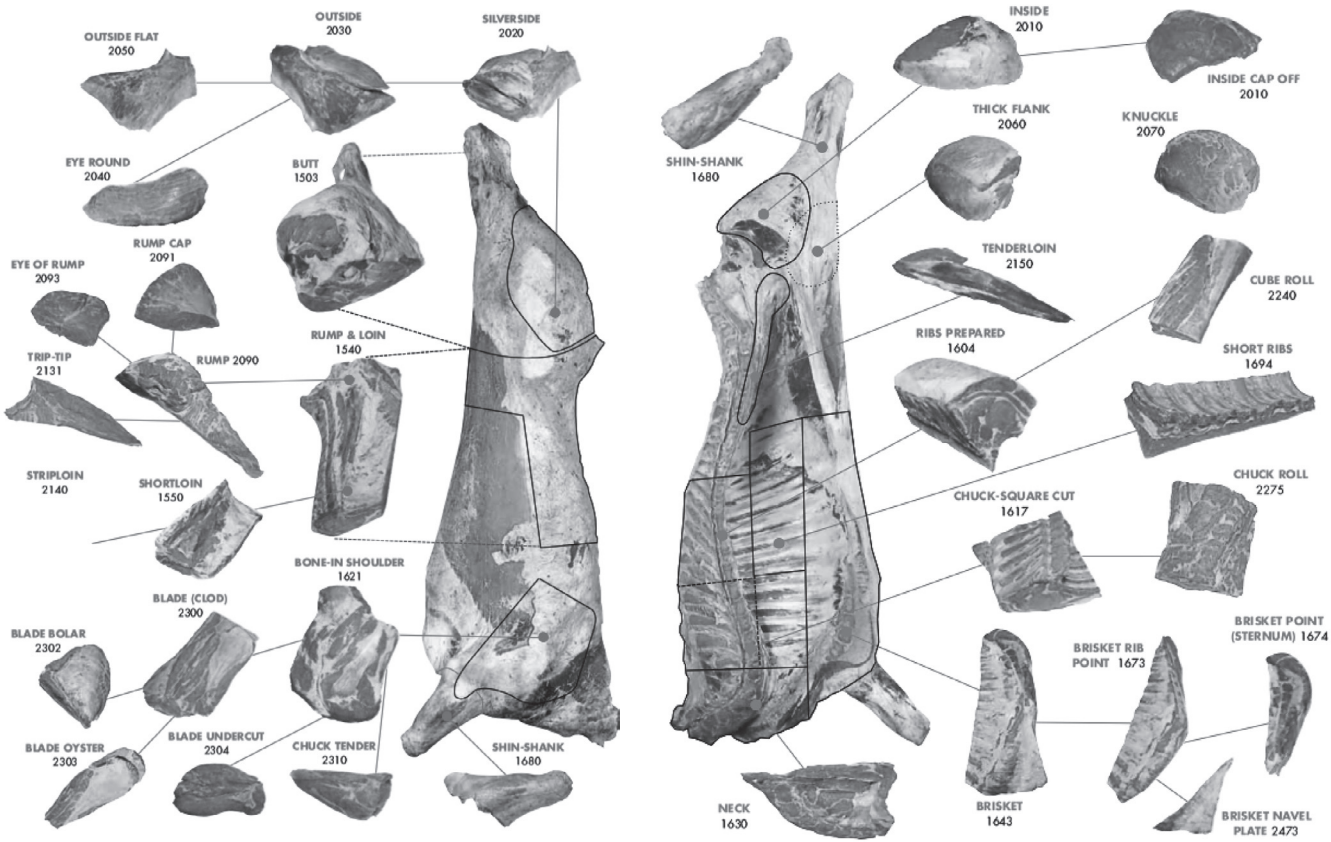


FIG. 3.1 A proposed international standard for meat cuts from the bovine carcass. *(Reproduced from UNECE, 2016. UNECE Standard Bovine Meat Carcases and Cuts. 2015 Revision. Geneva. ISBN 978-92-1-116885 https://unece.org/fileadmin/DAM/trade/agr/standard/meat/e/Bovine_326Rev2E_2016.pdf.)*

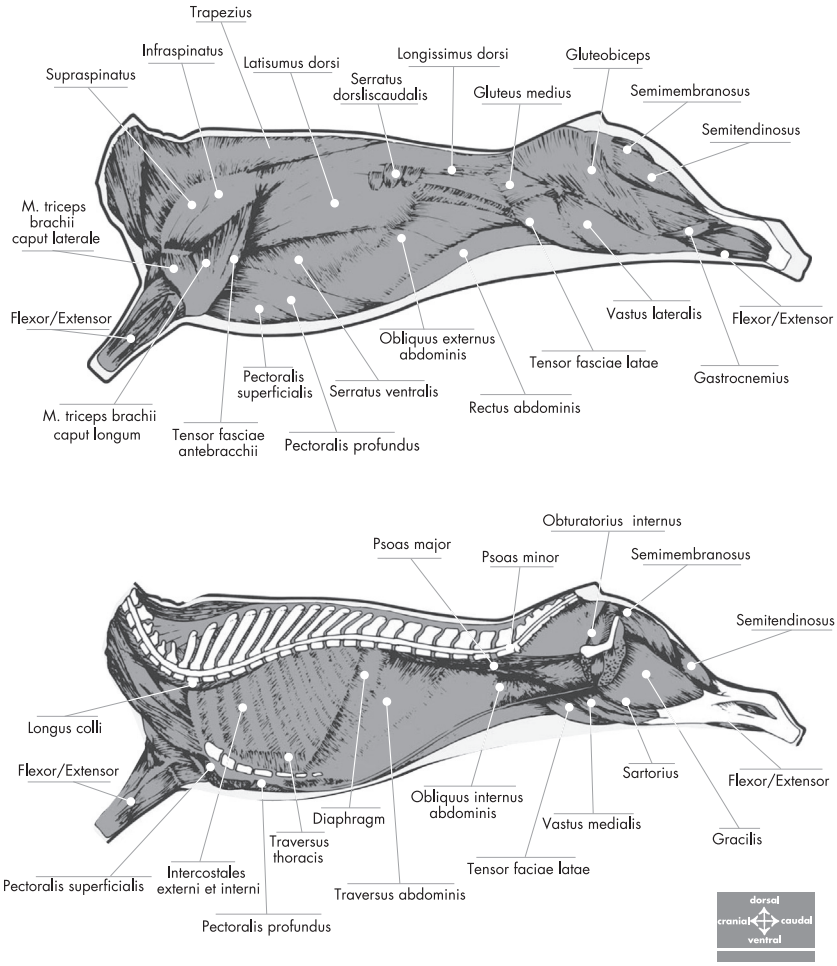


FIG. 3.2 Schematic diagram showing the position of major muscles in a beef half-carcass when viewed from the outside surface (*lateral view, top*) and the inside surface (*medial view, bottom*). *(Reproduced from UNECE, 2016. UNECE Standard Bovine Meat Carcases and Cuts. 2015 Revision. Geneva. ISBN 978-92-1-116885 https://unece.org/fileadmin/DAM/trade/agr/standard/meat/e/Bovine_326Rev2E_2016.pdf.)*

higher rate, so the amount of fat in the carcass increases substantially in later stages of growth (Berg and Butterfiled, 1976). The deposition of fat occurs earliest in the abdominal depot, followed by intermuscular fat, then subcutaneous fat and finally, intramuscular fat (marbling) is the late-maturing phase (Pethick et al., 2004). Fig. 3.3 summarizes data from a number of studies on cattle raised in feedlots and clearly demonstrates the later onset but faster deposition rate of fat.

From such considerations, it is apparent that the composition of the carcass depends on the age or live weight of the animal at slaughter. Berg and Butterfiled

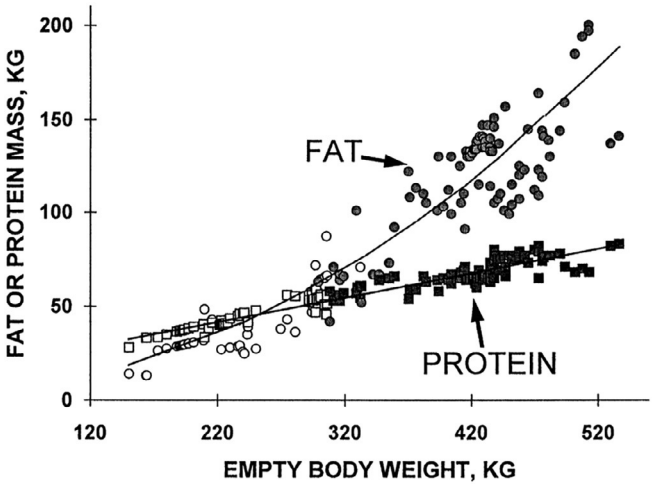


FIG. 3.3 Mass of fat (*circles*) and protein (*squares*) in empty body of feedlot cattle at various empty body weights (live weight minus blood and gut contents) in kilograms for cattle at the start of the feeding period (*open symbols*) and end of the feeding period (*filled symbols*). *(Reproduced from Owens, F.N., Gill, D.R., Secrist, D.S., Coleman, S.W., 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim. Sci. 73 (10), 3152–3172. https://doi.org/10.1021/1995.73103152x, with permission.)*

review data that show differences between on the ratios of fat:bone:muscle in various beef breeds and also between beef breeds and dairy cattle breeds (Berg and Butterfiled, 1976). There are also differences due to feeding. Fig. 3.4 demonstrates the difference in intramuscular fat levels between cattle raised on pasture or finished on a grain-based diet in a feedlot. Data for the percentage of muscle, bone and fat in eight European cattle breeds slaughtered at three different live weights are summarized by Lawrence et al. (2012). At 280 kg live weight, the composition ranges from 60% to 68% muscle, from 18% to 20% bone and from 12% to 20% fat. At 340 kg, the compositional ranges are 57%–69% muscle, 17%–18% bone and 16%–25% fat; whereas at 400 kg live weight, values of 53%–65% muscle, 15%–17% bone and 19%–30% fat are reported across the eight cattle breeds. It is apparent that, although there are big differences between breeds, the amount of fat increases substantially over this range of body weights, pushing down the percentages of bone and muscle. A study on Brazilian cattle revealed small differences in body composition between bulls and steers, and that composition varies in the range of 63%–67% muscle and 18%–22% fat across three levels of feed of concentrates in a feedlot, with the composition of bone remaining fairly steady at around 15% (Do Prado et al. 2015). This study also details the distributions of weights in the major cuts of the carcasses. A number of countries permit the use of hormonal steroids and beta-adrenergic agonists as growth promoters in cattle and pigs. Generally speaking, these growth promoters increase the accretion of

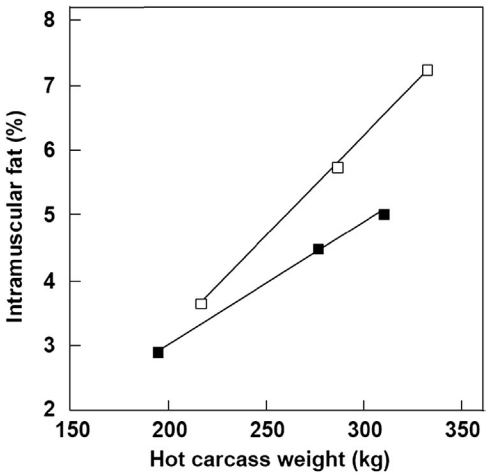


FIG. 3.4 Average levels of intramuscular fat as a percentage of hot carcass weight in Angus, Shorthorn, Murray Gray, and Hereford cattle killed at three market liveweights after finishing on either grass (*filled squares*) or in a feedlot (*open squares*). (*From Pethick, D.W., Harper,G.S., Oddy, V.H., 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Aust. J. Exp. Agric. 44 (7), 705e715. http://doi.org/10.1071/EA02165, with permission.)*

lean muscle tissue, increasing average daily gains in live weight and marginally increasing dressing-out percentages (Aroeira et al., 2021). Mechanisms of increased growth caused by steroid hormones are reviewed by Smith and Johnson (2020). Beta-adrenergic agonists have been known for at least four decades to be repartitioning agents, i.e., agents that divert nutrients from fat deposition to muscle deposition. Ractopamine hydrochloride and zipaterol hydrochloride are the most commonly used beta-adrenergic agonists at present in some countries, although the use of growth promoters is prohibited in many countries, including the European Union. With increasing lean meat yield, these agents may also decrease beef meat tenderness (Lean et al., 2014).

Variations in the amounts and distribution of lean muscle tissue and fat in the carcass result in differences in the total yield of meat and the amount of tissue distributed in higher-priced cuts. Together with the age of the animal, these factors are recognized in the various carcase grading schemes that exist in the world. Although originally designed to separate carcasses into classes of saleable yield, many of these carcass classification systems also divide carcasses into different quality classes, based mainly on animal age and degree of marbling. Bonny et al. (2017) describe a range of the classification and quality assurance schemes currently employed in major beef-producing countries.

The composition of pig carcasses also varies with breed, diet and slaughter weight. As pigs are monogastric animals, the composition of the fat (in terms of the proportions of different fatty acids) is highly influenced by diet. Fortin (1982) reported that carcasses of Yorkshire pigs slaughtered at live weights

between 85 and 112 kg contained 51%–53% muscle, 23%–28% fat and 14%–17% bone. DLY cross-bred pigs (Duroc × (Landrace × Yorkshire)) in the live weight range 107–125 kg in a study by Correa et al. (2006) had 42%–48% lean meat, 19%–24% fat and 7.7%–8.6% bone in their carcasses. The % of lean meat was lower in slow-growing vs fast-growing animals and also in barrows (males) vs gilts (females). Table 3.4 shows data taken from Wood et al. (2004) concerning the effect of diet and breed on carcass composition in four breeds of pigs; two "heritage" breeds (Berkshire and Tamworth) and two modern production breeds (Duroc and Large White).

Table 3.1 demonstrates that faster-growing modern production breeds such as Duroc and Large White have a higher percentage of lean meat and a lower percentage of fat than the two slower-growing, more traditional breeds. The low-protein diet also produced lower live weights at the given age and resulted in more fat and less lean tissue.

A variety of noninvasive or nondestructive techniques have been developed to estimate the proportion of fat and lean muscle tissue of cattle, pigs and sheep, both in vivo and in carcasses postslaughter. These include image analysis, hyperspectral imaging, ultrasound, bioimpedance measurements, magnetic resonance imaging, computer tomography scanning and dual-energy X-ray absorptiometry. A detailed description of these techniques is outside the remit of this chapter.

3.2.2 General anatomy of individual muscles

In terms of gross anatomy, the major skeletal muscles can be divided into six general types, as shown in Fig. 3.5; (a) long, strap like muscles (e.g., *psoas* and *sternomandibularis* muscles) with a fairly constant diameter along their length; (b) fusiform muscles (e.g., *biceps brachii* muscle) which tend to bulge in the midpoint between the tendons linking them to their origin and insertion points on bone; (c) unipennate muscles, where the muscle fibers lie slant-wise at an angle to the two tendons connecting them to their points of insertion and origin (e.g., *extensor digitorum longus* muscle); (d) bipennate muscles, where two sets muscle fibers are arranged like the flights on an arrow, slanting in opposite directions from a central tendon (e.g., *infraspinatus* muscle); (e) multipennate muscles, where there are multiple groups of fibers oppositely slanting from multiple branches of a central tendon (e.g., *deltoideus* muscle); and (f) fan-shaped or convergent muscles (e.g., *pectoralis* muscles) which have a wide, distributed origin, but narrow down to a small insertion. The only exception to these general shapes is the circular muscles surrounding the eye. The slanting muscle fibers in pennate muscles terminate on broad, flat tendons covering the muscle surface which are sometimes termed aponeuroses, to distinguish them from the denser, more concentrated tendons connecting nonpennate muscles to bones.

In all skeletal muscles, the muscle fibers are grouped together into muscle fiber bundles or fascicles. In pennate muscles, the fascicles lie at an angle to the long axis

TABLE 3.1 Composition of carcasses from four pig breeds fed either a conventional diet or a low-protein diet suitable for slower-growing animals.

	Conventional diet				Low-protein diet			
	Berk	Duroc	Large W	Tamwth	Berk	Duroc	Large W	Tamwth
Live weight/kg	64.3	85.2	86.8	66.5	61.0	74.4	72.3	60.6
% lean muscle	47.2	62.9	67.5	50.6	42.9	57.7	58.9	45.9
% subcutaneous fat	32.1	13.0	12.0	25.9	35.3	17.7	17.5	29.4
% intramuscular fat	7.6	5.7	3.8	7.4	9.0	6.5	5.8	7.8
% bone	13.0	14.5	16.8	16.1	12.8	18.2	17.8	16.9

Berk, Berkshire; Large W, Large white; Tamwth, Tamworth. Data based on dissection of the foreloin (5–13 ribs).
(Data taken from Wood, J.D., Nute, G.R., Richardson, R.I., Whittington, F.M., Southwood, O., Plastow, G., Chang, K.C., 2004. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. Meat Sci. 67 (4), 651–667. https://doi.org/10.1016/j.meatsci.2004.01.007.)

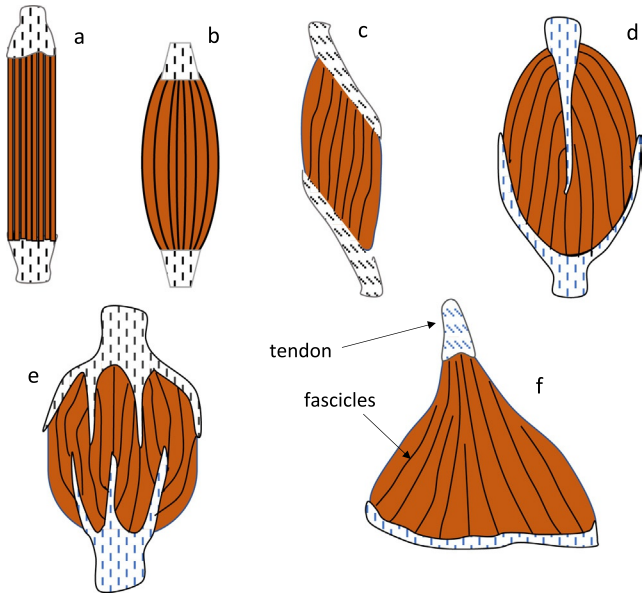


FIG. 3.5 Diagram representing the six general shapes of muscles. (A) strap like; (B) fusiform; (C) unipennate; (D) bipennate; (E) multipennate; (F) fan-shaped (or convergent). *Black lines* indicate the direction of fascicles in each muscle shape. *Gray stippled* areas represent the tensons at each end of the muscles.

of the muscle, but in nonpennate muscles, they run the length from muscle origin to insertion. It is commonly assumed that individual muscle fibers run the entire length of the fascicle, and that each end of the muscle fiber is attached to the tendon by a highly invaginated junction zone (the myotendinous junction) designed for efficient force transduction from the muscle fiber into the tendon (Charvet et al., 2012). However, this seems to be largely true only for humans and some monkeys, and for a large number of muscles in mammals, birds, reptiles and amphibians, this is not the case; the muscle fibers taper in diameter at each end and terminate within the body of the fascicles, with no connection to the tendons. Muscles with these intrafascicularly terminating fibers are called series-fibered muscles, as each fiber only runs for a short length of the fascicle. Series-fibered muscles have been found in pig, rabbit, horse, goat and cattle. The *pectoralis* muscle of 63 bird species (including chicken, pigeon, quail, turkey) are series-fibered (Gaunt and Gans, 1993). The incidence of series-fibered muscles is highly under-appreciated and has not been systematically investigated in muscles of meat animals. We do know that at least the *peroneus longus* muscle in pig, the *semitendinosus* muscle in goats and the *semimembranosus* and *sternomandibularis* muscles in cattle are series-fibered (Gans et al., 1989; Swatland and Cassens, 1971; Purslow and Trotter, 1994). There is evidence that the mechanism of hypertrophy in series-fibered muscles may be different from continuously fibered muscles.

With these caveats concerning general muscle architecture in mind, we can go on to discuss the general structure and composition of the skeletal muscle of meat animals.

3.2.2.1 Generalized skeletal muscle structure

Fig. 3.6 (from Listrat et al., 2016) summarizes the general structure of skeletal muscles. They are hierarchical fibrous structures, i.e., each level of structure being made up of several fibrous subunits. The largest level of structure is a whole muscle, whose outer surface is defined by a connective tissue layer, the epimysium. Internally, each muscle is divided into fascicles, or muscle fiber bundles, which are separated by another connective tissue layer, the perimysium. Larger (primary) fascicles may consist of a number of smaller (secondary) fascicles, separated by thinner layers of perimysium. Fascicles can be 1–5 mm in diameter and are easily seen by eye in transverse cuts of muscles. This can be referred to as the visual texture of the muscle. As animals mature, their fascicle size in muscles such as the *longissimus* tends to become larger. Hence, the difference between "coarse-textured" (larger fascicles) and "fine-textured" (smaller fascicles) is used as one indicator of animal maturity in the USDA beef quality grading system.

Fascicles are made up of numerous individual muscle fibers, typically 20–80 μm in diameter depending on species. Individual muscle fibers are multinucleate cells, each with their own basement membrane overlying the cell plasma membrane consisting of a phospholipid bilayer. Some authors refer to the plasma membrane as the sarcolemma, and some others include the overlying basement membrane together with the plasma membrane in their definition of the sarcolemma. The basement membrane is actually an extracellular matrix (or connective tissue) structure, but is a separate structure from another connective tissue layer, the endomysium, that lies between the basement membranes of adjacent muscle cells.

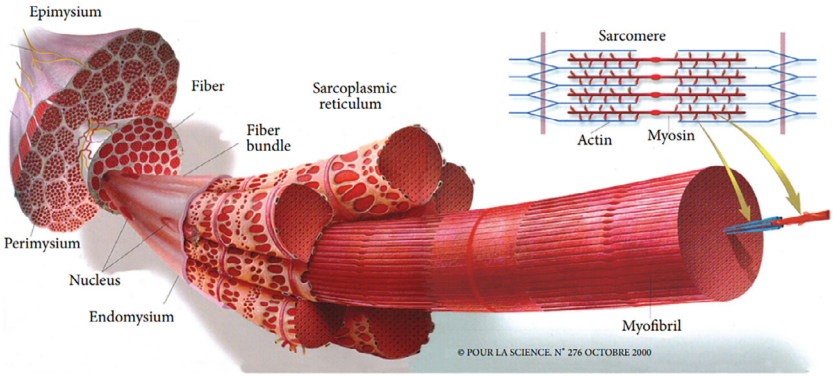


FIG. 3.6 Schematic diagram showing the general structure of skeletal muscles. *(From Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Bugeon, J., 2016. How muscle structure and composition influence meat and flesh quality. Sci. World J. http://doi.org/10.1155/2016/3182746, with permission.)*

3.2.3 The general structure of the striated (skeletal) muscle cell

Muscle fibers are individual cells. They are exceptionally large compared with other cell types (typically, most eukaryotic cellsare10–30 μm in size). Muscle cells can be up to 100 μm in diameter. The longest muscle fiber (cell) isolated intact (from the human quadriceps) was 34 cm in length. The muscle fibers in most muscles consumed as meat are many centimeters in length. This huge size of muscle brings about one of the peculiar characteristics of muscle cells; they are multinucleate. In mononuclear cells, typical values of the ratio of the volume of the nucleus to the volume of the cytoplasm it controls (N/C ratio) are between 1:1 and 4:1. Clearly, to control the huge volume of sarcoplasm within and individual muscle cell, large numbers of nuclei are required. Generally, nuclei are located peripherally in the muscle cells, just under the sarcolemma. Each muscle fiber is typically innervated just once, by a specialized nerve ending (motor end plate) that is somewhere within the central one-third of the muscle fiber. As the depolarization waves that cause calcium release and initiated muscle contraction spread out in both directions from the motor end plate, it is logical that the motor end plate is usually in the mid-length of the muscle fiber. Multiple staining for motor end plate bands along the length of the fascicle is a prime indicator that the muscle is series-fibered (Gans et al., 1989).

The major organelles within each muscle cell are the myofibrils. Myofibrils are approximately 1 μm in diameter, and there are up to 1000 of these contractive organelles packed side by side within each cell, occupying about 80% of the sarcoplasmic volume. The sarcoplasmic reticulum (SR) is a membrane loosely surrounding each myofibril, whose function is to store and release calcium ions. Extensions of the sarcoplasma extending perpendicularly from the cell surface into the center of the cell to reach the SR of all myofibrils form the T-tubules. Energy to the cell is provided by the mitochondria. The number of mitochondria in each muscle fiber varies, with more mitochondria in predominantly oxidative fibers and fewer in glycolytic fibers. The muscle cell also contains numerous soluble proteins (sarcoplasmic proteins) that are mainly composed of the enzymes responsible for the general metabolism of the cell.

3.2.3.1 Structures within the cell

The diagram in Fig. 3.7, reproduced from Gou and Greaser (2017), schematically represents the structures within the muscle cell. The long myofibrils are composed of proteins that are insoluble under physiological conditions. They are composed of longitudinally repeating units, the sarcomere. The sarcomere is considered the smallest contractile unit of the cell and runs from *Z*-line to Z-line. In the light microscope, myofibrils (and muscle fibers) have a banded or striated appearance due to the different optical properties of the I-band isotropic in polarized light) and the A-band (anisotropic in polarized light). Two sets of myofilaments are longitudinally arranged in the sarcomere; the thin filaments,

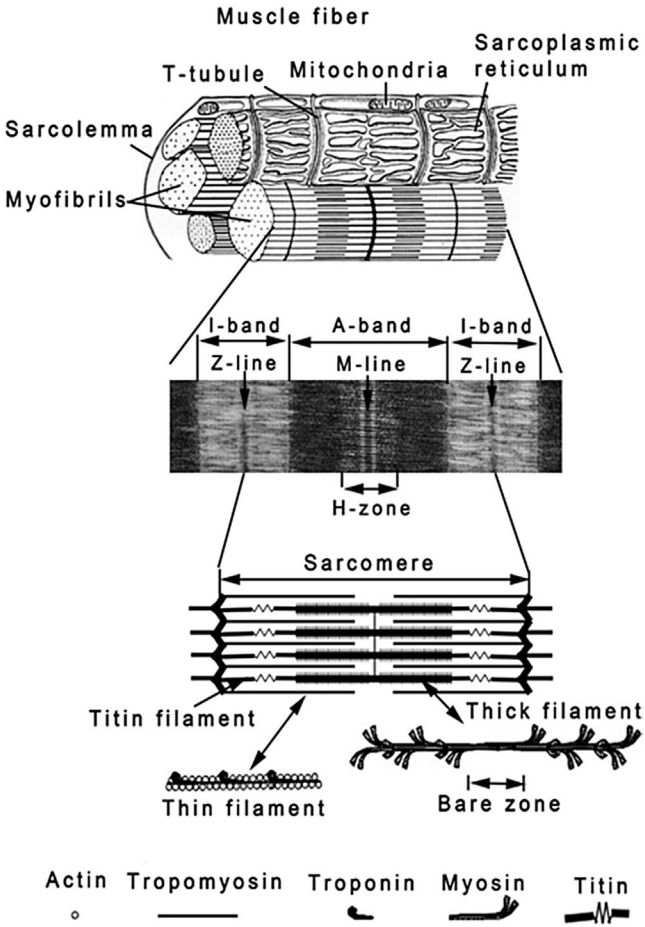


FIG. 3.7 Schematic representation of structures within a striated muscle fiber. *(From Gou, W., Greaser, M.L., 2017. Muscle structure, proteins, and meat quality. In: Purslow, P.P. (Ed.), New Aspects of Meat Quality e From Genes to Ethics. Woodhead Publishing, pp. 13e32. ISBN:9780081005934 (Chapter 2), with permission.)*

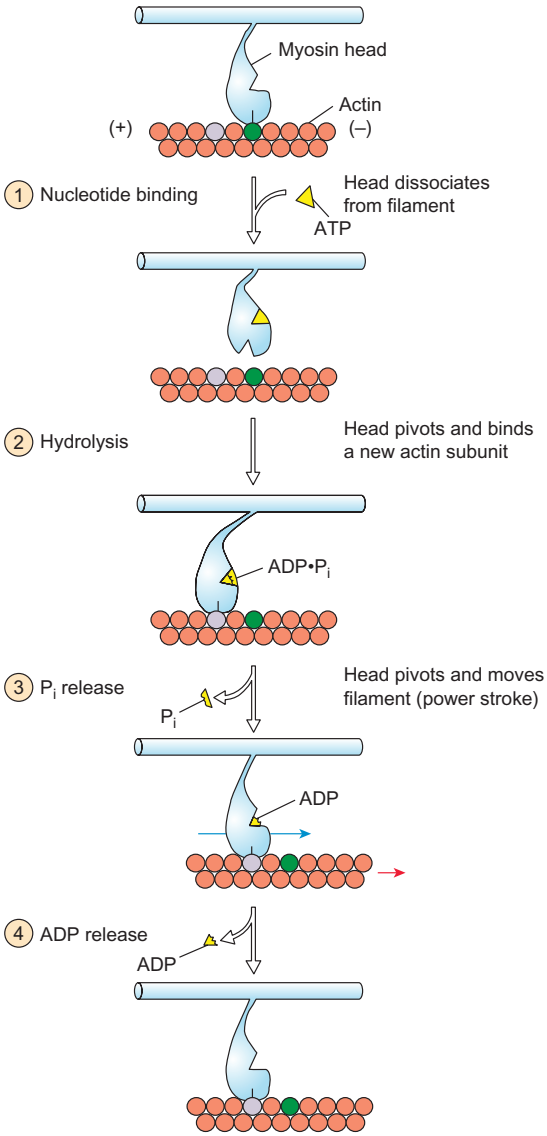
composed mainly of actin together with the regulatory proteins tropomyosin and troponins, and the thick filaments, composed mainly of myosin. These filaments interdigitate for a portion of their length and a cross section of the sarcomere where they do overlay reveals a hexagonal packing of thick filaments, with thin actin filaments lying equidistant from each thick filament on their own hexagonal array. The A-band is the length of the thick filaments and maintains a constant length in working muscle. The I-band is the distance between the point of overlap of the thin filaments in one half-sarcomere, through the *Z*-disc and up to the point of thin filament overlap in the next half-sarcomere. The length of the I-band varies with muscle contraction and relaxation. The H-zone is the slightly

less dense region of the A-band where thin filaments do not overlap with thick filaments, and again the length of this zone varies with sarcomere length as the muscle lengthens or contracts. At the middle of the A-band, there is a structure running transversely across the sarcomere, the M-line, that stabilized the thick filaments.

The sliding filament mechanism of contraction of muscle, by the repeated attachment and detachment of myosin heads along the actin filaments due to shape changes I, the myosin heads caused by the attachment of ATP, its cleavage into ADP + P, and the release of ADP are explained in depth in most biochemistry and physiology textbooks for the last half century and will not be treated in depth here; the process is summarized in Fig. 3.8. The process of contraction is triggered by action potentials running into the motor end plate, releasing acetylcholine, which binds to receptors in the sarcolemma causing sodium influx and potassium efflux in voltage-gated channels that causes an action potential wave to run along the fiber. Excitation–contraction coupling is achieved by this action potential running along the surface of the muscle fiber and spreading deep into the fiber via the T-tubule system, which causes calcium release from the sarcoplasmic reticulum via gated calcium channels (the ryanodine receptors, RyR, and the dihydropyridine receptors, DHPRs). Termination of muscle contraction is achieved by the pumping of calcium back into the sarcoplasmic reticulum. A mutation in the gene coding for RyR has been shown to be the cause of PSE.

3.2.3.2 Myofibrillar proteins

Almost all eukaryotic cell types have some form of myosin as a motor protein; there are currently on the order of 18 myosins known. The sarcomeric myosin responsible for skeletal muscle contraction is myosin type II, which is the most studied type. The myosin II molecule comprises two heavy chains (MyHC) and four light chains (MyLC). Each heavy chain has a bulbous head at the N-terminal end which contains an actin-binding site and an ATP-binding site. The ability of myosin to split ATP into ADP + P means that it has enzymic activity, i.e., it is an ATPase. The rest of the heavy chain is an alpha helical "tail," and the tails of the two heavy chains wind around each other in an alpha-helical coiled-coil. Two myosin light chains are associated with each heavy chain. They form rings around the "neck" region of the heavy chain where the heads merge into the tails. The myosin light chain with molecular weight of approximately 20 kDa (MyLC20) is also termed the regulatory light chain, and the myosin light chain with molecular weight of approximately 17 kDa (MyLC17) is also termed the essential light chain. Each MyHC chain is coded by a single MYH gene, and similarly, the MyLCs are products of MYL genes. Multiple isoforms of both light chains and heavy chains exist. In mammals, there are 11 genes that code for different isoforms of myosin heavy chains and four isoforms are known for each of the light chains (Schiaffino and Reggiani, 2011). The expression of different isoforms allows a fine-tuning of the function of this motor protein. Variations in the MYH genes are well studied as different isoforms of the heavy



(1) ATP binding to the myosin head releases it from binding to actin.

(2) The ATP is hydrolyzed to ADP and the head pivots to the left. In relaxed muscle, this is the position; the head is cocked, loaded with ADP and interaction with actin is blocked by the tropomyosin-troponin complex. Calcium released by the SR as a result of muscle activation binds to the troponin complex, moving tropomyosin and allowing the myosin head to bind to actin.

(3) Binding of the myosin head releases the inorganic phosphate (Pi), causing a change in the conformation of the head (in this diagram it pivots to the right). This is the power stroke, fulling the thick f ilament along the thin filament by 10 nm or so.

(4) The ADP nucleotide is released. In the presence of more ATP, the myosin head can bind to more ATP, causing its release from the actin and starting the cycle over again. Contraction is terminated by the sequestration of calcium back into the sarcoplasmic reticulum, causing the troponin-tropomyosin complex to sterically block myosin from binding to actin. In post-mortem muscle, low at ATP and high calcium levels due to the failure of ATP-dependent calcium pumps means that the cross-bridges formed between myosin heads and actin are stuck in the state shown at the top of the diagram.

FIG. 3.8 The molecular mechanism of muscle contraction. *(Diagram taken from Lodish, H., Berk, A., Zipursky, S.L., et al., 2000. Molecular Cell Biology, fourth ed. W. H. Freeman, New York. Section 18.3, Myosin: The Actin Motor Protein. Available from: http://www.ncbi.nlm.nih.gov/books/NBK21724/.)*

chains have different characteristics as ATPases. This topic is covered in more detail below, in the section on muscle fiber types.

Thick filaments also contain myosin binding protein-C (MyBP-C), a regulatory protein that modifies myosin-actin interactions. MyBP-C comprises 2% of the myofilament mass and is located only in the middle region of each half A-band (the c-region). There are two divergent forms (paralogs) of MyBP-C found in different proportions fast and slow muscles (as well as a third paralog found in cardiac muscle). MyBP-C modulates the interaction of thin and thick filaments by sensitizing the reactivity of the thin filament to calcium ions and modifying the kinetics of cross-bridge cycling so as to act as a brake to shortening (Li et al., 2019). The structure, location and functional roles of the MyBP-C paralogs are currently an active field of research which should clarify the roles of this protein family in fine-tuning the contractile properties of different muscles. Fig. 3.9 schematically shows the arrangement of MyBP-C within the sarcomere.

The principal protein of the thin filaments is actin, a globular protein (G-actin) that is polymerized into twisted chains of filamentous actin (F-actin). Each globular actin molecule is the product of the ACTA1 gene. It has a molecular weight of approximately 42 kDa and a diameter of 4–7 nm. In F-actin, the globular units form a twisted double strand with 2.17 actin units per turn. Wrapped together with these strands (as depicted both in Figs. 3.7 and 3.9) are the regulatory proteins tropomyosin and the troponin complex (comprising troponin T (TnT), which binds to tropomyosin, troponin I (TnI), which binds to both troponin T and C as well as actin and troponin C (TnC)). Calcium released from the sarcoplasmic reticulum as a result of excitation–contraction coupling binds to TnC. This strengthens the TnC-to-TnI interaction, resulting in a weakened TnI-to-TnT interaction. The result of these conformational changes is that the tropomyosin molecule is pulled aside to expose the binding sites on the surface of actin that interacts with myosin. The removal of calcium reversed this process, covering up the myosin-binding sites on the actin, so ending the process of contraction and returning the muscles to the relaxed state. Isoforms of troponin have found to be associated with different MyHC isoforms in a study on three bovine muscles (Oe et al., 2016).

3.2.3.3 Cytoskeletal protein structures

In addition to the major proteins of the thick and thin filaments, Wang (1985) identified a series of other filaments within the muscle cell that run either longitudinally or transversely.

Wang classified these as sarcomere-associated cytoskeletal lattices as either intrasarcomeric or extrasarcomeric. Fig. 3.10 schematically shows the connections that these structures make to form a continuous network linking the sarcolemma at the surface of the cell to the myofibrils and to the nuclear membrane, an emphasizes the role of these networks as both coordinators of the myofibrils and lines of mechanical communication between the cell surface and the nuclear membrane.

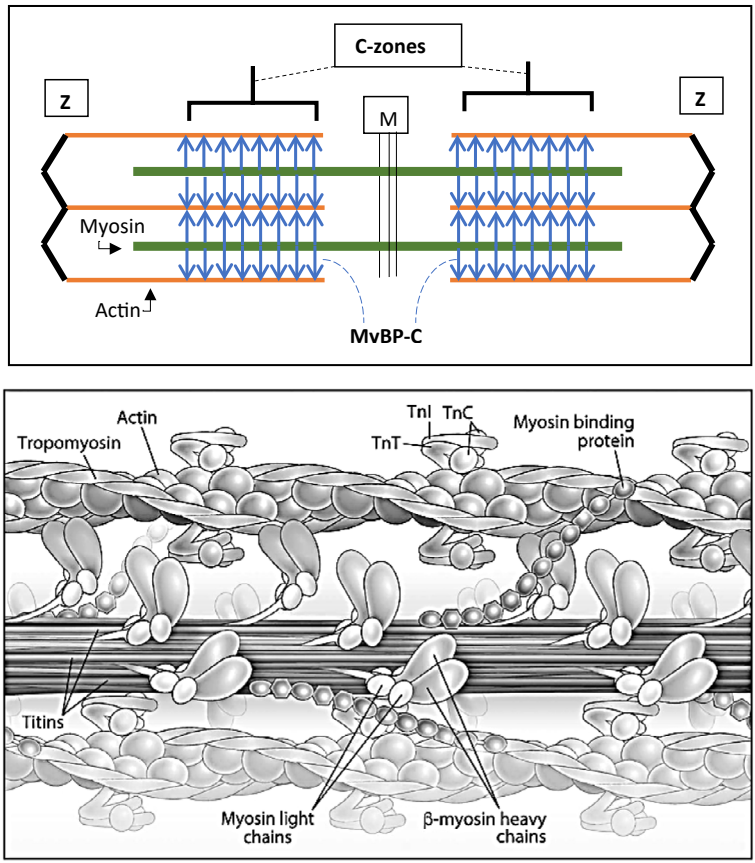


FIG. 3.9 Representation of location of myosin-binding protein C (MyBP-C) within the sarcomere. *Top panel*: Schematic diagram showing MyBP-C is localized within the C-zone on each side of the M-line of the sarcomere in seven to nine transverse stripes .MyBP-C interacts with both the thick and thin myofilaments filaments. Lower panel: Schematic diagram, reproduced from McNamara and Sadayappan (2018) with permission of Elsevier Ltd., showing location and interactions of MyBP-C in more detail. Both fast and slow paralogs of MyBP-C consist of seven immunoglobulin domains and three fibronectin-III domains plus an M domain.

Within the sarcomere, the giant protein titin runs the length of a half sarcomere from *Z*-disc to M-line. In the I-band, specific regions of the molecule can deform elastically. The amino terminus of the protein inserts into the Z-disc and interacts with the amino-terminus of titin running in the next sarcomere. In the older literature, there is mention of the protein zeugmatin being associated with the alpha actinin that comprises the bulk of the Z-disc; it is now known that zeugmatin is actually the part of the titin molecule running through the Z-disc. The carboxyl terminus of the protein ends at the M-line where it overlaps with the carboxy terminus of another titin molecule that runs until the next

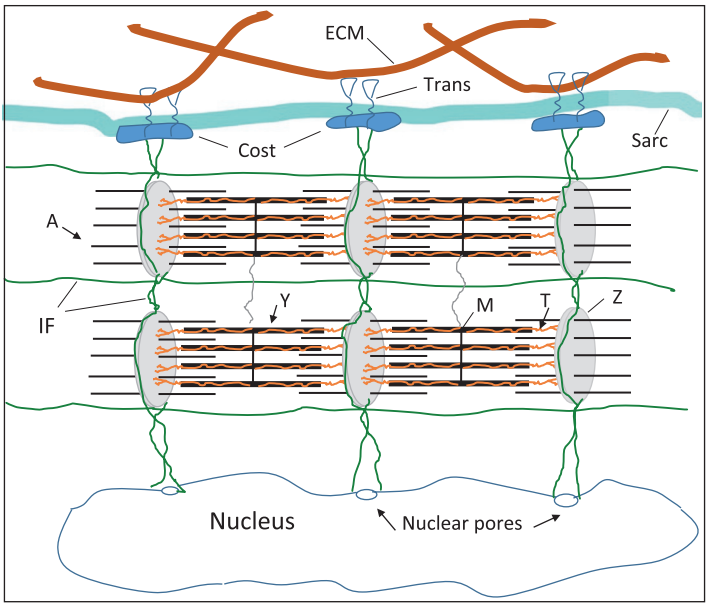


FIG. 3.10 Schematic model of the intermediate filament cytoskeleton in relation to the myofibrillar lattice of thick myosin filaments, thin actin filaments and titin filaments.

Z-line. Titin therefore makes a continuous connection that runs longitudinally through the myofibril. It is thought to help center the thick filaments in the sarcomere and to contribute to the passible elasticity of muscle fibers. Another giant protein, nebulin runs the length of the thin filaments and is capped by tropomodulin. Because titin and nebulin are very long protein molecules with distinct regions along their length, it had been postulated that they act as "protein rulers," guiding the polymerization of actin into thin filaments and of myosin into thick filaments, with titin dictating the precise length of the thick and thin filaments and nebulin that of the thin filaments (Tskhovrebova and Trinick, 2017). However, as nebulin may not run the length of the thin filament to where tropomodulin caps the actin polymer, it has been argued that other characteristics may dictate the exact thin filament length. It is now thought that nebulin strictly controls thin filament in fast muscle fibers, but has a less direct role in slow muscle fibers, where nebulin does not persist to the end of the thin filament and where another actin-binding protein, leiomodin-2 (which is also an analog of the capping protein tropomodulin) has a more definitive role (Kiss et al., 2020), In addition to its structural role, nebulin is thought to modulate myosin–actin interactions by affecting the location of the tropomyosin/troponin complex (Yuen and Ottenheijm, 2020).

Transverse cytoskeletal elements within the sarcomere comprise the *Z*-disc and the M-line, which anchor the thin filaments and thick filaments, respectively. The main component of the *Z*-disc is alpha-actinin, a member of the spectrin

superfamily of proteins that also includes spectrin and dystrophin. Alpha actinin is a short rod-shaped dimer with actin-binding domains at each end and forms a dense lattice structure that stabilizes both the thin filaments and titin filaments. The M-line contains the protein myomesin and M-line protein. M-line protein is only found in fast-twitch muscle fibers, whereas myomesin is found in both fast and slow fibers. In developing mouse embryos, myomesin appears at the same time as titin, whereas M-line protein expression is slightly delayed. This may point to a more critical role for myomesin as an anchor point for titin.

Exosarcomeric cytoskeletal structures

The proteins spectrin, ankyrin, vinculin and talin are found clustered with the intracellular components of the dystrophin-associated glycoprotein (DAG) complex (or dystroglycan complex) in the costameres. Costameres lie along the inside surface of the sarcolemma adjacent to the *Z*-discs of the most peripheral myofibrils. Intermediate filaments (diameter circa 10 nm) run transversely from the costameres to the Z discs and link z disks in adjacent myofibrils together. There is also a set of longitudinally running intermediate filaments peripheral to the myofibrils that bridge from Z-disc to Z-disc. These *exo*-sarcomeric intermediate filaments thus form a three-dimensional cytoskeleton that links and coordinates the myofibrils in a muscle fiber, keeping them in register and transmitting forces both into and out of the cell in a radial direction. The principal intermediate filament protein in adult muscle is desmin, a 53.5 kDa protein consisting of 470 amino acids and forming an alpha helical rod with nonhelical ends that allow the molecules to interact and form a chain in the intermediate filaments. Muscles in mice with gene knockout for desmin possess weak muscles with poorly aligned myofibrils.

Recent studies have also demonstrated that two forms of keratin are also present in intermediate filaments of muscle, although at very low concentrations compared with desmin (Muriel et al., 2020). The intermediate filaments and costameric structures perform two major functions. First, these structures link the myofibrils together so as to integrate their forces and deformations and transmit these to the cell membrane. This ensures that changes in sarcomere length are uniform during both contraction and elongation of the muscle cell. This lateral load sharing also explains how sarcomeres can be added or remodeled in individual myofibrils of the living muscle cell while the cell still maintains contractile abilities. Second, as shown in Fig. 3.10, the connection provided by the cytoskeleton from the cell membrane and into the nuclear membrane provides a pathway for external mechanical signals to be passed in to the nucleus so as to affect cell expression, a process known as mechaotransduction (Martino et al., 2018). This is a key signaling pathway in the response of muscle tissue to stimuli such as exercise. Cytoskeletal proteins are general targets for the calpains in all types of cells (see Chapters 5 and 12), and so it is not surprising that desmin, vinculin and nebulin are rapidly degraded by postmortem proteolysis (Huff-Lonergan et al., 1996). Calpain-1 (μ-calpain) is specifically

known to sequentially cleave the terminal regions of desmin, so effectively depolymerizing intermediate filaments.

Other proteins within muscle fibers: Soluble (sarcoplasmic) proteins

We have concentrated on the major myofibrillar and cytoskeletal proteins that make up filamentous structures within the muscle cell. These comprise approximately 19% of the wet weight of muscle tissue. A further 5.5% of the wet weight of muscle tissue is made up by a range of nonfibrous, soluble proteins that exist within the muscle cell, namely the sarcoplasmic (water-soluble proteins). These are mainly enzymes involved in cell maintenance, energy metabolism and general intracellular signaling, as well as the oxygen-storing protein myoglobin. As these are not strictly structural proteins, they will not be discussed here. Sarcoplasmic proteins are described in more detail in Chapter 4.

3.2.3.4 Muscle fiber types

Over more than a century ago, it was observed that slowly-contracting muscles are redder in appearance than fast-contracting muscles. Physiological measurements of the speed and force of contraction and the metabolism of muscle fibers show that the properties of each individual muscle fiber can vary across a broad range. Slow, red, muscles have a greater proportion of slow-type muscle fibers, whereas whiter muscles have a greater proportion of fast-twitch fibers. There has been a long history of grouping or classifying these variations into muscle fiber "types." During muscle contraction, ATP is broken down due to its interactions with myosin in the cross-bridge cycle, and myosin can therefore be termed an ATPase enzyme. Using histochemical staining of muscle cross sections with a stain for ATPase activity, Brooke and Kaiser, 1970) grouped fibers into type I fibers, which had light ATPase activity, and type II fibers, which had more. They divided the Type II category further, according to the pH range necessary to inhibit the ATPase activity, producing type IIa, IIb and a rare type IIc, with almost complete inhibition of ATPase activity and pH 4.5, 4.3 and 3.9, respectively. The lowest pH for inhibition in the type I fibers was about the same as for the type IIc. Peter et al. (1972) used histochemical staining of muscle sections to identify variations in the activity of enzymes used in the Krebs cycle to produce ATP (principally lactic dehydrogenase and succinic dehydrogenase) to classify fibers as either slow-twitch oxidative (SO), fast-twitch glycolytic (FG) or fast-twitch oxidative-glycolytic (FOG). They also correlated the proportion of these types with the myoglobin content of the muscle; red muscles with a higher SO population contain more myoglobin as an oxygen carrier. Peter et al. (1972) compared their classification to a host of previous schemes, including that of Brooke and Kaiser (1970), suggesting that their SO corresponded to type I muscle fibers, FOG to type IIa and FG to type IIb. Nemeth and Pette (1981) later used just succinate dehydrogenase (SDH) histochemistry and myosin ATPase activity to classify muscle fibers. They found that SDH activity was high in ATPase type I and IIa fibers, confirming their designation as SO and

FOG, but in ATPase type IIb fibers, there was a high variability in SDH activity. These histochemical studies demonstrate two points; first, that there are variations in metabolic factors between muscle fibers that may vary independently from other factors, so that there is not complete overlap between histochemical classification schemes, and second, that the classifications are arbitrarily grouped into "high," "low" and "intermediate" staining for the various attributes; in reality there is a continuous spectrum of enzyme activities in the type II fibers. Fig. 3.11 shows ATPase staining of sections from two pig muscles. In both the darkly stained type I fibers appear as islands surrounded by the type II fibers, but the size of the type I clusters is radically greater in the *rhomboideus* than in the *longissimus* muscle.

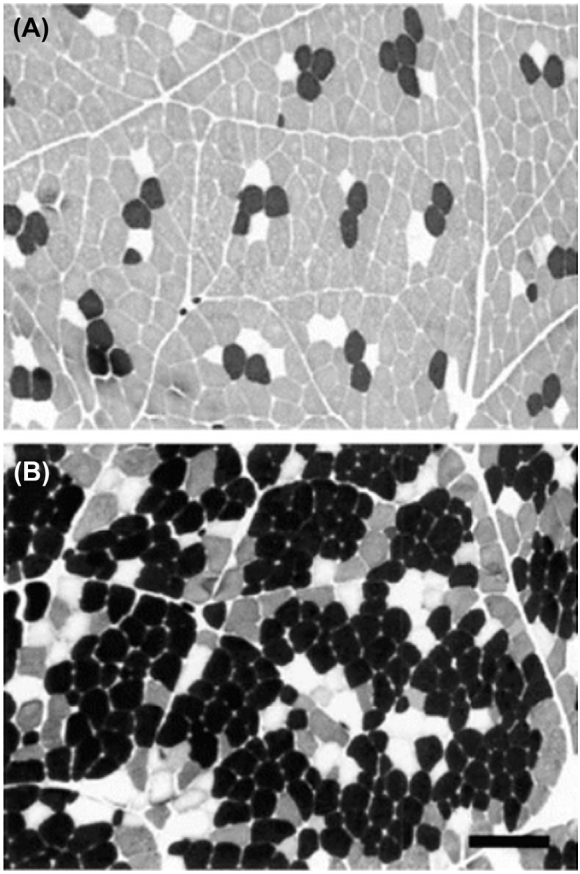


FIG. 3.11 ATPase histochemistry after preincubation at pH 4.35 in *longissimus thoracis et lumborum* (A) and *rhomboideus* (B) muscles of Large White pigs at slaughter weight of 100 kg. Islets of black Type I fibers are surrounded by unstained Type IIA and gray Type IIB fibers. Bar = 200 μm. *(From Lefaucheur, L., Ecolan, P., Plantard, L., Gueguen, N., 2002. New insights into muscle fiber types in the pig. J. Histochem. Cytochem. 50 (5), 719e730, with permission.)*

TABLE 3.2 Sarcomeric myosin isoforms in mammalian skeletal muscle.

Gene	Myosin heavy chain (MyHC) isoform expressed	Where expressed
MYH1	MyHC-2×	Fast type IIx fibers
MYH2	MyHC-2A	Fast type IIa fibers
MYH3	MyHC-emb	Developing muscle
MYH4	MyHC-2B	Fast type IIb fibers
MYH7	MyHC-1 (Slow)*	Slow type I fibers
MYH8	MyHC-neo	Developing muscle
	**also calledMyHC—in cardiac muscle*	

(Data from Schiaffino, S., Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. Physiol. Rev., 91(4), 1447–531. https://doi.org/10.1152/physrev.00031.2010.)

Schiaffino et al. (1989) revisited the ATPase-based classification of Brooke and Kaiser, equating type I, IIa and Type IIb fibers with different isoforms of myosin heavy chains in each and identifying a fourth isoform, IIx. Each isoform of the myosin heavy chain is a production of a different gene. Table 3.2 lists the genes and expressed isoforms found in skeletal muscle. This table is adapted from a larger version by Schiaffino and Reggiani (2011) which also shows the complete range of isoforms found in cardiac and extraocular muscles. It should be noted that the paralogs of myosin-binding protein C mentioned above also play a role in fine-tuning the contraction characteristics of fast and slow muscles.

It should be remembered that the majority of work in developing muscle fiber type classifications has been carried out in the skeletal muscles of humans and small laboratory rodents, and that the relative abundances of the various fiber types in farm animals may be different. Body size is a major factor determining the general composition of muscle fiber types, via consideration of its two major functions; generation of body heat and locomotion. The metabolic cost per unit body weight to generate body heat by muscle action is higher in small mammals than in larger ones. (Kleiber's law states that resting metabolic rate scales to the ¾ power of body mass.) The maximum metabolic rate per unit mass also goes down with increasing body size. This means that small mammals need fibers with more mitochondria and more capillaries surrounding them. Type IIb and IIx are common in small mammals but almost absent in most skeletal muscles in larger species, including humans, cattle, goats and horses. In terms of locomotion, muscles in a small animal have to contract more quickly

for a given speed of locomotion than in a larger animal. Schiaffino and Reggiani (2011) explain that the abundance of fast myosin types in the *soleus* muscle (normally considered a "slow" muscle) varies for this reason, from 100% in the shrew, 60% in the mouse, 20% in the rat and almost nil in the rabbit.

Large white pigs at a slaughter weight of 100 kg show high levels of type IIb fibers in some muscles.

Picard and Gagaoua (2020) provide an in-depth review of the variability of muscle fiber type proportions in cattle and the changes in fiber types during muscle growth and development. Variations in the development of meat quality post mortem that are related to muscle fiber type are further reviewed in detail by Matarneh et al. (2021).

Mitochondria

These intracellular structures or organelles deserve a special mention because they are vital to the energy metabolism of cells, and muscle cells require very substantial amounts of energy to perform their primary function (contraction). Mitochondrial proteins account for approximately 1% of the wet weight of muscle, which is on the same order as the wet weight of the IMCT proteins. Slow, red, oxidative muscle fibers contain more mitochondria than fast, white, glycolytic fibers (Yu et al., 2020). Mitochondria play critical roles in the postmortem metabolism of muscle during the conversion of muscle to meat, as well as the initiation of apoptosis. Mitochondrial metabolism postmortem is known to vary between different muscles, contributing to variations in postmortem quality characteristics (Yu et al., 2020).

3.2.4 Structure of the individual intramuscular connective tissues (IMCTs)

As shown schematically, in Fig. 3.6, there are three distinct connective tissue structures associated with each muscle. Previously, it was common to describe the whole muscle as being enveloped by the epimysium, divided into fascicles by perimysium and fascicles divided into individual muscle fibers by endomysium. However, this description of the perimysial and endomysial connective tissue networks as "dividing" the muscle gives a false impression of their function. Purslow and Delage (2021) describe the endomysium as a continuous network integrating the individual muscle fibers within a fascicle, so as to coordinate their forces and deformations, They also describe the perimysium as a continuous network throughout the muscle (and connected to the epimysium), which allows flexible movements between fascicles as the muscle changes shape on contraction.

This integrative aspect is clearly seen in Fig. 3.12, which shows a cross section of a muscle that has been digested with sodium hydroxide to remove all but the fibrous network of collagen in the IMCT. At high magnification (Fig 3.12B), the structure of the endomysium as a continuous network is clearly appreciated.

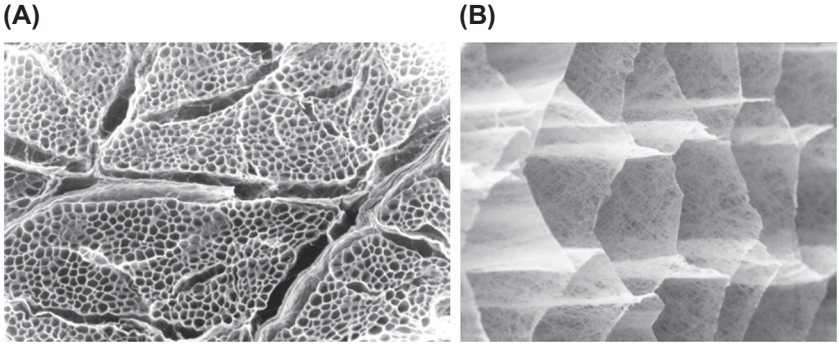


FIG. 3.12 Cross sections of bovine *sternomandibularis* muscle extracted with NaOH to show intramuscular collagen networks. (A) Low-magnification SEM showing continuous networks of endomysium bridging across fascicles, with thicker perimysial connective tissue lying between the fascicles. (B) Higher magnification of the surface of a fascicle, viewed at a slight angle. The semi-random feltwork of fine collagen fibers making up the walls of the continuous endomysial network can be seen. *(From Purslow, P.P., Trotter, J.A., 1994. The morphology and mechanical properties of endomysium in series-fibred muscles: variations with muscle length. J. Muscle Res. Cell Motil. https://doi.org/10.1007/BF00123482, with permission.)*

The vast majority of the thickness of the endomysium is made up of a near-random feltwork of fine, wavy collagen fibers lying in the plane of the endomysium. This collagen feltwork can easily reorientate to accommodate changing muscle lengths. The empty spaces in Fig. 3.12B represent the spaces occupied by the muscle fibers. The endomysium is a continuous structure spanning the entire fascicle which serves to integrate and coordinate all the muscle fibers in a fascicle. A lower-magnification view (Fig. 3.12A) of the transverse section clearly shows fascicles separated by the thicker perimysial network.

Collagen fibers in the perimysium are arranged in a crossed-ply arrangement of two sets of wavy collagen fibers which are larger in diameter than the fibrils in the endomysium, with the fibers in each ply parallel to each other but at an angle to the muscle fiber axis (typically ± 54 degrees in resting muscle). As with the endomysium, reorientation of this collagen network allows the perimysium to easily follow elongation or shortening of the muscle fascicles (Purslow, 1989). The perimysial strands continue out and fuse into the epimysium on the surface of the whole muscle. In many muscles (such as bovine *M. sternomandibularis*), collagen fibers in the epimysium take on the same crossed-2-ply arrangement as found in the perimysium. However, in the epimysium of pennate muscles (such as the *gastrocnemius*), or in muscles where the epimysium clearly participates in transferring load to adjacent structures (such as the bovine *semitendinosus*), the collagen fibers are more closely packed and longitudinally arranged, acting more like a tendon. At each end of the muscle, the endomysium, perimysium and epimysium blend into the tendons, which are large aggregates of longitudinally arranged collagen fiber bundles, often arranged into fascicles by a peritendinous sheath and surrounded by the epitendinous sheath.

Since the 1990s, there has been a growing body of evidence that not all of the force produced by muscle fibers is transmitted to tendons via the myotendinous junctions at the ends of muscle fibers, but that some transmission of contractile forces can be via this continual network of connective tissue structures, or fascia, within muscle, a process now known as epimuscular myofascial force transmission (Huijing, 2009). This would seem especially relevant in series-fibered muscles, where short muscle fibers in the middle of a fascicle have no connection to the tendons, and their only connections to the outside world are in fact through the endomysial–perimysial structures.

3.2.4.1 Variations in intramuscular connective tissue (IMCT) content between muscles

Given that each muscle in the body is fine-tuned to carry out different functional roles, it is no surprise that muscles vary in the composition and spatial distribution of their IMCT (as well as in the metabolic characteristics of their muscle fibers). It has long been recognized that variation in the IMCT content of muscles is one of the factors contributing to the toughness of different muscles after cooking. Bendall (1967) reports that, as a percentage of fat-free dry matter, the collagen content of 31 beef muscles varies between 1% and 15%, whereas the elastin content varies from 0.05% to 2%. Purslow (2005) summarized evidence that the amount of collagen in the perimysium varied by approximately 2.5 times as much as the amount of collagen in the endomysium of 14 beef muscles, and that the thickness of the perimysium in some muscles can be up to 2.5 times more than in others. The size and shape of the fascicles, as defined by the perimysial network, also show noticeable differences. For example, the fascicle size in bovine *pectoralis profundus* is much larger than in the *sternomandibularis* muscle. Given the different overall sizes and shapes (see Fig. 3.5) of individual muscles, and their different range of motions, it is not surprising that the division of muscle into fascicles by the perimysium is highly variable between functionally different muscles,

3.2.4.2 Composition of intramuscular connective tissues

The epimysium, perimysium and endomysium are distinctive connective tissue layers with different composition and morphologies, as described later. However, as with all connective tissues, they are extracellular matrices (ECMs) that are broadly comprised of fibrous proteins surrounded by an amorphous ground substance containing glycoproteins and proteoglycans. Of the fibrous proteins, the highest mass fractions belong to the collagen family.

3.2.4.3 Collagens in muscle

The most abundant proteins in the IMCT are the collagens. The following description is an abbreviated summary of the extensive description given by Birk and Bruckner (2011). Collagen molecules are assemblies of alpha chains.

Fig. 3.13 depicts the way in which amino acids (Fig. 3.13A) are assembled into polymers, commonly with a *Gly-Pro-X* repeating triplet of residues (Fig. 3.13B) which make up an individual alpha chain (Fig. 3.13C). Each collagen molecule has a helical central portion, bounded by nonhelical telopeptides. The alpha chains in the central region are each coiled into left-handed helices that lack

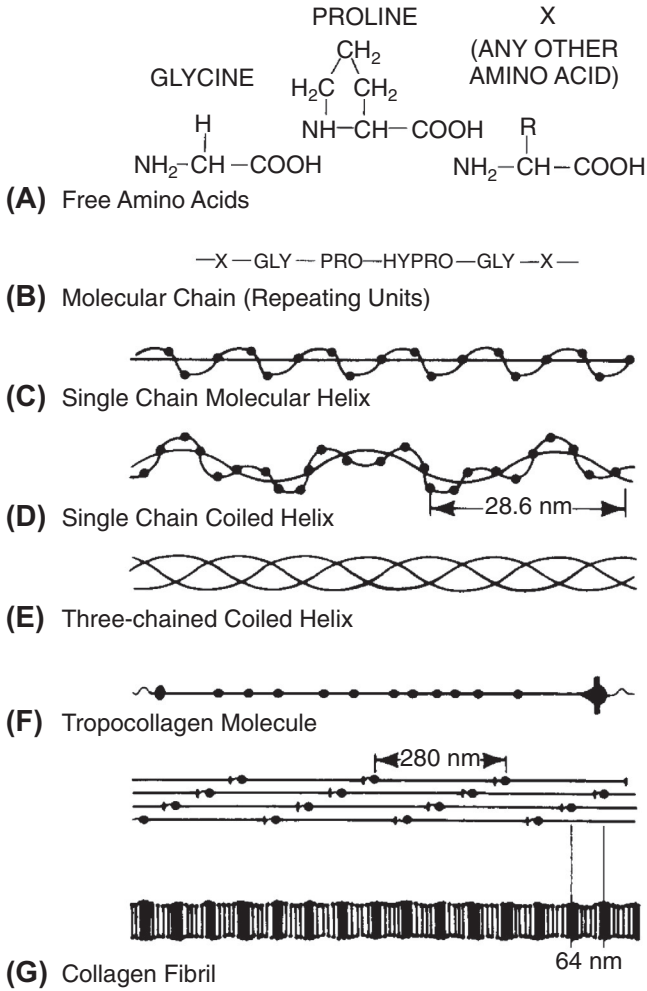


FIG. 3.13 Schematic illustration of (A) the amino acid sequence (B) molecular structure, (C) alpha helical structure, (D) single alpha helix coiled-coil configuration and (E) triple helical configuration of the collagen molecule. The assembled tropocollagen molecule (F) with nonhelical telopeptides is secreted and fibrillogenesis occurs extracellularly following scission of part of the nonhelical telopeptides by alignment of quarter-staggered molecules (G) to form fibrils with a characteristic banding pattern of 67 nm when wet, and 64 nm after dehydration for electron microscopy and collagen fibril formation. *(From 'Collagen', J. Gross. Copyright© May 1961 by Scientific American Inc. All rights reserved.)*

hydrogen bonds within the chain (Fig. 3.13D). The three chains are then supercoiled together for the triple helix characteristic of collagen (Fig. 3.13E). Collagen contains 18 of the 20 amino acids, with a high proportion of glycine, proline and hydroxyproline, a low content of methionine, cysteine and tyrosine and practically no tryptophan. The three alpha chains can be wound tightly together because glycine is present at each third position in the helix; the small hydrogen side chain of glycine is orientated toward the center of the helix and permits tight packing. The triple helical molecule with nonhelical telopeptides attached is termed tropocollagen (Fig. 3.13F). The nonhelical telopeptides prevent further aggregation of molecules within the fibroblast and are secreted as individual molecules. Extracellularly, substantial posttranslational modification of the molecules occurs. Cleavage of the nonhelical telopeptides removes their stearic hindrance of further aggregation. The fibril-forming collagens (principally type I and III in IMCT, together with a small proportion of type V) align laterally in a quarter-staggered overlap arrangement which maximizes the stability of the molecules due to charge distributions along their length, as depicted in Fig. 3.13G. This added stability increases the denaturation temperature of type I collagen from 37 °C in solution to 65–67 °C in the fibrous form found in IMCT.

In some collagen types, interruption of this Gly-X-Y repeating sequence results in an interruption of the helix. These are the FACIT collagens (fibril-associated collagens with interrupted triple helices). FACIT collagens are thought to limit the size of collagen fibrils by their interrupted helices interfering with further lateral packing of collagen molecules.

Genes coding for 28 different types of collagens has been found. Collagen types are assigned Roman numerals between I and XXVIII, depending on the chronological order they were discovered. There are actually 54 genes known to code for the different basic constituents of collagen, the alpha-chains. Collagen is typified by three alpha chains combining to make each collagen molecule. Different alpha chains that comprise the same collagen type are numbered with Arabic numerals (α 1, α 2, etc.), again in the order they were found (see examples in Table 3.1). The alpha chains in each type of collagen are different from each other and are coded by a different gene, so that α 1 in type I collagen (α 1(I)) is coded by the COL1A1 gene, whereas the α 1 in type III collagen (α 1(III)) is a different polypeptide and is coded by the COL3A1 gene.

The majority of collagen types have three identical chains (homotrimeric); for example, collagen type III is comprised of three identical α1(III) chains. However, some types have different chains; collagen type I, the most common type in IMCT, is comprised of two α1(I) chains and one α 2(I) chain (coded for by the COL1A2 gene). Types IV and V collagen have different isoforms, i.e., there are different combinations of alpha chains in different isoforms of the same collagen type. Type IV is found in basement membranes and does not form fibers, but rather meshwork-type structures.

At least seven collagen types have been found to exist in the IMCT of meat animals. The characteristics of these collagens are given in Table 3.3. It is likely

TABLE 3.3 Some characteristics of collagens found in intramuscular connective tissue.

Collage n type	Genes	Molecular structure (multiple lines = isoforms)			Classification	Location
I	COL1A1, COL1A2	$\alpha1(I)_2$	$\alpha2(I)$		Fibril forming	Perimysium, endomysium
III	COL3A1	$\alpha1(III)_3$			Fibril forming	Perimysium, endomysium
IV	COL4A1,COL4A2	$\alpha1(IV)_2$	$\alpha2(IV)$		Basement membrane	Immediately outside sarcolemma
	COL4A3,COL4A4	$\alpha3(IV)$	$\alpha4(IV)$	$\alpha5(IV)$		
	COL4A5	$\alpha5(IV)_2$	$\alpha6(IV)$			
	COL4A6					
V	COL5A1, COL5A2, COL5A3	$\alpha1(V)_2$ $\alpha1(V)_3$ $\alpha1(V)$	$\alpha2(V)$ $\alpha2(V)$	$\alpha3(V)$	Fibril forming	Perimysium, endomysium
VI	COL6A1	$\alpha1(VI)$	$\alpha2(VI)$	$\alpha3(VI)$	Beaded	
	COL6A2	$\alpha1(VI)$	$\alpha2(VI)$	$\alpha4(VI)$	filament	
	COL6A3	$\alpha1(VI)$	$\alpha2(VI)$	$\alpha5(VI)$	forming	
	COL6A4	$\alpha1(VI)$	$\alpha2(VI)$	$\alpha6(VI)$		
	COL6A5					
	COL6A6					
XII	COL12A1	$\alpha1(XII)_3$			FACIT	
X1V	COL14A1	$\alpha1(XIV)_3$			FACIT	

FACIT = fibril-associated collagens with interrupted triple helices.

that more than six collagens can be found in IMCT, but there has not been a systematic study to find all collagens in this tissue. Thin anchoring filaments containing type VII collagen have been found to link basement membranes to overlying connective tissue structures, and this led McCormick (McCormick, 1994) to speculate that type VII may anchor the basement membrane network to the endomysium. However, in one of the few studies of collagen type VII distributions in various organs, Wetzels et al. (1991) showed that adult (human) skeletal muscles were one of the few tissues not to contain type VII associated with basement membranes.

3.2.4.4 Collagen crosslinking

A further posttranslational modification of collagen in the extracellular space that quickly occurs is the oxidative deamination of specific lysine and hydroxylysine residues in the remaining short telopeptide domains of the collagen molecule to form allysines (aldehyde forms). The lysyl oxidase (LOX) family of copper-dependent oxido-deaminases is responsible for this. The quarter stagger alignment of collagen molecules in the fibril (Fig. 3.13F and G) allows these allysines (aldehydic forms) in the telopeptide region to interact lysine and hydroxylysine residues in the helical portion of adjacent molecules. Early formation of covalent crosslinks following LOX activity during fibrillogenesis imparts mechanical strength on the collagen fibrils and is necessary for the ordered formation and packing of fibrils. Crosslinks can form both intramolecularly (between alpha chains in the same molecule) and intermolecularly (Eyre and Wu 2005). Types III and IV collagen can also additionally form disulfide bonds because, unlike type I collagen, they contain cysteine. The divalent bonds formed between the alpha chains from lysine or hydroxylysine aldehydes are reducible in vitro; those and more complex bonds, joining more than two alpha chains, which arise during the aging of collagen (mature crosslinks) due to the condensation of divalent crosslinks. In addition to these physiologically regulated crosslinks, other, undesirable, crosslinks can form over time, including the action of reducing sugars such as glucose (nonenzymic glycosylation or glycation) and lipid oxidation products. Because of the long residence time of collagen in the body compared with other proteins, the mature covalent crosslinks can build up significantly as animals mature.

The formation of lysyl-oxidase-mediated crosslinks (both immature, divalent forms and their mature trivalent condensation products) in connective tissues generally is summarized in Fig. 3.14. Of the mature, trivalent crosslinks (double-lined boxes), the forms noted as predominant in skeletal tissues (III), are relevant to IMCT, with the pyridinoline and pyrrole crosslinks subject to the greatest amount of investigation. Pyridinoline crosslinks tend to increase in the IMCT of beef muscles with animal age, but vary between sexes and with feeding intensity (Bosselmann et al., 1995). Pyridinoline crosslinks have been found to increase with animal age in the IMCT of goat muscles, as did the thermal denaturation temperature of the collagen, while concentrations of the pyrrole

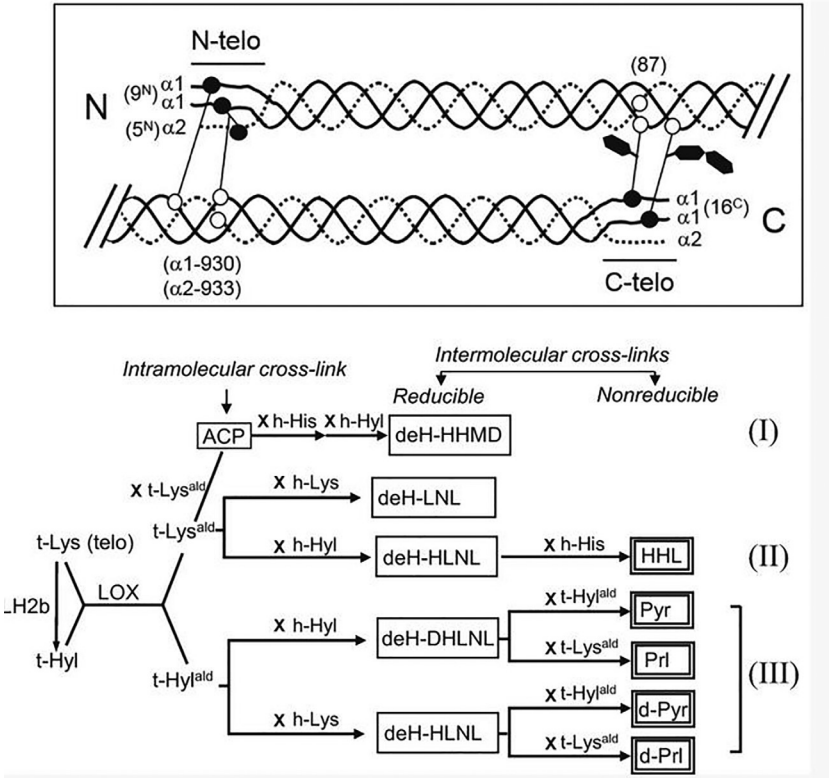


FIG. 3.14 Summary of collagen crosslinking. *Upper panel* illustrates collagen crosslinking between telopeptidyl lysine or hydroxylysine residue (*closed circles*) and the juxtaposed lysine or hydroxylysine residue (*open circles*). Closed hexagons indicate a galactose or glucose unit attached to hydroxylysine residues. N: amino-terminus, C: carboxy-terminus, telo: telopeptide. *Lower panel* illustrates the major collagen crosslinking pathways. Predominant in (**I**) soft tissues in general, (**II**) skin and cornea, (**III**) skeletal tissues. *LH2b* lysyl hydroxylase-2b, *LOX* lysyl oxidase, *ald*: aldehyde, *ACP* aldol condensation product (*intra*molecular crosslink), *deH* dehydro, *HLNL* hydroxylysinonorleucine, *DHLNL* dihydroxylysinonorleucine, *HHMD* histidinohydroxymerodesmosine, *HHL* histidinohydroxylysinonorleucine, *Pyr* pyridinoline, *d* deoxy, *Prl* pyrrole, *h* helical, *t* telopeptidyl. Crosslinking compounds are indicated in the rectangular boxes with a *single* (reducible with borohydride under mild conditions) or *double* (nonreducible) *line*. All compounds are intermolecular crosslinks except ACP. *(Reproduced from Yamauchi, M., Terajima, M., Shiiba, M., 2019. Lysine hydroxylation and cross-linking of collagen. In: Post-Translational Modification of Proteins. Humana, New York, NY, pp. 309–324. https://doi.org/10.1007/978-1-4939-9055-9_19, with permission of Springer Nature.)*

crosslink (otherwise known as Ehrlich Chromagen) diminished after 1 year of age (Horgan et al. 1991).

Although there is a correlation between the increase in thermally stable crosslinks in muscle collagen and increased toughness in the meat as animals mature, the differences in toughness which are observed between a given muscle

of animals of the same age cannot be readily explained. It has been shown that there is no correlation between toughness and the content of either immature (hydroxy-lysinonorleucine, dihydroxylysinonorleucine) or mature (hydroxylysylpyridinoline, histidinohydroxylysinonorleucine) crosslinks in the perimysial connective tissue isolated from the *longissimus lumborum* muscles of different pigs of similar maturity (Avery et al., 1996).

3.2.4.5 Turnover and remodeling of intramuscular connective tissue

There is a need for degradation and remodeling of IMCT during growth. As each muscle grows, the connective tissue networks within the muscle must expand to accommodate the hypertrophy of the individual muscle fibers and fascicles, and this involves degradation of existing structures as well as synthesis of new components. The principal proteases involved in the degradation of ECM components are the matrix metalloprotease (MMP) family. MMPs are secreted by both fibroblasts in the IMCT but also by muscle cells in latent (inactive) forms that undergo modification extracellularly to become active enzymes. The activity of these zinc-dependent proteases is regulated by their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). As discussion in the subsequent section on muscle growth and development, MMPs play key roles in myogenesis and the growth of muscle tissue as well as regulating the competing processes of fibrogenesis and adipogenesis. Additionally, the superfamily containing MMPs has important roles in cell signaling at the muscle cell–ECM interface, as well as cell signally roles within muscle cells, as active forms of MMPs can be seen around the nuclear membranes of myoblasts. These roles, as well as their function as enzymes remodeling IMCT in muscle repair and growth, are extensively reviewed by Christensen and Purslow (2016). As newly synthesized collagen is mechanically stabilized by the divalent crosslinks (discussed above) that form by posttranslational modification of collagen by lysyl oxidase immediately on fibril formation extracellularly, the possibility of stimulating collagen degradation and resynthesis so as to increase the heat solubility of IMCT in older animals has been the subject of some investigation. Multiple SNPs are known to exist in the principal collagenase (MMP-1). Some of these SNPs have been shown to reduce the raw strength of perimysial IMCT in beef muscle as well as to affect adipogenesis (Christensen et al., 2020).

3.2.4.6 Microfilaments and elastin

As well as fibers of collagen, IMCT also contains some fibers of elastic proteins. Elastic fibers in connective tissue are comprised of the protein elastin and associated microfilaments. In comparison to collagen, elastin is a minor component of IMCT and typically represents much less than 1% of the fat-free dry weight of muscle. Three exceptions to this reported for beef muscles are the *panniculus* muscle (1.13% of dry weight), the *semitendinosus* (1.82%) and the *latissimus dorsi* (2.0%) (Bendall, 1967). In contrast, the bovine *longissimus*

thoracis et lumborum muscle is reported to contain elastin at only 0.07% of dry weight. Bendall (1967) stated that, with the exception of the *semitendinosus* muscle, the majority of the high-value cuts in the beef hindquarter contain less than 02% of elastin (as % of dry fat-free matter), which corresponds to less than 5% of the total mass of IMCT. In mammals, the soluble precursor of elastin, tropoelastin, is the product of a single gene (ELN). There is a high degree of homology between elastins found in different species. Tropoelastin contains two hydrophobic domains rich in nonpolar amino acids such as *Gly, Val, Pro* and *Ala* and hydrophilic domains which are typically rich in *Lys* and *Ala* and are involved in crosslinking. These hydrophobic and crosslinking domains are encoded by separate, alternating exons. Alternative splicing leads to the expression of a number of isoforms of troponin in many species studied, including cattle and chicken (Vrhovski and Weiss, 1998). After secretion into the extracellular space, the process of aggregation of tropoelastin by interaction between hydrophobic domains is termed coacervation; this forms the insoluble elastin fibers, which are stabilized by the formation of crosslinks. Oxidative deamination of *Lys* residues by lysyl oxidase form allysine; lysine and allysine residues then form crosslinks that ultimately condense into two tetrafunctional crosslinks that are unique to elastin, desmosine and isodesmosine (Vrhovski and Weiss, 1998). The high stability of these tetravalent crosslinks results in elastin being very stable on heating, so that the stability of even small amounts of elastin fibers may affect the connective tissue contribution to the texture of cooked meat.

The principal components of the elastic microfilaments are the fibrillins, a family of glycoproteins that exist in an extensible, beaded molecular structure. Fibrillins 1 and 2 are known to exist in muscle, and the more recently discovered fibrillin-3 has been found in perimysium (Sabatier et al., 2011). It appears that fibrillin2 and -3 are expressed more during embryogenesis and early development, whereas fibrillin-1 persists into later stages of animal life. Fibrillin is ubiquitous to all connective tissues and forms the initial elastic microfilament network. In some connective tissues, this is supplemented by the thicker elastin fibers. The distribution of fibrillins and their role in the IMCT have not been studied extensively in the muscles of meat animals.

3.2.4.7 Glycoproteins and proteoglycans

These molecules contain both protein and carbohydrate units. The difference between them is in the relative amounts of each component. Glycoproteins have more protein and short glycan chains with no repeating unit. The glycans confer stability to the proteins. Glycoproteins are commonly transmembrane proteins that take part in cell communication and signaling.

Proteoglycans have much more carbohydrate units attached to the central protein backbone. The long unbranched glycan chains contain repeating disaccharide units. These long side chains are often charged and can stabilize large amounts of water. Proteoglycans are found in the IMCTs, where their gel-like structure provides a physical link between the fibrous proteins. Proteoglycans

can be categorized according to the types of glycans they contain, which include chondroitin sulfate, dermatan sulfate, heparin sulfate and keratan sulfate. In reality, proteoglycans can be considered a special class of glycoproteins. Both have roles in muscle development. Fig. 3.15 shows the repeating units in each of these glycan moieties.

Decorin is a small leucine-rich proteoglycan (SLRP) with side chains of either chondroitin sulfate units or dermatin sulfate repeating units. As with other SLRPs. Decorin is found at or near the muscle cell surface and interacts with other glycoproteins, including laminin and fibrillin. SLRPs are considered cell signaling molecules that affect both collagen fibril organization and the reaction of muscle cells to growth factors. Gene knock-out studies show that decorin-null mice have fragile skin, due to abnormal collagen fiber formation. Decorin and heparan sulfate-containing proteoglycans have been found in the basement membrane of muscle cells, whereas the proteoglycans of the perimysium contain more chrondroitin sulfate and dermatan sulfate. Two major groups of membrane-associated heparan sulfate-containing proteoglycans occur in muscle: the syndecans and glypicans. Syndecans have a core protein strand that inserts into the sarcolemma (i.e., it is a transmembrane protein), whereas glypicans attach to the cell surface via a glycosylphosphatidylinositol (GPI) anchor. All four types of syndecans have been found in muscle, but only one of the six types of glypicans (glypican-1). Both the syndecans and glypican-1 can affect the signaling of fibroblast growth factor 2 (FGF-2) in muscle. Both syndecan-4 and glypican-1 regulate satellite cell responsiveness to fibroblast growth factor 2 (Velleman and Song, 2017). FGF-2 acts to keep myoblasts in the proliferative phase and so affects cell number. In the early developmental stages of turkey *pectoralis* muscles, there are high levels of chondroitin sulfate-containing proteoglycans, whereas heparan-sulfate-containing proteoglycans dominate in later stages (Velleman, 2012).

3.2.4.8 Cell–matrix connectors: Laminin and fibronectin, dystrophin, and the integrins

For the efficient mechanical functioning of muscle cells, it is essential that there are good physical linkages between the muscle cell basement membrane (sarcolemma), the overlying basement membrane and the thicker network of collagen type I and III fibrils that comprise the reticular layer of the endomysium lying between adjacent cells. Additionally, the adaptation of muscle (growth/atrophy) to use (exercise) requires that mechanical signals be passed into the muscle cells from the ECM. Therefore, the molecules that principally provide the linkages between the muscle cells and their overlying connective tissues are of key functional significance.

Fibronectin is a dimeric glycoprotein consisting of two high-molecular-weight chains (~240 kDa per chain) covalently linked near their C-termini. There are three types of repeating subunit in each of the two chains which form various types of functional domains. Fibrillin contains domains for binding to

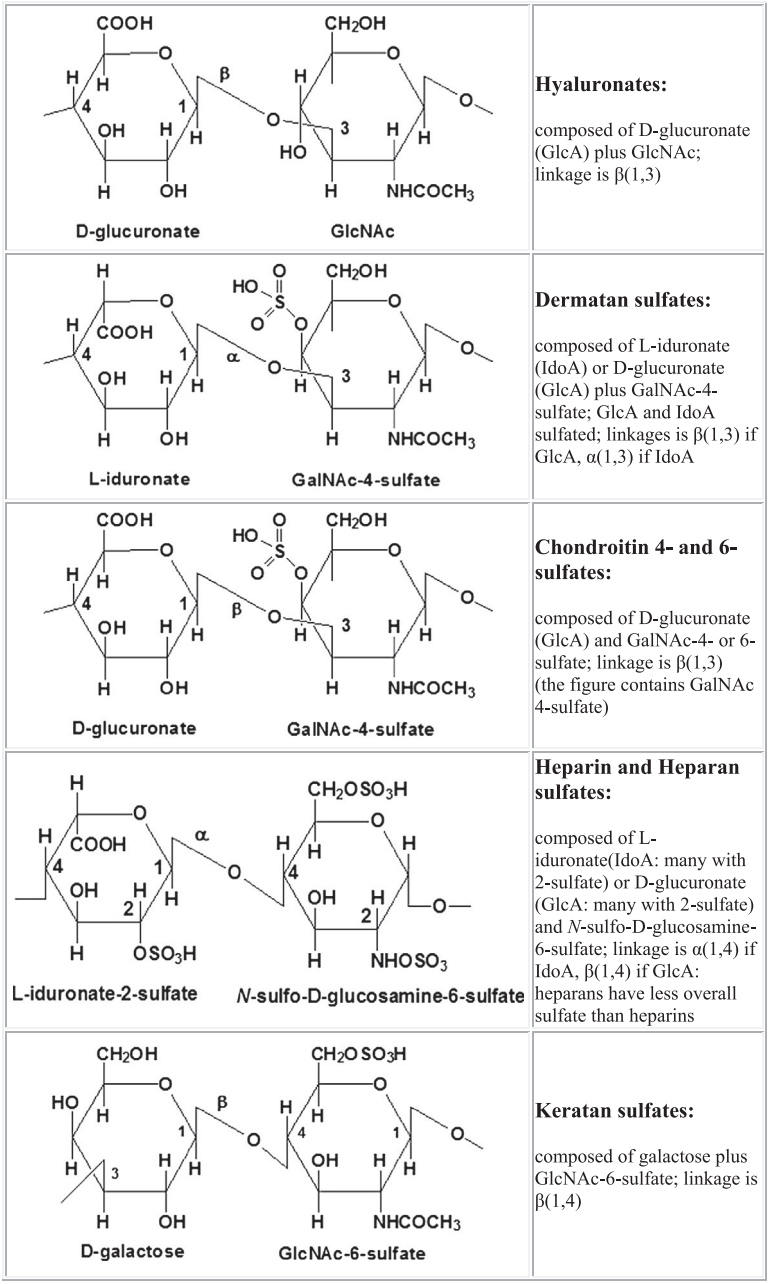


FIG. 3.15 Structure of side chain repeating units in various classes of proteoglycans. *(From http://themedicalbiochemistrypage.org/glycans.php.)*

other fibrillin molecules, for binding to collagen, for binding to heparan sulfate and a domain for binding to integrins on the cell surface. While some early studies reported the location of fibronectin in the perimysium, there is clear evidence that fibronectin is associated with the endomysium and basement membrane surrounding skeletal muscle cells (Purslow and Duance, 1990).

Laminin is another large glycoprotein found in the basement membrane that contains a number of domains whose function is to bind other ECM components and transmembrane cell surface molecules. Laminins are made up of three different polypeptide chains (α-, β- and γ-chains). There are 5 variants of the alpha-chain, four of the beta-chains and 3 of the gamma-chains, so giving rise to at least 15 different heterotrimeric molecules. Depending on the constituent chains, the molecule has either a cross-shape or a rod-shape or a Y-shape. Some domains bind to alpha-dystroglycan and integrin on the muscle cell sarcolemma, and at the other end of the molecules, there are binding domains to a variety of other ECM components. Fig. 3.16 simplistically depicts the interaction of a specific laminin with integrin and the dystrophyn–dystroglycan complex on the surface of muscle cells (Holmberg and Durbeej, 2013).

Integrins. The integrins are a family of glycoproteins that span the sarcolemma. They consist of two subunits (α and β). In mammals, 18 alpha units and 8 beta units are known to exist (Takada et al., 2007). The alpha chains are structurally similar to each other (homologous), and the beta chains are also homologous, but different in structure to the alpha units. Each integrin molecule is therefore an αβ heterodimer. Combinations of different alpha and beta units result in 24 distinct αβ heterodimers (24 types of integrins). Both of the integrin subunits have a large extracellular domain, a transmembrane domain and a cytoplasmic "tail" The cytoplasmic tail is quite short; usually less than 75 amino acids (with the exception of β4, which is 1008 amino acids in length).

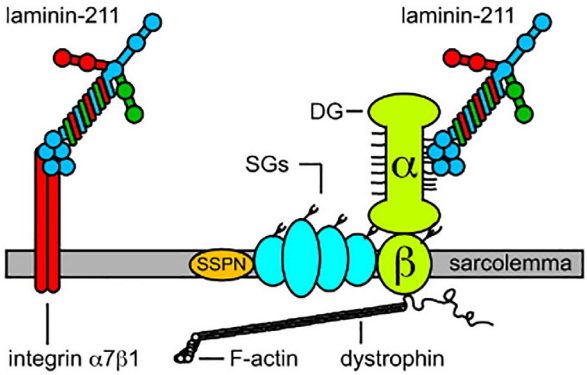


FIG. 3.16 Schematic diagram of laminin (type 211) binding to two major receptors in skeletal muscle, integrin α7β1 and dystroglycan (DG), a core component of the dystrophin-associated glycoprotein (DAG) complex. *(Adapted from fig. 5 in Holmberg, J., Durbeej, M. (2013). Laminin-211 in skeletal muscle function. Cell Adhes. Migr., 7(1), 111–21. https://doi.org/10.4161/cam.22618.)*

Internal to the muscle cell, integrins interact with microfilamentous actin, talin and vinculin, among other cytoskeletal proteins. (This actin is separate from the F-actin of the thin filaments in the sarcomere.) In terms of extracellular attachments to ECM molecules, each individual integrin has an affinity to one or more types of ECM molecule. The sequence arginine-glycine-aspartic acid (RGD) was first identified in fibronectin as a general integrin-binding motif and many ECM molecules contain this motif, but there are other binding sites specific to individual molecules. The affinity of integrins to specific ECM molecules also appears to be modulated by which cytoskeletal protein they are also interacting with inside the cell (Takada et al., 2007). Broadly speaking, integrins with the composition α1β1, α2β1, α3β1, α6β1, α7β1 and α6β4 are all capable of binding to laminin extracellularly, whereas α1β1, α2β1, α3β1, α10β1 and α11β1 integrins can bind to collagen. Integrins have two primary functions: to attach the cell to the extracellular matrix, and to transmit signals from the extracellular matrix into the cell. Although there are several cell signaling molecules that can bind to integrins, the majority of the inside-to-out and outside-to-inside transmissions via integrins in muscle cells are mechanical in nature. Integrins form a transmembrane pathway by which forces generated in the muscle cell can be transmitted to the overlying ECM, and, conversely, they also comprise the major pathway by which external forces are transmitted into the cell to affect cell expression and behavior). Mechanical cell–matrix interactions are important regulators of muscle growth or atrophy, as reviewed by Kjaer (2004).

The dystrophin-associated glycoprotein (DAG) complex. Integrins are common to all cell types in the body, but skeletal muscle cells have additional transmembrane structures that link the cytoskeleton within the cell to the extracellular matrix. Dystrophin is a 427 kDa rod-shaped protein that lies within the muscle cell and forms part of the costamere. The gene coding for dystrophin is the largest gene known. Costameres are assemblies of proteins including dystrophin, together with other proteins (such as α-dystrobrevin, syncoilin, synemin, sarcoglycan and sarcospan) that are attached to the inside surface of the sarcolemma in register with the *Z*-discs of the most peripheral myofibrils. The α and β dystroglycans form the transmembrane link between dystrophin (internally) and laminin (externally). This dystrophin-associated glycoprotein complex is another vital element in communications between the ECM and the cell. Deletion of the genes for α- and β-dystroglycans is fatal in embryo, as the basement membranes of the muscle cell fail to form. Although dystrophin is only about 0.002% of total muscle protein, mutations or deletions of the dystrophin gene cause myopathy (muscular dystrophy), which in the case of Duchenne muscular dystrophy can be severe, characterized by muscle fiber weakness and necrosis. Clearly, the DAG complex is important in cell–matrix signaling in muscle cells.

3.2.5 The structure of adipose tissues

Lipids are stored in the form of triglycerides in adipose cells or adipocytes. Mature adipocytes can be 100 μm in diameter and contain few organelles and

only a little cytoplasm; the great majority of the space within the cell is occupied by the triglyceride globule. Adipose tissue consists of adipocytes bound together by thin collagenous networks. Adipose tissue is well supplied by blood capillaries and can be arranged in lobules. Larger deposits of adipose tissue are termed fat depots. As well as being the major energy storage tissue, adipose tissue also has a major endocrine function. Adipose tissue secretes adipokines (bioactive peptides) that have paracrine and endocrine effects on appetite, lipid metabolism, insulin sensitivity and blood pressure, among other effects.

There are two types of adipose tissues: white fat and brown fat. Brown fat is specifically adapted for the generation of heat and is found in cold-adapted animals. In most farm animals used for meat, brown fat is prominent only in embryonic animals and will not be discussed further here.

White fat is the principal energy storage tissue and is aggregated in meat animals into four main locations: (1) subcutaneous fat, (2) visceral fat (around the intestines and internal organs), (3) intermuscular fat, lying in the spaces separating distinct muscles and (4) intramuscular fat, or marbling, which consists of streaks or lumps of adipose tissue within the muscle tissue, often situated at muscle fascicle boundaries. Three of these four fat locations are indicated in the meat cut shown in Fig. 3.17.

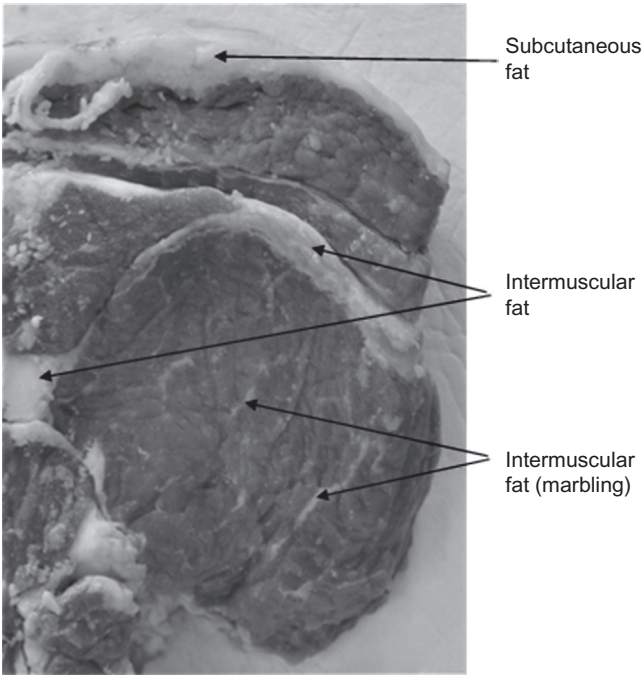


FIG. 3.17 Photograph of cross section through the rib region of a young bovine, showing fat distribution. The muscle where marbling is indicated is the *longissimus thoracis* muscle.

There are differences in the genetic factors controlling white adipose tissue growth in the visceral vs subcutaneous locations, and these two depots respond differently in their response to feed intake and the effect of male vs female sex hormones (Dodson et al., 2010). Differences also exist between patterns of adipose tissue development and lipid metabolism between ruminant meat species (cattle, goats, sheep) and monogastrics (pigs, poultry). However, in both ruminants and monogastrics, the composition of the fatty acids held within the adipose tissue affects the firmness and the melting point of the fat, attributes that affect both sensory and technological aspects on meat quality. The heritability of fat deposition in specific locations can be quite high (e.g., almost 50% for subcutaneous fat in pigs). In cattle, it is known that quantitative trait loci (QTL) associated with the development of subcutaneous fat are mostly different from those associated with intramuscular marbling fat or heart, kidney and pelvic fat (Dodson et al., 2010). The various fat depots within meat animals have different economic significance. Generally, high levels of visceral fat depress the lean:fat ratio of an animal and represent an economic waste. In beef meat, the inclusion of a certain level of intramuscular fat (marbling) is associated with good eating quality and is therefore of some economic value.

3.3 Muscle development and growth

Having established an overall picture of the general structure of meat and its components and some of the sources of variability in these structures, we can now turn our attention to the processes of muscle development and growth.

These can be divided into three stages: (1) embryonic formation of muscle and its associated tissues, (2) fetal muscle development and (3) postnatal growth. Generally speaking, the prenatal stages (1) and (2) determine the number of muscle fibers in each muscle, and stage (3) involves the growth of muscles by increasing the volume (hypertrophy) of the muscle fibers present at birth, although in some cases muscle fibers can be added (or destroyed) postnatally. The number of muscle fibers developed in the fetus is therefore an important determinant of the growth potential of the animal in later life. One of the most important determinants on muscle fiber number is the nutrition that the developing animal receives in utero, so maternal nutrition is a key factor. The effects of poor in uterine nutrition are evident in runt piglets, whose lack of in uterine nutrition determines that they will have a lower growth potential than their litter mates.

3.3.1 Muscle development in embryogenesis and prenatal growth

The general process of embryonic development of muscle is shown in Fig. 3.18. Early in embryonic development, mesodermal progenitor cells divide rostrocaudally into somites. The skeletal muscles of the tongue, limbs trunk and diaphragm (i.e., the muscles usually sold as meat) develop from these, whereas the

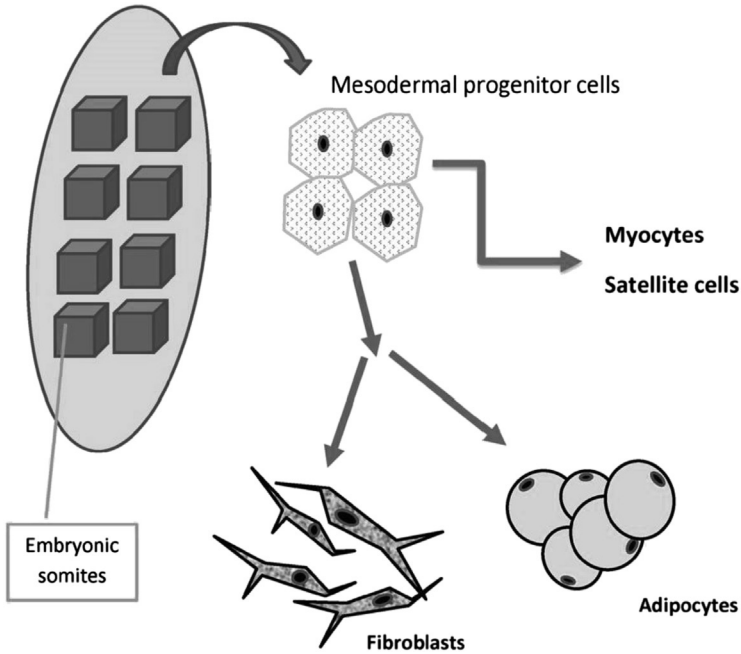


FIG. 3.18 Formation of major cell types in muscle from mesodermal cells in the embryonic somites. One line of progenitor cells forms satellite cells or the myocytes that fuse to form myotubes and subsequently muscle fibers. The other mesodermal progenitor line forms either fibroblasts (connective tissue-forming cells) or adipocytes. *(From Christensen, S., Purslow, P.P., 2016. The role of matrix metalloproteinases in muscle and adipose tissue development and meatquality: a review. Meat Sci. http://doi.org/10.1016/j.meatsci.2016.04.025, with permission.)*

muscle of the head develops from prechordal and unsegmented paraxial mesoderm. Muscle cells, adipose tissue and IMCT all derive from these somites. The development of limb muscles is a process that is under a slightly different pattern of regulation to the development of trunk muscles (Braun and Gautel, 2011), but the essential stages of development are the same.

Myogenesis refers to the formation of muscle fibers during embryonic development, whereas adipogenesis and fibrogenesis refer to the formation of adipocytes and fibroblasts, respectively. In this section, we will consider only the formation of intramuscular adipocytes that develop into intramuscular fat (IMF). Fibroblasts are the cells responsible for synthesis of connective tissues, and here we are concerned with the intramuscular fibroblasts that synthesize the components of IMCT.

In myogenesis, a subset of the mesodermal progenitor cells develops into the myogenic cell line, i.e., the cells that will go on to form muscle cells and satellite cells. Cells that become committed (determined) to produce muscle cells are termed myoblasts. The myoblasts proliferated (multiply) under the influence of growth factors and subsequently differentiate and fuse to form multinucleate

myotubes. These precursor muscle fibers then align with each other, partly under the influence of ECM factors. The process of myogenesis is very finely coordinated by a range of myogenic regulatory factors (MRFs). MRFs are basic helix-loop-helix (bHLH) proteins that bind to specific DNA sequences and regulate the expression of the genes they interact with by promoting or impeding the recruitment of RNA polymerase, which is the enzyme that facilitates the copying of DNA to messenger RNA.

In all skeletal muscles, the different stages of myogenesis are heavily regulated by four members of the MRF family: myogenic factor 5 (Myf5), myoblast determination protein (MyoD), Myogenin (MyoG) and muscle-specific regulatory factor 4 (MRF4). Myf-5 is the earliest transcription factor to be expressed in embryogenesis, and together with myoD, it promotes myoblast commitment (determination) and proliferation, and myogenin and MRF4 (also confusingly sometimes called Myf6) promote differentiation into myotubes and the subsequent maturation of these into muscle fibers (Braun and Gautel, 2011; Bentzinger et al., 2012). These act as a concerted network of coordinators whose activities are in turn affected by other transcription factors. In limb muscles, sine oculis homeobox homolog proteins (Six1 and Six4) regulate paired box protein 3 (PAX3), which in turn has downstream effects on the actions of a cascade of MRFs. In trunk muscles, PAX3 has downstream effects on MyoD, which acts in parallel to Myf5 and MRF4 in activation of myogenin (Braun and Gautel, 2011).

Myogenesis occurs in two stages. Primary myogenesis occurs in the embryonic stage, early in gestation. The primary muscle fibers formed in this stage tend to remain smaller during subsequent postnatal growth. These primary muscle fibers form templates around which more muscle fibers are added in a second wave of myogenesis later in the gestation period. These secondary muscle fibers go on to form the bulk of the muscle. Table 3.4, abridged from a

TABLE 3.4 Timing of primary (P) and secondary (S) myogenesis in a range of meat animals.

Species	P-fiber formation, days of gestation	S-fiber formation, days of gestation	Gestation length, days
Pigs	25–50	50–80	113
Sheep	32–38	38–80/125	145
Cattle	20–100	75–225	280
Chicken	4–7	8–16	21
Turkey	5–8	8–16	28

(Adapted from Oksbjerg, N., Therkildsen, M., Ma, 2017. Myogenesis and muscle growth andmeat quality. In: Purslow, P.P. (Ed.), New Aspects of Meat Quality e From Genes to Ethics. Woodhead Publishing, pp. 33e62. ISBN: 9780081005934 (Chapter 3).)

more comprehensive table by Oksbjerg and Therkildsen (2017), shows the timing of these two stages in the gestation of typical farm animals. A small wave of tertiary myogenesis late in gestation has also been observed in sheep and pigs (Bérard et al., 2011).

3.3.2 Adipogenesis and fibrillogenesis

As mentioned previously, intramuscular adipocytes share common progenitor cells with myocytes. The differentiation of adipocytes takes place later than that of myocytes, and adipocytes first form in the subcutaneous and intermuscular locations before finally being detectable intramuscularly at around 180 days of gestation in cattle. Du et al. (2012) suggest that, because of the different origin and behavior of intramuscular adipocytes, the development and growth of marbling fat may be manipulated independently from fat in other depots. As with myogenesis, differentiation of progenitor cells into adipocytes is regulated by transcription factors; in the case of adipogenesis, it is peroxisome proliferator-activated receptor γ (PPARγ) that acts in concert with members of the of the CCAAT/enhancer-binding protein (C/EBP) family. C/EBPβ and C/EBPδ are expressed early and trigger expression of PPAR, whereas other members of the C/EBP family are expressed later. Lefterova et al. (2008) suggest that most of the genes expressed in adipogenesis are bound by both PPARγ and C/EBPα or C/EBPβ so that these transcription factors act in concert to determine the biology of adipose tissue.

Fibrillogenesis is the process whereby other progenitor cells differentiate into fibroblasts, which lay down connective tissue (ECM) components and especially the fibrous collagens. Contact with ECM components and direct contact with fibroblasts aid the aligned formation of myotubes, and in mature muscle, ECM components guide the regeneration of damaged muscle fibers. Differentiation of fibroblasts, which goes on throughout development and growth, is one of the many processes under the control of the transforming growth factor beta (TGFβ) signaling pathway. TGFβ is a cytokine secreted by many cells including macrophages that binds to a cell surface receptor, triggering phosphorylation of receptor-regulated SMADs (R-SMADs). These bind with other SMADS to form transcription factors, binding to specific DNA regions and controlling gene transcription. As well as the major effect of TGFβ-SMAD signaling, Miao et al. (2015) list 11 other factors that either upregulate or downregulate fibrogenesis. Adipocytes and fibroblasts in muscle are both derived from the same common mesodermal progenitor cells, and in this sense, the processes of fibrogenesis and adipogenesis compete with each other. Thus, enhancing adipogenesis within muscle may reduce the formation of IMCT, and vice versa, although this has not been experimentally demonstrated to date.

3.3.3 Fetal development

In later stages of gestation, formation of new muscle fibers slows and muscle growth from then on is mostly by hypertrophy (i.e., the muscle fibers increase in

diameter and length) rather than new fibers being added. Most authors are of the opinion that the total number of fibers in any muscle is fixed at birth, although this number can vary with genetics and maternal nutrition. However, there is evidence that the total number of fibers in pig muscles can increase for up to 28 days postnatally by tertiary myogenesis (Bérard et al., 2011).

Initially, all myotubes formed during myogenesis contain a fetal type of myosin, but this is replaced by adult forms of fast and slow myosin during gestation. The muscle fibers formed in the primary wave of myogenesis go on to contain the adult form of slow type I myosin, whereas the fibers formed in secondary myogenesis can develop to contain either type I or any of the type II myosin heavy chains (Gagnière et al., 1999).

As the amount of connective tissue and the ratio collagen types varies between muscles, it follows that patterning of the development of muscles occurs differentially in different muscles. The organization of muscle fibers into fascicles or muscle fiber bundles is established within the first 6 months of gestation in cattle (Albrecht et al., 2013). Fibroblasts extracted from different bovine muscles are partially differentiated from each other, but it is not known at which stage this occurs. Different types of collagens appear early in development and while the absolute amounts of intramuscular collagen vary between muscles at any stage of development, in all bovine muscles, the amount of type I and type III collagen increases during the period of primary myogenesis and decreases thereafter (Listrat et al., 1999). Hypertrophy of muscle fibers increases the amount of myofibrillar proteins vs IMCT but at birth, fetal muscles still contain higher levels of connective tissue per unit weight than adult tissues. In the developing chicken embryo, by day 10 (i.e., approximately half-way through the 21-day period of development *in ovo*), the concentration of types I and III collagen in the *quadriceps* (thigh) is already higher than in the *pectoralis* (breast) muscles, and this difference is maintained at all times up until hatching (Lawson and Purslow, 2001).

3.3.4 Postnatal muscle growth

The growth rates of modern farm animals produced for meat are high. An average daily weight gain in intensively reared pigs of 1 kg per day and in cattle of up to 1.5 kg/day is possible. A chicken grows from an egg to a slaughter wright of around 2 kg in 6–8 weeks. Although not all of this growth is in muscle tissue, the rate of growth of muscle in farm animals is remarkable.

With the possible exception of new muscle fibers being added in pigs up to 28 days postnatally mentioned above, the total number of muscles fibers in animals used for meat is fixed at birth, and muscle growth in the postnatal or posthatch period is a process of hypertrophy of those fibers. An increase in the diameter of each muscle fiber involves the splitting apart of lateral connections between myofibrils and the synthesis of new myofibrils. An increasing length involves the addition of new sarcomeres, which again involves breaking

structures within the myofibril and between adjacent myofibrils in order to achieve this. Outside the muscle cells, the IMCT networks of the perimysium and endomysium must be remodeled to accommodate the widening muscle fibers and fascicles and to accommodate length changes. Muscle growth is therefore a balance between new protein synthesis and protein degradation. In order for growth to occur, the rate of protein synthesis must be faster than the rate of degradation. As growth slows and stops, these rates balance out, and conditions of poor food supply, illness, parasitism, high environmental stress or old age, muscles can atrophy, meaning that protein degradation outweighs synthesis. The fractional synthesis rate (synthesis rate expressed as a fraction of the total amount present) of different proteins varies. Oddy et al. (2001) review evidence which suggests that lack of nutrition (e.g., in extensive rearing systems where grass quality or availability is poor) followed by catch-up, or compensatory, growth and in weight loss/weight gain cycles, may produce a smaller connective tissue contribution to meat toughness due to connective tissue degradation in periods of limited growth, followed by synthesis of new connective tissue in catch-up growth. The same effects are not seen on the myofibrillar contribution to toughness, probably because the effects of postmortem proteolysis of myofibrillar proteins overwhelm any effects.

Major factors affecting muscle growth rates are genetic effects, the level of nutrition, muscle activity (exercise), stress hormones (corticosteroids, e.g., cortisol, epinephrine) and growth promoters such as the naturally occurring growth hormone, or somatotropin, or artificial growth promoters such as β-adrenergic agonists (β-agonists).

With the advent of genome-wide association studies (GWAS), there have been many attempts for find QTLs that related to growth rate (e.g., Gutierrez-Gil et al., 2009; Saatchi et al., 2014). It is fair to say that transcriptomes from animals within one breed are fairly noisy, and that there are obvious difficulties in trying to find common QTL clusters for growth rate across different breeds of cattle. It is perhaps not surprising than many QTLs that have been identified in study populations of cattle are pleiotropic (i.e., they affect many traits of the animal simultaneously).

The main signaling pathways controlling muscle growth are summarized in Fig. 3.19. More detailed description of these is given by Schiaffino et al. (2013).

Growth hormone (somatotrophin) is released from the pituitary gland and in many tissues (especially the liver) binds to external cell receptors triggering secretion of Insulin-like Growth Factor-1 (IGF1) which is an autocrine and endocrine signaling molecule; it can either bind to the same tissue/cells that secrete it or travel through the circulation to affect distant issues. As well as binding insulin receptors, in muscle it binds to the IGF1 receptor (IGF1R) on the surface of the muscle fiber which initiates by phosphorylation a signaling pathway involving Phosphoinositide 3-kinase (PI3K), Protein kinase B(PKB), more commonly called AkT and ultimately the mechanistic target of rapamycin (mTOR). This PI3K/AkT/mTOR pathway is a major signaling highway

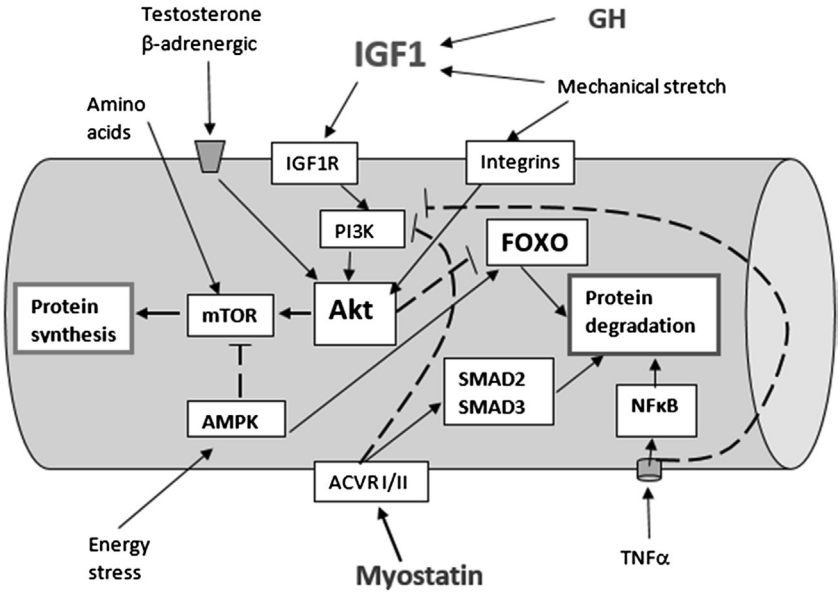


FIG. 3.19 Schematic representation of major pathways regulation muscle fiber growth.

for protein synthesis in the cell. A number of other signaling processes also involve AkT, so there is considerable cross talk between many signaling pathways involving AkT. AkT inhibits the breakdown of protein by inhibiting the FOXO signaling pathway that promotes protein degradation. FOXO is a member of the Forkhead family of transcription factors that gets the first part of its name from a conserved DNA-binding domain, the "forkhead-box" (FOX), and the "O" designation signifies a member of the FOX family that is inhibited by PI3K/Akt signaling. Sex hormones (testosterone) and β-agonists bind to a different class of cell receptors but also promote protein synthesis by upregulations AkT/mTor signaling. As muscles in the growing animal are subject to greater forces from postural requirements or locomotory forces, mechanical signaling through the integrins also promotes protein synthesis. The principal signaling pathway that balances the IGF1 promotion of growth is the myostatin pathway that stimulated protein degradation. Myostatin (also known as growth differentiation factor 8) is an autocrine protein secreted by muscle cells. It is a member of the transforming growth factor beta (TGF-β) family. Myostatin is coded for by the MSTN gene. In Belgian Blue and Piedmontese breeds of cattle, there are various mutations in the MSTN gene that lead to the secretion of nonfunctional myostatin. These "double muscles" animals have increase muscle fiber numbers and approximately 40% more muscle tissue than normal. Treatment of animals with follistatin (FSH-suppressing protein) also increased muscle mass by blocking the myostatin receptors, the activin A receptors (ACVR type I or ACVR type II) on the surface of the muscle fiber. Binding of myostatin to the ACVR

receptor triggers a pathway involving SMAD signaling that upregulated protein denaturation and also inhibits PI3K, so reducing protein synthesis.

AMP-activated protein kinase (AMPK) is an enzyme that is central to the energy metabolism of the cell. It regulates glycolysis and lipid metabolism in muscle. Any stress (including exercise, lack of food, hypoxia, oxidative stress, hormonal stress) that reduced ATP levels in the muscle cell turns on the AMPK cascade, resulting in increased glucose and lipid metabolism, but also inhibiting protein synthesis via an inhibitory effect on mTOR.

Tumor necrosis factor alpha (TNFα) is a cytokine that is systemically elevated in diseased involving inflammation or in muscle injury. It is associated with pathological conditions involving muscle wasting, but is also active in muscle regeneration following injury. TNFα induces protein degradation through the NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway. NFκB is a transcription factor that upregulates protein degradation through the apoptotic pathway (see below).

There are several proteolytic systems involved in the degradation of proteins that are active in muscle tissue during the constant remodeling that is part of muscle growth. The major proteolysis systems within the muscle cells are the calpains, the ubiquitin-proteosome system, the cathepsins and the caspases of the apoptotic (cell death) process. External to the muscle cells, the major family of proteases that degrade IMCT structures are the metallopreoteinases, which include the matrix metalloproteinases (MMPs) and the ADAMs (A Disintegrin and a Metalloproteinase) families. As discussed above, the major function of the MMPs is to degrade ECM components, but they also have cell signaling roles and have some roles in controlling adipogenesis. ADAMs are "signaling scissors"; these membrane-bound proteases cleave receptors, including IGFR and integrins, so turning off their signaling action. In addition to disposing of abnormal proteins that are produced in the ribosomes and of short-living normal proteins (regulatory proteins and rate-limiting proteins), the proteasome system can also contribute to degradation of long-living contractile proteins and is the best studied system in relation to muscle wasting (Mitch and Goldberg, 1996). Activation of the ubiquitin-proteasome pathway in fasting muscle requires the absence of insulin and the presence of glucocorticoids such as cortisol. Insulin normally suppresses proteolysis. Cortisol acts to increase the glucose levels in the blood. The calcium-activated calpains are best known for their role in postmortem proteolysis leading to development of meat tenderness. Oddy et al. (2001) summarize (in their table 1) much of the evidence for the involvement of calpains (especially calpain-1) in development of postmortem tenderness. In the living muscle, these proteases break down cytoskeletal proteins and destabilize large sections of myofilaments, which are then broken down either by the ubiquitin-proteasome system or by the apoptotic caspase cascade. Single-point nucleotide mutations (SNPs) of the inhibitor of calpains (calpastatin) exist in pigs and cattle and are associated with increased muscle yield due to supressed proteolysis.

3.3.5 Muscle cells grow by incorporating satellite cells

As mentioned above, the process of muscle cell hypertrophy requires the addition of many nuclei to the growing muscle cells in order to keep the nuclear/cytoplasmic volume ratio (N/C ratio) in reasonable bounds. Nuclei within muscle fibers are not mitotically active, and the new nuclei are recruited by the incorporation of satellite cells into the muscle fiber. Satellite cells lie in specialized niches between the plasmalemma (sarcolemma) of the muscle cell and the basement membrane (Dumont et al., 2015). These normally quiescent cells can be characterized by their expression of paired box protein Pax7, a transcription factor which regulates its gene expression. When activated, satellite cells can multiply rapidly. The satellite cell population can reproduce in two ways: to replenish the number of pluripotent stem-cell-like progenitor satellite cells or to produce satellite cells committed to fusing with muscle cells, as depicted in Fig. 3.20. Cells committed to the myogenic progenitor line express Myf5 and/or MyoD, in contrast to the cells in the quiescent pool (Dumont et al., 2015).

3.4 Conclusions and future trends

While the basic structure and composition of skeletal muscle tissue are well understood, new details of the complexity and plasticity of this tissue continue to come to light, especially in relation to the precise basis of the in vivo functions

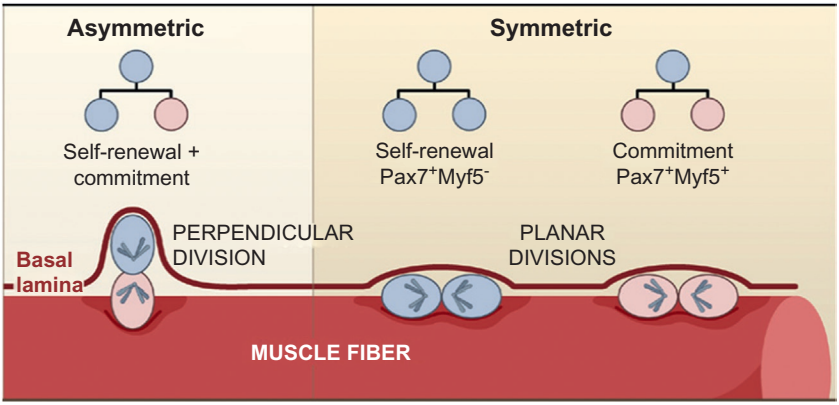


FIG. 3.20 Satellite cell proliferation. Division of stem cells generates either two self-renewing cells that are Pax7+/Myf5− (*blue*) or two committed cells that are Pax7+/Myf5+ (*pink*). In adult skeletal muscle, symmetric divisions (where the mitotic spindle is oriented parallel to the muscle fiber axis) generate two identical (either stem or committed progenitor) daughter cells that both contact the basal lamina and the plasmalemma. Asymmetric division (where the mitotic spindle is oriented perpendicular to the fiber axis) generates one of each type. *(Adapted from Cossu, G., Tajbakhsh, S., 2007. Oriented cell divisions and muscle satellite cell heterogeneity. Cell 129(5), 859e861. http://doi.org/10.1016/j.cell.2007.05.029.)*

of muscle (Knight, 2016) and the fine-tuning contractile characteristics by regulatory proteins such as MyBP-C (Li et al., 2019).

Modern genomics and transcriptomics continue to yield information on optimizing animal production efficiency, and meta-analysis of proteomics is increasingly increasing our understanding of the complex interactions between variations in the amount of individual proteins and their modification postmortem related to meat quality parameters such as tenderness, color and water-holding capacity (Gagaoua et al., 2020, 2021; Huang et al., 2020). Clearly, a good deal of clarification on the structural and molecular basis of eating quality traits is still to be provided by such approaches.

In terms of advances in the understanding and manipulation of muscle development and growth, maximizing the genetic potential of animals by fetal programming (Du et al., 2015) is a promising area of continuing work. However, we must ask just how far increased productivity and manipulation of muscle growth and composition can be pushed in the living animal without compromising the in vivo functions of muscle and consequently animal welfare. There is debate as to whether we are already approaching some limits in poultry and the effects that growth-related myopathies can have on poultry meat quality (Petracci and Cavani, 2012).

The cultivation of muscle tissue in vitro as a means of production of muscle biomass for human consumption is a rapidly growing area of both research and commercial development (see Chapter 23). Without the restrictions of growth imposed, the functional demands of a growing animal or variations in structure and contractile characteristics required by different muscles in a whole organism or restrictions due to issues of animal welfare, it is possible to envisage the manipulation of cultured meat constructs to provide consistent and desirable eating quality. Although some extracellular "scaffolding" is required to emulate the textural quality of whole meat as an aligned, hierarchical composite, it appears possible to substitute materials other than connective tissues (with their negative contributions to meat tenderness) for this scaffolding function, although this area of cultivated meat production is currently the least well-defined. Future production of cultured meat may therefore be able to avoid some of the variability in structure and properties of natural meat. Chriki and Hocquette (2020) discuss the advantages and remaining problems in this rapidly changing field.

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Chapter 4

Chemical and biochemical constitution of muscle

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4.1 General chemical aspects

Meat is the postmortem aspect of a complicated biological tissue, muscle, which is formed by highly specialized contractile fibers bound together in a complex network of connective tissue, which merges at each end to form tendons and adhesions connected directly or indirectly to bones (see Chapter 3). In a broad sense, the composition of meat can be approximated to 75% of water, 19% of protein, 3.5% of soluble, nonprotein, substances including inorganic compounds, and 2.5% of fat (Table 4.1).

4.1.1 Muscle proteins

The proteins in muscle (Table 4.1) can be broadly divided into those that are soluble in water or dilute salt solutions (the sarcoplasmic proteins), those that are soluble in concentrated salt solutions (the myofibrillar proteins), and those that are insoluble in the latter, at least at low temperature (the proteins of the connective tissue and other formed structures).

4.1.1.1 Myofibrillar proteins

Myofibrillar proteins are long fibril proteins organized into repeated sections (sarcomeres) to contract by sliding the thick (myosin) and thin (actin) filaments along each other.

Most abundant myofibrillar protein is myosin. The molecule of myosin has a molecular weight (MW) near 500,000 and a ratio of length to diameter about 100:1. Because of its high content of glutamic and aspartic acids, and of dibasic amino acids, it is highly charged and has some affinity for calcium and magnesium ions. Myosin is composed of two heavy polypeptide chains and four light polypeptide chains. Heavy meromyosin (HMM) and light meromyosin (LMM) are proteolytic fragments of myosin.

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TABLE 4.1 Chemical composition of typical adult mammalian muscle after rigor mortis but before degradative changes postmortem.

Components			Wet%	Weight
1.	Water			75.0
2.	Protein			19.0
	(a) Myofibrillar		11.5	
	Myosin[a] (H- and L-meromyosins and several light chain components associated with them)	5.5		
	Actin[a]	2.5		
	Connectin (titin)	0.9		
	N2 line protein (nebulin)	0.3		
	Tropomyosins	0.6		
	Troponins, C, I and T	0.6		
	a, b and g actinins	0.5		
	Myomesin, (M-line protein) and C-proteins	0.2		
	Desmin, filamin, F- and I-proteins, vinculin, talin, etc.	0.4		
	(b) Sarcoplasmic		5.5	
	Glyceraldehyde phosphate dehydrogenase	1.2		
	Aldolase	0.6		
	Creatine kinase	0.5		
	Other glycolytic enzymes especially phosphorylase	2.2		
	Myoglobin	0.2		
	Hemoglobin and other unspecified extracellular proteins	0.6		
	(c) Connective tissue and organelle		2.0	
	Collagen	1.0		
	Elastin	0.05		
	Mitochondrial, etc. (including cytochrome c and insoluble enzymes)	0.95		
3.	Lipid			2.5
	Neutral lipid, phospholipids, fatty acids, fat-soluble substances		2.5	

TABLE 4.1 Chemical composition of typical adult mammalian muscle after rigor mortis but before degradative changes postmortem—cont'd

Components			Wet%	Weight
4.	Carbohydrate			1.2
	Lactic acid	0.90		
	Glucose-6-phosphate	0.15		
	Glycogen	0.10		
	Glucose, traces of other glycolytic intermediates	0.05		
5.	Miscellaneous soluble nonprotein substances			2.3
	(a) Nitrogenous		1.65	
	Creatinine	0.55		
	Inosine monophosphate	0.30		
	Di- and tri-phosphopyridine nucleotides	0.10		
	Amino acids	0.35		
	Carnosine, anserine	0.35		
	(b) Inorganic		0.65	
	Total soluble phosphorus	0.20		
	Potassium	0.35		
	Sodium	0.05		
	Magnesium	0.02		
	Calcium, zinc, trace metals	0.03		
6.	Vitamins			
	Various fat- and water-soluble vitamins, quantitatively minute			

[a] *Actin and myosin ate combined, as actomyosin in postrigor muscle.*
Reproduced from Greaser, M., Wang, S.M., Lemanski, L.F., 1981. Proceedings 34th Annual Reciprocal. Meat Conference vol. 34, 12 and Lawrie's Meat Science, 7th ed., 2006, with permission from Elsevier.

LMM contains parts of both heavy chains, and HMM comprises parts of the two heavy chains and the four light chains. H-meromyosin, which contains all the ATPase and actin-combining properties of myosin, is sited on the periphery of the myosin filaments.

The other major myofibrillar protein is actin, which can exist in two forms: G-actin, which consists of relatively small globular units having an MW of

about 42,000, and F-actin, in which these globular units are aggregated end to end to form a double chain. It is F-actin that combines with myosin to form the contractile actomyosin of active or prerigor muscle and the inextensible actomyosin of muscle in rigor mortis.

Tropomyosin is a rod-shaped molecule that extends along the helical groove in the actin filament. Tropomyosin has a structure similar to that of the myosin tail, being a coiled unit of two protein chains. Each tropomyosin molecule is in contact with seven actin units. Thus, the troponin complex promotes the aggregation of tropomyosin, binds calcium, and prevents actomyosin formation; a-actinin promotes the lateral association of F-actin; b- and g-actinins inhibit polymerization of G-actin. Tropomyosin B is the term now given to the protein remaining after troponin has been removed from tropomyosin as it occurs naturally. Because of its high content of a-helix, tropomyosin B is capable of contributing mechanical stability to the muscle filaments. The proteins of the M-line substance represent at least two molecular species. One of these (myomesin) promotes the lateral polymerization of L-meromyosin but not that of H-meromyosin. It has a subunit weight of 165,000 Da and may bind creatine kinase to the M-line. The proteins of the M-line appear to be essential for controlling the polarity of the myosin molecules in each half of the sarcomere.

Troponin is composed of three major members, referred to as C, I, and T, which are concerned with the contractile process. The amino acid sequence in each has been determined. Troponin C (MW 18,000, 159 amino acid residues) has four binding sites for Ca2þ ions and forms an equimolar complex with troponin I. It is phosphorylated neither by 3,5-cyclic-AMP-dependent protein kinase nor by phosphorylase b kinase. Troponin I (MW 21,000, 179 amino acid residues) inhibits actomyosin ATPase.

Apart from desmin, small quantities of additional proteins have been isolated from skeletal muscles, which also appear to be involved in forming the *Z*-line lattice, such as Eu-actinin, filamin, synemin, vimentin, and zeugmatin.

Connectin (variously referred to as "gap filament," T-filament, or as a mixture of titin and nebulin), nebulin, and desmin have already been described as forming a filamentous cytoskeleton in the muscle; their arrangement in the sarcomere and their relationship with myosin and C-protein are also considered in some detail. The very large MW of titin and nebulin makes them susceptible to destruction by ionizing radiation. Such findings emphasize the importance of these large proteins in cytoskeletal structure.

In that portion of muscle, which is insoluble in concentrated salt, are the mitochondria, containing the enzymes responsible for respiration and oxidative phosphorylation, the formed elements of the muscle membrane (sarcolemma) and the collagen, reticulin, and elastin of connective tissues.

4.1.1.2 Sarcoplasmic proteins

The sarcoplasmic proteins are a mixture of several hundred molecular species (Bendixen, 2005; Fig. 4.1). Enzymes of the glycolytic pathway constitute the

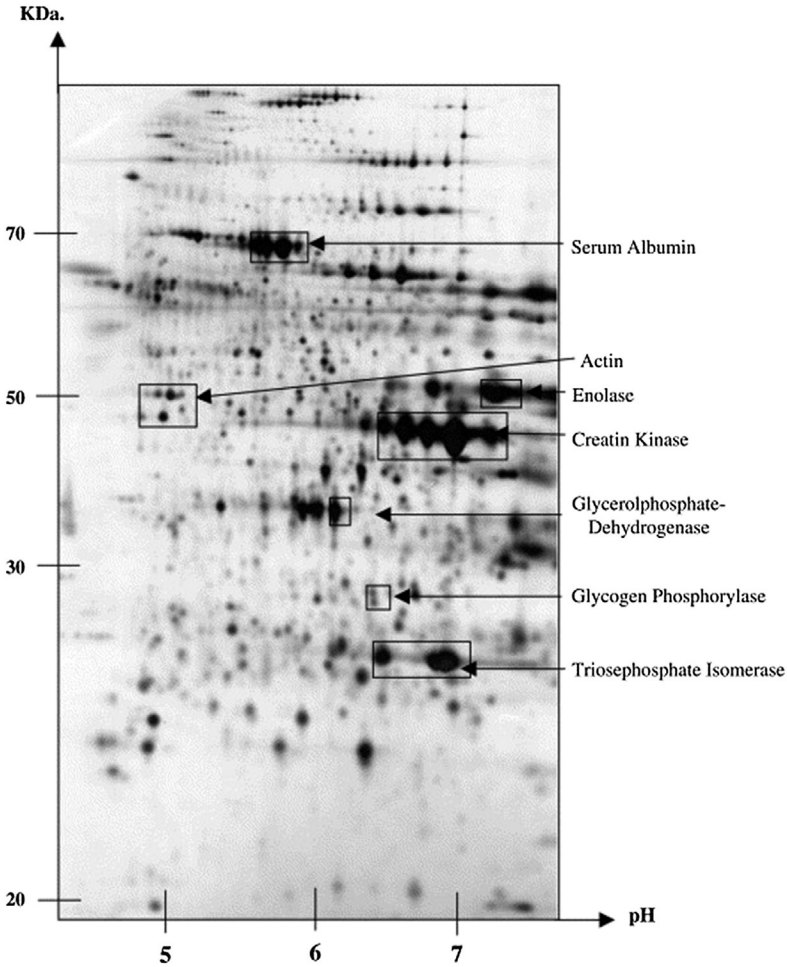

FIG. 4.1 Proteins separated by two-dimensional electrophoresis from exudates of porcine muscle. *Reproduced from Bendixen, 2005, Meat Sci., 71, 138–149 with permission from Elsevier.*

major proportion of the sarcoplasmic proteins and may be present in more than one form (isozymes). Glycolytic enzymes are bound to the myofibrillar protein actin, the proportion bound increasing on stimulation.

of glycolysis. It is evident, however, that some of the glycolytic enzymes bind not to F-actin, but to enzymes, which are already bound to the latter. Thus, triose phosphate isomerase binds to aldolase and triose phosphate dehydrogenase (both of which are bound to F-actin). It is interesting to note that these enzymes are arranged spatially in their correct sequence in the glycolytic pathway, the clusters of enzymes forming separate metabolic compartments wherein metabolites are transferred from the enzymes.

Glycolytic enzymes bind also to other locations in the muscle cell, including the sarcolemma, the sarcoplasmic reticulum, and the membranes of the nuclei and mitochondria. Much of the AMP-deaminase activity of muscle is located at the ends of the myosin filaments on the A/I junction. Phosphorylase b appears to be localized both at the Z-disk and the M-line. The M-line is also the location of creatine kinase.

4.1.1.3 Connective tissue

Intramuscular connective tissue (IMCT) is a complex network of extracellular proteins that maintains muscle structure and carries contraction forces into tendon and bones (see Chapter 3). Besides its structural role, IMCT is important in the regulation of muscle cells growth by direct cell signaling and by modulation of growth factors (Nishimura, 2015; Fig. 4.2).

The IMCT develops synchronously with muscle fibers and adapts through growth and intramuscular fat (IMF) deposition (Fig. 4.3) (Nishimura et al., 2009). There are different layers of connective tissue. The external epimysium surrounds the whole muscle and is made of thick sheets of collagen fibers. The perimysium separates muscle into bundles and also contains sheets of collagen fibers. These bundles are formed by several muscle fibers, which are surrounded by the endomysium, composed also of a collagen fibril networks surrounding each muscle fiber (Nishimura et al., 2009; Nishimura, 2015; Fig. 4.3). Finally, the basement membrane joins the connective tissue to the muscle cell membrane, which contains collagen (40%) and some complex polysaccharides. Epimysium, perimysium, and endomysium converge to form aggregates of connective tissue, known as tendons that attach to the skeleton and transfer the forces. It is

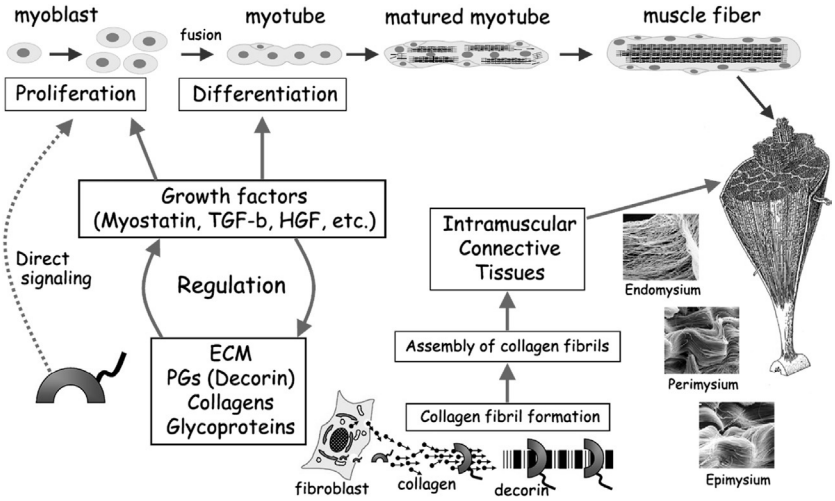

FIG. 4.2 Structural and regulatory role of extracellular matrix in skeletal muscle. *Reproduced from Nishimura, 2015, Meat Sci. 109: 48–55 with permission from Elsevier.*

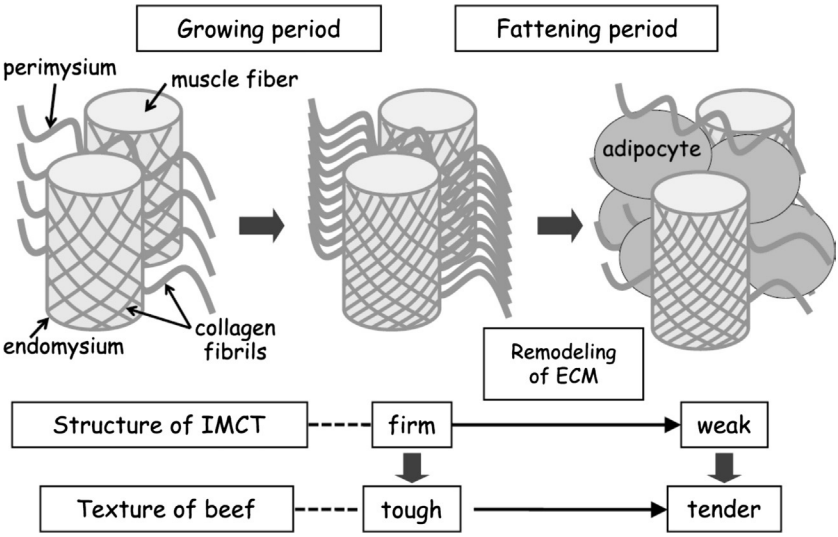

FIG. 4.3 Effect of intramuscular fat deposition on the structure of intramuscular connective tissue along the growing and fattening period. *Reproduced from Nishimura, 2015, Meat Sci. 109: 48–55 with permission from Elsevier.*

estimated that over 90% of intramuscular collagen is located in the perimysium (McCorninck, 1994). The connective tissue is mainly formed of collagen followed by elastin, proteoglycans, and glycoproteins. These latter two proteins have strong negative charges allowing repulsion and an extended structure capable of retaining water. Collagen has a regular sequence of amino acids, with a very high proportion of glycine, proline, and hidroxiproline, which are nonessential amino acids with limited nutritional value. This particular arrangement allows stabilization of molecules and intermolecular cross-links over time, thus increasing meat toughness and decreasing digestibility. The collagen content and collagen fiber diameter of both perimysia and endomysia, and the ratio of heat-stable to heat-labile cross- links in epimysial, perimysial, and endomysial connective tissue are each correlated positively with toughness, but the perimysium appears to be the most implicated in accounting for textural differences between muscles. The IMF deposition, mainly between adipocytes located between muscle fibers, produces a disorganization of the perimysium, which causes a remodeling of extracellular matrix and leads to a tender meat (Fig. 4.3).

4.1.2 Water

In spite of its solid structure, living muscle and meat contain a high proportion of water (Table 4.1). As water is a dipolar molecule, close interaction with proteins and other charged molecules may take place. Thus, water can be classified as bound and nonbound water, which in turn can be either immobilized in cell structures or free.

Since proteins form about one-fifth of wet weight of meat, and the binding of water to them is equivalent to about half of the weight of proteins, water bound to protein is about 13% of total amount of water in meat (Lopez-Bote et al., 1989). Bound water is tightly bound to proteins and does not easily move between compartments, even applying heat of freeze-drying. Protein denaturation affects external exposure of charged groups and markedly leads to a loss in their ability to bind water. Both myofibrillar and sarcoplasmic components denature to different degrees after death depending mainly on the combination of pH decline, temperature, and oxidative deterioration, thus loosing part of their ability to bind water (Honikel et al., 1983; Lopez-Bote et al., 1989). On the other hand, proteolytic degradation of meat along aging increases the number of charged groups and the ability to retain water.

The major part of the water (up to 85%) is contained within the protein-dense myofibrillar network (intramyofibrillar), which is usually referred as entrapped or immobilized water. This water is located between the thin and thick filaments, and it is retained by capillarity forces (Offer and Trinick, 1983). Ability to retain water between myofibrils is affected by meat ultimate pH (pHu). Space between myofibrils is minimal at pH near to isoelectric point of proteins (5.5), and a marked increase in water retention occurs at higher pH. The remaining water is located in the nuclei, organelles, sarcoplasm, between muscle fibers (in the interfascicular space), and between muscle fasciculi (extrafascicular). This water is usually referred as free water and may be lost easily (Huff-Lonergan and Lonergan, 2005).

Depending on its degree of freedom, water may altered by a radiofrequency pulse under a magnetic field, giving different relaxations in two planes. Thus, nuclear magnetic resonance relaxometry allows studying the behavior of three different populations of water (Pearce et al., 2011). The P21 represents 80%–95% of the transverse relaxation and corresponds to water in organized protein structures, and the P′′ population is the slow relaxing water and represents 5%–15% of the transverse relaxation, which is believed to correspond to free water. A third group with the fastest relaxation time is speculated to be closely associated to macromolecules (Bertram et al., 2004; Fig. 4.4).

Water is a dynamic component in living muscle, since contraction produces a reduction of intramyofibrillar volume, which leads to a displacement of the water to the extramyofibrillar space, where it is retained temporarily in cell structures until it recovers the original location when muscle relaxes (Kristensen and Purslow, 2001; Fig. 4.5).

Postmortem initial steps of myofibril shrinkage produce also an outflow of water from intra- to extracellular compartment that may either be retained by membrane structures or lost (see Chapter 14). An alteration of the cellular membranes may also take place, allowing the water to flow out of the cell, phospholipid composition and antioxidant status playing a key role in maintaining membrane structure postmortem (Monahan et al., 1994). Interesting to note that not only membrane integrity is required, but also an active proteolysis of the cytoskeleton during initial postmortem stages, which breaks the myofibrillar strain to the cell

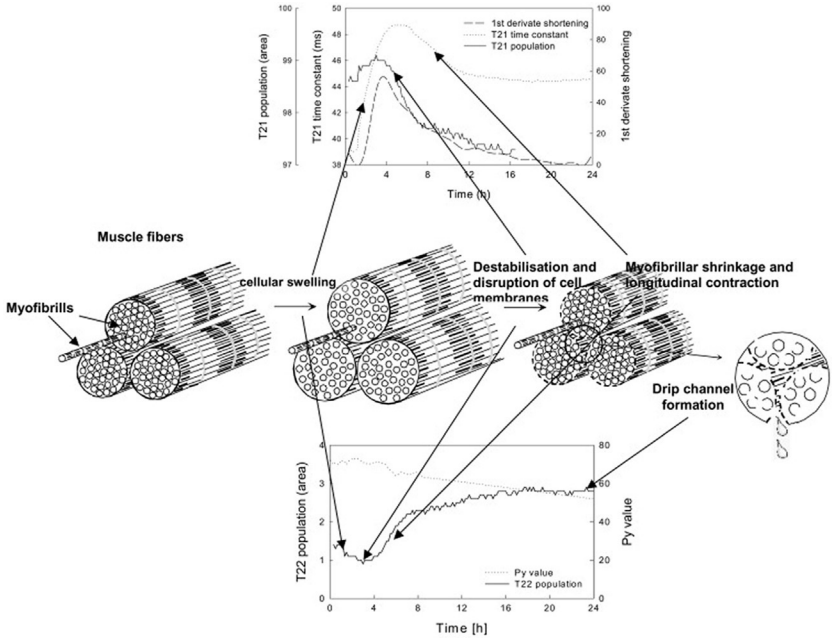

FIG. 4.4 Proposed mechanism in the postmortem reorganization of muscle fluids and its relation to observed changes in NMR T2 characteristics. *Reproduced from Bertram, H.C., Schafer, A., Rosenvold, K., Andersen, H.J., 2004. Physical changes of significance for early post mortem water distribution in porcine M. Longissimus. Meat Sci. 66, 915-924 with permission from Elsevier.*

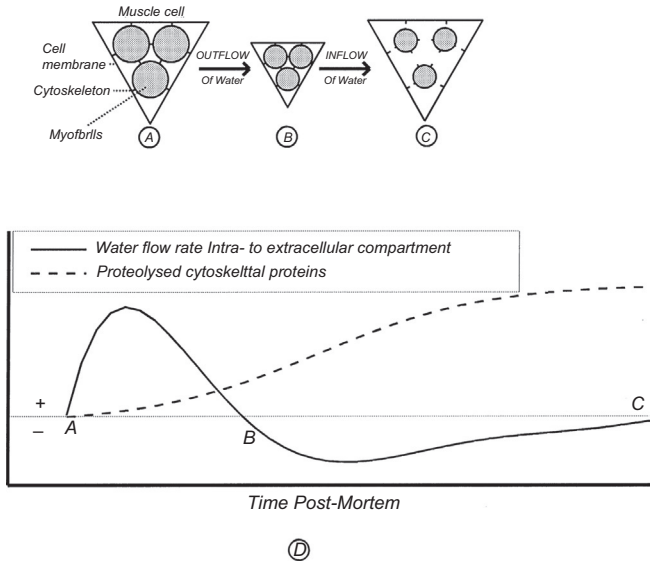

FIG. 4.5 The changes in water distribution in muscle and meat. (A) A simplified prerigor muscle cell with three myofibrils connected to each other and to the cell membrane by the cytoskeleton. (B) Postmortem shrinkage of myofibrils leads to shrinkage of the whole muscle cell, which causes an outflow of water from intra- to extracellular compartment. (C) Proteolysis of the cytoskeleton removes the myofibrillar strain on the cell membrane, which results in a flow of extra-myofibrillar water to the muscle cell. (D) Relationship between water flow rate, time of postmortem, and quantity of proteolyzed cytoskeletal proteins. *Reproduced from Kristensen, L., Purslow, P., 2001. The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. Meat Sci. 58, 17–23 with permission from Elsevier.*

membrane and allows detachment of both structures, thus providing a space capable of retaining water (Kristensen and Purslow, 2001). If myofibril contraction takes place without previous detachment to membranes, cell shrinkage produces a marked exudation of water (Kristensen and Purslow, 2001). On the other hand, some studies show that the water-holding capacity of pork meat increases along aging (Joo et al., 1999; Kristensen and Purslow, 2001; Fig. 4.5).

Therefore, water-holding capacity of meat depends on factors that affect rigor mortis development and proteolysis, the pH drop and the pHu playing a key role together with the antioxidant status.

4.1.3 Carbohydrates

The amount of carbohydrates in meat is very low (0.2%–0.4% weight). However, muscle in vivo stores glycogen at 60–150 mmol glucose/kg wet weight concentration (equivalent to 1%–2.7%) as a highly available source of energy readily available in demanding situations, such as stress, exercise, or fasting. Glycogen is a branched chain polymer of glucose, analog of starch in plants. It has a similar structure, but is more extensively branched, thus allowing more hydration (approximately 3–4 parts of water per part of glucose) and metabolic availability. Glycogen synthesis is initiated by a protein, glycogenin, the molecule of which combines autocatalytically with eight glucose molecules to form a glycosyl-protein and constitutes a primer for glycogen synthesis. Each linear glucose chain contains 13 units, bound together with α-1,4-glycosyl bonds. At the fourth and eighth glucosyl units of each chain, there are 1,6-bonds, which gives rise to new linear chains of 13 units (Fig. 4.6; Pösö and Puolanne, 2005). Glycogen is present in the cytosol in the form of granules (10–40 nm). There are two forms of glycogen, the proglycogen (MW 4×10^5 Da) and the

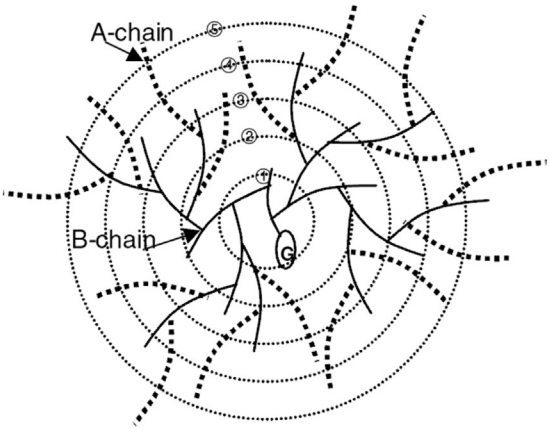

FIG. 4.6 Scheme showing the structure of the glycogen molecule. *Reproduced from Pösö and Poulanne (2005) Meat Sci. 70, 423–434 with permission from Elsevier.*

macroglycogen (10^7 Da). Metabolic differences and implications are still to be fully clarified. Importance of carbohydrate in meat goes far beyond its tiny concentration, as it may dramatically affect perimortem metabolic features with critical importance in meat properties. Therefore, attention has been devoted to understand and control adequate levels of glycogen in the liver and muscle. As explained in Section 4.3, the amount of muscle glycogen is affected by species (horse > beef and pig) and fiber type (Type IIb > Type I) (Pösö and Puolanne, 2005) and a number of extrinsic factors.

4.1.4 Intramuscular fat

IMF is composed of polar (phospholipid) and neutral (mainly triglyceride) lipids. Cell membranes are formed by a bilayer structure of phospholipids of about 5 nm thick, which provides a barrier that constitutes the cell boundaries. Phospholipid includes phosphoglycerides, plasmalogens, and sphingomyelin. In the phosphoglycerides, one of the three hydroxyl groups of glycerol is combined with choline, ethanolamine, serine, inositol, or glucose. In the plasmalogens, the second hydroxyl group of glycerol is esterified with a long-chain fatty aldehyde instead of with fatty acid; and in sphingomyelin, the amino alcohol sphingosine is bound by an amide link to a fatty acid and by an ester link to phosphorylcholine. There are also present in muscular tissue complex sugar-containing lipids and glycolipids. Membrane plays an essential structural and regulatory role in living cells. Phospholipids may form these structures thanks to its amphiphilic characteristics, as the two hydrophobic fatty acids are located in the extreme opposite to the hydrophilic phosphate end.

As membrane structures are essential in all living cells, the amount of polar lipids is very constant (approximately 0.8% in weight of the muscle) (Warren et al., 2008; Fig. 4.7). Fatty acid of membranes contains a high proportion (above 30%) of essential polyunsaturated fatty acids (PUFAs), including long-chain PUFA (2:20 carbon atoms) (Table 4.2), which are precursors of eicosanoids, such as prostaglandins, thromboxanes, etc., and play a role in cell communication and other regulatory aspects. Fatty acid composition of membrane phospholipids is regulated to maintain a certain proportion of classes (saturated, $n-6$, and $n-3$), which allow adequate structural and regulatory function. However, diet and other extrinsic factors may produce alteration in these proportions.

On the other hand, adipocytes are cells specialized in storing energy by accumulating fatty acids into acylglycerols. This is a high molecule containing three fatty acids without any charge (nonpolar or neutral lipids), which makes it highly hydrophobic. Therefore, lipid droplets in adipocytes contain no water.

Intramuscular adipocytes are located into a connective tissue matrix near a blood capillary network, where they get the circulating fatty acids or its precursors (see Chapter 3). Marbling refers to a group of adipocytes forming visible white flecks or streaks irregularly distributed along the muscle, with a higher density near main blood vessels (Faucitano et al., 2005; Fig. 4.8). In fact, a

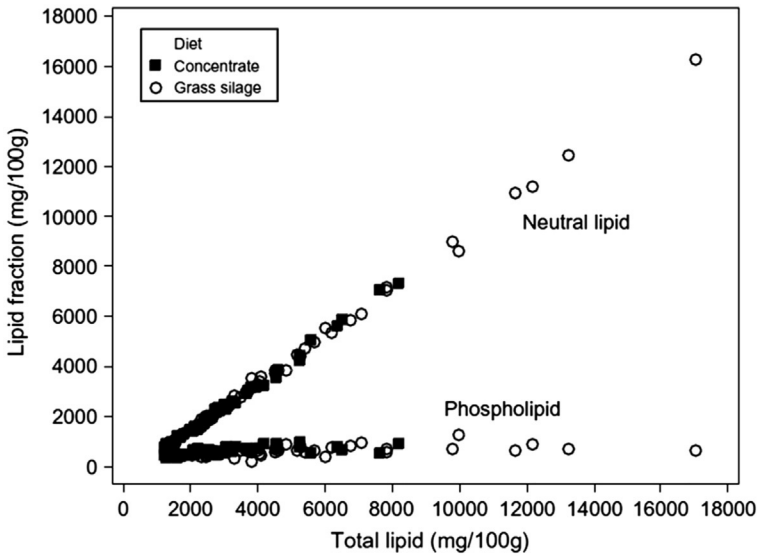

FIG. 4.7 Concentration of neutral lipid and phospholipid (mg/100 g muscle) plotted against total lipid in *longissimus dorsi* muscle of steers given a concentrated grass silage diet and slaughtered at 14, 19, or 24 months of age. *Reproduced from Warren et al., 2008, Meat Sci. 78: 256–269 with permission from Elsevier.*

TABLE 4.2 Fatty acid composition (%) of *longissimus* muscle triacylglycerol (neutral lipid) and phospholipid in pigs, sheep, and cattle.

	Neutral lipid			Phospholipid		
	Pigs	Sheep	Cattle	Pigs	Sheep	Cattle
14:0	1.6	3.0	2.7	0.3	0.4	0.2
16:0	23.8	25.6	27.4	16.6	15.0	14.6
16:1*cis*	2.6	2.2	3.5	0.8	1.5	0.8
18:0	15.6	13.6	15.5	12.1	10.4	11.0
18:1*cis*-9	36.2	43.8	35.2	9.4	22.1	15.8
18:2*n*-6	12.0	1.5	2.3	31.4	12.4	22.0
18:3*n*-3	1.0	1.2	0.3	0.6	4.6	0.7
20:4*n*-6	0.2	ND	ND	10.5	5.9	10.0
20:5*n*-3	ND	ND	ND	1.0	4.1	0.8

Reproduced from Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Science 78, 343–358, with permission from Elsevier.

FIG. 4.8 Intramuscular fat distribution in *longissimus dorsi* muscle of pigs. *Reproduced from Faucitano, F., Huff, P., Teuscher, F., Gariepy, C., Wenger, J., 2005. Application of computerized image analysis to measure pork marbling characteristics. Meat Sci. 69, 537-543 with permission from Elsevier.*

relationship exists between intercellular signaling of skeletal muscle fibers, angiogenesis, formation of extracellular matrix, and development of IMF adipose tissue. Total number of adipocytes is affected by species, sex, breeds, genetics, and epigenetics factors and by dietary intervention. Filling of preexisting adipocytes ultimately depends on the nutritive status of the animal, as only energy, which exceeds requirements for maintenance and production, is stored. Main fatty acids in neutral lipids are oleic, palmitic, and estearic, which usually comprise more than 75%–80% of total fatty acids. Compared with polar lipids, there is a wide range of variation, particularly in monogastric animals (Fig. 4.9).

Depending on the metabolic circumstances, the animals can synthesize fat from carbohydrates, volatile fatty acids, or proteins, but it may also deposit directly absorbed fatty acid with minor metabolic modification (see Chapter 2). Therefore, fatty acid composition in neutral lipids depends on the proportion of those produced endogenously (de novo synthesis) or absorbed from the digestive system (direct deposition). Several studies have shown that fatty acid composition of endogenously synthesized fat is relatively constant and is equivalent to approximately 45% saturated fatty acids and 55% monounsaturated fatty acids (Mitchaothai et al., 2008).

On the other hand, direct deposition of fatty acids from the diet is carried out through a complicate sequence of processes including digestion, absorption, and transport. In ruminant animals, rumen hydrogenation of fatty acids diminishes the proportion of unsaturated fatty acids and produces saturated fatty acids and some derivatives, such as conjugated linoleic acids (CLA). Moreover, branched chain fatty acids may also be produced by microorganism and be absorbed (Lopez-Bote et al., 1997). Therefore, in ruminants, a marked difference

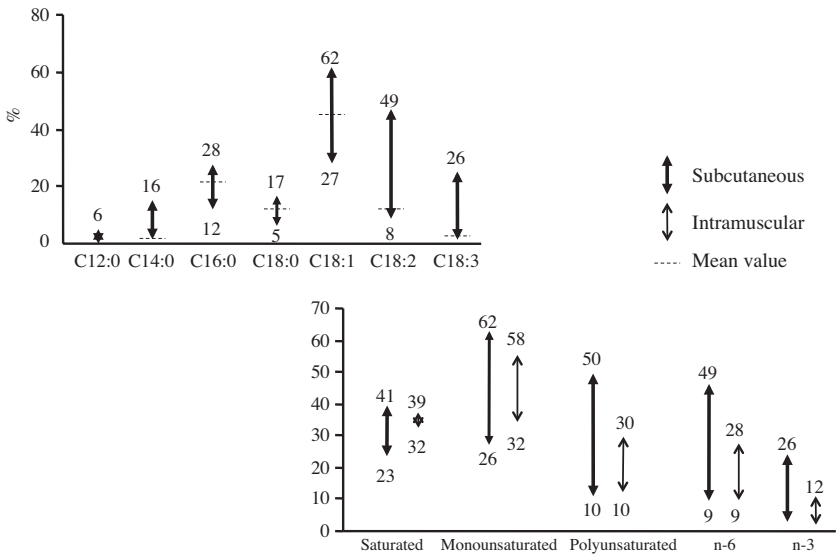

FIG. 4.9 Fatty acid concentration ranges in subcutaneous and intramuscular fat in pigs (Lopez-Bote, original data).

may exist between ingested and absorbed fatty acids. On the other hand, in most monogastric animals, there is a close relationship between ingested and absorbed fatty acids, and thus, a highly different lipid profile from dietary lipids may occur. It is interesting to note that most common feedstuffs (cereals, soybean, sunflower) have a high proportion (near 50%) of linoleic acid (C18:2, $n-6$), thus leading to a high proportion (>15%) of these fatty acids in neutral lipids in many cases.

The relative importance of endogenous synthesis and direct accumulation of fatty acids from the feed greatly depends on the energy balance and the dietary fat content. Under productive circumstances, where meat-producing animals are in positive balance, absorbed fatty acids are preferentially accumulated in adipocytes rather than metabolized to obtain energy. In pigs, it has been reported that when intake of energy from nonfat nutrients is enough to support maintenance and growth (as is the case in most productive circumstances), most ingested fat is accumulated in tissues with little modification (Chwalibog and Thorbek, 1995). If energy from carbohydrates, volatile fatty acids, or proteins surpasses the requirement of the animal, endogenous lipid synthesis also occurs with the production (and accumulation) of saturated and monounsaturated fatty acids.

Fatty acid composition of fat affects the melting point (MP) and rheological properties of adipose tissue. This in turn affects technological properties of meat, such as salt distribution and water migration. In a saturated fatty acid chain, all carbon bonds are in *cis* configuration and orientated alternatively to produce a lineal structure. When there is a double bond, two *cis* bonds are

orientated consecutively in the same way to produce a bend structure, much more difficult to arrange in organized crystalline-like structures (hydrophobic bonds, etc.). Therefore, the energy needed to disorganize (melt) the fat is lower as the number of unsaturation rises. The MP for tristearin (three molecules of C18:0 esterified to glycerol) is around 70°C, while for trilinolein (C18:2), it is below 0°C. The relationship between number of double bonds (x) and MP of 18 carbon fatty acids triglycerides can be estimated by means of the following equation: $MP = -3.8x^3 + 29.5x^2 - 80.7x + 69$.

The relationship between MP and number of double bonds follows a similar pattern for all fatty acid classes. Of course, under natural circumstances, triglycerides are formed by a wide range of fatty acids. Therefore, animal fat has not a single MP, but different triglycerides melt over a wide range of temperature. When the fat is mostly in liquid state at temperatures at which meat is normally commercialized and processed, consistence is low and appearance is oily. It should be noted that solid triglycerides of IMF at a given temperature markedly affect lean firmness, which contribute to differences between spices (ruminant having more saturated fat than pigs or chicken) and individuals.

Among fatty acids present in pig tissues, stearic acid (C18:0) and linoleic acid (C18:2) show the highest correlations (positive and negative, respectively) with fat consistency (Wood et al., 2008). In some extreme cases, when a high proportion of PUFA exists, fat may be melted even at temperatures below 0°C. Fatty acid MP also affects fat color: while solid fat has a white color, melted fat shows a gray-dark appearance, probably because it becomes transparent and therefore allows the observation of capillary tubes and connective tissue (Zhou et al., 1993). Fatty acid composition in meat-producing animals is also a matter of interest because animal fat has some negative health implications for consumers, which is negatively affecting consumer view. Attention is being paid to limit the concentration of saturated fatty acids, to enhance concentration of monounsaturated and $n-3$ (particularly long chain) fatty acids and the ratio $n-6/n-3$ (Lopez-Bote et al., 2002). On the other hand, demonstrated health benefits of CLA, naturally present in ruminant fat, have opened new insight on the beneficial nutritive aspects of meats (Park and Pariza, 2009). Strategies to control fatty acids composition are described below (see Section 4.3) and are mainly based on dietary manipulation, which can be provided either for a short or a long period before slaughter.

Fatty acids are not randomly orientated in animal fats. In beef fat, saturated acids are found preferentially in external Sn-1 and Sn-3 positions of the triglyceride and unsaturated fatty acids in the internal Sn-2. In vegetable oils, a similar distribution occurs, saturated fatty acids being preferentially located in external positions. However, in pig, most palmitic acid is found in the Sn-2 position (Segura et al., 2015). It is generally believed that palmitic acid accounts for most of the cholesterol-raising effect of diets rich in saturated fatty acids. However, evidence that palm oil, which has a 44% palmitic acid concentration, does not raise cholesterol accordingly suggests that this different behavior might be explained by the position of palmitic acid in palm oil triacylglycerol

(approximately 10% of total palmitic acid in the Sn-2 position), thus pork fat being apparently particularly undesirable from the nutritional point of view. A possible explanation is that digestibility of saturated fatty acids is considerably higher when located in the internal position of the glycerol. Certainly more research effort is needed in this topic.

Physical properties of adipose tissue are also affected by positional distribution of fatty acids within the triacylglycerol structure, those located in the external position of the molecule (Sn-1 and Sn-3) having a higher influence on consistency and MP, while adhesiveness is preferentially affected by fatty acids in Sn-2 position (Segura et al., 2015). Moreover, some evidence exists that dietary treatment may alter the proportion of fatty acid in external (Sn-1 and Sn-3) and internal (Sn-2) positions of the triacylglycerol.

Accompanying the triglycerides are small quantities of liposoluble substances, e.g., vitamins A, D, E, and K and cholesterol derivatives.

4.2 Biochemical aspects

Muscle metabolism is a very dynamic adaptive process that may lead to dramatic differences in muscle composition and metabolic profile. Thus, depending on intrinsic and extrinsic factors, marked differences may exist on composition and quality attributes of meat.

In the following lines, some characteristics and aspects of muscle contraction are reviewed, which are determinant of muscle metabolic features (see Section 4.2.1). Then, some particular aspects of carbohydrate, protein, and lipid metabolism in living muscle are reviewed focusing on questions that may affect meat properties. Particular attention is paid to oxidative status. Handling prior to slaughter, stress, nutritive status, dietary constituents, and many other productive circumstances may differentially affect muscle composition and metabolism, altering, for example, enzyme activity or concentration at slaughter, which in some cases may be active for a long period after death. Therefore, these aspects are also discussed in the postmortem period (see Section 4.2.2).

4.2.1 Muscle function in vivo

The thick filaments consist essentially of myosin and the thin filaments of actin. The latter are continuous through the *Z*-line, but do not traverse the H-zone, which bounds each sarcomere; the myosin filaments traverse the A-band only. The three-dimensional (3D) aspect of this arrangement is outlined in Chapter 3 and shown diagrammatically in Fig. 4.10 where there are six straight rows of projections running longitudinally along the side of each myosin filament, the sets of projections being symmetrically distributed around the periphery of the latter, so that one set of projections is opposite one of the six filaments of actin, which surround each myosin filament. The two-dimensional aspect of this arrangement is shown in Fig. 4.10. This shows, in diagrammatic form, a

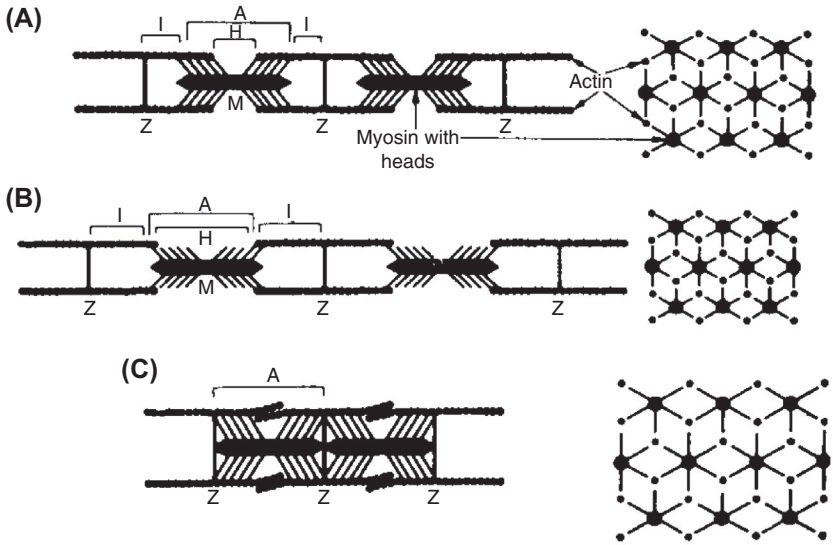

FIG. 4.10 Schematic representation of fine structure of ox muscle in longitudinal and cross sections. *Courtesy the late Dr. J. R. Bendall. Reproduced from Lawrie's Meat Science 7th ed., 2006, with permission from Elsevier.*

longitudinal section of the sarcomeres (1) at rest length, (2) when extended, and (3) during contraction. The thick myosin filaments are depicted with (above and below) two of the six actin filaments with which they are associated. The degrees of interdigitation, and of linkage between the myosin heads and the actin, in each condition will be apparent.

Fig. 4.10 also depicts the patterns seen in cross sections corresponding to these sarcomere lengths. It will be apparent that, as the muscle is extended beyond rest length, it becomes narrower, and the hexagonal array of myosin and actin filaments becomes tighter. Conversely, when the muscle contracts, the cross-sectional area and the distance apart of the myosin and actin rods both increase. In muscle at rest in the living animal (or in the prerigor state in the dying muscle), the beads comprising the actin filaments are prevented from combining with the corresponding projections on the myosin by the magnesium complex of adenosine triphosphate ($MgATP^{2-}$); the latter acts as a plasticizer.

In the myofibril, the contractile proteins are associated with the regulatory complex of troponins (troponins C, T, and I) and tropomyosin. These confer sensitivity to Ca2þ ions on the hydrolysis of $MgATP^{2-}$ by the actomyosin ATPase.

A possible sequence of events in contraction starts by nerve stimulus that produces a reverse polarization of the sarcolemmal surfaces. The sarcolemma temporarily loses its impermeability to potassium and sodium ions, and Ca2þ ions dissociate from the calsequestrin by which they are normally bound in the sarcotubular system and equilibrate with those in the sarcoplasm. As a result, the Ca2þ ions concentration rises from about 0.10 to 10 mM. This saturates

troponin C, the calcium-binding member of the troponin complex, causing a configurational change whereby the inhibitory protein, troponin I, no longer prevents actin from interacting with the $MgATP^{2-}$ on the H-meromyosin heads of the myosin molecule. The contractile ATPase in the vicinity of the linkage is thus strongly activated, splitting $MgATP^{2-}$ to $MgADP^{-}$ at a high rate and providing the energy for the actin filament to be pulled inward toward the center of the sarcomere, i.e., the portion of the myofibril involved con tracts. The link between actin and myosin is simultaneously broken, although tension will remain as there are 5.4×10^{16} cross-links per milliliter of muscle; and some will be bearing tension at any given moment in contracting muscle. The $MgADP^{-}$ on myosin is recharged to $MgATP^{2-}$, either by direct exchange with cytoplasmic ATP, or by the action of ATP:creatine phosphotransferase, or by the action of ATP:AMP phosphotransferase.

The process is repeated so long as an excess of Ca^{2+} ions saturates troponin C and myosin cross-bridges link with the myosin-binding sites on actin at successively peripheral locations as the interdigitation continues.

$MgATP^{2-}$ is bound to the H-meromyosin cross-bridge by a polypeptide chain. Its helices are extended at rest by mutual repulsion generated between the negative charge on $MgATP^{2-}$ at one end and a net negative charge at the other, where the polypeptide joined the H-meromyosin. On stimulation, Ca2þ ions annulled the negative charge on $MgATP^{2-}$ and this eliminated the repulsive effect on the coils of the polypeptide, causing it to assume the α-helical configuration by the energy of formation of about 46 hydrogen bonds- and through the link with actin, pulling the latter inward by an amount equivalent to the distance between successive myosin-binding sites on actin. On the $MgADP^{2-}$ being recharged to $MgATP^{2-}$, the polypeptide is reextended, but now to a position opposite to the next distal myosin-binding site on actin.

When the stimulus to contract ceases, the concentration of Ca2þ ions in the sarcoplasm is restored to rest level (approx. 0.10 mM), being reabsorbed into the sarcotubular system by the sarcoplasmic reticulum pump, which depends on ATP for the necessary energy. ATP is also needed to restore the differential distribution of sodium between the two surfaces of the sarcolemma, which provides the action potential on nerve stimulation. This probably requires only about one-thousandth, and the calcium pump one-tenth, of the energy required in contraction per se (Bendall, 1973).

Being no longer saturated with Ca2þ ions, troponin C and troponin I return to their resting configurations whereby the latter prevents interaction of myosin and actin. It is feasible that the gap filaments, which have some elasticity, assist in pulling the actin filaments outward from their interdigitation with those of myosin so that the sarcomeres' resting length is reestablished. Apart from the major involvement of an elevated sarcoplasmic concentration of Ca2þ ions in the interaction of actin and myosin during muscular contraction, it also activates myosin light chain kinase (which phosphorylates one of the light chain components of myosin) and phosphorylase b kinase (which phosphorylates troponins T and I). Purified troponin requires the addition of tropomyosin for

its inhibitory action on actomyosin ATPase. It has been demonstrated that muscular contraction changes the angles of the lattice of the perimysial connective tissue and the crimp length of the collagen fibers, therefore playing also a role in contraction.

Most other aspects of muscle contraction concern the mechanism of ensuring an adequate supply of energy. The most immediate source of new ATP is resynthesis from ADP and creatine phosphate (CP), by the enzyme creatine kinase, which is one of the soluble proteins of the sarcoplasm: $ADP + CP \leftrightarrow creatine + ATP$.

The level of CP quickly falls along sustained muscle contraction and under anaerobic conditions. Initial ATP level is maintained while CP is depleting, but has to be resynthesized from ADP thereafter. As previously stated, skeletal muscle stores glycogen as source of energy, and therefore, muscle glycogen plays a critical role in maintaining homeostasis, as it has to be oxidized to provide energy. However, other metabolites, such as acetate, nonesterified fatty acids, and ketone bodies are also oxidized. It is estimated that at rest the contribution of glucose to oxidations accounts to nearly 50%, but the proportion decreases to 25%–30% in exercised conditions (Hocquette et al., 1998). However, the absolute amount of glucose oxidized increases with exercise. When energy is needed above the capacity of the respiratory system to generate ATP, the process of anaerobic glycolysis starts, thus producing energy through the glycolytic pathway, which implies the production of lactic acid. This is a fast but less efficient way of obtaining energy and produces a concomitant decline in intramuscular pH due to lactic acid accumulation.

Myoglobin acts as a short-term oxygen store in muscle, therefore muscles with a higher concentration of this pigment are able to maintain aerobic metabolism for a longer period.

Another critical aspect that determined metabolic features of muscle is carbohydrate availability, which is strictly regulated in large animals, as central nervous system requires a continuous supply of glucose to fulfill energy requirements. That is the main priority in carbohydrate metabolism regulation.

Glucose requires protein carriers for active transport across lipophilic cell membranes (GLUT). Several transporters and isoforms have been described. GLUT1 (present in central nervous system) is not regulated by insulin and has a constant basal activity. Muscle GLUT1 activity incorporates glucose into cell in a small and continuous regular manner. To supply glucose requirement to the central nervous system, a strict range (approximately 2.5–5 mmol in ruminant and 4.7–8.6 mmol in monogastric animals) of plasma glucose concentration is required.

This complicated task is carried out by the liver, which actively incorporates glucose from the portal blood in the postprandial state and stores it as liver glycogen, which is steadily mobilized when glucose peripheral concentration is low. Glucose can also be produced in the liver by endogenous synthesis from proteins or volatile fatty acids (mainly propionic acid), the latter being particularly active in ruminants. Liver glucose uptake from portal blood largely

depends on GLUT2 transport, which is not regulated either by insulin. When a high amount of glucose from digestion is coming in a short period, glucose uptake capacity of liver GLUT2 may be surpassed, and an outflow of glucose may reach peripheral circulation, thus producing a peak in peripheral blood concentration over the upper limit of the optimal range. This happens frequently in monogastric animals. Excess of plasma glucose concentration triggers insulin production and GLUT4 activation. GLUT4 is present in muscle and adipose tissue and is located intracellularly while inactive, but responds to insulin stimulus and translocates to cell membrane, thus markedly increasing cellular glucose uptake. This allows both recovering plasma glucose level in the adequate range and increasing energy storage as glycogen is muscle cells (or as endogenously produced fat in adipocytes) for further requirements.

Glycogen synthase increases and glycogen phosphorylase diminishes muscle glycogen concentration, with a complex allosteric regulation of these processes. While the phosphorylated form of glycogen phosphorylase is active in degrading glycogen, the phosphorylated form of glycogen synthase is inactive, and the contrary occurs with nonphosphorylated forms. Therefore, reciprocal control takes place, which is ultimately regulated by phosphorylation. As phosphorylase hydrolyzed only lineal glucose chains, glycogen breakdown requires also the activity of glycogen debranching enzyme to remove branches, thus allowing phosphorylase to continue its activity (Pösö and Puolanne, 2005). Debranching enzyme activity is affected by temperature (Kyla-Puhju et al., 2005; Fig. 4.11). This is an important issue in slaughter technology.

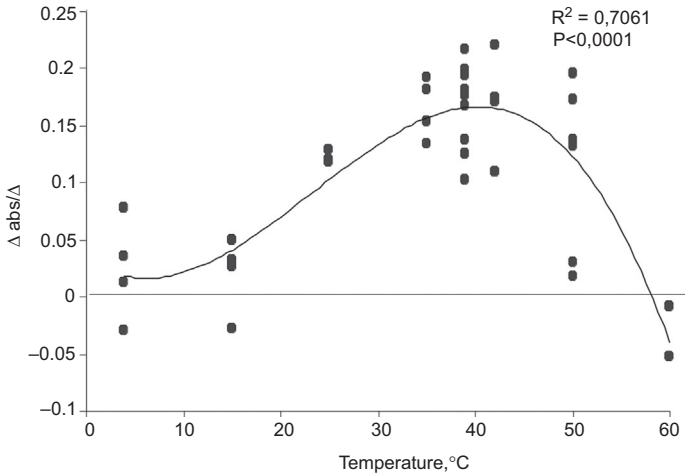

FIG. 4.11 Effect of temperature on activity of glycogen debranching enzyme from porcine *Longissimus dorsi*. *Reproduced from Kyla-Puhju, M., Ruusunen, M., Puolanne, E., 2005. Activity of porcine muscle glycogen debranching enzyme in relation to pH and temperature. Meat Sci. 69, 143-149 with permission from Elsevier.*

Finally, glucose 1 phosphate released by glycogenolysis requires enzyme phosphoglucomutase to enter glycolysis. The glycogen synthase and phosphorylase enzymes are differentially expressed between fiber types (see Section 4.3.1) and may be affected by extrinsic factors, such as exercise.

The oxygen-utilizing enzymes of respiration, in particular the cytochrome system, and those required to convert pyruvic acid to carbon dioxide and water and to form ATP, are located in mitochondria, which are distributed in the sarcoplasm. Most of the glycolytic and respiratory enzymes require cofactors, which are either vitamins or trace metals.

Meat-producing animals along growth are most of the time in an anabolic state, which implies that in general terms anabolism predominates over catabolism, but this does not mean that catabolism is residual. As indicated for carbohydrates, proteins are also continuously degrading by a complex combination of endogenous proteases. It can be quantitatively estimated that in growing pigs more than 70% of synthesized protein is hydrolyzed to produce energy or rebuild new proteins. Four proteolytic systems can be identified in muscle tissue: calpains, cathepsin, proteasome, and caspases (see Chapters 5 and 12). While sarcoplasmic proteins catabolism is similar to most cells, highly specialized myofibrillar proteins require a specific protease system.

This complex process of breakdown is poorly understood, but there are some plausible hypotheses for it (Koohmaraie et al., 2002). The first step is a disassembly of the myofibrils into myofilaments. Only the calpains are capable of making the specific cleavages in the myofilaments. Then the lysosomal enzymes, peptidases linked to membranes, and/or the proteasome complex would go further degrading the proteins into amino acids (Goll et al., 2003).

The calpain system is formed of several isoforms of the calcium-dependent cysteine proteinases (calpains) and their specific inhibitor calpastatin (Goll et al., 2003). In vivo calpain activity is considered a modulator protease that governs different functions such as signal transductions and cell morphogenesis. The rules of its activity are not fully elucidated. It seems that calpain can recognize the 3D structure of its substrates (protein kinases, phosphatases, phospholipases, cytoskeletal proteins, membrane proteins, cytokines, and calmodulines have been suggested to be in vivo substrates). There are three forms of calpains in muscle, μ-calpain, m-calpain, and P68. All of them have optimal activity near neutral pH (Lonergan et al., 2010). μ- and m-calpain degrade desmin, synemin, talin, vinculin as well as titin and nebulin, key cytoskeleton proteins without significantly affecting amounts of actin or myosin. These enzymes are mainly located in the *Z*-line. They differ in the calcium requirements for half-maximal activity, being it from 5 to 65 mM Ca^{2+} for μ-calpain and between 300 and 1000 mM Ca^{2+} for m-calpain (Goll et al., 2003). The maximum calcium concentration expected in meat is between 210 and 230 mM, not enough for the m-calpain, thus making the m-calpain the most active in the postmortem period. Activity abruptly decreases as postmortem conditions involve a low pH and increased ionic strength.

Calpastatin also requires calcium to bind the calpain, but the concentration required to reach its half-maximal activity is lower than that of the non-autolyzed calpains. Calpastatin has the ability to bind several molecules of calpain, but this binding is reversible as lower Ca2+ concentrations would release the calpain (Otsuka and Goll, 1987).

A number of cathepsins with endopeptidase activity have been described in muscle tissue (B, D, E, F, H, K, L, and S), which are located in lysosomes and are cysteine, aspartic, or serine proteases, mainly active at low or slightly low pH (3.0–6.5). Their activity is controlled by the ratio of their precursor zymogen cathepsin (procathepsins) and inhibitors as cystatin (Toldrá and Reig, 2015). Cathepsins are thought to fully degrade protein in the lysosomes, but they might also contribute to apoptosis by the activation of caspases and tissue regeneration after injury. Cathepsins activity can be limited at initial steps postmortem since the lysosomes membrane maintains its barrier properties, although it plays an important role in meat products, even after very long period of processing (Toldrá et al., 1993).

The system of caspases is a family of cysteine aspartate specific proteases responsible for apoptosis (the cell's programmed death). The current hypothesis states that after slaughter, the cell conditions (hypoxia and ischemia) promote them to engage into the programmed cell death (apoptosis). The caspase family includes some members involved in inflammatory response and others in apoptosis. Caspases are synthesized in the cytosol as inactivated zymogens.

The proteasome system, also known as the multicatalytic protease complex, is a threonine protease. It has an important role in the degradation in living muscle, aiding finalizing the breakdown of myofibrillar renewal. In short, after ubiquitin (a small protein) is activated with the usage of ATP, it binds to a lysine residue of an NH_2 terminal amino group of the targeted protein. This ends up generating a polyubiquitin chain, which is bound by the proteasome allowing the protein degradation and releasing the ubiquitin chain.

Lipid accretion in meat-producing animals, either by direct deposition or synthesis, usually increases in late fattening, while mobilization is scarce. Only in some particular cases lipid mobilization takes place through lipolysis, thus releasing free fatty acids from triglycerides and phospholipids. This process may be triggered by feed deprivation, as it is the case in many cases during preslaughter handing (Nielsen et al., 2011). Also exercise may enhance muscle esterase and lipase activity (Daza et al., 2009). Muscle lipase activity at slaughter affects postmortem lipolysis and has important implications in meat and meat products' technological properties and quality attributes (Gandemer, 2002).

On the other hand, muscles are a complex matrix with a delicate balance between antioxidant and prooxidant factors that can be easily altered in vivo. The continuous production of reactive oxygen species (ROS) is matched in living organism by a number of enzymatic and nonenzymatic antioxidants, which in most cases allow it can recover a healthy redox balance, but in some cases severe oxidative stress may cause permanent DNA, protein, or lipid deterioration

and loss of functionality. Diet may provide a number of exogenous antioxidants including soluble and liposoluble compounds, thus ultimately oxidative deterioration both in vivo and postmortem is closely related to nutrition.

4.2.2 Muscle function postmortem

Since postmortem muscle function reflects the basic physiology of muscle, it is appropriate to briefly consider first of all the irreversible anaerobic glycolysis that takes place when oxygen is permanently removed from the muscle at death, although the more general consequences of circulatory failure are outlined in Chapter 5. The sequence of chemical steps by which glycogen is converted to lactic acid is essentially the same postmortem as in vivo when the oxygen supply may become temporarily inadequate for the provision of energy in the muscle; but it proceeds further. Except when inanition or exercise immediately preslaughter has appreciably diminished the reserves of glycogen in muscle, the conversion of glycogen to lactic acid will continue until a pH is reached when the enzymes affecting the breakdown become inactivated. In typical mammalian muscles, this pH is about 5.4–5.5 (Bate-Smith, 1948). Muscle glycogen concentration below approximately 0.8% markedly affects postmortem pHu, but little effect is found with concentrations over this value (Warriss, 1990; Fig. 4.12). A high meat pHu seriously compromises microbial deterioration along storage and quality attributes, producing meat that is dark, firm, and dry. There is a quantitative relationship between pHu and these characteristics, thus exists a wide range of intermediate values in color and exudation. A pH above 6 is generally considered undesirable. Therefore, strategies should be implemented to guarantee this minimum glycogen concentration at slaughter. There is some evidence that residual glycogen in meat increases the water-holding capacity and tenderness of the muscles when cooked.

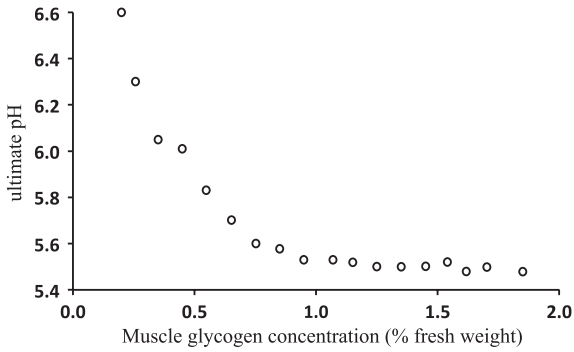

FIG. 4.12 Relationship between ultimate pH and slaughter concentration of glycogen in *Longissimus lumborum* muscle in beef. *Reproduced from Warriss, 1990, Appl. Animal Behav. Sci. 28, 171–186 with permission from Elsevier.*

Lactic acid production is virtually the only event causing the pH fall during postmortem glycolysis. The isoelectric point of many muscle myofibrillar proteins is 5.5. This is reflected in the low electrical impedance of the muscle at normal pHu, and its high electrical resistance at high pHu, and this has been related to the mechanical resistance of the muscle fibers.

As postmortem glycolysis proceeds, the muscle becomes inextensible: this is the stiffening long referred to as rigor mortis. The onset of rigor mortis is correlated with the disappearance of ATP from the muscle: in the absence of ATP, actin and myosin combine to form rigid chains of actomyosin (Bendall, 1973). The loss of extensibility that reflects actomyosin formation proceeds slowly at first (the delay period), then with great rapidity (the fast phase): extensibility then remains constant at a low level. The time to the onset of the fast phase of rigor mortis (at a given temperature) depends most directly on the level of ATP, which, in the immediate postmortem period, is being slowly lowered by the surviving noncontractile ATPase activity of myosin (Bendall, 1973). Under local control, the latter operates in an attempt to maintain the structural integrity of the muscle cell.

The level of ATP can be maintained for some time by a resynthesis from ADP and CP. When the store of CP is used up, postmortem glycolysis can resynthesize ATP, but only ineffectively and the overall level falls. Stress at death will lower the initial pH and shorten the time until the fast phase, as will depletion of glycogen by other means (starvation, insulin tetany).

Rigor begins in normal meat when pH is near 6.0 (Hannula and Puolanne, 2004), and it is manifested by a decrease in extensibility (Bendall, 1973; Honikel et al., 1983). Shortening of muscles starts before the onset of rigor mortis since contraction requires a minimum concentration of ATP and an increased level of Ca2þ ions around the myofibrils (Honikel et al., 1983).

There are marked differences between spices and individuals in the rate of pH decline (Fig. 4.13), and a number of intrinsic and extrinsic factors have been identified. Among them, it is important to emphasize the importance of fiber type, as red oxidative fiber contains more myoglobin and maintains aerobic conditions for a prolonged period, thus showing a steady and slow pH decline in comparison to glycolytic fibers. Accordingly, in pig, carcass pH 6 in *Longissimus dorsi* muscle is usually reached at 2–6 h after slaughter, while in lamb and beef carcasses, this period usually extends to more than 8–10 h.

Postmortem metabolism and pH decline in the carcass are not uniform either, with marked differences between muscles and locations (Fig. 4.14).

In pigs, postmortem metabolic processes may develop very actively in some cases, with an abnormally high temperature and a quick pH decline, reaching a value below 6 within the first 45 min (pH_{45}) after slaughter, thus leading to a massive denaturation of proteins, which produces meat that is pale, soft, and exudative (PSE). Elevated levels of Ca2þ ions are found in the muscles of such pigs postmortem. These would activate the ATPase of actomyosin and hence accelerate the rate of postmortem glycolysis. PSE meat in pigs has been a matter of high interest in meat industry, and much attention has been devoted to understand and control this problem, as prevalence may be in the range 10%–30% and in some

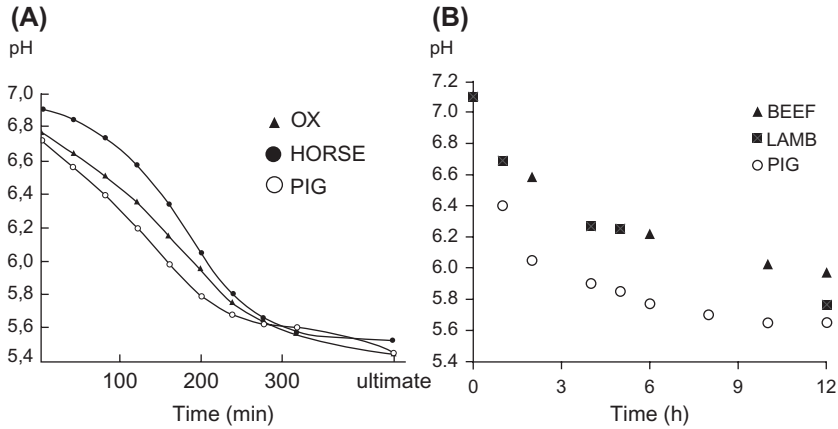

FIG. 4.13 The effect of species on the rate of postmortem pH fall. (A) Muscle *longissimus dorsi* at 37°C (zero is the time 1 h post mortem). (B) Muscle *longissimus* dorsi in carcasses in commercial slaughter houses (original data). *Panel (A) reproduced from Lawrie's Meat Science 7th ed. (2006), with permission from Elsevier.*

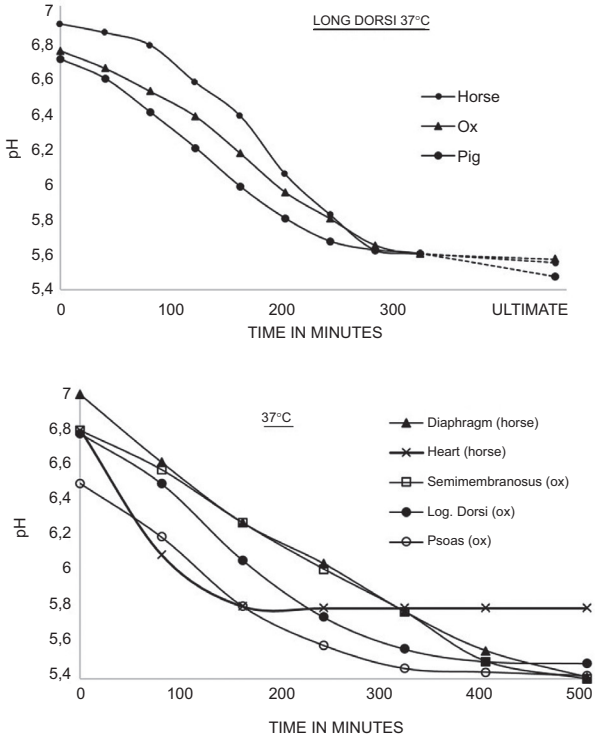

FIG. 4.14 The effect type of muscle on the rate of postmortem pH fall at 37°C (zero is the time 1 h post mortem). *Reproduced from Lawrie's Meat Science 7th ed., 2006, with permission from Elsevier.*

cases may reach values up to 60% (Lee and Choi, 1999). Pigs with a mutation at nucleotide 1843 in the homozygous recessive (nn) ryanodine receptor 1 gene develop a stress syndrome (PSS) characterized by labored breathing, muscle rigidity, acidity, and in some cases death. This is produced when exposed to halothane and under stress situations, as usually occurs prior to slaughter. Therefore, halothane-sensitive pigs are particularly prone to produce PSE meat.

The pale and exudative meat is not limited to halothane-sensitive pigs. Meat with similar characteristics has been described also in halothane-free pigs, broiler, turkey (Malila et al., 2013), rabbit (Kowalska et al., 2011), and cattle (Hunt and Hedrick, 1977).

Moreover, the incidence cannot be established on qualitative ($PH_{45}<6$), but on qualitative terms, thus exists a wide range of intermediate value.

Surveys in the United States showed that more than 40% of carcasses present red, soft, and exudative pork (Kauffmann et al., 1993), which is characterized by lower solubility of phosphorylase sarcoplasmic protein than normal red, firm, and nonexudative pork (Joo et al., 1999).

Denaturation of myofibrillar and sarcoplasmic proteins is produced when high temperature and low pH occur simultaneously and increases exponentially as muscle pH drops below 6. Rigor development in pork muscles at elevated postmortem temperatures of 37°C always resulted in PSE meat characteristics. Therefore, muscle temperature at pH<6 rather than time after slaughter (pH_{45}) is of importance in controlling protein denaturation. Warner et al. (2014) proposed that to minimize protein denaturation, pH 6 should be reached when muscle temperature is already below 30–35°C. Fig. 4.15 shows the percentage of beef carcasses with meat color score for a range in muscle temperature when pH 6 is reached.

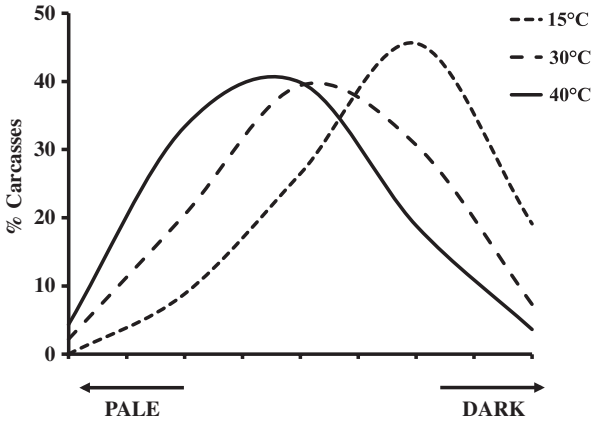

FIG. 4.15 Rate of temperature and pH decline for pork *longissimus thoracis et lumborum* muscle with temperature chilling at 2°C or 14°C, with or without electrical stimulation (200 mA, 14 Hz, 60 s). *Adapted from Rees, M.P., Trout, G.R., Warner, R.D., 2003. The influence of the rate of pH decline on the rate of ageing for pork. II. Interaction with chilling temperature. Meat Sci. 65, 805-818 with permission from Elsevier.*

The importance of the relationship between postmortem muscle pH and temperature goes beyond protein denaturation, as muscle temperature largely affects metabolic processes. Marsh (1954) observed that the rate of postmortem glycolysis increases with increasing muscle temperature (Fig. 4.16), and Kyla-Puhju et al. (2005) observed a marked decrease in the activity of muscle glycogen debranching enzyme at temperatures below 30°C (Fig. 4.11). It should be noted that circulatory system cannot remove heat after slaughter, but active postmortem metabolism still occurs for some hours, therefore the ability to dissipate heat largely depends on external temperature and evaporation. During the initial steps at the slaughterhouse (exsanguination, scalding, skinning, evisceration), limited chilling can be applied. It will be obvious that, in the carcasses of meat animals, various muscles will have different rates of fall of temperature postmortem, according to their proximity to the exterior and their insulation. As a result, the rates of postmortem glycolysis and pH drop will tend to be higher in muscles, which are slow to cool. The rate of temperature decline is therefore

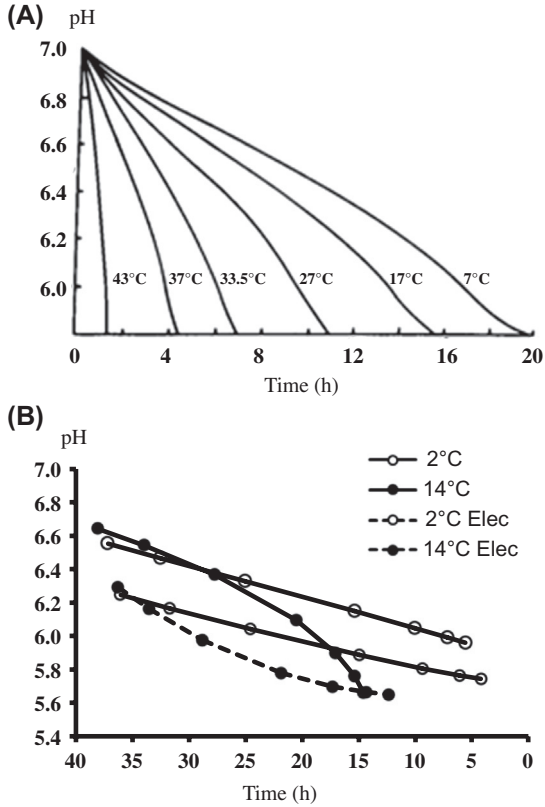

FIG. 4.16 Predicted percent of beef carcasses with meat color score in the *longissimus thoracis* for a range in temperature at pH 6. *Adapted from Hughes, J.M., Kearney, G., Warner, R.D., 2014. Improving beef meat colour scores at carcass grading. Anim. Prod. Sci. 54, 422-429.*

a critical aspect, particularly in carcasses isolated by a fat cover in thick muscle areas, such as the hindquarter of pigs, where muscle temperature 20 min post-mortem may easily be over 40–41°C.

Chilling temperature after slaughter may be effectively used to reduce metabolic processes and rate of pH decline (Fig. 4.17). However, low temperature prior to rigor mortis has to be applied cautiously, as an excessive chilling rate may lead to sarcomere shortening and a concomitant loss of quality.

Contractile system is stimulated by Ca2þ, but sarcoplasmic reticulum may actively pump these ions out of the sarcoplasm while there is still ATP availability, thus temporarily relaxing actomyosin bridges until new Ca2þ ions enter into the cell. This iterative event explains the limited (20%–25%) shortening, which is observed during "normal" rigor. However, in some cases, unusual shorter sarcomere length and higher muscle fiber diameter have been observed, which produce tough and exudative meat (Honikel et al., 1983).

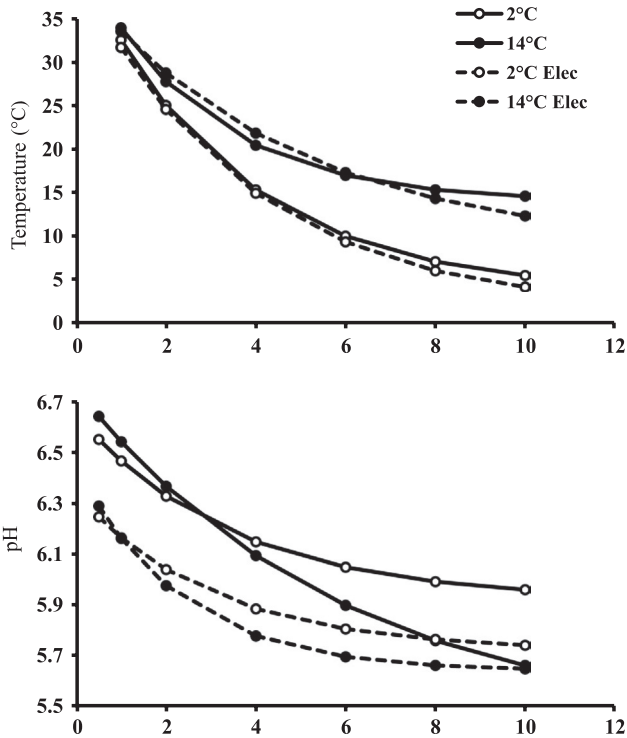

FIG. 4.17 The effect of environmental temperature on the rate of postmortem pH fall in: (A) beef *longissimus dorsi* muscle chilled at 7–43°C (Marsh, 1954), (B) muscle *longissimus dorsi* in pig carcasses chilled at 2°C or 14°C, with or without electrical stimulation (200 mA, 14 Hz, 60s). *Adapted from Rees, M.P., Trout, G.R., Warner, R.D., 2003. The influence of the rate of pH decline on the rate of ageing for pork. II. Interaction with chilling temperature. Meat Sci. 65, 805-818 with permission from Elsevier.*

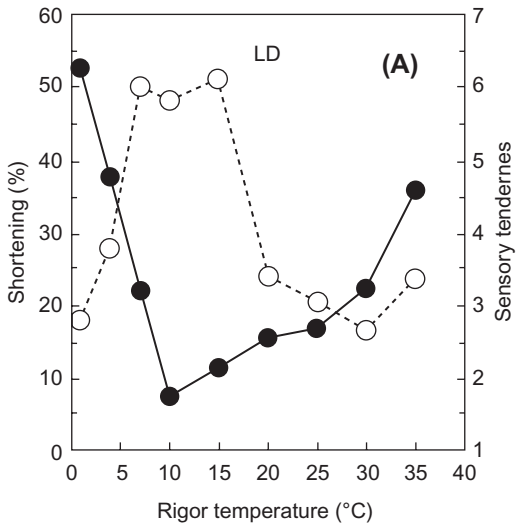

FIG. 4.18 Shortening (o) and sensory tenderness (●) (14 days) as function of constant rigor temperatures. *Reproduced from Tornberg, 1996, Meat Sci. 43, S175–S191 with permission from Elsevier.*

Shortening is minimum at 15–20°C (Fig. 4.18), and cold shortening occurs when muscles are exposed to low temperatures (below 10–15°C) early postmortem, as the ability of the sarcoplasmic reticulum pump to recapture Ca2þ is greatly diminished by the low temperature. Moreover, if muscle is frozen while the ATP level is at the prerigor value, an exceedingly fast rate of ATP breakdown and of rigor onset ensues on thawing (thaw rigor). The muscle may contract to 50% of its initial length and exude much drip. It has been suggested that the contractile actomyosin ATPase, which is not normally responsible for ATP depletion postmortem, is activated on freezing and thawing (Bendall, 1973). Muscle types vary in their potential to cold shorten, with red being more susceptible than white (Bendall, 1973).

Therefore, according to carcass characteristics (muscle type, anatomical location, and fat cover), an accurate control of temperature may allow an adequate pH drop, which markedly affects quality attributes and technological properties of meat.

Electrical stimulation accelerates biochemical processes and increases the rate of pH decline, thus reducing the overall time of rigor mortis and preventing cold shortening (Olsson et al., 1994; Fig. 4.15). Carcass suspension may also help to reduce sarcomere shortening (Liu et al., 2016).

All these evidences indicate that there is a critical period in the postmortem state when pH is near 6. If temperature is too low, the resulting meat might result tough, while an excessive temperature may lead to massive protein denaturation and dramatic loss of quality. The term "pH-temperature window"

strategy for beef carcasses has been proposed by an Australian scientist, which states that muscle reaches pH 6 between 12°C and 30°C (Webster et al., 1999).

Soon after slaughter, the breakdown of the cytoskeletal proteins by calpains, cathepsins, caspases, and multicatalytic proteinase complex thus initiates the process of rigor resolution. Changes begin in muscle almost immediately following slaughter and continue at varying rates until tissue is completely degraded. The extent and speed of these reactions depend on several factors, including temperature, level, and activity of proteolytic enzymes, oxidative status, and pH.

It has been traditionally believed that connective tissue is stable during postmortem aging compared with myofibrils (Nishimura et al., 2009) as there is no increase in water-soluble hydroxyproline. However, it has been demonstrated by studies that breaking strength decreases postmortem. The mechanism is unknown though some enzymes (lysosomal enzymes and metalloproteinases) have shown in vitro activity (Bailey and Light, 1989). The type and quantity of associated proteoglycans are important in determining the level of susceptibility of collagen to enzymatic digestion. Proteoglycans in the basement membrane and the perimysium are degraded during aging.

On the other hand, after slaughter, cellular mechanism for controlling lipid oxidation no longer works and lipid peroxidation may take place. Primary products of lipid autoxidation are hydroperoxides, which can further decompose to form a large variety of volatiles including alkanes, aldehydes, ketones, alcohols, esters, and carboxylic acids. Both, the nature and the relative proportion of the volatile compounds in the muscle food, depend on several factors, among which the fatty acid profile of the raw material is the main one. Since desmin may serve to connect adjacent myofibrils to the sarcolemma, degradation of this protein may compromise structure of the muscle fiber. Calpain, which can degrade desmin, is a susceptible subtract to oxidation, leading to a loss in its proteolytic activity, which is a key issue in initial steps of proteolysis after slaughter (Melody et al., 2004). Therefore, muscle pH, temperature, and oxidative status interact continuously during initial steps after slaughter.

Both the rate and the extent of the postmortem metabolic changes are influenced by intrinsic factors such as species, muscle cell type, the type of muscle, anatomical location, carcass characteristics (size, fatness, thickness), and variability between animals and by extrinsic factors such as animal feeding, preslaughter handling, preslaughter administration of drugs, salts, or active compounds and by the environmental temperature. A holistic view is needed in each particular case to optimize animal production system and slaughter technology to maximize meat quality (see Section 4.3).

It is necessary to acknowledge also that the situation is dynamic, and the biochemical profile within the muscle may change drastically if any of these factors is modified, needing a readjustment of procedures taking place at slaughter (cold load, speed, etc.). These adjustments are specially required if meat is aimed to be processed for quality products. A good example of this is probably the problem of destructured meats, which is becoming an important issue in recent years

in cured ham production. The problem is characterized by pale and soft zones that can be observed inside the ham (particularly at muscles semimembranosus and Biceps femoris) in the green state and lead to the production of hams with pale areas, which are unsuitable for mechanical slicing. Glycolytic potential and thermosoluble collagen are higher in these areas (Minvielle et al., 2001). It is observed in 5%–20% fresh hams (50% in some cases), and it is positively related to a low pH and high internal muscle temperature (particularly at initial steps postmortem), a high weight gain at fattening, and a high slaughter weight and lean carcass. The effect is particularly important in cooked cured hams, in which 7%–8% of slices are affected and resulted in significant economic losses (Hugenschmidt et al., 2010). Clinquat et al. (2021) observed a quantitative response in the proportion of slices with defects (cohesion, ointment, holes) and exudative losses according to initial visual classification of fresh muscles.

4.3 Factors affecting muscle function with possible consequences on meat quality

4.3.1 Fiber type, muscle, and anatomical location

The skeletal muscle contains a heterogeneous population of cells, with marked morphological and metabolic characteristics. Schiaffino and Reggiani (1996) differentiated four muscle fiber types: type I or slow-oxidative, type IIA or fast oxido-glycolytic, and fast glycolytic IIB and IIX fibers (see Chapter 3). Type I fibers are usually smaller in diameter and have higher concentration of heme pigments and mitochondria, as well as higher vascularization than type IIB fibers. This enables them to depend on aerobic metabolism for energy production and to perform sustained exercise without lactic acid accumulation. So-called red muscles tend to have a greater proportion of narrow, myoglobin-rich fibers; so-called "white" muscles have a greater proportion of broad, myoglobin-poor fibers.

Muscle fibers are originated from myoblast, which fuse to myotubes. Along development, myotube formation takes place in at least two phases, giving rise to primary (embryonic) and secondary (fetal) muscle fibers. It is generally believed that maturation of primary fibers produces a high proportion of type I fibers, while secondary give rise to IIB fast glycolytic fibers. Apparently, the number of primary myofibers is under genetic control, while those coming from ulterior phases may be regulated by epigenetic factors. Maternal dietary energy intake affects differentiation and maturation in skeletal muscle fiber of the fetus (Zhao et al., 2016). Undernutrition of pregnant sows or low-birth piglet coming from hyperprolific sows is associated with lower number of secondary fibers. Moreover, a third generation of fibers have also been described at mid- and late-gestation period in large animals, such as bovines, sheep, pigs, or humans (Picard et al., 2002).

Dark, aerobic muscles contain mainly oxidative type I and oxidative/glycolytic type IIA fibers with high myoglobin content, while light muscles contain glycolytic type IIB fibers with low myoglobin content (Morita et al., 1970).

Muscle fiber proportion varies also by factors such as species and breed, gender, diet, and muscle location (Zhao et al., 2016). Muscles of domestic pigs generally contain more type IIB fibers and less type IIA fibers than those of wild pigs (Essen-Gustavsson and Lindholm, 1984). Moreover, as meat animal growth and efficiency have been major goals in intensive selection in meat-producing animals, a concomitant effect on muscle fiber composition has been produced along generations, thus promoting a higher proportion of larger type IIB fibers (Ruusunen and Puolanne, 2004). In domestic pigs, the cross-sectional area of type IIB fibers is markedly larger than the cross-sectional area of type I and IIA fibers. But in wild pigs, the cross-sectional area of all fiber types is about the same (Ruusunen and Puolanne, 2004).

On the other hand, muscle fiber proportion determines not only contractile properties but also metabolic features. The concentration of the dipeptides, carnosine, and anserine, which act as buffers in muscle, appears to bear an inverse proportionality to respiratory activity.

In assessing the significance for meat quality of differences in the constitution of anatomically defined muscles, it should be appreciated that, in the past, both wholesale and retail cuts of meat-producing animal were relatively large and thus represented aggregates of a number of specific muscles. With the increasing tendency for the centralized preparation and prepackaging of portions of meat for the individual consumer, these may be derived from a single muscle or part thereof. With pork, the individual joints are frequently processed (e.g., cured) after separation from the carcass. Their specific composition may thus be of great interest.

Collagen content of the beef forequarter was significantly greater than that of the hindquarter, the shin having the highest value. In pork, collagen was significantly highest in the hand (i.e., the lower forequarter) (Table 4.3). Bailey and Light (1989) demonstrated that muscles differ not only in their total content of connective tissue, but also in respect of the types of collagen molecule present, in the ratio of heat-stable (oxo-imino) to heat-labile (aldimine) cross-links in these collagens, and in their histological distribution.

TABLE 4.3 Total collagen and its histological distribution in bovine muscles.

Muscle	Total collagen (% dry weight)	Percentage in epimysium	Percentage in perimysium	Percentage in endomysium
Psoas major	2.24	15	79	24
L. dorsi	2.76	13	80	34
Pectoralis profundus	4.96	22	98	42
Semitendinosus	4.75	29	54	41

Reproduced from Bailey, A.J., Light N.D., 1989. Connective Tissue in Meat and Meat Products. Elsevier Science Publishers Ltd., London, 355 pp., with permission from Elsevier.

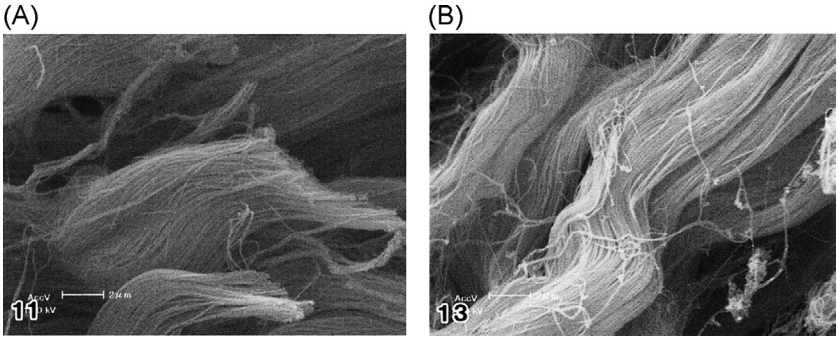

FIG. 4.19 High-magnification scanning electron micrographs of the perymisium from (A) porcine *longissimus lumborum* and (B) porcine *pectoralis profundus* muscle. Bar = 2 μm. *Reproduced from Nishimura and Tabaka, 2003, Meat Sci. 64, 43–40 with permission from Elsevier.*

In a study of porcine muscles by scanning electron microscopy, Nakamura et al. (2003) showed that the higher collagen content of *pectoralis profundus* in comparison with *Longissimus lumborum* could be related to the more complex structure of the perimysium of the former, which involved longitudinal, circular, and oblique collagen fibers crossing over one another in several bands; whereas the perimysium of *I. lumborum* was markedly simpler in its architecture (Fig. 4.19).

Certain differences between muscles in fat unsaturation have been attributed to corresponding differences in local temperature, a higher temperature being associated with a higher fat saturation, and vice versa.

It is particularly interesting to note that, as judged by the hydroxyproline content, the connective tissue concentration is greater in most pig muscles than in corresponding beef muscles. Since pork is generally tender, this again emphasizes that the quality of connective tissue must be considered as a factor additional to its quantity.

Studies of the fatty acid compositions of neutral lipids and phospholipids of different muscles have revealed a number of distinctions between them.

The content of $n-3$ fatty acids is greater in red muscles than in white (Wood et al., 2008). Differences in the fatty acid pattern between anatomical locations are more marked when muscles are fractionated into such functional components as mitochondria, and the phospholipids are considered separately from the total and neutral lipids.

There are also qualitative differences in proteins between muscles within a given species and at a given age. Thus the proteins of beef *Longissimus dorsi* and psoas muscles differ in their susceptibility to freezing damage. The susceptibility of myoglobin to oxidize during chill or frozen storage differs between different muscles (Ledward, 1971).

In addition to purely chemical differences, muscles also vary in their enzymic constitution (Table 4.4). The bovine *masseter* muscle has a particularly high capacity for respiratory metabolism and a very low titer of glycolytic enzymes

TABLE 4.4 Malate (MDH) and lactate (LDH) dehydrogenase activity mean values (expressed as U/g) and MDH/LDH ratios in oxidative muscle masseter, intermediate muscle trapezius and glycolytic muscles biceps femoris and *Longissimus dorsi* of the assayed animal species.

Animal species	Muscle type	LDH (U/g)	MDH(U/g)	MDH/LDH ratio
Pork	Masseter	$150^{a} \pm 20$	$390^{a} \pm 50$	2.6
	Trapezius	$590^{b} \pm 75$	$240^{b} \pm 20$	0.4
	L. dorsi	$970^{c} \pm 85$	$100^{c} \pm 10$	0.1
	B. femoris	$720^{b} \pm 90$	$120^{c} \pm 15$	0.2
Lamb	Masseter	$130^{a} \pm 10$	$380^{a} \pm 45$	2.9
	L. dorsi	$630^{b} \pm 75$	$290^{b} \pm 30$	0.5
	B. femoris	$300^{c} \pm 25$	$220^{b} \pm 35$	0.7
Rabbit	Masseter	$140^{a} \pm 15$	$510^{a} \pm 60$	3.6
	L. dorsi	$1010^{b} \pm 90$	$150^{b} \pm 20$	0.1
	B. femoris	$760^{c} \pm 95$	$80^{c} \pm 10$	0.1
Beef	Masseter	$70^{a} \pm 5$	$310^{a} \pm 35$	4.4
	L. dorsi	$890^{b} \pm 95$	$230^{b} \pm 25$	0.3
	B. femoris	$840^{b} \pm 85$	$200^{b} \pm 25$	0.2

[a,b,c]*Different letters within the same column for a given animal species indicate statistical significant difference ($P < .05$).*
Reproduced from Reig, M., Aristoy, M., Toldra, F., 2015. Sources of variability in the analysis of meat nutrient coenzyme Q(10) for food composition databases. Food Control 48, 151–154, with permission from Elsevier.

(Talmant et al., 1986). Thus, of 18 muscles studied by these workers, the *masseter* possessed the highest cytochrome oxidase activity (and the highest pHu) and the *semimembranosus* the lowest; whereas the latter had the greatest buffering capacity, and the highest ATPase and phosphorylase activities, these features being lowest in *masseter*. The oxidative capacity of masseter was even greater than that of the diaphragm.

As a further reflection of the predominant respiratory metabolism of "red" muscles, their store of initial glycogen tends to be less, their pHu higher, and their buffering capacity lower than those of "white" muscles. Muscles that were composed predominantly of "red" fibers had significantly higher pHu, and lower acid buffering capacity, than those of which were composed predominantly of "white" fibers. The higher buffering capacity of the latter appeared to be due to their higher contents of inorganic phosphorus and of the dipeptide carnosine. The content of various other chemical compounds differs between "red" and "white" muscles.

Thus the "red" (*masseter*) muscles in beef have 10 times as much taurine and three times as much coenzyme Q10 (ubiquinone) (but only a 10th of the carnosine content), as the relatively "white" *semitendinosus* muscle (Reig et al., 2015).

There are differences in glycogen metabolism between "red" and "white" muscles, the pathway from glycogen to glucose being more active in the former. The activity of glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and glycogen synthetase is also more marked in "red" muscles. On the other hand, the enzymes in the pathway from glycogen to lactic acid are more active in "white" muscle. The activity of glycogen debranching enzyme diminishes and thereby the rate of postmortem glycolysis by a rapid fall in temperature (Kyla-Puhju et al., 2005). Enzymes involved in the complete oxidation of fat and carbohydrate are more prevalent in red muscles (e.g., b-hydroxy acyl CoA dehydrogenase, citrate synthetase, isocitric dehydrogenase, malic dehydrogenase, succinic dehydrogenase, and cytochrome oxidase). Such differences are reflected in their relative postmortem susceptibility to the benefits of incorporating antioxidants in the feed.

Red muscles tenderize less markedly than white muscles during conditioning, and it might be supposed, therefore, that they contain a lower concentration of proteolytic enzymes, including those activated by calcium ions (calcium-activated sarcoplasmic factors, calpains). The differences between types of muscle in proteolytic capacity are complex. There are at least two types of calcium-activated factors, referred to as calpains I and II (also referred to as μ- and m-calpains), which are, respectively, activated by micromolar or millimolar concentrations of Ca^{2+} ions, as well as an inhibitor, calpastatin. While the ratio of calpain II/calpastatin is greater in white muscles than in red—an observation that accords with the greater susceptibility of the former to undergo conditioning changes—the levels of calpain II and of calpastatin are both greater in red muscles (Ouali and Talmant, 1990).

Even within a single muscle, there may be systematic differences in composition and constitution. Lawrie and Gatherum (1962) observed marked differences in pHu and pigmentation along the *Longissimus dorsi* muscle in Large White and Landrace pigs; and Lundstom and Malmfors (1985) showed that this was reflected in corresponding variations in the light-scattering and water-holding properties of this muscle in Swedish Landrace x Yorkshire crossbred pigs. It is of interest that the rate of postmortem glycolysis was greatest at the caudal end of *Longissimus dorsi*. In the *semimembranosus* muscles of pigs, areas only 19 cm apart may be pale and exudative and have a pHu of 4.9 on the one hand or be pink and dry and have a pHu of 5.6 on the other.

Preslaughter stress depletes glycogen from muscle fibers according to their nature and to its mode of inducement. Thus, mixing stress causes a greater depletion from fast fibers (types IIA and IIB) than from slow fibers (type I), whereas depletion of glycogen by adrenaline injection occurs more severely from slow fibers. Severe cold reduces the glycogen more in red fibers than in white since red muscles are those mainly responsible for shivering. Thus the

susceptibility of different muscles to develop dark-cutting character will be affected by the relative proportion fibers they contain.

The problems of PSE pork and of other undesirable quality conditions have stimulated investigation of the relative susceptibilities to them of different muscles of the porcine carcass. Thus, Warner et al. (1993) compared the quality attributes of *Longissimus lumborum* with those of muscles from the loin, ham, and shoulder. They found that when *Longissimus lumborum* was classed as dark and nonexudative, the other muscles were also dark and their pHu was high. When *Longissimus lumborum* was pale and exudative, however, only the ham muscles (other than *rectus femoris*) were similarly defective.

Young and Bass (1984) showed that there was a markedly higher proportion of type IIB fibers in the muscles of steers than in those of bulls, and a higher proportion of type IIA fibers in the latter, suggesting that serum androgens exert a differential control on the growth of muscle. Wild pigs have more oxidative type IIA fibers than domestic pigs, especially in fibers with less myoglobin (i.e., more glycolytic), and the cross-sectional area of types I, IIA, and IIB is similar; whereas in domestic pigs, the cross-sectional area of IIB fibers is much greater than those of types I and IIA (Ruusunen and Puolanne, 2004).

4.3.2 Species, breed, and lines

Species is perhaps the most easily appreciated factor affecting the composition of muscle; but its effect is conditioned by the simultaneous operation of many of the intrinsic and extrinsic factors already mentioned (see Chapter 2). In comparisons between species, it is thus desirable to choose definite values for these other variables.

Although it is obvious that the contents of water, of total nitrogen, and of total soluble phosphorus are similar in all five species, there are marked differences in the other characteristics. The low myoglobin content of rabbit and pig muscle accords with the superficial paleness of the flesh of these animals. The myoglobin of pig muscle also differs qualitatively from that of ox muscle. The results obtained using freshly cut surfaces of *Longissimus dorsi* suggest that the rates of oxygenation of myoglobin are the fastest in pork, intermediate in lamb, and the slowest in beef.

As previously mentioned, initial pH in living muscle is in the range 7–7.2, while that of beef, lamb, and pig loin is usually near 5.5. However, there is a marked difference in pH decline after slaughter. While pig *Longissimus dorsi* pH drops acutely (approximately 1.1 units in the first 2 h postmortem, thus reaching a pH near 6), lamb and beef decrease is slower (0.85 and 0.7 units, respectively), thus reaching this pH around 8 and 12 h postslaughter (Fig. 4.13). Of course, pH drop is affected by a number of factors, including season, diet, handling, carcass fatness, postmortem temperature, etc., but this different physiological behavior of meat species markedly affects all technological procedures after slaughter. To minimize shortening and protein denaturation and to optimize

meat technological properties, pH 6 should be reached when the carcass temperature is between 18°C and 25°C as measured in *Longissimus dorsi* (Thompson et al., 2005). Therefore, very different recommendations for cooling should be applied in each case.

Although the nature of these differences is not yet understood in detail, they are most important in relation to the texture and appearance of meat postmortem. Under controlled conditions, muscles may differ in the times to the onset of rigor mortis, in their initial and pHu, in their content of initial and residual glycogen, in the rates of pH fall aerobically and anaerobically, in their initial store of energy-rich phosphate (i.e., the labile phosphorus of ATP and CP), in their capacity for energy-rich phosphate resynthesis, and in their capacity of the sarcoplasmic reticulum to remove Ca2þ ions from the system (Table 4.5). In intact carcasses or cuts of meat, the environment of individual muscles clearly varies. Thus, temperature differences will affect the rates of postmortem glycolysis; but, after correction for such circumstances, systematic intrinsic differences between muscles are still apparent.

The time to the onset of the fast phase of rigor mortis under anaerobic conditions at 37°C tends to be proportionate to the initial store of energy-rich phosphorus and of glycogen. On the one hand, *Longissimus dorsi* has the characteristics of a "white" muscle, capable of short bursts of activity, this being aided by the relatively large store of energy-rich phosphorus and a low capacity for the aerobic resynthesis of ~P.

To achieve these targets, in ruminant, intervention such as electrical stimulation is often recommended to accelerate processes. On the other hand, in pigs, pH drop is usually faster than ideal, and effort is being paid to slow down processes. This includes optimizing preslaughter handling, dietary manipulation, and applying cold as soon as possible. PSE meat surpasses any possibility of applying cold and leads to a marked protein denaturation, paleness, and drip loss, but in halothane-free pigs, it may be also usual that temperature and pH decline are not adequately matched.

TABLE 4.5 Time to onset of fat phase rigor mortis and pH in the *Longissimus dorsi* of different species maintained at 37°C.

	Time to onset of fast phase of rigor mortis (min)	Initial pH	pH at onset	pH (ultimate)
Horse	238	6.95	5.97	5.51
Ox	163	6.74	6.07	5.50
Pig	50	6.74	6.51	5.57
Lamb	60	6.95	6.54	5.60

Reproduced from Lawrie's Meat Science, 7th ed. (2006), with permission from Elsevier.

Dietary fat has relatively little influence on the depot fat of all species of ruminant since ingested fatty acids are hydrogenated by rumen microorganisms (which may also effect a change in the length of the fatty acid chains). Although ruminant muscle thus has normally a low ratio of polyunsaturated to saturated fatty acids, it does contain various C_{20} and C_{22} PUFAs of the $n-6$ and $n-3$ series, which are valuable nutritionally to human consumers.

The potential benefits for human health of CLA, especially the isomers *cis*- 9, *trans*-10 linoleic acid and *trans*-10, *cis*-12 linoleic acid, have been investigated in recent years. Hydrogenation by rumen microorganisms converts ingested linoleic to stearic acid and produces CLA as intermediates. Supplementation of ruminant diets with fat sources rich in C18:2 $n-6$ leads to an increase in the production of *cis*-9, *trans*-10 linoleic acid in the flesh (Noci et al., 2005). Nevertheless, there are seasonal alterations in the degree of unsaturation of the depot fats of ruminants, probably because of the ingestion of octadecatrienoic acid during pasture feeding; and there are distinct differences due to breed. Cattle regulate the degree of unsaturation of their fat by interchange between stearic and hexadecenoic acids, whereas pigs do so by an exchange between stearic and oleic acids. With pigs the ratio of $n-3$ to $n-6$ PUFAs is readily amenable to dietary manipulation; and indeed, this has a greater influence on the ratio than genetic factors. The nutritional value of pork, as represented by the ratio, can be increased by feeding linseed at a level that has no adverse effect on eating quality.

Rabbits lay down much more polyunsaturated fatty acids than other herbivorous such as horses. The fats of rabbit flesh are comparatively richer in palmitic, linoleic, and myristic acids and poorer in stearic acid, than the meat of other domestic species (Cambero et al., 1991).

After species, breed exerts the most general intrinsic influence on the biochemistry and constitution of muscle. A particularly striking effect of breed is found in the horse. The percentage of myoglobin in the *Longissimus dorsi* of thoroughbreds, which arch their backs strongly in running, is considerably higher than that in this same muscle of the draught horse in which the *Longissimus dorsi* is moved relatively little. On the other hand, the psoas muscles show no such difference.

By using halothane-negative British Landrace pigs and those of the resistant Duroc breed, Cameron and Enser (1991) were able to assess the effect of breed on the IMF of *Longissimus dorsi* muscles without confounding the issue by stress susceptibility. The IMF of Duroc pigs had a higher content of saturated and monounsaturated fatty acids and a lower concentration of PUFAs, than the British Landrace; and there was a higher level of fat in the former. From an investigation of the back fat from 11 breeds of pig, Warriss (1990) found that the characteristics of the fat (e.g., firmness) were largely determined by the level of fatness rather than by inherent breed factors. In a subsequent study of the fatty acid characteristics of porcine *Longissimus dorsi*, Wood et al. (2008) found highly significant differences between breeds in respect of the contents of linoleic and linolenic acids in both neutral lipids and phospholipids (Table 4.6).

TABLE 4.6 Breed differences in fatty acid composition of neutral lipids and phospholipids of porcine *Longissimus dorsi* muscle.

	Berkshire	Duroc	Large white	Tamworth
Neutral lipid fatty acids				
Weight (as % of muscle)	2.29	1.98	0.94	0.91
Linoleic	6.78	10.14	10.49	8.09
Linolenic	0.58	0.87	0.78	0.67
Phospholipid fatty acids				
Weight (as % of muscle)	0.45	0.45	0.39	0.40
Linoleic	29.10	30.10	29.35	31.40
Linolenic	0.70	0.67	0.61	0.75

Reproduced from Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M., 2003. Effect of fatty acids on meat quality: a review. Meat Science 66, 21–32, with permission from Elsevier.

Because of interest in pale exudative pig musculature, the effect of breed on muscle composition in this species has been investigated. At all locations from the fifth thoracic to the sixth lumbar vertebrae, the *Longissimus dorsi* muscle of pigs of the Large White breed has more myoglobin and a higher pHu than the corresponding muscle from pigs of Landrace breed (Lawrie and Gatherum, 1962).

Genetic selection also leads to compositional and metabolic differences, including enzymatic activity (Claeys et al., 2001) (Table 4.7).

4.3.3 Sex

In general, males have less IMF than females, whereas the castrated members of each sex have more IMF than the corresponding sexually entire animals and higher concentration of saturated fat (Wood et al., 2008).

As assessed from a representative sample of UK animals, entire pigs were found to have a greater concentration of heme pigment (myoglobin plus hemoglobin) in their *Longissimus dorsi* muscles than castrates (Warriss, 1990). Because the meat of mature boars can be associated with an unpleasant odor, it is important to be able to detect the presence of meat from male pigs in various products.

4.3.4 Age

Irrespective of species, breed, or sex, the composition of muscles varies with increasing animal age, although the rates of increment are not identical in all

TABLE 4.7 Enzyme activities in trasversus abdominis muscle samples (3 h postmortem) in two pig lines.[a]

Pig line	Lean (*n* ¼ 16)	Growth (*n* ¼ 16)	*P*
Fat tissue gain 20–80 kg (g/day)	46 (4)	105 (5)	.01
Lean tissue gain 20–80 kg (g/day)	592 (36)	628 (44)	.01
Age at slaughter (days)	190 (10.8)	176 (14.7)	.005
Carcass weight (kg)	78.9 (7.7)	83.7 (8.7)	.142
Carcass lean meat (%)	63.2 (2.8)	55.4 (4.6)	.000
Carcass weight gain (g/day)	411 (41)	471 (56)	.001
Carcass lean meat gain (g/day)	259 (26)	260 (35)	.961
M. longissimus thoracis			
pH 1 h postmortem	5.71 (0.32)	6.32 (0.26)	.000
M. transversus abdominis			
pH 1 h postmortem	6.06 (0.30)	6.43 (0.21)	.001
Dry matter content (%)	26.1 (0.89)	26.7 (0.89)	.016
Intramuscular fat content (%)	2.75 (1.03)	3.27 (1.03)	.078
m-calpain[b]	2.4 (4.6)	8.6 (7.2)	.013
m-calpain[b]	33.4 (7.2)	42.3 (5.7)	.001
Calpastatin[b]	848 (89)	894 (173)	.247
Cathepsin (B þ L)[c]	2.23 (0.51)	2.34 (0.74)	.721
Cathepsin D[d]	46.1 (5.2)	45.3 (4.1)	.728
Dipeptidyl peptidase IV[c]	1.67 (0.37)	1.87 (0.30)	.130
Pyroglutamyl aminopeptidase I[c]	3.38 (1.36)	4.92 (1.13)	.002
Acid lipase[e]	3.80 (0.80)	4.61 (0.77)	.015
Acid phospholipase[e]	1.19 (0.28)	1.35 (0.16)	.116
Neutral phospholipase[e]	121 (15)	149 (25)	.000

[a]*Mean values (standard deviation).*
[b]*mg casein hydrolyzed/min/g muscle.*
[c]*nmol 4-amido-7-methyl coumarin released/min/g muscle.*
[d]*mg hemoglobin hydrolyzed/min/g muscle.*
[e]*nmol methylumbelliferon released/min/g muscle.*
Reproduced from Claeys, E., De Smet, S., Demeyer, D., Geers, R., Buys, N., 2001. Effect of rate of pH decline on muscle enzyme activities in two pig lines. Meat Sci. 57, 257–263, with permission from Elsevier.

muscles. Moreover, different components reach adult values at different times. Thus, in bovine *Longissimus dorsi*, those nitrogen fractions representing myofibrillar and sarcoplasmic proteins have reached 70%–80% of their mean adult value by birth, and their subsequent rates of increase become asymptotic at about 5 months of age. Nonprotein nitrogen, however, does not attain its characteristic adult value until about 12 months of age; and the concentration of myoglobin increases rapidly until about 24 months of age. IMF appears to increase and moisture content on a whole tissue basis to decrease, up to and beyond 40 months of age. On a fat-free basis, however, the moisture content remains fairly constant after 24 months of age. The great increase in IMF and in myoglobin content, the lesser increase in total and sarcoplasmic nitrogen, and the decrease in moisture and in stroma with age are evident. Much of the increased saturation of intramuscular lipid in heavier pigs is due to an increase in the ratio of C18–C18.1 fatty acids in the neutral lipid fraction. It is interesting to note that odd-numbered fatty acids (C11, C13, C15, and C17) are quite prevalent in the phospholipid fraction of porcine IMF. In respect of the lipids of bovine fatty tissues, however, while fat unsaturation has been shown to decrease with increasing animal age and level of fatness (Wood et al., 2008), the ratio of linoleic to stearic acids, and the softness of the fat, increases. It is feasible that this apparent contradiction may reflect age-related changes in branched-chain fatty acids. In this species, one of the important factors involved is the development of the rumen microflora, which hydrogenates dietary fats.

Myoglobin concentration appears to increase in a two-phase manner, an initial swift rate of increment being followed by one that is more gradual. The fast phase lasts about 1, 2, and 3 years in pigs, horses, and cattle, respectively. Reflecting the increases in myoglobin with age, there is a concomitant two-phase increment in the activity of the enzymes, which govern respiration and, thereby, in energy production potential.

These activities are based on the rate of oxygen uptake of mitochondrial membrane preparations from the muscles concerned and are thus relative. They are proportional, however, to the absolute values for the muscles themselves.

The connective tissue content of muscle is greater in young animals than in older ones. The concentrations of both collagen and elastin diminish with increasing animal age, which is attributed to differences in solubility. The degree of intra- and intermolecular cross-linking between the polypeptide chains in collagen increases with increasing animal age (Nishimura, 2015). The investigations of Bailey and his colleagues have provided detailed information on the age-related changes in the collagen of tendon, muscle, and the other tissues. While, in young animals, most of the cross-links are reducible, heat- and acid-labile and increase up to 2 years of age, thereafter they are gradually replaced by linkages, which are thermally stable (Bailey and Light, 1989). The change with maturation is greatest in the epimyseal connective tissue, which in young animals consists mainly of thermally labile cross-links, whereas the endomysium already consists of thermally stable cross-links (Bailey and Light, 1989).

Even in mature animals, collagen is known to have a significant turnover, and the rate differs according to the nature of the collagen. Thus, type III precursors turn over at a slower rate than those of type I (Bailey and Light, 1989). A high rate of collagen turnover, as in rapidly growing pigs, can have detrimental effects on the strength of the collagen laid down and can lead to lameness in the live pig, to a separation of fat from lean in the fresh meat, and to "lacy" bacon in the cured product.

4.3.5 Training and exercise

Implicit in the differences in constitution in a given muscle between active and inactive species and breeds, between young and old animals, and between "red" and "white" muscles in a given animal, is the concept that constant usage can cause a development of certain features and that, conversely, disuse can cause a reversion of such factors. Systematic usage over a period ("training") as opposed to fatiguing exercise immediately preslaughter, or cessation of activity in a previously active muscle, causes opposing changes in constitution. The most obvious alteration in constitution is the elaboration of myoglobin during systematic exercise. This is logical if myoglobin functions as a short-term oxygen store in muscle, which facilitates its ability to develop power. There would appear to be a concomitant increase in the activity of the respiratory enzymes. Both training on a treadmill and spontaneous activity decreased the level of lactic dehydrogenase in porcine muscles, indicating an increase in the capacity for aerobic metabolism.

Other features of training are the elaboration of increased stores of muscle glycogen, which, of course, leads to a lower pHu postmortem (Bate-Smith, 1948), and the enhanced muscle esterase and lipase activity (Daza et al., 2009).

4.3.6 Animal welfare, stress, and oxidative status

The science of animal welfare has developed very rapidly in recent years with the aim of assessing how stress and its adaptative response affect the animal homeostasis and its biological functioning. This includes changes in the energy metabolism of the cell, ion dynamics, enzyme regulation and proteolysis, DNA expression, and changes in the microbiome (Xing et al., 2019), which may alter meat structure, chemical constitution, fate of muscle to meat conversion, and consequently, technological properties and quality characteristics. Implications of short-term preslaughter stress on pH drop and consequences on meat quality are reviewed in Chapter 5.

Challenge to physiological homeostasis and adaptation is something continuous in living organism but in some cases may severely disturb animal equilibrium, as it is the situation in mixing, noise, fear, transport, fighting, hunger, dehydration, fatigue, high density, extreme temperature, injury, pathology, and so on. Reaction to stress is complex and with many interactions among different

factors and individuals, thus generating marked variations in response. A decrease in productive efficiency is generally observed mediated by lower feed intake and digestibility, impaired metabolic efficiency (osmotic, acid-base, and redox disbalance, metabolic pathways, and enzyme functioning alteration), thus leading to higher energy and nutrient expenditure, higher susceptibility to infectious diseases, and altered peri-mortem metabolism. The conditions before harvest are also reviewed in Chapter 5.

The AMP-activated protein kinase (AMPK) has an important role in cellular energy homeostasis, being activated by all the means that require an increase in the ATP (starvation, hypoxia, excessive muscle contraction (Xing et al., 2019); once activated, it enhances glucose uptake (GLUT4 exposure), promotes glycolysis (activating glycogen phosphorylase), lipolysis and inhibits anabolic pathways. Thus, an increased activation of the path leads to accelerated pH drop.

Moreover, apoptosis, or programmed cell death, has been shown to be a key factor during the conversion of muscle to meat and comprises ion channels, mitochondria mediating signals, ROS overproduction, high calcium levels that activate calpain, unfolded protein accumulation.

Acetylation and phosphorylation play a key role in several biological pathways, including glycolytic, oxidative metabolism, and proteolysis. As such, its role is not fully elucidated and heavily depends on the extent in which the different pathways are modulated. Protein posttranslational modifications (PTMs) regulate structures and functions of proteins in living tissues (Li et al., 2021). Nitrosilation is also a known PTM. Higher nitric oxide synthase (NOS) has been observed in RFN pork than PSE pork; further, RFN pork has lower activities and higher nitrosylation of glycogen phosphorylase, phosphofructokinase, and pyruvate kinase.

Heat shock proteins (HSPs) are ubiquitous family of proteins that can be rapidly synthesized under various stressors and usually serve as chaperones aiding proteins achieve its intended configuration. They were first identified as HSPs given that they were quickly synthesized after the exposure of the cells to heat but are also highly expressed under other stressful conditions such as hypoxia, exercise, cold, UV light, or tissue remodeling. Given this, HSPs tend to modulate apoptosis regulating protein degradation, maintaining their assembly and structure in ATP devoid conditions (Hendrick and Hartl, 1993). Some of the key functions to meat development are maintaining calcium channels by the HSP70, interaction with phospholipids avoiding oxidation, modulating energy metabolism through the modulation of AMPK (HSP90), and the glycolytic enzymes, pyruvate kinase, HSP90 interacts (HSP70), and even modulating calpain (HSP90). HSPs are also associated with exercise and can bring new lights to muscle transformation (Xing et al., 2019).

One common feature of stressful situations is free radical formation. ROS interact with macromolecules, such as proteins, DNA, RNA, and lipids leading to a broad range of actions and reaction products. The oxidative modification of proteins affects its physical properties and functionality, outstanding among them the role in aconitase activity, an enzyme containing an iron-sulfur cluster

sensitive to oxidation by superoxide. Oxidized forms lead to cessation of citrate isomerization to isocitrate and consequently to tricarboxylic acid cycle disruption, thus leading to anaerobic glycolysis and pH drop. Long-term oxidative stress may also lead to higher fat accumulation, as excess of acetyl CoA may be used for fatty acid synthesis. ROS can also affect proteases and particularly μ-calpain, as will be discussed later. On the other hand, calcium signaling is also affected in many ways by the oxidative status, giving that ROS disrupts the cell membrane potential inducing Ca^{2+} overflow into the cytoplasm through the calcium channels; further, increased Ca^{2+} concentration increases oxygen consumption and ROS creation through energy production (Brookes et al., 2004).

It has been shown that chronic glucocorticoid treatment can reduce the activity of specific mitochondrial electron transport chain complexes and increase mitochondrial ROS production. In broiler chicken, exogenous glucocorticoids produce an increase of TBARS in muscle (Gao et al., 2010), thus suggesting a critical role of mitochondria in ROS production under stress.

4.3.7 Diet, plane of nutrition, and fasting

Diet and feeding strategy largely affect meat composition and metabolic properties of muscle, thus affecting meat quality in many ways. This is an active area of research, and many possible orientations have been tested (e.g., altering major nutrients, including minor components to alter metabolic regulation, enrichment of specific nutrients, etc.), paying attention not to reduce productivity and efficiency (see Chapter 2). Moreover, as diet represents main cost of production, any change in diet formulation aimed to enhance quality usually produces a higher productive cost. Therefore, much effort is being paid to optimize time of administration prior to slaughter, in some cases even some minutes may be effective. An outline of effective strategies is presented here briefly.

A wide number of studies have focused on altering carbohydrate composition, postmortem metabolism, and meat quality, particularly orientated to optimizing pH drop. Supplementation of diets or drinking water with sugar or other digestible carbohydrate was reported to increase glycogen stores and to reduce pH. On the other hand, diets with low-digestible carbohydrate (high in fat and protein) have been used to reduce glycogen stores with the aim of modulating pH drop (Rosenvold et al., 2001; Fig. 4.20). In this case, a similar pHu was obtained, but water-holding capacity was increased. Cretin increases availability of phosphocreatine for ATP production thus sparing glycogen, which may reduce pH decline during initial postmortem steps and enhance water retention. Including chromium, a regulator of glucose metabolism, in pig diets at very low concentration (200 ppb) produced a higher pHu and water retention (Matthews et al., 2005). Dietary vitamin E administration also affects muscle glycogen concentration (Lauridsen et al., 1999). Some feed components have been reported to reduce stress in meat- producing animal, with concomitant effects on meat quality. Magnesium was reported to

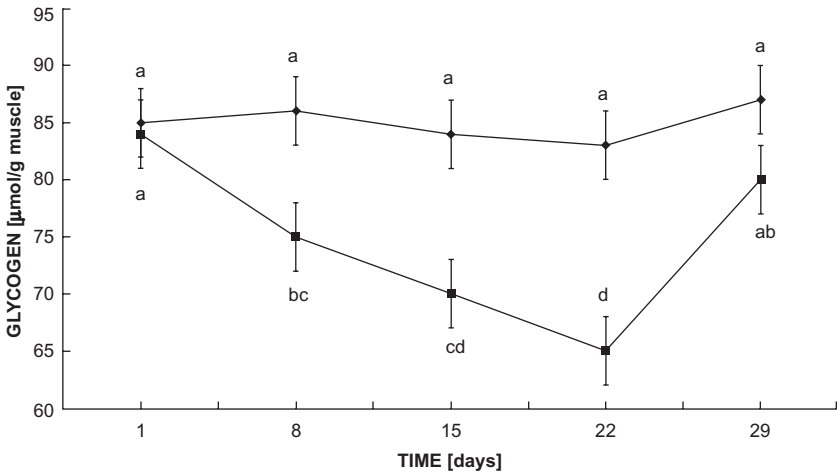

FIG. 4.20 Evolution of muscle glycogen concentration from pigs fed a control diet (●) or an experimental diet (■) containing a low level of digestible carbohydrates and high fat concentration. *Reproduced from Rosenvold et al., 2001, J. Animal Sci. 79, 382–391.*

reduce catecholamine concentration and to improve water-holding capacity and some other quality characteristics (D'Souza et al., 1998). Also dietary amino acid tryptophan enrichment decreases stress response and aggression as it is a precursor of serotonin.

On the other hand, preslaughter fasting reduces glycogen concentration and increases pHu, delays activation of calpains, and accelerates release of lysosomal enzymes (Wang et al., 2013; Fig. 4.21).

Much attention has been devoted to modifying concentration of some particular components to enhance nutritive value of meat (Reig et al., 2015). Main strategies were to increase $n-3$ PUFAs, monounsaturated fatty acids or to alter $n-6/n-3$ fatty acid ratio. Fish oil, linseed oil, and flaxseed, sunflower oil high in monounsaturated, by-products of the olive oil, acorns, and a number of other fat sources at varied including level were included. A good response was generally obtained within wide ranges of variation in each animal species (Fig. 4.9), but saturation over the time was observed, thus reaching a plateau. Endogenous synthesis may be altered by some specific regulators (copper, vitamin A, CLA, etc.), which modulates delta-9 desaturase activity (Cordero et al., 2010).

Deleterious effects of oxidation on meat and meat products have been a line of active research. Vitamin E reduces oxidation and rancid flavor in a quantitative manner, stabilizes color, and reduces exudation, probably by enhancing cell membrane integrity (Asghar et al., 1991; Isabel et al., 2003). More recently, a piece of evidence suggests that a role of vitamin E in preserving calpain activity postmortem may be responsible for reducing water loss (Huff-Lonergan and Lonergan, 2005). A similar effect may be produced by dietary organic selenium supplementation (Calvo et al., 2016; Fig. 4.22).

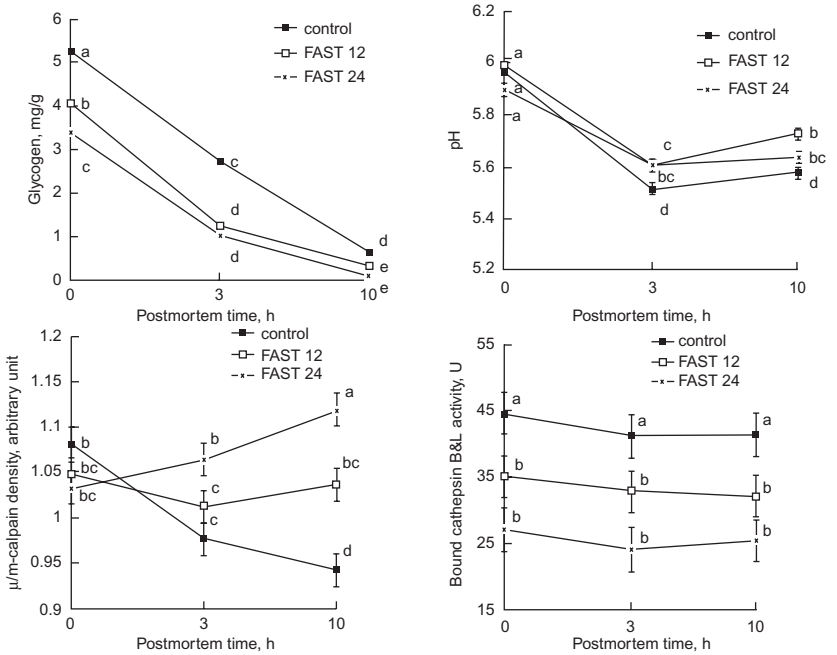

FIG. 4.21 Effect of fasting for 12 or 24 h of muscle glycogen concentration, pH calpain density, and free cathepsin activity at slaughter and 3 and 10 h thereafter in chicken breast. *Source: Wang et al., 2013, Meat Sci. 93, 865–872 with permission from Elsevier.*

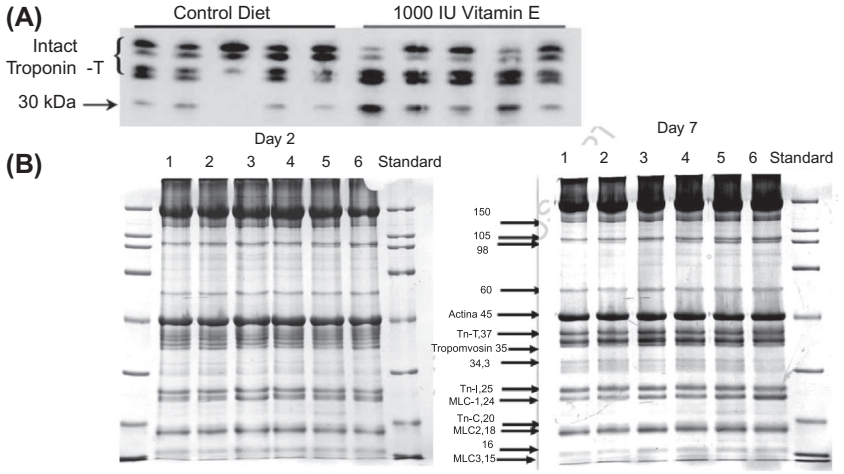

FIG. 4.22 (A) Western blot of Troponin-T in purified myofibrils from control and a-tocoferol enriched diet steers steaks after 2 days of aging. An increased in the density of the 30 kDa band (degradation products from troponin-T) indicates more degradation. (B) Electrophoresis of myofibrilar proteins and degradation products after 7 days of storage in meat from pigs receiving a control diet (band 1) or diets enriched in sodium selenite (bands 2–3), organic form of selenium (bands 5–6), or a supranutritional concentration of vitamin E. *Reproduced from Huff-Lonergan, E., Lonergan, S., 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Sci. 71, 194–204 and Calvo, L., Toldra, F., Aristoy, M.C., Lopez-Bote, C.J., Rey, A.I., 2016. Effect of dietary organic selenium on muscle proteolytic activity and water-holding capacity in pork. Meat Sci. 121, 1–11 with permission from Elsevier.*

The content of iron in pork meat is quite relevant as well as its content in trace elements such as selenium, magnesium, and zinc. Iron content is higher in oxidative than in glycolytic muscles. The selenium content in meat may be enriched through supplementation with sodium selenite or selenium-rich yeast (Calvo et al., 2016).

IMF enrichment has also been an object of interest. Saturated fats, lysine reduction, protein reduction, leucine supplementation, CLA inclusion, or removing dietary vitamin A (Hyun et al., 2003; Cordero et al., 2010).

Attempts to reduce the fat content of porcine carcasses by genetic or nutritional manipulation can cause an increased softness and loss of cohesiveness in fatty tissue, leading to the separation of subcutaneous fat into layers. This undesirable effect is due to a decrease in the size of the fat cells and an increase in the ratio of linoleic to stearic acids in the fat (Wood et al., 2008).

As the contents of linoleic and acid increase, the fat becomes soft and more translucent (Maw et al., 2003). Ingestion of high levels of unsaturated fat will cause the deposition of unsaturated IMF in pigs but not in ruminants unless the feed has been protected against the reducing action of rumen microorganisms. Even in ruminants, however, when the feed is not so protected, the ingestion of different feeds can effect some changes in the fatty acid pattern of the fats laid down. Plane of nutrition affects fatty composition and lipogenic enzyme activity.

4.3.8 Interanimal variability

The least understood of the intrinsic factors, which affect the constitution of muscle, is the variability between individual animals. Even between litter-mates of the same sex, considerable differences are found in the percentages of IMF, of moisture, and of total nitrogen and in the distribution of nitrogen between sarcoplasmic, myofibrillar, and stroma proteins. Such differences may be adventitiously determined by early stages of development (see Chapter 2). Currently, special attention is given to variation in birth weight, as some evidences suggest an increased within-litter birth weight variation in modern sows. Understanding the factors that can influence the events that occur during gestation and that have an impact on the fetal growth and development is important to achieve increased within-litter uniformity weight, performance, and quality characteristics. The detrimental effects of protein restriction on fetal growth during early gestation may be due to altered placental and endometrial angiogenesis and growth, which leads to a reduction in placental-fetal blood flow, nutrient supply from mother to the fetuses, and ultimately to fetal growth retardation.

Intrauterine growth retardation is a significant problem in livestock production. It adversely affects neonatal survival, postnatal growth performance, efficiency of feed utilization, tissue composition (including protein, fat, and minerals), meat quality, long-term health of offspring, and adult onset of disease. Genetic, epigenetic, and environmental factors (including nutrition), as

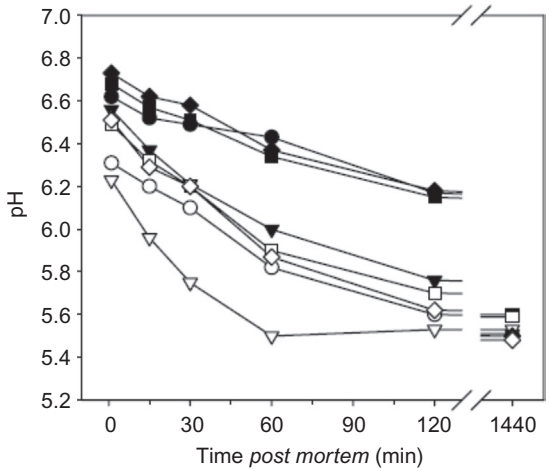

FIG. 4.23 Variability in the fall of postmortem pH in Longissimus dorsi in pigs of different genotypes (shape of the labelling) subjected to CO_2 or electrical stunning either with minimum stress prior to slaughter (back filling) or moderate exercise (white filling). *Reproduced from Lindahl et al., 2006, Meat Sci. 613–623 with permission from Elsevier.*

well as maternal maturity, impact oil the size and functional capacity of the placenta, placental vascular growth, uteroplacental blood flows, transfer of nutrients from mother to fetus, the endocrine milieu, as well as embryonic development of myocytes, adipocytes, and other cell types. Growing evidence suggests that arginine-derived signaling molecules (nitric oxide and polyamines) play an important role in regulating these key physiological and biochemical processes.

Recessive genes no doubt account for apparently sporadic differences in the composition of muscles of animals within a given breed. The muscles of adult cattle, which exhibit the double-muscling condition, appear to contain an unusual form of myosin (Picard et al., 2002).

Interanimal variability in the rate of postmortem glycolysis and pH drop in pigs may be considerable (Fig. 4.23). There is some evidence that a slow rate may be associated with the presence of the enzyme phosphoglucomutase in a dephosphorylated form.

Clearly, substantial differences in the composition of muscle are caused by factors that are, as yet, unexplained: their elucidation will no doubt form the subject for a wide area of future research.

4.4 Conclusion and future trends

Meat science was based from the very beginning on a deep knowledge of metabolic features that take place in the muscle and the complex mechanism that occurs after slaughter. This has been particularly evident in Prof. Lawrie's career and was covered in all editions of Meat science, which included basic and

applied aspects. Progress in physiology, biochemistry, and other branches of science is active in providing fully understanding of biochemical processes and its regulation. Any technological approach to meat should stand on these complex mechanisms. Animal production technologies are increasingly focusing on quality characteristics of meat, including nutritive aspects, which is a matter of growing concern. Animal farming has to recover a positive link to society and rural development, thus attention should be paid to the interaction between animal and environment and the quality attributes of traditional-based production systems. Optimization of preslaughter technology, including welfare aspects, is also a matter of growing interest and social concern. A holistic view, based on a deep knowledge of biochemical aspects, is still required.

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Chapter 5

The conversion of muscle to meat

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5.1 Introduction

During the postmortem period, a complex cascade of energetic, biochemical, and physical changes occur in muscle that results in its conversion to meat. The process begins shortly after harvest when many of the homeostatic mechanisms of the animal are disrupted. As the animal succumbs to exsanguination and resulting anoxia, skeletal muscle continues to synthesize and utilize adenosine triphosphate (ATP) in a futile attempt to sustain cellular homeostasis. Shortly after exsanguination, glycogen and high-energy phosphate compounds present in the muscle at the time of death are anaerobically metabolized for the sole purpose of ATP production. Anaerobic metabolism is significantly less efficient at generating ATP than aerobic metabolism. As a result, the rate of ATP hydrolysis exceeds its generation, which triggers the onset of rigor mortis (Latin for "stiffness of death"). As postmortem metabolism proceeds, the muscle gradually loses the ability to generate ATP and eventually all ATP is depleted. In the absence of ATP, myosin binds irreversibly to actin, leading to the completion of rigor mortis and the loss of muscle excitability and extensibility. The completion of rigor mortis occurs at 1–24 h postmortem, depending mainly on the species, muscle fiber type, and ante- and postmortem conditions. During postmortem storage (aging or maturation), the proteolytic degradation of myofibrillar proteins causes the loss of muscle structural integrity and thus, a decrease in muscle tension (resolution of rigor mortis).

Another significant change that occurs in postmortem muscle under anaerobic conditions is acidification. The end product of postmortem glycolysis and ATP hydrolysis, lactate, and hydrogen ions (H^+), respectively, accumulate in the muscle due to the lack of an effective elimination mechanism. As a result, muscle pH gradually declines from around 7.2 in living tissue to an ultimate pH near

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5.6. The rate and extent of postmortem metabolism significantly influence the development of meat quality attributes. Hastened, extended, or insufficient postmortem pH decline adversely influences meat color, texture, and water-holding capacity (Fig. 5.1). Factors such as environmental conditions and pre- and postslaughter handling can significantly alter postmortem pH decline. Therefore, understanding the factors that control the rate and extent of postmortem metabolism can influence the probability of producing high-quality products.

5.2 Rigor mortis

During muscle contraction, myosin heads of the thick filament form cross-bridges with the actin monomers of the thin filament, a process driven by ATP hydrolysis by the myosin ATPase activity. When the myosin head initially binds to an active site on actin, the actomyosin cross-bridge is in a weakly bound state. The subsequent release of inorganic phosphate (P_i) followed by adenosine diphosphate (ADP) transforms the cross-bridge into a tightly bound state (rigor). This rigor complex is maintained until a new ATP binds to the myosin head, allowing it to detach from actin and start a new cycle (cross-bridge cycle; Fig. 5.2). The cycle is repeated as long as actin active sites are exposed and ATP is available.

Postmortem stiffness (rigor mortis) is one of the most dramatic physiochemical changes that occur in the muscle during its transformation to meat. It was shown very early that stiffening observed in postmortem muscle is directly related to the loss of ATP concentration (Bendall, 1951). As postmortem ATP levels continue to decline, ATP-dependent muscle relaxation through breaking

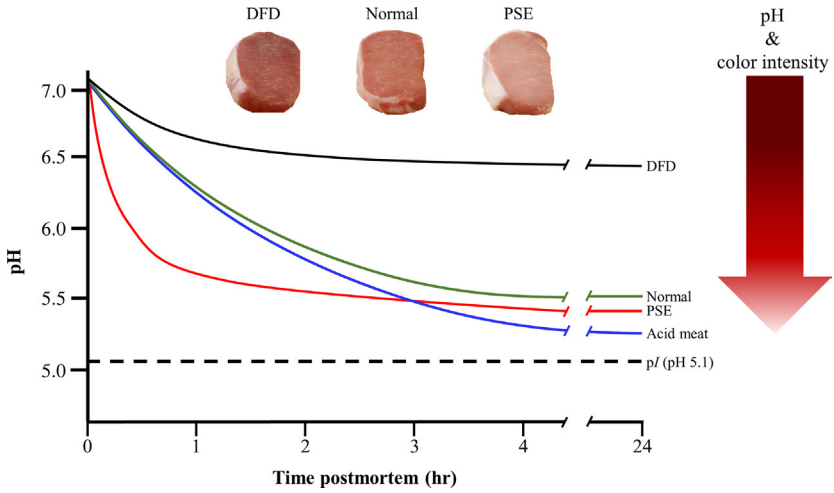

FIG. 5.1 The influence of the rate and extent of postmortem pH decline on meat quality characteristics. *DFD*, dark, firm, and dry; p*I*, isoelectric point; *PSE*, pale, soft, and exudative. *(The pork chop pictures were used with permission from the National Pork Board.)*

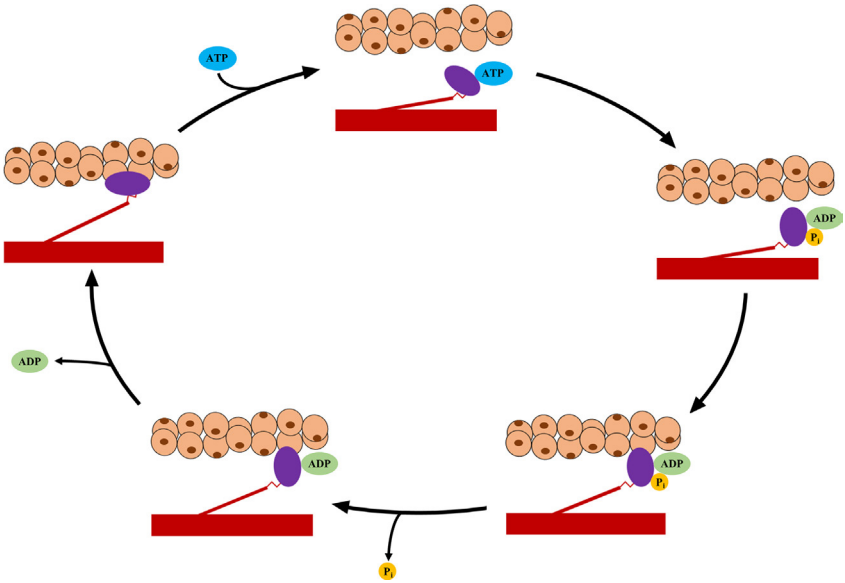

FIG. 5.2 The cross-bridge cycle.

the actomyosin cross-bridges also declines, leading to a gradual formation of irreversible cross-bridges. These linkages result in tension development within the entire muscle, and even physical shortening if the muscle is not restrained.

The phenomenon of rigor mortis can be divided into four phases: delay, onset, completion, and resolution (Fig. 5.3). The delay phase is an early postmortem stage in which the muscle remains fully extensible (stretchable). During this "lag" phase, ATP is sustained at or near antemortem levels by the phosphagen system and aerobic metabolism, thereby no permanent cross-bridges are formed. Indeed, an early postmortem muscle strip is extremely extensible when a force is applied to it and returns to its original length when the force is removed (Bendall, 1973a). However, once ATP falls below a certain level, postmortem muscle begins to lose extensibility, which signals the onset of rigor mortis. The completion of rigor mortis is reached when ATP is completely depleted from the muscle. In this phase, maximum number of rigor linkages is formed, and extensibility reaches its minimum. Hence, meat is least tender when consumed immediately after the completion of rigor mortis.

The length of time required for rigor completion varies between species and muscles and is closely controlled by factors dictating the rate of ATP hydrolysis. In general, time to rigor completion decreases as the proportion of fast-glycolytic fibers increases in the muscle, primarily owing to their greater ATP hydrolysis rates. This is evidenced by significantly shorter rigor completion times in chicken and pork (2–8 h) compared with beef and lamb (~ 24 h). Muscle temperature is another factor influencing the length of time to rigor completion.

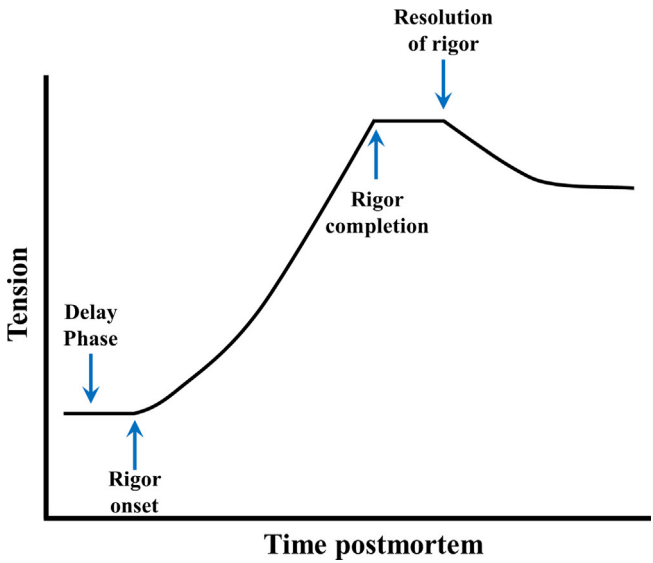

FIG. 5.3 Isometric tension development in postmortem muscle. *(Reproduced from Aberle, E., Forrest, J., Gerrard, D., Mills, E., 2012. Principles of Meat Science, fifth ed. Kendall Hunt Publishing, Dubuque, IA, with permission.)*

ATP hydrolysis rate increases with increasing temperature from 15°C to 37°C, which markedly shortens the rigor process (Locker and Daines, 1975). It is noteworthy that pH decline and rigor mortis are intimately correlated, as both are function of ATP hydrolysis/availability. Ultimate pH and rigor completion are reached when ATP is completely exhausted from the muscle; thus, muscle that undergoes hastened or limited pH decline exhibits rapid development of rigor mortis, whereas extended pH decline is associated with a longer time of rigor mortis development.

During the postrigor period, muscle gradually losses rigidity and becomes relatively stretchable. This phenomenon is referred to as "resolution" of rigor mortis and is mediated by enzymatic degradation of muscle proteins. The term rigor resolution could be misleading because the decrease in muscle tension is not due to breaking the actomyosin cross-bridges. Rather, the reduction in muscle stiffness is a function of proteolytic degradation of myofibrillar proteins in the Z and M lines of the sarcomere, which disrupts muscle structure. This, in turn, increases meat tenderness, as will be described later in the chapter (Section 5.10).

5.3 Postmortem metabolism

5.3.1 ATP homeostasis

Skeletal muscle is a highly dynamic tissue and exhibits a remarkable ability to change its metabolic capacity to meet demands. The energy requirement of

skeletal muscle remains relatively low under resting conditions, but can quickly increase up to 100-fold during periods of intense activity. The nucleotide coenzyme ATP is the primary currency of cellular energy transfer. Energy liberated from the hydrolysis of the terminal phosphate group of ATP (reaction 5.1) is used to perform cellular functions. This includes muscle contraction, active ion transport, cell signaling, and biosynthesis of macromolecules. Given these vital functions, muscle tissue must maintain ATP homeostasis over a wide range of cellular challenges and circumstances. Yet, skeletal muscle stores a limited amount of ATP (5–8 μmol/g of muscle tissue); only enough to maintain muscle energetic demands for no longer than a few seconds of high-intensity work. Therefore, ATP must be continuously generated by other mechanisms that involve the catabolism of stored energy compounds such as carbohydrates and lipids.

$$\mathrm{ATP} + \mathrm{H_2O} \xrightarrow{\mathrm{ATPase}} \mathrm{ADP} + \mathrm{P_i} + \mathrm{H^+} + \mathrm{energy} + \mathrm{heat} \tag{5.1}$$

Many of these same cellular functions operate seamlessly during the conversion of muscle to meat and essentially involve the synthesis, hydrolysis, and availability of ATP during the cataclysmic event of tissue death. There are three major energetic pathways by which muscle generates ATP: the phosphagen system, glycolysis, and oxidative phosphorylation. Understanding the role of each pathway in postmortem metabolism is fundamental to understanding the process of converting muscle to meat.

5.3.2 The phosphagen system

During early postmortem metabolism, muscle ATP concentration remains stable through the utilization of a high-energy phosphate compound known as phosphocreatine (PCr), also known as creatine phosphate. PCr serves as an immediate energy source that rapidly buffers ATP levels during high-energy demands. The enzyme creatine kinase (CK) catalyzes the reversible transfer of a P_i from PCr to ADP to form ATP and creatine. While under resting conditions, the reaction favors the formation of PCr, during high-ATP demands, similar to that occurring postmortem, PCr is degraded and the free energy released is used to regenerate ATP. Because muscle stores of PCr are also limited (~ 25 μmol/g of muscle tissue), PCr can only sustain postmortem cellular ATP levels for a brief period. Creatine kinase is one of the three enzymes that comprise the so-called phosphagen system (Fig. 5.4), with the other two enzymes being adenosine monophosphate deaminase (AMPD) and adenylate kinase (AK).

As soon as the majority of PCr has been degraded in postmortem muscle, the rate of ATP hydrolysis has likely exceeded the rate of resynthesis, leading to an excessive formation of ADP. This activates AK, which buffers the drop in ATP by converting two molecules of ADP into an ATP and adenosine monophosphate (AMP). Subsequently, AMP is irreversibly deaminated by AMPD to form

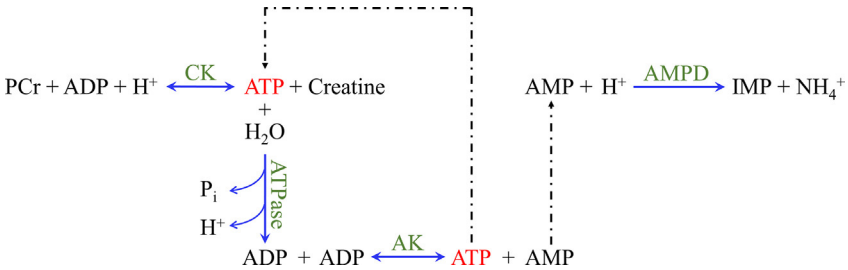

FIG. 5.4 Contribution of the phosphagen system to ATP and H^+ production. *AK*, adenylate kinase; *AMPD*, AMP deaminase; *CK*, creatine kinase.

inosine monophosphate (IMP), which accumulates in the muscle. The AMPD reaction is important to shift the equilibrium of the AK reaction in the direction of ATP formation. However, deamination of AMP is responsible for the drop in the total adenine nucleotide pool (ATP, ADP, and AMP) because IMP is unable to contribute to ATP synthesis.

It is important to note that the net production of H^+ by the phosphagen system during postmortem metabolism is zero. Protons released through the hydrolysis of ATP generated by the phosphagen system are consumed by the CK and AMPD reactions (Fig. 5.4). Nonetheless, metabolites produced by the phosphagen system (AMP, ADP, P_i) function as activators for rate-limiting enzymes in the glycolytic pathway. Using an in vitro glycolyzing model, England et al. (2015) found that elevating AMP concentration by inhibiting AMPD increases the rate and the extent of glycolysis. On the other hand, alteration to the antemortem concentration of total muscle creatine pool (PCr + creatine) was shown to be associated with higher ultimate pH and enhancements in meat quality (Scheffler et al., 2013a).

5.3.3 Glycogenolysis and glycolysis

The capacity of the phosphagen system to maintain postmortem ATP homeostasis is limited to the available PCr and adenine nucleotides at the time of harvest. As PCr concentration drops below 4 μmol/g of muscle, the catabolism of muscle glycogen through glycogenolysis (glycogen degradation) and glycolysis becomes the dominant pathway for ATP production (Bendall, 1973b).

Glycogen is the predominant storage form of carbohydrate in skeletal muscle and represents 1%–2% of the total muscle mass. It is a highly branched polymer of glucose residues held together linearly by α-1,4-glycosidic bonds. At every 8–12 residues, a branch is formed by α-1,6-glycosidic bond. Branching is important because it increases the solubility of glycogen and the number of nonreducing terminal glucose residues (glucose with a free hydroxyl [OH] group at C-4), thus increasing the number of sites accessible to the enzymes involved in glycogen degradation. Glycogen exists in the form of granules (10–40 nm) in the sarcoplasm of muscle fibers that contains the enzymes needed for glycogenolysis.

Complete degradation of glycogen requires a combined action of two enzymes: glycogen phosphorylase (GP) and glycogen debranching enzyme (GDE). GP catalyzes the sequential phosphorolytic cleavage of the α-1,4-linkages at the nonreducing ends of the glycogen chains. Inorganic phosphate splits the glycosidic bond between the terminal glucose moiety and the adjacent one to yield glucose 1-phoshate, thus leaving the glycogen chain one glucose residue shorter (reaction 5.2). Because GP is incapable of cleaving α-1,6-linkages at the branch points and indeed stops cleaving four residues away, the action of another enzyme is required. GDE has two distinctive catalytic activities: transferase and α-1,6-glucosidase. The transferase exposes the α-1,6-linkages by transferring the terminal three glycosyl residues to an adjacent chain, thereby extending it. The last glucose residue remaining at the branch point is hydrolyzed by α-1,6-glucosidase and released as a free glucose molecule. Glucose 1-phosphate molecules resulting from the action of GP can be readily converted to glucose 6-phosphate by phosphoglucomutase (PGM; reaction 5.3). Free glucose molecules are either converted by the enzyme hexokinase to glucose 6-phosphate (reaction 5.4) or accumulate in postmortem muscle.

$$\text{Glycogen}_n + \text{P}_\text{i} \xrightarrow{\text{GP}} \text{glycogen}_{n-1} + \text{glucose 1} - \text{phosphate} \tag{5.2}$$

$$\text{Glucose 1} - \text{phosphate} \xrightarrow{\text{PGM}} \text{Glucose 6} - \text{phosphate} \tag{5.3}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose 6} - \text{phoshate} + \text{ADP} + \text{H}^+ \tag{5.4}$$

Glucose 6-phosphate obtained through glycogenolysis, in addition to glucose 6-phosphate stored in the muscle, can directly enter the glycolytic pathway (glycolysis); a sequence of 10 reactions, all of which occur in the sarcoplasm of the muscle fiber (Fig. 5.5). During glycolysis, each six-carbon glucose moiety of glycogen is metabolized into two three-carbon pyruvate molecules, in addition to a net yield of three molecules of ATP, two molecules of reduced nicotinamide adenine dinucleotide (NADH), one H^+, and two water molecules (reaction 5.5).

$$\begin{aligned} &\text{Glycogen}_n + 3\text{ADP} + 3\text{P}_\text{i} + 2\text{NAD}^+ \underset{2\text{H}_2\text{O}}{\rightarrow} \\ &\text{Glycogen}_{n-1} + 2\text{pyruvate} + 3\text{ATP} + 2\text{NADH} + \text{H}^+ + 2\text{H}_2\text{O} \end{aligned} \tag{5.5}$$

Under anaerobic conditions, pyruvate is reduced to lactate by the enzyme lactate dehydrogenase (LDH; reaction 5.6). This reaction is crucial to regenerate NAD^+ that is required for the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reaction, thereby allowing glycolysis to continue under anaerobic conditions. Due to the absence of circulation, lactate accumulates in the muscle postmortem. Yet, lactate itself is not responsible for the drop in postmortem muscle pH. Indeed, the LDH reaction functions as a buffer by consuming one

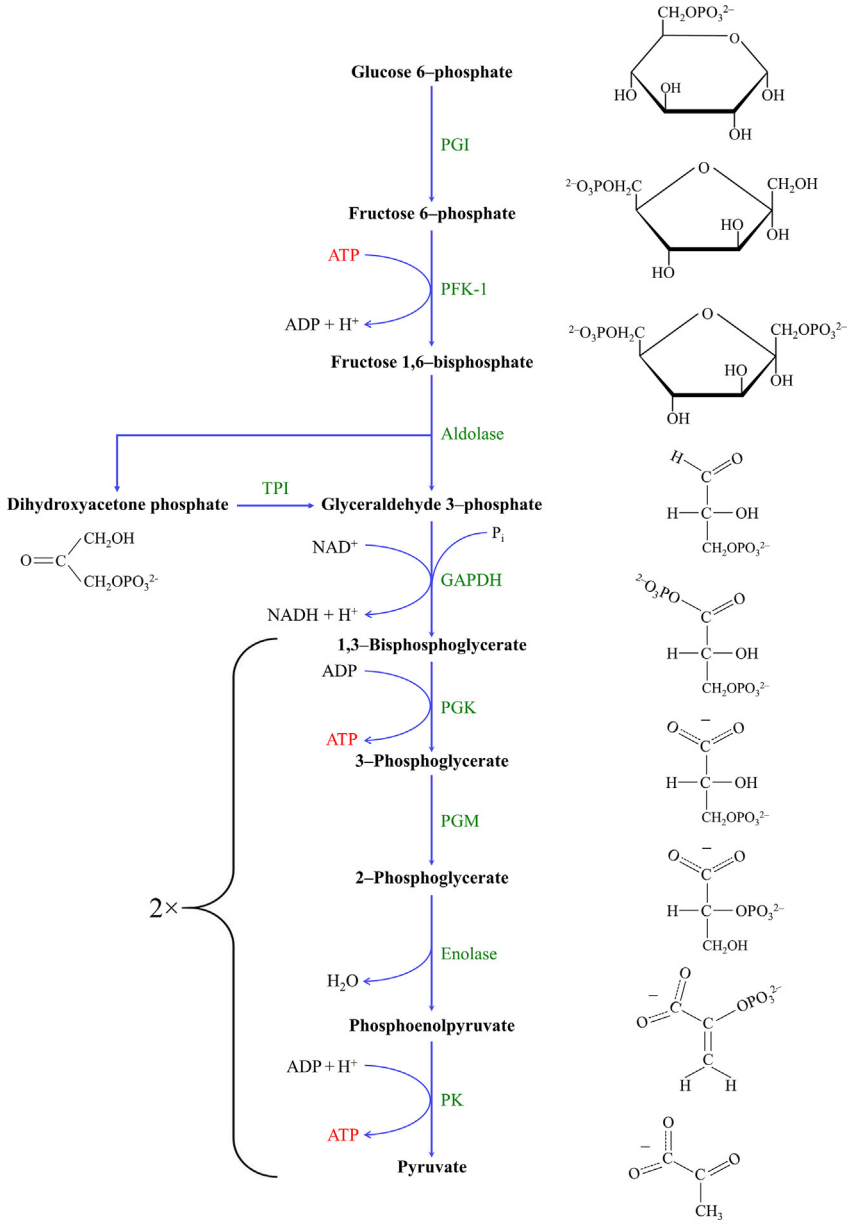

FIG. 5.5 The glycolytic pathway. *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *PFK-1*, phosphofructokinase-1; *PGI*, phosphoglucose isomerase; *PGK*, phosphoglycerate kinase; *PGM*, phosphoglycerate mutase; *PK*, pyruvate kinase; *TPI*, triosephosphate isomerase.

H^+ from the system for every pyruvate molecule converted to lactate (reaction 5.6). Consequently, when glycolysis is coupled to lactate formation, a net decrease of one H^+ occurs for each glucose moiety metabolized (reaction 5.7). Instead, the hydrolysis of the ATP yields H^+ (reaction 5.1) that accumulate in the muscle postmortem and lower the pH. Honikel and Hamm (1974) indicated that the hydrolysis of ATP generated through postmortem glycolysis is responsible for 90% of the total H^+, while the remaining 10% originated from the hydrolysis of ATP present in the muscle at the time of death. When glycolysis is combined with lactate formation and ATP hydrolysis, a net of two lactate and two H^+ are produced (reaction 5.8). Because glycolysis generates H^+ and lactate at 1:1 ratio, a negative linear relationship is often observed when postmortem muscle pH values are plotted against lactate values measured at the same time, making lactate a good indicator for the extent of postmortem metabolism.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \tag{5.6}$$

$$\text{Glycogen}_n + 3\text{ADP} + 3\text{P}_\text{i} + \text{H}^+ \rightarrow \text{Glycogen}_{n-1} + 2\text{lactate} + 3\text{ATP} + 2\text{H}_2\text{O} \tag{5.7}$$

$$\text{Glycogen}_n \rightarrow \text{Glycogen}_{n-1} + 2\text{lactate} + 2\text{H}^+ \tag{5.8}$$

The flux through the glycolytic pathway is regulated by three rate-limiting enzymes: GP, phosphofructokinase-1 (PFK-1), and pyruvate kinase (PK). The concentration of allosteric regulators, feedback mechanisms (end-product regulation), and covalent modification (phosphorylation or dephosphorylation) have been shown to regulate the activities of the aforementioned enzymes. These regulatory mechanisms allow the glycolytic pathway to accommodate changes in energy demand by increasing or decreasing the activities of the enzyme. Understanding mechanisms controlling the flux through the glycolytic pathway is essential to understand postmortem metabolism and ultimately fresh meat quality.

GP, the key enzyme in glycogenolysis, exists in two interconvertible forms: the less active *b* (dephosphorylated) form and the more active (phosphorylated) *a* form. Phosphorylase *b* is allosterically activated by AMP, IMP, and high levels of P_i and inhibited by ATP and glucose 6-phosphate. In response to AMP, phosphorylase *b* undergoes conformational changes that enhance the enzyme-substrate binding affinity (i.e., decreasing Michaelis constant [K_m]). Phosphorylation of the Ser^{14} residue in phosphorylase *b* by the enzyme phosphorylase kinase converts it to phosphorylase *a*, the fully active form independent of AMP stimulation.

PFK-1 is the key regulatory enzyme of glycolysis and one of the most tightly regulated enzymes in metabolism. PFK-1 catalyzes the irreversible phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate, a reaction that consumes one molecule of ATP. The activity of PFK-1 is regulated by

allosteric regulators, pH, and the formation of oligomer structures. PFK-1 is allosterically stimulated by different ligands, including AMP, ADP, and fructose 2,6-bisphosphate, while ATP, PCr, and citrate act as inhibitors. The enzyme exists in different oligomeric structures, including dimers, tetramers, and larger multimers. PFK-1 dimers exhibit minimal catalytic activity, while tetramers (dimer of dimers) and higher aggregates are fully active. Low pH and high concentration of allosteric inhibitors favor the formation of dimers, whereas higher pH and greater concentration of allosteric activators stabilize the tetramer form.

The last rate-limiting enzyme of glycolysis is PK, which irreversibly catalyzes the conversion of phosphoenolpyruvate to pyruvate. This reaction is coupled to ATP synthesis and accounts for two ATP molecules produced by postmortem glycolysis for each molecule of glucose metabolized. PK is allosterically activated by AMP and the upstream intermediate fructose 1,6-bisphosphate and inhibited by ATP and acetyl-CoA.

5.3.4 The role of mitochondria

Mitochondria are often referred to as the "powerhouse" of the cell because they generate the majority of ATP needed for cellular functions through a process called oxidative phosphorylation. Mitochondria consist of two membranes, inner and outer, hence creating two separate mitochondrial compartments: the intermembrane space (the space between the outer and the inner membrane) and the matrix, which is bound by the highly convoluted inner membrane. The outer membrane is highly porous and permeable to most ions and small molecules. In contrast, the inner membrane is impermeable to the passage of nearly all ions and molecules unless mediated by a membrane transport protein. The matrix is the site for the tricarboxylic acid cycle, where energy liberated from the oxidation of acetyl-CoA is conserved in the structure of the reducing equivalents NADH and flavin adenine dinucleotide ($FADH_2$). Embedded in the inner mitochondrial membrane are components of the electron transport chain (ETC). During cellular respiration, electrons are released from NADH and $FADH_2$ to the ETC and finally to O_2 to yield H_2O. The movement of electrons through the ETC is coupled with the pumping of H^+ from the matrix to the intermembrane space. These protons build up and create an electrochemical potential gradient across the inner membrane (i.e., proton motive force). The flow of protons down their electrochemical gradient back into the matrix through the F_1F_0 ATP synthase drives the synthesis of ATP.

The cessation of oxygen delivery to the muscle following exsanguination impedes mitochondrial ability to produce ATP through oxidative phosphorylation. Therefore, mitochondria are often considered irrelevant to postmortem metabolism. However, mitochondria do not "die" immediately; rather, they maintain functionality and structural integrity for several hours postmortem (England et al., 2018). Additionally, mitochondria play a major role in cellular calcium homeostasis (Dang et al., 2020), an element known to modulate

postmortem metabolism (Section 5.4). Therefore, mitochondria may influence postmortem metabolism by altering the biochemical and energetic properties of the muscle.

As the oxygen supply is removed due to lack of blood circulation, skeletal muscle utilizes oxygen bound to myoglobin for energy production. Because oxidative phosphorylation generates 10 times more ATP than anaerobic glycolysis (30 vs 3, respectively), aerobic activity for even a short period of time postmortem would significantly improve the maintenance of cellular ATP levels. Indeed, we showed in multiple studies that adding functioning mitochondria to an in vitro model simulating postmortem metabolism reduces the rate of ATP hydrolysis during the first 30 min of 24 h incubation period (Scheffler et al., 2015; Matarneh et al., 2017). Pösö and Puolanne (2005) estimated the amount of postmortem aerobic ATP production using oxygen stored in the muscle to be between 0.4 and 6 μmol/g of muscle, depending on animal age, species, and muscle fiber type. Curiously, England et al. (2018) indicated that skeletal muscle maintains measurable oxygen levels within the first 2 h postmortem, which suggests that residual oxidative metabolism could occur later postmortem. In support of this notion, an in vitro study by Matarneh et al. (2021) demonstrated that mitochondria are capable of metabolizing a portion of glycolytic pyruvate even when they are mechanically disrupted. Because maintaining adenine nucleotides for longer periods of time postmortem can extend glycolysis (England et al., 2015), differences in mitochondrial oxidative capacity between muscles could influence the onset and the extent of anaerobic metabolism.

ATP depletion under postmortem anoxic conditions impairs calcium uptake by the sarcoplasmic reticulum calcium ATPase pump, resulting in a cytosolic calcium overload. As a part of the calcium buffering system, mitochondria sequester large quantities of calcium in an attempt to maintain cellular calcium homeostasis. It has recently been shown that inhibition of mitochondrial calcium uptake elevates cytosolic calcium concentration (Dang et al., 2020), indicating that mitochondria contribute to cellular calcium homeostasis in postmortem muscle. Mitochondrial calcium overload increases the formation of reactive oxygen species (ROS), which together stimulate mitochondrial permeability transition pore (mPTP) opening. As a result, apoptosis-inducing proteins, such as cytochrome *c*, are released into the cytosol and activate the downstream caspases leading to cell death. In addition, the opening of mPTP is associated with the loss of the inner mitochondrial membrane potential. To avoid the collapse of the proton motive force, the F_1F_0 ATP synthase switches roles and commits "treason" by coupling the pumping of H^+ from the matrix to the intermembrane space to ATP hydrolysis (St-Pierre et al., 2000). The energetic need for this function is obtained from ATP produced by glycolysis, which, in turn, exacerbates cellular energy deficit. The ATP-driven proton pumping activity of the F_1F_0 ATP synthase could increase the rate of ATP hydrolysis and subsequently postmortem metabolism, which has the potential to adversely influence meat quality. Our previous in vitro studies showed that mitochondria increase the rate

of ATP hydrolysis and glycolytic flux after 2 h of incubation (Matarneh et al., 2017, 2018a,b). This effect is attributed to enhanced ATP hydrolysis by the F_1 domain of mitochondrial F_1F_0 ATP synthase at pH values below those permissible for other muscle ATPases.

5.4 Factors controlling the rate of postmortem metabolism

The rate of pH decline during the conversion of muscle to meat reflects the intensity of postmortem metabolism. Typically, pH gradually declines from around 7.2 to 5.8 within 8 h postmortem, with an ultimate pH of about 5.6 achieved at 24 h. The rate of postmortem pH decline is influenced by many factors, including species, genetics, muscle fiber type, and pre- and postmortem handling conditions. In general, the rate of postmortem pH decline differs among meat species in the following order: poultry > pork > beef > lamb. Scopes (1974) concluded that the rate of ATP hydrolysis by muscle ATPases drives the rate of postmortem metabolism. In addition, he indicated that mechanisms controlling the flux through the rate-limiting enzymes of glycolysis must be determined by the activity of ATPases. Several enzyme systems present in the muscle, such as myosin ATPase, sarcoplasmic reticulum calcium ATPase, Na^+/K^+ ATPase, and mitochondrial ATPase require the hydrolysis of ATP to perform cellular functions. Because myosin is the most abundant protein in the muscle, myosin ATPase is considered to be the major ATPase responsible for ATP hydrolysis postmortem (Hamm et al., 1973). ATP splitting rate by muscle ATPases at 38°C is approximately 0.5 μmol/min per g of muscle (Scopes, 1973). The rate of ATP hydrolysis decreases with decreasing temperature from 38°C to 15°C; however, ATP depletion rate rises again around 0°C (Newbold and Scopes, 1967; Bendall, 1973b). At lower temperatures, calcium release from the sarcoplasmic reticulum is enhanced while calcium sequestering is impaired, thereby increasing the cytosolic concentration of calcium.

The rate of ATP hydrolysis dramatically increases as cytosolic calcium rises. Under a high cytosolic concentration of calcium, the inhibition of muscle contraction by the troponin-tropomyosin complex is removed, allowing the interaction between myosin heads and adjacent actin active sites. Approximately 10-fold increase in myofibrillar ATPase activity was observed when the level of calcium increases from pCa^7 to pCa^6 or less (Bowker et al., 2004). Additionally, calcium ATPase catalyzes the breakdown of ATP in an attempt to sequester calcium back into the sarcoplasmic reticulum, which further increases the rate of ATP hydrolysis. High sarcoplasmic calcium can also increase the rate of postmortem glycolysis through the activation of phosphorylase kinase by binding to its calmodulin subunits. Activated phosphorylase kinase subsequently phosphorylates and activates the enzyme GP. Thus, calcium plays a significant role on defining the rate of postmortem metabolism. Passing an electric current through the carcass early postmortem (electrical stimulation) accelerates

postmortem metabolism by triggering massive liberation of calcium from the sarcoplasm reticulum. Electrical stimulation has been widely used in red meat (mainly beef and lamb) to accelerate ATP depletion and rigor mortis completion. This process reduces the incidence of cold shortening, in addition to improving meat tenderness, color, and sensory characteristics (Nazli et al., 2010; Cetin et al., 2012).

5.5 Factors controlling the extent of postmortem metabolism

The extent of postmortem acidification is a key factor determining meat quality development as it influences meat color, texture, water-holding capacity, and shelf-life. The normal ultimate pH value of meat in most meat species ranges between 5.5 and 5.7, and meat within this range possesses the most desirable quality characteristics. Meat at pH 6.0 or greater appears darker in color and has a shorter shelf-life, while meat of pH < 5.4 has a pale color, impaired water-holding capacity, reduced protein extractability, and poor processing yield. Therefore, ultimate pH is widely regarded as an indicator of fresh meat quality. The ultimate pH of meat is a function of many factors (Fig. 5.6). However, none of the factors is sufficient in predicting more than 50% of the variation in ultimate pH (Van Laack et al., 2001a). Understanding the factors determining the ultimate pH is key to minimizing variations in meat quality.

Recall, anaerobic degradation of glycogen during postmortem metabolism drives pH decline. Therefore, the extent of postmortem metabolism would be expected to be a function of muscle glycogen content at the time of slaughter. In other words, the pH of the muscle should continue dropping as long as glycogen is available in the muscle. This, however, is not the case; postmortem glycolysis

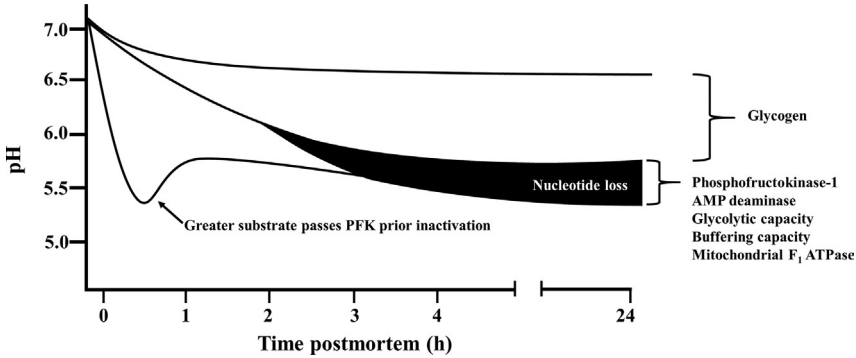

FIG. 5.6 Working model of the factor controlling the extent of postmortem metabolism. *(Reproduced from Matarneh, S.K., Beline, M., Silva, S., Shi, H., Gerrard, D.E., 2018. Mitochondrial F1-ATPase extends glycolysis and pH decline in an in vitro model. Meat Sci. 137, 85–91, with permission from Elsevier.)*

usually stops in the presence of residual glycogen in muscle of certain species. Indeed, the relation between muscle glycogen content and ultimate pH is curvilinear instead of linear. Ultimate pH decreases as glycogen increases until a plateau is reached. Henckel et al. (2002) found that the extent of pH decline is determined by glycogen content if glycogen levels fall between 0 and 53 μmol/g of muscle. Any further increase in glycogen levels beyond 53 μmol/g of muscle is not associated with a further decline in pH. Van Laack et al. (2001a) showed that differences in muscle glycogen account for less than 40% of the variation in ultimate pH.

The cessation of postmortem metabolism in the presence of residual glycogen and glycolytic intermediates suggests that other biochemical mechanisms are involved in determining the ultimate pH of meat. Two hypotheses have been proposed; pH-mediated inactivation of one or more glycolytic enzymes and/or the loss of adenine nucleotides through the phosphagen system (Kastenschmidt et al., 1968; Bendall, 1973b). England et al. (2014) indicated that PFK-1 begins to lose activity around pH 5.9 and becomes completely inactive at pH 5.5, while other enzymes, including GP and PK, maintain functionality at pH 5.5. The authors suggested that PFK-1 may function as a substrate "gate." Essentially, glycogen can be converted to lactate and H^+ provided PFK-1 maintains activity. As pH declines, the ability of this process to occur becomes limiting, and postmortem glycolysis eventually stops. Thus, the more substrate passes PFK-1 before inactivation, the lower the ultimate pH. However, PFK-1 inactivation may not explain why some muscles produce meat with a high ultimate pH (pH > 5.9) in the presence of residual glycogen. In this case, the complete conversion of adenine nucleotides to IMP by AMPD arrests glycolysis (England et al., 2016). Anaerobic glycolysis and phosphagen system work synergistically in an attempt to maintain cellular ATP homeostasis postmortem. While glycolysis preserves adenine nucleotides by converting ADP to ATP, the phosphagen system is responsible for the loss of adenine nucleotides (Section 5.3.2). Oxidative (red) muscle has less glycolytic enzymes abundance and activity (i.e., less glycolytic capacity) and thereby a slower rate of postmortem glycolysis compared with glycolytic (white) muscle. Therefore, oxidative muscle is unable to "keep up" with the phosphagen system, resulting in the loss of nucleotides and the cessation of metabolism while glycogen is still available. The activity of AMPD can influence the two aforementioned mechanisms responsible for the termination of postmortem metabolism. Lower AMPD activity increases AMP concentration, which, in turn, activates the rate-limiting enzymes of glycogenolysis and glycolysis; thus, leading to an increased flux through PFK-1 and extended pH decline. Moreover, lower AMPD activity may delay the loss of adenine nucleotides and therefore extend metabolism (England et al., 2016).

Mitochondria have been recently identified as modulators of the extent of postmortem metabolism. In several in vitro studies (Matarneh et al., 2017, 2018a,b), it was shown that incorporating isolated mitochondria into a bioassay designed to mimic postmortem metabolism increases lactate accumulation and

extends pH decline. This is achieved by increasing the rate of ATP hydrolysis by the F_1 domain of mitochondrial F_1F_0 ATPase (F_1 ATPase), which subsequently promotes glycolytic flux to cope with the rapid decrease in ATP. Consistent with this, chicken breast is particularly poor in mitochondria and possesses elevated ultimate pH (~pH 5.9) (Matarneh et al., 2018b).

Muscle acidification during the postmortem period is the net result of total H^+ produced minus those bound and neutralized by buffering systems in the muscle. Nearly 50% of the muscle buffering capacity is due to myofibril proteins, whereas phosphate compounds, histidine containing dipeptides (carnosine and anserine), and lactate contribute to the other half (Honikel and Hamm, 1974). The buffering capacity of skeletal muscle among meat species varies between 40 and 60 μmol H^+ pH^{-1} g^{-1}, depending on the type of muscle. Typically, buffering capacity is greater in white muscles than those of red muscles. This is likely due to the greater contents of inorganic phosphate and carnosine. Van Laack et al. (2001b) suggested that buffering capacity might explain differences in ultimate pH between muscles with similar lactate levels. While differences in buffering capacity may help to explain the extent of pH decline, buffering capacity does not appear to be a major determinant of ultimate pH (Puolanne and Kivikari, 2000).

In summary, the extent of postmortem metabolism is dictated by the glycogen content of the muscle as long as levels are ≤ 53 μmol/g of muscle. If glycogen is >53 μmol/g, PFK-1 brackets ultimate pH into a fairly consistent range near pH 5.6. A unique situation exists in some oxidative muscles, where metabolism is terminated while PFK-1 is presumably still functioning. While most believe that glycogen depletion arrests pH decline, our data suggest that glycogen content alone is unable to explain the full limitation in pH decline. Rather, the loss of adenine nucleotides arrests glycolysis (England et al., 2016). Finally, buffering capacity and the activity of AMPD and mitochondrial F_1 ATPase may help to explain variations in ultimate pH (pH 5.4–5.8) in the same muscle between animals of similar genetic background (Fig. 5.6).

5.6 Abnormal postmortem metabolism

5.6.1 Pale, soft, and exudative meat

Pale, soft, and exudative (PSE) is the term used to describe meat with abnormally light color, soft texture, and impaired ability to hold water. This defective condition is caused by excessively rapid metabolism immediately following slaughter when carcass temperature is still elevated. The pH of PSE meat drops to a value around the ultimate pH of muscle within the first hour postmortem. The combined action of low pH and high temperature leads to an extreme denaturation of many sarcoplasmic and myofibrillar proteins in the muscle. It should be noted that the rate of pH decline associated with PSE meat may not necessarily produce meat with a lower ultimate pH.

Intensive selection for high body weight and meat yield in pigs and poultry shifts muscle fiber toward a more glycolytic type. Typically, glycolytic muscle of pigs and poultry exhibits rapid glycolysis and thus greater susceptibility for PSE. Due to the high incidence (up to 40%), PSE has become one of the biggest challenges facing the meat industry worldwide, in particular pork and poultry (Petracci et al., 2009).

The development of PSE conditions is mainly associated with genetic factors and preslaughter stressors, including environmental stressors, improper handling, and mixing unfamiliar animals. Stressful conditions activate the sympathetic nervous system, which, in turn, prompts the secretion of epinephrine from the adrenal medulla. Once released, epinephrine binds to β-adrenergic receptors on skeletal muscle cells, triggering a signaling cascade that stimulates glycogenolysis to sustain high glucose levels. The stimulation of β-adrenergic receptors activates cAMP-dependent protein kinase A, which successively phosphorylates and activates phosphorylase kinase. Activated phosphorylase kinase phosphorylates the less active form GP *b* to the more active GP *a* form, enhancing glycogen degradation. In addition, protein kinase A phosphorylates and activates ryanodine receptor type 1 RYR1, the calcium release channel on the sarcoplasmic reticulum of muscle cells, causing a rapid release of calcium into the cytosol. High-amplitude calcium release accelerates glycolysis by increasing muscle ATPase activities and the flux through glycolysis (Section 5.4).

In pigs, PSE meat is often associated with porcine stress syndrome (PSS), a condition analogous to human malignant hyperthermia. Pigs with PSS lack the ability to adapt to environmental stressors, leading to severe muscle contractions, a rapid increase in body temperature, cardiac arrest, and death (Ball and Johnson, 1993). PSS is greatly induced by a mutation known as halothane mutation (HAL or HAL-1843), a single base substitution (C1843 to T) mutation in the RYR1 gene (halothane gene). The mutation results in a replacement of arginine by cysteine at residue 615 at the protein level. Halothane gas was used as a screening method to identify animals susceptible to PSS, and since then, the genetic component has been referred to as the HAL gene. HAL-positive pigs are hypersensitive to agents that stimulate the opening of RYR1 channels, leading to an excessive release of calcium into the cytosol.

Because of the apparent similarity of PSE condition between pigs and poultry, a genetic component was thought to be responsible for PSE susceptibility in poultry. However, to date, evidence for genetic basis of PSE poultry is limited. Oda et al. (2009) found lower RYR β-isoform (β-RYR) gene expression in PSE chicken breast compared with non-PSE. This finding suggests that the differential expression of β-RYR could contribute to the development of PSE in chicken. PSE in poultry has been associated with antemortem stress and, in particular, heat stress that can cause accelerated postmortem metabolism. Improper chilling of carcasses postmortem can also develop PSE conditions in poultry. Certainly, the incidence of PSE has been reduced through eradicating the RYR1 mutation and implementing proper animal handling practices and rapid carcass

chilling techniques. Even so, however, PSE remains a major problem for the pork and poultry industries, with an incidence rate of up to 40%.

5.6.2 Acid meat

Another porcine genetic mutation that often leads to abnormally low ultimate pH meat (acid meat; pH < 5.4) is AMPKγ_3^{R200Q}, also known as Rendement Napole (RN^-) mutation. AMPKγ_3^{R200Q} is a point mutation in the PRKAG3 gene that encodes the γ3 regulatory subunit of AMP-activated protein kinase (AMPK), resulting in a single amino acid substitution at residue 200 from arginine to glutamine. This mutation is most notably found in Hampshire pigs and renders a constitutively active AMPK in muscle tissues. The gain-in-function mutation results in an approximately 100% increase in glycogen content and enhanced mitochondrial oxidative capacity in glycolytic muscles.

The pH decline of muscle from AMPKγ_3^{R200Q} pigs is characterized by a normal rate, but continues to drop for a longer time. The resulting ultimate pH is near the isoelectric point (p*I*), or the pH at which the positive and negative charges of muscle protein side groups are approximately equal (pH 5.1–5.2), thereby reduces the ability of muscle to bind water. Low ultimate pH associated with AMPKγ_3^{R200Q} mutation adversely influences meat water-holding capacity, protein content and functionality, and processing yield. The abnormally low ultimate pH of AMPKγ_3^{R200Q} mutant pigs is usually attributed to the fact that these pigs deposit greater muscle glycogen when compared with wild-type pigs. Yet, muscle from AMPKγ_3^{R200Q} pigs accumulates similar lactate levels at 24 h postmortem to that of muscle from wild-type pigs. Scheffler et al. (2013b) concluded that glycogen content does not have a direct effect on the extent of postmortem pH decline of RN^- pigs. Rather, we showed that greater glycolytic flux coupled with a lower postmortem buffering capacity explains the lower ultimate pH of meat from AMPKγ_3^{R200Q} pigs (Matarneh et al., 2015). However, reasons beyond the greater flux in the AMPKγ_3^{R200Q} pigs are not well understood. Scheffler et al. (2011) proposed that other metabolic properties such as mitochondria, PCr, and glycolytic enzymes content and activity could play participating roles in the determination of ultimate pH in the AMPKγ_3^{R200Q} mutant pigs. However, these factors might work through the activation of glycogenolysis and glycolysis as evidenced by the greater glycogen degradation and net lactate accumulation in the AMPKγ_3^{R200Q} pigs (Matarneh et al., 2015). England et al. (2015) observed that activity and abundance of the enzyme AMPD are lower in the AMPKγ_3^{R200Q} pigs, which results in greater AMP levels late postmortem. In addition, muscle from pigs harboring the AMPKγ_3^{R200Q} mutation contains more mitochondria, which may contribute to their extended metabolism via increasing the rate of ATP hydrolysis (Matarneh et al., 2018a). At present, both RN^- and HAL mutations have been eliminated from commercial swine industry, and as such, RN^- and HAL-mediated reductions in pork quality have been reduced dramatically. However, these two mutations remain valuable as models for the study of the rate and extent of postmortem pH decline.

5.6.3 Dark, firm, and dry meat

Dry, firm, and dark (DFD) is a meat quality defect observed predominantly in beef (dark cutting) and to a lesser extent in lamb and pork. As the name implies, DFD meat is characterized by its abnormal dark color, firm texture, and dry sticky surface. This condition is a function of muscle glycogen deficiency and results from chronic exposure to preslaughter stresses. Inadequate glycogen leads to early termination of postmortem metabolism, therefore limiting the drop in pH (ultimate pH > 6.0). Meat color is directly correlated to the ultimate pH; color progressively darkens as pH increases from 5.8 to 7.0. High ultimate pH minimizes meat pigment losses and denaturation, thereby increasing light absorbance, which gives the meat darker appearance. Meat of elevated pH also exhibits higher water-holding capacity as the pH is further from the p*I*. This increases the negative charges on protein side groups available for water binding. Although the high water-holding capacity makes DFD meat a superior raw material for use in processed meat products, the difficulty to retail and the higher susceptibility to microbial spoilage make DFD a major concern for the beef industry in particular.

The exposure to prolonged antemortem stresses triggers glycogen degradation to meet increased energy demands under stress. DFD conditions are developed when an animal fails to restore muscle glycogen reserves prior to slaughter. The repletion of muscle glycogen during the recovery period is typically a slow process, particularly in ruminants. This is usually attributed to the relatively low blood glucose level in ruminants, as very little is absorbed from their gastrointestinal tract, and therefore the potential for greater incidence of DFD. McVeigh and Tarrant (1982) reported that glycogen restoration rate in beef *longissimus* muscle following epinephrine-induced glycogen depletion was 1.5, 6.1, and 7.6 μmol/g of muscle per day for fasted, hay fed, and barely fed animals, respectively.

The energy density of a bovine finishing diet can affect muscle glycogen content, an effect emphasized by the comparison between grain and grass-fed cattle. When compared with grain-fed cattle, grass-fed cattle possess higher susceptibility to dark cutting. In general, feeding cattle a low energy diet (grass-based) reduces the production of the volatile fatty acid propionate, a major precursor for hepatic gluconeogenesis, which, in turn, decreases the capacity for glycogen deposition in the muscle (Daly et al., 1999). Curiously, however, a recent study by Apaoblaza et al. (2020) reported a normal ultimate pH value (pH ~ 5.6) of beef from grass-fed cattle. Those authors indicated that darker lean color of grass-fed cattle is a function of greater proportions of oxidative muscle fibers (dark color fibers) rather than insufficient pH decline.

5.7 Presalughter stress

When an animal is stressed prior to slaughter, meat quality oftentimes suffers as a result. However, the timing of the stress preslaughter and duration both impact the observed effect on meat quality. The problems that arise from stress

primarily occur from acute (short-term) stress immediately prior to slaughter or chronic (long-term) stress. These detrimental effects of stress on meat quality both arise from the release of stress hormones such as epinephrine. Once released, these hormones initiate a series of biochemical reactions designed to mobilize energy to meet the demands of the stressors. Specifically, epinephrine converts GP to the active form ($b \rightarrow a$) through a series of biochemical reactions. By doing so, GP degrades glycogen stores in the muscle to produce ATP. In the acute stress situation, this activation of GP accelerates postmortem glycolysis and subsequently pH decline. The acute stress results in meat that tends to be PSE and is most commonly exhibited by nonruminants. Chronic stress prior to slaughter also activates the enzymes responsible for metabolizing glycogen. However, the primary difference is the depletion of stored muscle glycogen prior to slaughter resulting in high ultimate pH meat. Therefore, many of the antemortem handling practices are designed to limit or counteract the stress on animals.

5.7.1 Transport and lairage

Most animals are raised in a separate location from where they will be slaughtered, which requires a transportation step. Moving animals from one facility to another is stressful due to unfamiliar mixing, hot or cold temperatures, humid environments, poor ventilation, and handling by humans (Schwartzkopf-Genswein et al., 2012). These stressors affect the living animal's welfare and meat quality. Furthermore, during transportation, animals may experience bruising, a reduction in weight, or even death. Therefore, a strong research emphasis has and continues to investigate ways to reduce the stress associated with transport prior to slaughter.

To counteract the stress associated with transport, animals are typically given a lairage period afterward. Lairage can range from a few hours to overnight and allows the animal to recover from the stress of transport. During this period, muscle is able to remove the H^+ produced during the stress and replenish depleted glycogen stores. Glycogen is replenished through the mobilization of stored glycogen in the liver.

5.7.2 Fasting

Prior to or during lairage, animals are given access to water, but not food. This process is known as fasting. Food is limited for two reasons. First, it limits the visceral contents in an attempt to prevent intestinal rupture that would lead to microbiological contamination of the carcass. Secondly, fasting reduces glycogen content in the muscle. This goal may seem counterintuitive to the previous discussion on DFD meat. However, in pigs and poultry, glycogen is regularly deposited in muscle at a higher concentration than necessary to produce a normal pH decline. Furthermore, following transport or other stressors, the liver is able to quickly replenish the glycogen stores to normal levels in muscle of these

species. Thus, to protect against acute stress immediately prior to slaughter, glycogen levels can be reduced to limit the extent of pH decline. To date, fasting is advantageous up to 24 h, but no further benefit is gained from additional fasting (Wittmann et al., 1994).

5.7.3 Stunning

Following lairage, animals are slaughtered. The first two steps of slaughter are comprised of two processes: stunning and exsanguination (blood released from the circulatory system). Stunning is designed to render the animal unconscious and insensible to pain without stopping the heart to allow for complete blood removal. Most stunning methods allow for the animal to regain consciousness if not exsanguinated shortly thereafter. Stunning methods vary between species. Physical methods, such as captive bolt or nonpenetrating concussive stunning, are used for larger animals such as cattle, whereas electrical stunning and gas stunning (also known as controlled atmosphere stunning) are used for pigs and poultry. While the mechanism responsible is unclear, recent evidence has suggested that stunning methods may affect meat quality in certain species. For instance, gas stunning reduced drip loss in pork when compared with electrical stunning (Channon et al., 2000, 2002). This may be due to the ability of an electrical current to initiate muscle contraction and accelerate the rate of pH decline. Following stunning, the animals are exsanguinated, which is responsible for the animal's death. Exsanguination should occur rapidly after stunning to prevent a defect known as blood splash (small drops of blood) in the meat.

5.8 Development of meat quality attributes

During the postmortem period, muscle undergoes physical, biochemical, and energetic changes that result in its conversion to meat. The innate characteristics of muscles, as well as extrinsic conditions during slaughter, influence the development of key traits important to fresh meat quality: water-holding capacity, color, and texture. These fresh meat quality characteristics impact perception of freshness and consumer appeal, thereby influencing purchase decisions. Further, quality characteristics affect the utility of fresh meat in further processed products and relate to the palatability of cooked meat.

5.8.1 Water-holding capacity

Water constitutes approximately 75% of muscle. Of this water, roughly 85% is held within the myofibrillar protein network or between the thick and thin filaments; and the remaining water is distributed between myofibrils, between muscle cells, and between fascicles (Offer and Trinick, 1983). Due to its dipolar nature, water is attracted to electrically charged groups. Bound water is tightly associated with charged constituents, such as reactive groups on proteins, and

not easily removed by physical force. Immobilized or entrapped water is attracted to the bound water layer and is the most affected by physical and biochemical changes postmortem. Lastly, free water is loosely held in meat by weak capillary forces, and its flow from tissue is not hindered. Free water is not readily observed in prerigor meat, but can result from conditions that allow movement of immobilized water.

Structural changes in myofibrils influence the movement and binding of water during the conversion of muscle to meat. At physiological pH, there is an overall net negative charge of proteins; after slaughter, muscle pH declines and approaches the p*I* (Fig. 5.7). The attraction between positive and negative charges reduces the amount of sites available for water binding by the protein. Additionally, as net charge is reduced, there are fewer like charges. This limits electrostatic repulsion between structures, leading to myofilaments packing more closely together and reducing space for water to be trapped. This "net charge" effect primarily influences the capacity of meat to hold immobilized water.

Moreover, the decline in ATP postmortem causes myosin heads to become permanently bound to actin. Formation of rigor bonds causes shortening of the sarcomere and shrinkage of the myofibrillar lattice. Consequently, there is less space for water between myofilaments, and fluid moves to extramyofibrillar spaces. If cytoskeletal linkages are intact during the development of rigor, this shrinkage is transmitted to the muscle cell and, in turn, results in a reduced volume of the muscle cell and formation of gaps between muscle fibers and muscle bundles. In fact, muscle cell cross-sectional area may decrease in postmortem muscle (Offer and Cousins, 1992). Ultimately, shrinkage of muscle cells causes water to be expelled from the extramyofibrillar space into the space between muscle cells. Gaps between fascicles are the primary "drip channels" that permit

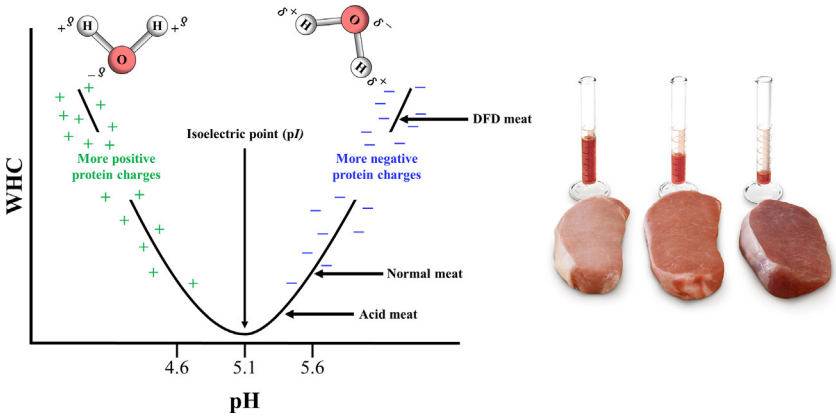

FIG. 5.7 The relationship between muscle pH and water-holding capacity. *(The pork chop pictures were used with permission from the National Pork Board.)*

the flow of water or purge from meat. The degradation of cytoskeletal proteins responsible for maintaining cell integrity, as well as changes in membrane permeability, facilitates the movement of extracellular water back into muscle cells (Kristensen and Purslow, 2001). Additionally, proteolysis of structures within the myofibril allows for water redistribution within the cell and swelling of the intramyofibrillar space, leading to an improvement in water-holding capacity (Melody et al., 2004).

The rate and extent of postmortem metabolism and pH decline are intimately related to water-holding capacity (see Chapter 14). If postmortem metabolism is limited and meat pH remains high, properties of proteins are more similar to living muscle, and thus water-holding capacity is high. Conversely, a low ultimate pH, such as the pH typically observed in RN^- muscle, is at or near p*I* of muscle proteins and reduces water-binding ability. In comparison, rapid pH decline while the muscle is still warm contributes to denaturation and reduced solubility of proteins. This loss in functionality diminishes water-holding capacity and is associated with high drip losses observed in PSE meat (Fig. 5.7).

5.8.2 Color

Myoglobin is the primary pigment responsible for meat color. In well-bled muscle, myoglobin constitutes 80%–90% of total pigment, and other proteins, such as hemoglobin and cytochrome *c*, play a relatively minor role (see Chapter 11). The content of myoglobin in muscle varies and is affected by several factors, including species, age, sex, and muscle. Red meat species, such as beef and lamb, have greater myoglobin content than poultry, while pork is intermediate. The physiological role of myoglobin is to bind oxygen and deliver it to mitochondria in muscle; thus, myoglobin content corresponds to metabolic and functional demands of muscle fibers. Myoglobin content is greater in mitochondria rich, oxidative fibers, and as a result, muscles with a high proportion of oxidative fibers appear darker. For instance, chicken thigh muscles contain more oxidative fibers, lending to "dark" meat, while the less oxidative, more glycolytic breast muscle is "white" meat.

Myoglobin is a water-soluble protein composed of eight α-helices and a nonprotein heme group. The heme prosthetic group lies within the hydrophobic pocket and contains a centrally located iron atom. This iron participates in six chemical bonds: four are with pyrrole groups of the heme ring, one is to a histidine residue of the protein, and one reversibly binds various ligands. The redox state of the iron and the ligand bound at the last site play a key role in determining the color of meat. For example, in the interior of meat or meat in vacuum packages, iron is reduced (Fe^{2+}) and has no ligand bound; this form is referred to as deoxymyoglobin, and color is dark purplish-red or purplish-pink. Once the meat is exposed to oxygen or allowed to "bloom," color becomes a bright red or pink color. This relatively stable form, oxymyoglobin, results from diatomic

oxygen (O_2) binding to the ligand site while iron remains in the reduced state. In comparison, iron in the oxidized state (Fe^{3+}) cannot bind ligands. Formation of metmyoglobin contributes to an undesirable brown color.

Inherent enzyme systems contribute to oxidation and reduction of myoglobin and hence, influence color and color stability. Mitochondria remain intact postmortem and can continue to metabolize oxygen (Cheah and Cheah, 1971; England et al., 2018). Oxygen consumption by mitochondria competes with myoglobin, and greater mitochondrial respiration decreases oxygen partial pressure, resulting in oxygen transfer to mitochondria, formation of deoxymyoglobin, and dark color. Further, mitochondria may promote reduction by transferring electrons from cytochrome to metmyoglobin (Aalhus et al., 2001; Tang et al., 2005), which improves meat color stability. Metabolites, such as lactate, succinate, and malate, can regenerate reducing equivalents and protect the stability of oxymyoglobin (Mohan et al., 2010; Ramanathan et al., 2011). However, free radicals generated by mitochondrial metabolism can promote metmyoglobin formation. Greater contents of antioxidant enzymes and chaperone proteins may relate to improved color stability (Joseph et al., 2012).

The rate and extent of pH decline postmortem influence meat color development and are linked to how water is held and distributed within the meat. For example, in PSE meat, there is more free extracellular water; this contributes to a more open structure that increases light reflectance. Precipitation of sarcoplasmic proteins onto myofibrillar proteins also contributes to pale color observed in PSE meat (Joo et al., 1999). In contrast, in DFD meat, there is a high proportion of intracellular water. This enhances light absorption by tissue and results in dark color.

5.8.3 Texture

Textural properties of fresh meat relate to the structure, consistency, and appearance of the cut surface. As evidenced by PSE and DFD meat, texture can vary considerably and may be closely related to postmortem pH decline and water binding. In extreme cases, PSE meat appears extremely soft and wet, with muscle separation and coarse texture. In contrast, DFD meat has a firm, rigid structure that retains its shape, and its high water-binding capacity gives the cut surface a sticky texture.

Postmortem events, as well as intrinsic factors such as fat and connective tissue, affect meat textural attributes (see Chapter 12). At rigor, permanent actomyosin cross-bridges decrease muscle extensibility and increase rigidity. Additionally, chilling causes fat within and between muscles to solidify, enhancing firmness. Eventually, proteolysis during meat aging resolves rigor and contributes to some loss in rigidity. The amount and strength of connective tissue influence the texture of fresh meat and, subsequently, are important factors dictating tenderness and palatability. The main component of connective tissue is collagen; cross-linking of collagen increases as an animal ages and imparts

increased mechanical strength and coarser texture. Within an animal, connective tissue content is greater in muscles that are heavily used for locomotion, and these muscles are coarser-textured and tougher. Conversely, muscles in the back, such as the tenderloin (*psoas major*), contain relatively low amounts of connective tissue and are finer-textured. Considering that connective tissue exists throughout muscle, thickness and cross-linking of perimysium and size of muscle bundles are the most relevant aspects of connective tissue that affect texture and palatability differences. Greater deposition of marbling (or intramuscular fat) between bundles may contribute to weakening of connective tissue structures and improvements in tenderness.

5.9 Postmortem handling and meat quality

The pH-temperature relationship early postmortem significantly influences the conversion of muscle to meat. These parameters are dependent on a combination of intrinsic and extrinsic factors. Specifically, muscle fiber properties and ante- and postmortem slaughter conditions affect energy metabolism and rate of ATP disappearance (e.g., stress, Section 5.7). In conjunction, temperature modulates postmortem metabolism by affecting the rate of enzymatic reactions. Muscle temperature is a function of carcass attributes, including size, muscle location, and fat cover, as well as postslaughter procedures and chilling. These interrelated factors determine the course of rigor development and, in turn, meat quality development.

5.9.1 Actomyosin toughening and sarcomere shortening

During rigor development, permanent actomyosin cross-bridges form and contribute to the loss of extensibility and the "stiffness of death." These stable cross-bridges, along with sarcomere shortening, are the basis for toughening of meat during the first several hours postmortem. The reported length for resting sarcomeres is ~2.5 μm, while those in rigor are generally 1.8–2.0 μm. Decreases in sarcomere length during the first 24 h postmortem coincided with increases in shear force in lamb *longissimus*, but toughening did not occur when the muscle was prevented from shortening (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). In stretched muscles, the sarcomeres are longer, and there is less overlap between myosin and actin. Consequently, fewer myosin heads can form cross-bridges with actin. In shortened muscles, proteins are more tightly packed, which facilitates protein-protein interactions. As a result, more force is required to shear through cooked meat.

The relationship between muscle shortening and toughness exhibits two phases. Shortening up to 20% of initial length has little effect on the shear force, but as shortening increases from 20% to 40%, shear force increases appreciably (Marsh and Leet, 1966). The myosin filament is about 1.6 μm; so, at 35% shortening, myosin filaments encroach the *Z*-disk, and there is significant overlap

and interaction with actin. In cases of maximum toughness, myosin appears to penetrate the Z-line and associate with actin in the neighboring sarcomere. The formation of rigor bonds between adjacent sarcomeres extends along the length of the myofibril, thus creating a uniform and strong structure resistant to shear forces (Marsh et al., 1974), with further increases in shortening up to 60%, toughness decreases and approaches initial values (Marsh and Leet, 1966). In this case, the sarcomeres are not uniformly shortened, rather some areas may be highly contracted (up to 80%), while others are less shortened (<35%) (Voyle, 1969; Marsh et al., 1974). In areas with less shortening, there are "weak" zones near the Z-line that are susceptible to fracturing when nearby myofibrils are highly contracted. In turn, this fracturing destabilizes the structure and contributes to the paradoxical decrease in toughness at high shortening values.

A muscle's capacity to shorten during rigor development is related to its orientation and attachments to the skeleton. The traditional method of suspending carcasses by the Achilles tendon stretches certain muscles, whereas others are permitted to shorten. For example, Achilles suspension stretches the *psoas major* and lengthens sarcomeres (~3.4 μm), whereas the *longissimus* is allowed to contract, resulting in shorter sarcomeres (1.9 μm). Conversely, suspending carcasses by the obturator foramen (hip suspension or tender stretch) restrains the *longissimus* and prevents sarcomere shortening (2.4 μm), while the *psoas major* shows greater shortening (2.4 μm) relative to Achilles suspension (Hostetler et al., 1970). Stretching muscles during rigor development limits cross-bridge formation and contraction, resulting in longer sarcomeres and improved tenderness.

After completion of rigor, further changes in muscle ultrastructure follow. Endogenous proteases in meat are responsible for degrading Z-disks and myofibrillar proteins, leading to a decrease in muscle tension and "resolution" of rigor. Protein degradation, leading to fragmentation of the muscle structure, increases tenderness (Section 5.10).

5.9.2 Muscle temperature

Muscle shortening is a temperature-dependent phenomenon. Locker and Hagyard (1963) demonstrated that minimal shortening (roughly 10%) occurred in excised muscle held at temperatures between 14°C and 19°C. As the temperature increased or decreased from this optimal range, the degree of shortening also increased. Colder temperatures contribute to greater shortening than higher temperatures (~50% shortening at 0°C vs ~30% at 40°C). Thaw rigor, cold shortening, and heat rigor are conditions caused by extreme muscle temperatures at the time of rigor development.

Thaw rigor occurs when muscle is frozen prerigor and then thawed. Perry (1950) observed that frog *sartorius* that had been frozen before rigor exhibited a 70% increase in shortening and substantial loss of water upon thawing. In muscles frozen prerigor, ice crystals form and damage the sarcoplasmic reticulum.

Thawing results in a sudden release of calcium in the presence of ATP. This hastens actomyosin ATPase activity, resulting in rapid and significant shortening. Slowing the rate of thawing reduced the degree of shortening and decreased drip loss (Marsh and Thompson, 1957). These authors also observed that shortening up to 50% was associated with relatively low drip loss, whereas faster thawing resulted in extreme shortening (up to 80%), rupture of the sarcolemma, and appreciable fluid loss. Imposing a load on muscle strips during thawing limits the effects of thaw rigor (Perry, 1950), whereas maintaining muscle attachments to the skeleton prevents thaw rigor in carcasses (Marsh and Thompson, 1958).

Cold shortening is similar to thaw rigor but less severe; it occurs when muscle is chilled to less than 14°C before the onset of rigor mortis (Locker and Hagyard, 1963). The lower the temperature, the greater the degree of muscle shortening. The rate and extent of cold shortening are also dependent on the degree of rigor onset at the time of cooling (Marsh and Leet, 1966). Greater cytosolic calcium concentration and greater ATP levels result in a more rapid and severe shortening. Increases in cytosolic calcium are attributed to anoxia-induced calcium release by mitochondria (Buege and Marsh, 1975) and reduced capacity of the sarcoplasmic reticulum to sequester calcium at colder temperatures (Cornforth et al., 1980). These observations are consistent with a greater propensity for cold shortening in red muscles. Red, oxidative muscles contain more mitochondria and less developed sarcoplasmic reticulum; the combination of augmented calcium release to the cytosol and limited calcium removal instigates cold shortening.

Other factors also contribute to the propensity for cold shortening. Species that have a longer delay time prior to the onset of rigor, such as beef and lamb, are at greater risk. Certain muscles, including the *longissimus*, are more prone to shortening because of fiber attachment, as fibers are anchored to the skeleton at one end only. However, even if the muscles are restrained by attachment to the skeleton, regions within a muscle that are exposed to cold temperatures may exhibit shortening (Marsh and Leet, 1966). Accordingly, the susceptibility of a muscle or muscle region to cold shortening also depends on fat cover and location on the carcass.

Maintaining muscle at elevated temperatures (up to 43°C) causes significant shortening. Heat rigor is more similar to postmortem events in PSE muscle. Higher temperatures hasten metabolism and ATP depletion, leading to early onset of rigor while the carcass is still warm (Marsh, 1954). This combination of warm temperature and low pH diminishes protein functionality by causing protein denaturation and reducing solubility. The extent of shortening at high temperatures (25–43°C) is less than that observed for cold shortening.

Clearly, temperature decline has an important impact on toughening during the conversion of muscle to meat. These defects and species-specific quality concerns should be taken into account when designing chilling procedures. For beef and lamb, the longer delay period prior to rigor increases the probability of cold shortening; chilling regimes to enhance quality aim to hasten metabolism and pH decline and avoid low muscle temperature at high pH. Delayed

chilling, which involves holding carcasses at ambient temperatures for several hours prior to chilling, promotes faster postmortem metabolism. In addition to preventing cold shortening, delayed chilling may also improve tenderness by expediting aging and proteolysis. The potential benefit of delayed chilling on tenderness depends on the temperature and duration of delayed chilling, as well as carcass characteristics (e.g., size or fat cover). Regardless, delayed chilling is not practical in high volume commercial settings due to production constraints and potential food safety concerns.

On the other hand, rapid chilling is more favorable for hog carcasses, which are more prone to quality issues associated with accelerated postmortem metabolism (i.e., PSE). Several parameters of chilling, including temperature, air velocity, and duration, can be modified to facilitate faster heat removal than conventional chilling. This rapid or blast chilling hastens muscle temperature decline, thereby slowing postmortem metabolism and pH decline. As a result, rapid chilling reduces the incidence of PSE pork and improves water-holding capacity. There are conflicting reports regarding the effect of blast chilling on pork tenderness. However, these inconsistencies may be related to variations in chilling parameters, as well as the interaction of chilling with other factors, including genetics, stunning method, and carcass size.

5.9.3 Electrical stimulation

Electrical stimulation involves applying an electrical current to a carcass early postmortem in order to induce muscular contractions. Electrical stimulation accelerates pH decline and ATP utilization, which permits earlier onset and completion of rigor. This is advantageous in species such as cattle, which have a longer delay period before rigor, but electrical stimulation is not recommended in swine because accelerated postmortem metabolism exacerbates pork quality problems. Electrical stimulation improves tenderness in beef and lamb, which has led to widespread use in these industries. Enhanced tenderness has been attributed to the prevention of cold shortening, increased activity of enzymes that mediate proteolysis, and physical disruption and fracturing of muscle structure by intense contractions. Electrical stimulation is an effective means for improving the tenderness acceptability of carcasses, particularly for beef carcasses that are inherently tough (Ho et al., 1997; Roeber et al., 2000). Other benefits of electrical stimulation include more rapid development of quality characteristics, including more desirable lean color and more youthful appearing lean. Electrical stimulation followed by blast chilling of beef carcasses can maintain beef quality while also reducing chilling times and shrink loss (Aalhus et al., 2001).

5.9.4 Accelerated processing

Accelerated processing involves conducting processing steps such that the slaughtering, boning, and packing of meat are performed within a single day.

Removal of bone and fat while the meat is still warm reduces the space and energy required for cooling and increases throughput. However, tenderness of hot or warm boned cuts is a major concern; muscles that are stretched on the carcass are allowed to shorten when removed from bone, and they will cool more rapidly. The risk of cold and rigor-induced shortening can be reduced by applying electrical stimulation to carcasses to accelerate the onset of rigor mortis and by maintaining temperatures that minimize shortening during rigor development (14–19°C).

Prerigor meat has many advantages in sausage production. Meat is removed from carcasses within 1 h after slaughter and immediately followed by grinding, blending with salt, and chilling. These steps disrupt the muscle structure, increase protein solubility, and result in a higher pH (>6.0) than postrigor meat. Higher pH and improved protein solubilization enhance water binding, which positively impacts yield and eating characteristics.

5.10 Aging and proteolysis

Once an animal is harvested for meat, an aging period (maturation) typically follows. An aging period is the time between the slaughter of the animal and the consumption of the meat. Typically, aging occurs under refrigeration temperatures (2–4°C). During the aging period, a number of chemical reactions occur that change the texture, taste, and aroma of meat. However, the primary goal of aging is to tenderize meat. In general, as meat is aged, tenderness improves. This aging period varies based on species, primarily based on the need for tenderization and the ability to age the meat without creating off-flavors from lipid oxidation. Beef and lamb are typically aged for longer periods compared with pork and poultry. Thus, much of our understanding of postmortem tenderization was determined in beef and lamb.

Postmortem tenderization arises from a process known as proteolysis (see Chapter 12). Proteolysis is the breakdown of proteins into smaller constituents comprised of polypeptides and amino acids. Typically, this process is accomplished by enzymes known as proteases. The proteases responsible for aging of meat are naturally present and function in living muscle. However, it is still not fully clear what proteases are responsible for the tenderness improvements during aging. In order for a specific protease to be potentially involved in postmortem proteolysis, it must meet three criteria outlined previously (Goll et al., 1983; Koohmaraie, 1994). These requirements state that any participating protease must (1) be localized within the skeletal muscle cell, (2) have access to the myofibrillar and/or costameric proteins, (3) have the ability to degrade the same proteins that are degraded during postmortem proteolysis. From these requirements, multiple protease systems may contribute to postmortem proteolysis. These include the lysosomal cathepsin system, the calpain system, the caspase system, and the proteasome system.

5.10.1 The calpain system

The calcium-activated cysteine protease or calpain system is thought to be the protease primarily responsible for postmortem tenderization. At least three isoforms have been identified in skeletal muscle: calpain-1 (μ-calpain), calpain-2 (m-calpain), and calpain-3 (p94). Little is known about calpain-3 outside of its association with titin in the myofibrillar structure. The other two calpain proteases are named for the level of calcium required for their activation; μ-calpain requires micromolar (μM) levels of calcium, whereas m-calpain requires millimolar (mM) levels of calcium. Because the calcium is not thought to increase to millimolar levels postmortem, most researchers have identified that calpain-1 is the primary calpain protease active postmortem. Though, new research suggests that calpain-2 may play a role in postmortem tenderization in pork and beef (Pomponio and Ertbjerg, 2012; Colle and Doumit, 2017).

Calpain-1 is a heterodimer composed of an 80 kDa catalytic subunit and a 28 kDa regulatory subunit. This protease targets specific myofibrillar and costameric proteins at specific amino acid sequences (cleavage sites). These proteins include titin, nebulin, troponin-T, tropomyosin, desmin, talin, vinculin, and filamin. However, calpain-1 has limited (if any) activity on the two most abundant proteins in muscle, actin and myosin. Calpain-1 is unable to degrade proteins into their amino acid components.

Calpain-1 experiences an autolytic cleavage of its amino acid structure, which ultimately reduces the 80 kDa subunit to a 76 kDa fragment and the 28 kDa subunit to an 18 kDa fragment. Both forms of the protease exhibit proteolytic activity, but autolysis reduces the calcium requirement necessary for activity (Huff Lonergan et al., 2010). The activity of calpain-1 is pH- and temperature-dependent. Therefore, the rate of pH decline and carcass chilling both impact the ability of calpain-1 to tenderize meat. For instance, as postmortem muscle pH and carcass temperature drop, the sarcoplasmic reticulum loses its ability to sequester calcium. Thus, calcium in the cytosol increases to levels required for calpain-1 autolysis.

The calpain system is also comprised of an additional protein, calpastatin, which is a specific inhibitor of calpain-1 and -2 that limits activity. By limiting activity, this inhibitor limits the ability of calpain-1 to improve tenderness postmortem. Hence, as calpastatin increases, abundance meat tenderness tends to decrease. Particular breeds of cattle express increased levels of calpastatin. For instance, *Bos indicus* cattle (e.g., Brahman breed) tend to be tougher than *Bos taurus* cattle (e.g., Angus), and this is due, in part, to the increased levels of calpastatin in the *Bos indicus* muscles (Ferguson et al., 2000; Wright et al., 2018). Furthermore, feeding growth promotants (i.e., β-agonists) increases the calpastatin level in muscle and decreases tenderness (Koohmaraie and Shackelford, 1991; Geesink et al., 1993). For the most part, calpain-1 is considered the primary protease responsible for postmortem proteolysis. However, calpain-1 is not the sole protease active in postmortem muscle.

5.10.2 The caspase system

The caspase enzymes are a family of proteases that participate in programmed cellular death (apoptosis). Apoptosis can be executed via two main signaling pathways, namely the extrinsic pathway and the intrinsic pathway. The former pathway is triggered by the binding of death ligands to their respective cell membrane death receptors, whereas the intrinsic pathway, also known as the mitochondrial pathway, is owing to an increase in mitochondrial permeability. Regardless of the means by which apoptosis is initiated, ultimately a number of caspases are activated. Apoptotic caspases can be divided into two general classes: effector caspases (e.g., caspase-3, -6, and -7) and initiator caspases (e.g., caspase-2, -8, -9, and -10) that cleave and activate downstream effector caspases. Once activated, effector caspases target several myofibrillar proteins, including actin, myosin, desmin, α-actinin, and troponin-T, resulting in disassembly of myofibrillar structures.

While extrinsic and intrinsic apoptotic pathways are partially linked, a recent study by Huang et al. (2016) indicated that the mitochondrial pathway, involving caspase-3 activation, is the main apoptotic pathway active postmortem. Postmortem mitochondrial calcium overload increases mitochondrial ROS production, which, in turn, results in mitochondrial swelling and eventually the rupture of the outer membrane. As a result, many of the loosely held membrane-bound proteins and those located in the intermembrane space, such as cytochrome *c*, are released into the cytosol. Cytosolic cytochrome *c* triggers the activation of the initiator caspase-9, which then activates downstream caspases including caspase-3, the main effector caspase involved in the execution phase of apoptosis.

The potential role of caspase-3 in meat tenderization has been previously investigated, showing that in vitro incubation of porcine myofibrils with recombinant caspase-3 enhances the degradation of various myofibrillar proteins (Kemp and Parr, 2008). Further, the degradation of titin, nebulin, desmin, and troponin-T was significantly inhibited by DEVD-CHO, a specific inhibitor for caspase-3 (Huang et al., 2009). In a more recent study, Dang et al. (2022) indicated that caspase-3 may contribute to improved proteolysis and tenderness of beef steaks that were subjected to power ultrasound treatment. Caspase-3 can also indirectly contribute to postmortem proteolysis by degrading and deactivating calpastatin, which allows calpain-1 to maintain activity (Huang et al., 2014). In line with these findings, proteomic-based research showed that enhanced beef tenderness is associated with greater susceptibility to apoptosis (Gagaoua et al., 2015). Moreover, the expression of heat shock proteins, a family of proteins that possess an antiapoptotic effect, was found to be negatively correlated to meat tenderness (Bjarnadóttir et al., 2011). Although there are considerable observations suggesting that the caspase system can participate to postmortem proteolysis and tenderization, the role the caspase system plays in postmortem tenderization is at best limited compared with calpain-1.

5.10.3 The cathepsin and proteasome systems

Cathepsins are ubiquitous lysosomal proteolytic enzymes that are classified according to their catalytical mechanism into: cysteine (cathepsins B, H, L, and X), aspartic (cathepsins D and E), and serine (cathepsins A and G) proteases. Cathepsins require acidic pH for optimal activity, similar to that found in fresh meat; thus, cathepsins have been recognized as potential proteases involved in postmortem proteolysis. Moreover, exogenous cathepsins have been shown able to degrade several myofibrillar proteins in vitro, including actin and myosin. However, based on several observations, researchers have largely concluded that cathepsins play little to no role in postmortem proteolysis. First, cathepsins are confined within the lysosomes, and disruption of lysosomes does not seem to occur during meat aging. Second, although ubiquitously expressed, cathepsins show variable expression in different tissues, with relatively low expression levels in skeletal muscle (Bechet et al., 2005). Third, there is no convincing evidence suggesting that actin and myosin undergo proteolytic degradation postmortem, these being primary substrates for cathepsins. In addition, inhibition of cathepsins had no effect on postmortem proteolysis and tenderization (Hopkins and Thompson, 2001).

The proteasome is a protease capable of degrading both sarcoplasmic and myofibrillar proteins into their amino acid components. Proteasomes are abundantly localized both in the sarcoplasm and nuclei of mammalian skeletal muscle fibers. In living tissue, the proteasome is part of the ubiquitin system and involved in protein turnover. Under simulated postmortem conditions (temperature, pH, and ionic strength), the proteasome is capable of degrading muscle proteins. Koohmaraie (1992) indicated that calpain-1 initiates the degradation and disassembly of the myofibrillar structure, which allows the proteasome to subsequently act on the partially degraded proteins. However, the same author concluded that the proteolytic activity of the proteasome does not contribute to the overall improvement in meat tenderness during aging.

5.11 Conclusions

The conversion of living muscle to consumable meat is a complex process that involves energetic, chemical, and physical changes. pH decline is the most significant change occur during the postmortem period. This is because the drop in pH is fundamental for the development of meat characteristics and microbial resistance of the fresh product. The rate and extent of postmortem pH decline can drastically influence meat quality. The rate of postmortem metabolism is mainly controlled by the activity of muscle ATPases, while substrate availability (glycogen and adenine nucleotides), glycolytic enzymes abundance and activity, and buffering capacity are factors dictating the extent of postmortem metabolism. PSE, DFD, and acid meat are examples of how abnormal rates and extents of postmortem metabolism can negatively influence meat quality. Proper

management to minimize antemortem animal stress and optimize postmortem handling of carcasses can efficiently reduce the incidence of meat defects.

The conversion of muscle from highly excitable and extensible tissue into a rigid tissue as rigor mortis develops is the major physical change that occurs postmortem. Fortunately, during the aging period, enzymatic degradation of myofibrillar proteins improves meat tenderness and develops meat flavor. Calpain-1 is the primary protease responsible for postmortem tenderization, while the role of calpain-2, caspases, cathepsins, and proteasomes is not well understood.

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Chapter 6

Meat microbiology and spoilage

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6.1 Introduction

A large diversity of meat products exist on the market that are dedicated to human consumption. Meat products originate from various birds (mainly chicken, turkey, ducks, …) or mammals (mainly pork, beef, lamb) whose carcasses and primary cuts, after slaughtering, are processed to a large variety of raw or processed food stuffs. Consequently, the nature of the microbial communities present on meat is very diverse as it depends on the animal it is issued and on the nature of the subsequent processing steps. Fresh meat is a substrate enabling microbial growth as it provides nutrients (sugars, amino acids, vitamins or cofactors, …), and it possesses pH and Aw values usually compatible with microbial development. Therefore, after initial contamination of raw meat, the number of microbial cells may rapidly increase. Then, the physicochemical parameters encountered during processing steps and storage can shape the microbial communities in multiple ways influencing their dynamics and then the amount of the final population reached by each of the initial contaminants. Indeed, production processes such as smoking, fermenting, or drying, the addition of various preservatives, and temperature or atmosphere packaging procedures in usage differently influence growth of microorganisms during the shelf life of meat products. In addition, storage conditions, such as the use of modified atmosphere packaging, also influence metabolic activities of bacteria. Consequently, meat can also be considered as an environment that presents stressing or unfavorable conditions the microbial communities have to cope with. While species belonging to firmicutes and proteobacteria are commonly found in the dominant microbiota of meat, their relative abundance may vary depending on the type of meat product, processing, or storage conditions and lead to the occurrence of various spoilage manifestations.

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6.2 Recent advances in meat microbiology and identification of the main bacterial species involved in spoilage

During processing and storage, bacteria present in meat microbiota interact together (biotic interactions) and with meat substrate (abiotic interactions). Meat enables microorganisms to grow and consequently to express many metabolic functions. Among the diverse microorganisms that can develop on meat, only a small proportion can spoil the products through their metabolic activities. Microbial metabolism is characterized by the consumption of compounds and the production of new ones. The best example is that of lactic acid bacteria (LAB), which produce lactic acid out of the carbon sources they consume from meat. Lactic acid may have different effects, desirable or undesirable, depending on the type of meat product. It is one of the important steps of fermented sausage manufacturing: it can influence the texture by inducing protein precipitation and improve the microbial safety due to the acidic nature of lactic acid and the pH drop, which exert an antimicrobial effect. Lactic acid also participates to acidic taste, an important trait of fermented meat but unwanted in other products. The consequences of microbial metabolic activity may lead to the spoilage of different raw or cooked meat products. Therefore, microbial spoilage must be considered as a complex process, which depends both on the microorganisms and on their biotic and abiotic interactions. Several microbial metabolic pathways leading to identified spoiling molecules that may affect the color, the texture, and/or the odor and taste of meat and meat products are known. However, in many cases, even when defects are observed and correlated to the presence of identified bacterial species and to the production of known spoilage molecules, the exact mechanism leading to spoilage is still unknown or only speculated. Thus, for a better understanding of meat spoilage, knowledge on the microbial actors present in meat, their interactions, and their metabolic activities is required. In the past few years, several studies contributed to better assess the question. The development of new methods for describing the microbial species composing meat microbiota, mainly based on DNA sequencing, gave a better description on who is there, with the identification of new or unsuspected species. Genome analyses also revealed functions that are potentially expressed by meat contaminants. And finally, using meat products or simplified models (meat matrix models and/or simple bacterial consortia), metabolic functions contributing to bacterial adaptation or metabolism were emphasized.

6.2.1 Microbiota of various meat products, origin, and shaping

Meat microbiology studies based on cultural methods using different more or less selective media are still numerous. These bring useful information on the microbial counts of various populations, as total aerobic, anaerobic, psychrophilic, or mesophilic counts, for instance. Selective media enable

enumeration of microbial families (yeasts or molds, LAB, enterobacteria as examples) or are dedicated to a more or less specific counting of species or genera (*Brochothrix thermosphacta*, *Pseudomonas* sp.). Such approaches can be associated to the subsequent identification of isolates through various molecular methods (see as examples Fuertes-Perez et al., 2019 or Hilgarth et al., 2018a, 2019). Although powerful and informative, cultural-based methods may suffer from biases due to the fastidious growth of some bacteria on the media used for enumeration and also to the "a priori" of the study design due to the media that are chosen. On the other hand, these analyses led to the isolation and characterization of species of interest. This is the case of a novel *Pseudomonas* species (*Pseudomonas carnis*) initially isolated on STAA plates, dedicated to the isolation of *B. thermosphacta*, during a routine analysis of refrigerated poultry processing meat and also isolated from spoiled pork shop (Lick et al., 2020). As well *Lactococcus carnosus* and *Lactococcus paracarnosus*, two novel species, close to *Lactococcus piscium*, issued from minced beef were recently described (Hilgarth et al., 2020).

In less than a decade, the development of noncultural methods initially used to describe environmental and digestive tract microbiota benefited also to food microbiology investigation. Based on DNA extraction without any prior cultivation step, such techniques escape the above mentioned bias and will undoubtedly benefit the generation of knew knowledge of meat microbial contaminants (Jagadeesan et al., 2019). Yet, most of these recent reports used amplicon sequencing, but still some used other methods as PCR-DGGE (Wang et al., 2019). Amplicon sequencing was performed using mainly the V3-V4 variable regions of 16S rDNA, often enabling identification at genus or even species level. Targeting *gyrB* was also successfully used for an improved resolution at species level and for ensuring more reliable results (Poirier et al., 2018b). Metagenomics (whole DNA sequencing) and reconstruction of pangenomes, although less frequently used, provide information at strain level and on the potential metabolic activities generated by meat microbiota. Table 6.1 summarizes the main questions that could be addressed through non-cultural DNA-based analyses. Almost all types of meats (poultry, pork, lamb, and beef) have been reanalyzed such a way, and most studies focused on the growth dynamics during storage or compared different types of packaging. This gave a better view on the bacteria present in meat and their relative abundance along the shelf life or regarding storage conditions. Most results, in accordance with previous cultural methods, identified the major taxa occurring in meat products. Although taxa assignment depends on the nature and the length of genes used for amplicon sequencing, a core microbiota common to all meat products can be drawn. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria are the main phyla composing meat bacterial ecosystems as previously reported (Chaillou et al., 2015). A complete overview of the genera observed in various raw and processed meat products using amplicon sequencing methods has been recently published (Van Reckem et al., 2020). The dominant genera shared by different meat products as are listed below.

TABLE 6.1 Survey of most recent studies dedicated to meat microbiota and new information generated.

Type of meat product	Addressed question	Methodology	Conclusion	Reference
Mutton chop rolls	Storage over time	16S amplicon sequencing	Microbiota dynamics and meat color	Zhang et al. (2020a)
Lamb meat	Impact of atmosphere packaging	PCR-DGGE	Microbiota is shaped by vacuum packaging	Wang et al. (2019)
Vacuum packed lamb meat	Origin and dynamics of bacterial communities	16S amplicon sequencing	Skin microbiota persisting in abattoir as origin, microbiota dynamics over storage time	Kaur et al. (2017)
Chicken legs	Origin of microbiota diversity	16S amplicon sequencing	Atmosphere packaging and production line influence microbiota	Rouger et al. (2018)
Chicken cuts	Impact of broiler production system	16S amplicon sequencing	Production line but not production system influence microbiota	Lee et al. (2019)
Broiler carcasses	Impact atmosphere packaging	Metagenomics	Microbiota is shaped by packaging atmosphere	Wang et al. (2017)
Chicken carcasses	Contamination routes	16S amplicon sequencing	Contamination of the feather follicles	Zhang et al. (2020b)
Chicken carcass/ production line	Contamination routes	16S amplicon sequencing	Identification of putative contamination steps and microbiota dynamics over storage time	Lauritsen et al. (2019)
Chicken carcass/cuts	Impact of season, region, and producer	16S amplicon sequencing and metagenomics	Seasonal effect more important than region or producer effect	Kim et al. (2019)

TABLE 6.1 Survey of most recent studies dedicated to meat microbiota and new information generated—cont'd

Type of meat product	Addressed question	Methodology	Conclusion	Reference
Chicken breast	Impact of broiler production system	Metagenomics	Packaging type and processing environment shape microbiota	Li et al. (2020)
Roast chicken	Impact of atmosphere packaging	16S amplicon sequencing	Microbiota is shaped by MAP	Huang et al. (2020)
Ground beef, raw pork and turkey sausages	Description of microbiota at species level	16S + *gyrB* amplicon sequencing	*gyrB* amplicon sequencing accurate for firmicutes and proteobacteria	Poirier et al. (2018a, b)
Beef meat	Impact of production lots	16S amplicon sequencing	No incidence of production, contamination route may be similar for different taxa	Säde et al. (2017)
Beef meat	Impact of air or vacuum-packaging	Metagenomics (pangenome)	Microbiota (at *Pseudomonas fragi* strain level) is shaped by packaging atmosphere	De Filippis et al. (2019)
Beef steaks	Impact of atmosphere packaging	16S amplicon sequencing	Microbiota is shaped by packaging atmosphere	Yang et al. (2018)
Beef steaks	Impact of atmosphere packaging	16S amplicon sequencing	Microbiota is shaped by packaging atmosphere and it impacts color and myoglobin status	Yang et al. (2020)
Beef steaks	Impact of sanitizing treatments	16S amplicon sequencing on cDNA	Microbiota dynamics over storage time and treatment effects	Botta et al. (2018)

Continued

TABLE 6.1 Survey of most recent studies dedicated to meat microbiota and new information generated—cont'd

Type of meat product	Addressed question	Methodology	Conclusion	Reference
Minced beef	*Pseudomonas* diversity	MALDI-TOF identification of isolated colonies	Adaptation of *Pseudomonas* sp. to meat	Hilgarth et al. (2019)
Raw/minced beef	Seasonal and production region effect	16S amplicon sequencing	A shared core microbiota with seasonal and geographical variations	Hwang et al. (2020)
Raw pork sausage	Impact of salt reduction and atmosphere packaging	16S amplicon sequencing	Microbiota shaping and spoilage	Fougy et al. (2016)
Raw pork sausage	Storage over time and spoilage	16S amplicon sequencing	Microbiota dynamics and spoilage	Raimondi et al. (2018)
Pork meat	Storage over time	16S amplicon sequencing	Microbiota dynamics and quality	Li et al. (2019)
Minced pork meat	Impact of atmosphere packaging	16S amplicon sequencing	Microbiota is shaped by MAP	Cauchie et al. (2020)
Vacuum-packed pork	Cause of blown pack spoilage	16S amplicon sequencing	Evidence for *Clostridium estertheticum*	Zhang et al. (2020c)
Cooked ham	Impact of processing steps	*gyrB* amplicon sequencing	Processing line shapes microbiota and type of spoilage	Zagdoun et al. (2020)
Pig carcasses	Contamination routes	16S amplicon sequencing	Identification of taxa specific contamination points	Zwirzitz et al. (2020)

Among proteobacteria, *Pseudomonas* (with mainly *Pseudomonas fragi*, *Pseudomonas lundensis, and Pseudomonas fluorescens*), *Acinetobacter* (*Acinetobacter lwoffii*), and *Psychrobacter* were reported in all studies. Other genera such as *Shewanella, Serratia, Janthinobacterium* (*Janthinobacterium*

lividum), *Iodobacter, Rahnella*, and *Citrobacter* were present in a majority, and *Photobacterium and Budvicia* only in some. The firmicutes *B. thermosphacta, Carnobacterium* (*Carnobacterium divergens*, *Carnobacterium maltaromaticum*) appear to be shared by all meat products, whereas lactobacilli (*Latilactobacillus sakei*, *Latilactobacillus curvatus*, *Dellagliosa algida*—formerly *Lactobacillus algidus* (Zheng et al., 2020), *Lactococcus* (*L. piscium*), and *Vagococcus* (*Vagococcus fluvialis* and *Vagococcus salmoninarum*) by most. Globally, lactobacillales were systematically present in all types of meat. Among *Bacteroidetes*, the genus *Chryseobacterium* was depicted in most studies, *Myroides* and *Flavobacterium* only in some, and *Arthrobacter* was the common genus belonging to *Actinobacteria* present in meat.

The origin of contamination could be assessed in several studies. For instance, the composition of chicken meat microbiota mainly depends on slaughtering and packaging conditions (Rouger et al., 2018; Lauritsen et al., 2019; Lee et al., 2019; Li et al., 2020). Slaughtering processing steps of chicken carcasses and cuts appear to be the most probable contamination route of *J. lividum* (Lauritsen et al., 2019). Compared with that of mammals, poultry slaughtering process involves water bathes, and meat (carcasses and cuts) is sold with the skin. Feather follicles present on the skin can be contaminated by microbiota from the gut during the evisceration, defeathering, chilling processes, and by the chilling water (Zhang et al., 2020b). Lamb meat initial contamination seemed also to originate from bacteria subsisting in the abattoir environment but issued from animal skin and gut (Kaur et al., 2017) as shown for *Carnobacterium* (Mills et al., 2018). A complete survey of a pork-processing plant also identified animal and surfaces of equipment or employees gloves as contaminant sources and could associate some processing steps as contamination routes for specific taxa (Zwirzitz et al., 2020). Concerning processed pork meat, the slicing line of cooked ham was demonstrated as an important contamination route that impacts microbiota composition during subsequent storage with the dominance of specific species (*C. divergens* or *Leuconostoc carnosum* plus *Serratia proteamaculans* depending on the slicing line) and consequently the development of different types of spoilage (Zagdoun et al., 2020). These results confirm meat processing environments as important contamination sources. This may be correlated to the presence of multispecies biofilms as shown in beef, pork, and poultry processing environments, with *Brochothrix, Pseudomonas*, and *Psychrobacter* as the main genera composing those (Wagner et al., 2020).

To date, many studies have monitored the microbiota composition during storage. Most observed an increase of bacterial loads overtime and a concomitant decrease in the number of dominant species, i.e., species evenness, richness, and diversity. Actually, the most competitive species become dominant to the detriment of the others, which become then undetectable through amplicon sequencing methods. A large part of the recent literature on meat microbiota has been dedicated to evaluate the influence of atmosphere packaging. Depending on meat type and geographical usages, vacuum or modified atmosphere packaging

is often used for ensuring longer shelf life of meat. Oxygen-enriched atmosphere and vacuum packaging are often used for red meat in particular beef, and vacuum packaging associated to chilling for long-distance export of lamb, whereas carbon-dioxide-enriched atmosphere is more common for processed poultry or pork meat such as cooked ham or raw sausages. Intensive research has been devoted to study the influence of atmosphere on raw beef safety and quality in the past and often extrapolated to other meat although what was observed for beef might be not true for other types of meat.

Recent studies have shown that gas mixtures differently influence bacterial communities in different meat products. Applying carbon-dioxide-enriched atmosphere packaging on raw pork sausages with reduced salt lowered bacterial diversity overtime but induced a more important spoilage than vacuum packaging (Fougy et al., 2016). In minced pork meat, *Pseudomonas* sp. were more abundant under air packaging than under a gas mixture composed of 30% CO_2–70% O_2, while the opposite situation was observed for *B. thermosphacta* and *Leuconostoc* sp., and the dominance of *Photobacterium* sp. was more depending on samples than on packaging mode (Cauchie et al., 2020). In beef, O_2-enriched atmosphere, compared with $CO + CO_2$ atmosphere, favored *B. thermosphacta* and *Pseudomonas* sp., whereas $CO + CO_2$ favored *Vagococcus* sp., *Leuconostoc* sp., and *Lactococcus* sp. overtime (Yang et al., 2018). As well O_2–enriched atmosphere was more favorable to *Pseudomonas* sp., mostly *P. fragi*, compared with vacuum packaging, whereas under vacuum packaging *Leuconostoc* sp., *L. sakei*, and *Photobacterium* sp. were more abundant (De Filippis et al., 2019). In lamb, air packaging was more favorable to *P. fluorescens*, *P. fragi*, and *Acinetobacter* sp. and vacuum packaging to *Carnobacterium* (*C. divergens* and *C. maltaromaticum*) and *L. piscium* (Wang et al., 2019). Thus, globally the presence of oxygen is associated to pseudomonads, but not only, and the absence of oxygen is associated to LAB, but not only, showing the difficulty to draw general rules and suggesting various bacterial interactions depending on gas present in packs.

6.2.2 New evidences, new genomes

The genome sequence of many species involved in meat spoilage is now available. Although several Gram-positive and Gram-negative species were described since a long time as meat spoilers, their genome sequence has been determined in the last years. Other species were reported only recently as belonging to the dominant microbiota of spoiled meat products by using abovementioned methods without any prior cultural step. For instance, *Photobacterium* species such as *Photobacterium phosphoreum, Photobacterium iliopiscarium*, which were previously described as emblematic of sea food spoilage, have been reported in various meat products (Chaillou et al., 2015; Li et al., 2019) as a potential novel species, *Photobacterium carnosum* (Hilgarth et al., 2018b) that still requires validation (Oren and Garrity, 2018). Other species,

for instance, *D. algida*, which was undetected by cultural methods because of its poor growth in the media routinely used was revealed as an important bacterium to consider in some meat products (Säde et al., 2020).

Table 6.2 summarizes the articles dedicated to the determination of genome sequence from strains involved or putatively involved in meat spoilage. In some cases, genome sequences being available from different strains, comparative genomics enabled to identify functions related to meat adaptation or spoilage.

TABLE 6.2 List of published genome sequences issued from bacterial species involved in meat products spoilage.[a]

Species	Number/origin of sequenced strains	Reference
Brochothrix thermosphacta	12/pork, beef, veal, and lamb meats	Stanborough et al. (2017)
	4/shrimp, chicken, beef slaughterhouse, salmon	Illikoud et al. (2018a, b)
	1/horse meat	Poirier et al. (2018a)
	1/beef meat	Kolbeck et al. (2019)
Carnobacterium divergens	1/beef meat	Poirier et al. (2018a)
	1/chicken meat	Kolbeck et al. (2019)
Carnobacterium maltaromaticum	1/chicken meat	Kolbeck et al. (2019)
Clostridium sp.	2/lamb and venisson meats	Palevich et al. (2020a, b)
Clostridium algidicarnis	2/lamb meat	Wambui et al. (2020c)
Clostridium estertheticum	1/beef meat	Yu et al. (2016)
	1/beef meat	Palevich et al. (2019)
	1/lamb meat	Palevich et al. (2020)
	1/lamb meat	Wambui et al. (2020d)
Clostridium gasigenes	3/lamb and veal meats	Wambui et al. (2020a, b)
Dellagliosa algida	1/beef meat	Poirier et al. (2018a)
	1/beef meat	Hultman et al. (2020)
	2/pork and beef meats	Säde et al. (2020)
Latilactobacillus fuchuensis	1/beef meat	Poirier et al. (2018a)

Continued

TABLE 6.2 List of published genome sequences issued from bacterial species involved in meat products spoilage—cont'd

Species	Number/origin of sequenced strains	Reference
Lactococcus piscium	2/beef meat	Poirier et al. (2018a)
	1/chicken meat	Andreevskaya et al. (2015)
Leuconostoc carnosum	12/pork meat	Candeliere et al. (2020)
Leuconostoc gasicomitatum	1/beef meat	Poirier et al. (2018a)
	1/beef meat	Kolbeck et al. (2019)
	1/chicken meat	Johansson et al. (2011)
Leuconostoc gelidum	1/beef meat	Kolbeck et al. (2019)
Photobacterium carnosum	1/chicken meat	Hilgarth et al. (2018a, b)
Pseudomonas fragi	10/milk, beef and lamb meats	Stanborough et al. (2018a, b)
Pseudomonas lundensis	8/milk, beef meat	Stanborough et al. (2018a, b)
	1/beef meat	Poirier et al. (2018a)
Weissella viridescens	1/beef meat	Poirier et al. (2018a)

[a]*Only references of articles targeting meat spoilage have been selected.*

Several authors noticed cell surface components, transporters, transcription regulators, and meat resource utilization as the main functions representing intraspecies variability. Wambui et al. (2020a, b, c, d) noticed a large variability among *Clostridium* genomes associated in particular to carbon source utilization, but also to protein degradation. As well, Palevich et al. (2021) noticed a large and variable number of degradative carbohydrate-active enzymes (CAZymes) among *Clostridium* species, suggesting a different ability to cope with meat carbohydrates depending on strains or species. Conversely the comparison of 17 strains of *Leuconostoc carnosum* revealed a poor intraspecies diversity at the chromosome level and a shared adaptation of strains to nitrogen sources of meat (Candeliere et al., 2021). Nevertheless, these authors observed a large diversity in the plasmid content (1–4 per strain) and in the plasmid encoded functions, which were associated mainly to amino acid metabolism or transport, exopolysaccharides, and stress resistance. *B. thermosphacta* comparative genomics also showed a poor genomic variability, limited to transport and cell surface functions, transcriptional regulators, and plasmid, phage and restriction/modification systems (Stanborough et al., 2017; Illikoud et al., 2018b). *B. thermosphacta* genome comparison also suggested a strain-dependent adaptation to

the meat environment and single nucleotide polymorphism (SNP) modulating spoilage ability (Illikoud et al., 2018b). The adaptation of bacteria from the *Pseudomonaceae* family through gene acquisition for ensuring growth under cold and hostile conditions encountered during meat storage has been proposed, based on a large comparative genomics analysis of bacteria known as meat contaminants (Saenz-García et al., 2020). Comparative genomics of *P. fragi* and *P. lundensis* indeed showed the large genome size (about 5 Mb) encountered in these species (Stanborough et al., 2018a, b). These authors also noticed a large intraspecies diversity with an accessory genome (genes that are not shared by all strains) encompassing functions involved in transcription, cell surface, transport of nutriments present in meat, and prophage/transposon content. Nevertheless, *P. fragi* accessory genome was more dedicated to energy production and amino acid and carbohydrate transport and metabolism than that of *P. lundensis* (Stanborough et al., 2018a, b). These genome analyses thus revealed the great adaptation of these different bacteria to the meat environment (ability to cope with nutrient resources and stress conditions) whatever the species or genus considered.

6.3 The main microbial contaminants of meat involved in spoilage

Most of the microbial species associated to meat spoilage are known, as well as the defects observed in their presence, but the description of metabolic pathways leading to different spoilage manifestation still requires investigation. Spoilage is a complex phenomenon that results from the metabolic activity of microbes that interact together and whose metabolic activities depend both on abiotic and biotic parameters as schematized in Fig. 6.1. Thus the knowledge on spoilage caused by genera or species, taken individually, is described below, as well as new information taking into account these interactions.

6.3.1 Yeasts and molds

When thinking of spoilage in meat, yeast is not usually the first actor that comes to mind, unlike in dairy or plant products. The literature on their presence in these products is also scarce. The species most often isolated from meat belong to the genera *Candida*, *Rhodotorula*, *Debaryomyces,* or *Cryptococcus* (Odeyemi et al., 2020). These species are involved in spoilage when high numbers are reached, i.e., 10^7 CFU/g. However, their presence depends on the nature of the meat products. In products with low water activity, such as salted meat products, a greater diversity of species can be found, including species of the genera *Yarrowia* or *Trichosporon* (Nielsen et al., 2008). In cured meat, *Debaryomyces hansenii*, *Trichosporon ovoides*, *Trichosporon beigelii*, *Cryptococcus albidus* and *Rhodotorula mucilaginosa* have been identified (Nielsen et al., 2008). Vacuum-packed beef has also been described as harboring yeasts such as *Candida zeylanoides*, *Candida sake,* or *Kazachstania psychrophila* (Kabisch

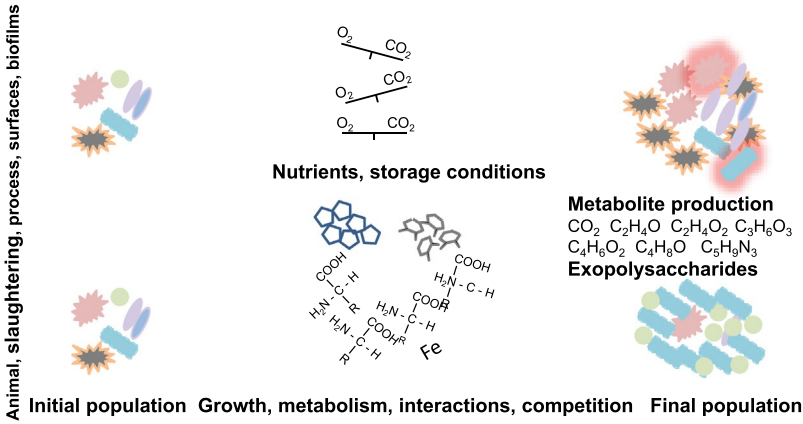

FIG. 6.1 Schematic representation of factors influencing the nature of the initial population of meat and its dynamics during storage, leading to various types of spoilage. Initial population is composed of various genera, various strains belonging to the same species. Meat is a source of nutrients enabling microbial growth and storage conditions impact microbial dynamics. Abiotic and biotic interactions of the microbial communities shape the final population, with genera, species, or strains that become dominant and some that are overgrown. Various metabolic activities of the components of meat microbiota lead to different types of spoilage.

et al., 2016). The first description of some yeast species came out after their isolation from meat. This is the case of *K. psychrophila*. Indeed, from a large survey on vacuum-packed beef, five isolates were found to belong to a new clade of the genus *Kazachstania* for which the name *K. psychrophila* was proposed (Kabisch et al., 2013). The spoilage power of this species was then proven when used for artificial contamination of meat, and this resulted in the production of gas after 16 days when yeasts had reached up to the level of 10^7 CFU/g, associated with discoloration phenomena (Kabisch et al., 2016). Similarly, Nagy et al. (2014) characterized 11 yeast strains previously isolated from meat in Hungary and also from river sediment in Brazil. They were able to demonstrate that these strains did not belong to known yeast species and that two new species of the *Yarrowia* clade could be described for which they proposed the names *Yarrowia porcina* and *Yarrowia bubula*. Of the seven isolates of *Y. porcina*, six had been isolated from meat (pork/beef) while all five *Y. bubula* were of meat origin.

Whole microbiota analyses usually only target bacteria using 16S, and it is likely that the diversity of yeasts in meat products has been underestimated.

6.3.2 Bacteria

6.3.2.1 Pseudomonas

Pseudomonas is a very ubiquitous and diverse genus comprising more than 250 species. Among them, the psychrotrophic *Pseudomonas* species that are able to grow and lead to meat spoilage are mainly represented by *Pseudomonas putida,*

P. fragi, P. lundensis, and *P. fluorescens* (Wickramasinghe et al., 2019; Odeyemi et al., 2020). Recently, the new species *Pseudomonas carnis* was described from two *Pseudomonas* strains isolated independently in different laboratories; one was isolated from spoiled pork and the other from poultry (Lick et al., 2020). On the basis of their average nucleotide index values, these two isolates could not be assigned to known species and were only 94% related to their closest relatives, *Pseudomonas lactis* and *Pseudomonas paralactis.* No other studies have yet reported the identification of these species in other meat samples, but it is likely that this will happen in the future and that it will open the way for a new taxonomic consideration of meat spoilage *Pseudomonas.*

Taking advantage of the great potential offered by genomic mining tools such as metagenomics, studies have also characterized species diversity at the strain level from metagenomic data. Indeed, De Filippis et al. (2019), using in situ reconstruction of the pangenome from metagenomic data derived from the analysis of beef meat microbiota, showed that several *P. fragi* strain profiles, characterized by different gene repertoires, were present in the meat. Furthermore, this exploration also provided keys to a better understanding of the involvement of this species in spoilage. It showed that while *Pseudomonas* are highly dominant when meat is stored in air, they are also present, even in low numbers, under vacuum and that different strains are selected by the packaging conditions. Regarding the metabolic pathways represented in the metagenomes, functional profiling indicates carbohydrate metabolism under vacuum, whereas air is more characterized by amino acid catabolism. *P. fragi* strains dominant in vacuum-packed meat had less prevalence of genes involved in oxidative stress, and strains present in air had higher lipolytic potential. On the other hand, it was also shown that *P. fragi* cellular metabolism was affected during growth under modified atmosphere. Indeed, under these storage conditions, the physiological state of the cells (evaluated through ATP synthesis and membrane potential) as well as their extracellular proteolytic potential was altered (Wang et al., 2018) with consequences on the spoilage capacities under these environmental storage conditions. Stanborough et al. (2018a) also highlighted the genetic diversity of *P. fragi* among the 12 sequenced strains, and the genetic diversity of *P. ludensis* was also noticed, although only seven genomes were sequenced.

This genetic diversity of *Pseudomonas* species in meat products is thus certainly a strong asset for their adaptation to meat and environmental conditions and may explain their importance in meat spoilage phenomena. One of these fitness traits lies in their ability to develop biofilms, which has been considered key to their persistence in the meat environment (Wickramasinghe et al., 2020, 2021; Ripolles-Avila et al., 2019). An in-depth analysis was performed through transcriptome profiling to identify the genetic functional basis of this highly relevant phenotype in the meat environment. The authors studied the different stages of the biofilm of *P. fragi* 1793. Among the differential gene expression patterns, it can be noted that some functions related to meat fitness (taurine utilization, iron acquisition) are downregulated during biofilm

dispersal. Understanding the gene expression of this critical stage of biofilm dispersal would certainly help to develop possible control strategies. Regarding iron utilization by *P. fragi*, if it was known to be a typical feature of this species, the genetic mechanisms involved had not yet been demonstrated. The use of extensive phenotypic analysis and culturomic conditions (Stanborough et al., 2018a) enabled showing siderophore production by this species, which was not reported up to this date.

Besides these recent genetic and ecological explorations of the fitness of *Pseudomonas* in meat, progress has been made in understanding their spoilage potential and the associated metabolic activities. Strains isolated in monocultures in sterile beef meat samples, followed by sensory analyses, revealed that *P. fragi* is clearly correlated with 2-butanone, 3-carene, diacetyl, and acetaldehyde (Papadopoulou et al., 2020). The strain-dependent nature of the spoilage potential was also highlighted.

It is therefore tempting to say that, if *Pseudomonas* sp. have long been recognized as spoiling actors in aerobic stored meats, their versatility and genetic diversity need to be further investigated in order to determine their role in all meat microbial communities whatever the conditions of meat storage.

6.3.2.2 Lactic acid bacteria

Several studies reported spoilage production by various LAB species, either by correlating the dominance of some species with the presence of volatile compounds or spoilage manifestations in meat products or by experimental trials showing spoilage occurrence after inoculation of meat or media mimicking it with various strains. For instance, different spoilage characteristics were reported for *Leuconostoc mesenteroides*: in raw pork meat inoculated by *L. mesenteroides*, the production of 2-butanone, diacetyl, 2-pentyl-furan, 2-ethyl-furan, and different alcohols and carbonyls was observed (Papadopoulou et al., 2020), while ropy slime producing *L. mesenteroides* strains were isolated from spoiled vacuum-packed cooked ham (Cenci-Goga et al., 2020). *L. gelidum* can provoke off-odors, discoloration, but also gas production and slime (Andreevskaya et al., 2018). The metabolic activity of *L. gelidum* and *Leuconostoc gasicomitatum* is modulated by the nature of the gas used in modified atmosphere packaging. In particular, pentose and citrate utilization, acetolactate synthase, and lactate dehydrogenase, all associated to carbon metabolism and putatively to spoilage occurrence, were expressed differently, depending on the O_2/CO_2 ratios (Kolbeck et al., 2020). *L. gasicomitatum* is also responsible for the production of unwanted buttery-odor compounds as diacetyl and acetoin resulting from pyruvate metabolism through acetolactate synthase (Jääskeläinen et al., 2015). *L. sakei* has been associated to the detection of 2-butanone when inoculated in raw pork meat (Papadopoulou et al., 2020), and some ropy slime producing strains have been reported in the past in packaged Frankfurters (Björkroth and Korkeala, 1997). More recently, *D. algida*, a species poorly described as meat spoiler although its role has certainly been underestimated, has been shown as

a putative biogenic amine producer (Säde et al., 2020) in addition to the production of butanoic acid correlated to its presence in beef meat (Mansur et al., 2019). *L. piscium* considered for a long time as spoiling seafood was revealed as an important member of meat microbiota and involved in the production of buttery-odor compounds from its carbohydrate catabolism (Andreevskaya et al., 2015). *C. maltaromaticum* was described as producing acetoin and butanoic acid, depending on storage atmosphere of beef meat, but its contribution to spoilage was judged negligible (Casaburi et al., 2011), possibly because of poor competitive fitness or *Carnobacterium* compared with other LAB species (Jääskeläinen et al., 2016). *C. divergens* was reported as a putative biogenic amine (cadaverine and tyramine) producer in chicken meat (Höll et al., 2020).

6.3.2.3 *Brochothrix thermosphacta*

B. thermosphacta is known as one of the major species involved in meat spoilage (Gribble and Brightwell, 2013; Gribble et al., 2014). Recently, the genome of several strains from various origins has been sequenced. *B. thermosphacta* is a clonal species with a restricted intraspecies biodiversity (Stanborough et al., 2017; Illikoud et al., 2018b). Interestingly, no ecotype could be observed, i.e., phenotypic and genotypic traits of strains are not correlated to their ecological origin, whatever they are issued from spoiled or nonspoiled food, and whatever the type of food matrix (Illikoud et al., 2018a). It seems that the different ability of strains to spoil meat products can be related to SNP or mutations in genes encoding enzymes responsible for the synthesis of some metabolites as acetoin, 2-methylbutanol, and 3-methylbutanal (Illikoud et al., 2018b). Although *B. thermosphacta* when inoculated alone was able to highly spoil seafood products through the production of malodorous compounds, it appeared that its metabolic activity was not sufficient for clear spoilage detection of beef meat, suggesting the requirement of interaction with other microbial species for spoilage occurrence in its presence (Illikoud et al., 2019). Indeed, in naturally contaminated chicken breast, its spoilage potential seemed to be amplified by *C. divergens* and *Photobacterium* sp. (Höll et al., 2020). This species shows high ability to colonize meat through cell surface proteins and adhesion (Stanborough et al., 2018b; Illikoud et al., 2018b). In addition, *B. thermosphacta* modulates its transcriptome to take advantage of the nutrients present in beef meat such as pyruvate, ethanolamine, and *myo*-inositol, catabolism of which may produce spoilage molecules (Illikoud et al., 2019). To summarize, the spoilage potential of *B. thermosphacta* is strain-dependent and driven by meat components and also by storage conditions that influence both bacterial community composition and bacterial metabolism.

6.3.2.4 *Clostridium* sp.

Psychrophilic and spore-forming *Clostridium* species such as *Clostridium estertheticum*, *Clostridium frigidicarnis*, *Clostridium gasigenes,* or *Clostridium algidicarnis* are responsible for gas production and mainly associated to the

so-called "blown-pack spoilage." These species are of special concern for vacuum-packed meat products since the production of CO_2 causes a visible pack distention and malodorous gases may also be produced, even at low level of *Clostridium* counts. In addition to CO_2, *C. estertheticum* can produce H_2, butyrate, acetate, formate, 1-butanol, and ethanol (see Wambui and Stephan, 2019 for a review). While most reports on *Clostridium* spoilage concerned lamb, and to a lesser extend beef meat, and were associated to the abovementioned species, *C. algidicarnis* seems specific to pasteurized vacuum-packed "foie gras" spoilage (André et al., 2017). From comparative genomics, it appeared that *C. gasigenes* strains possess an important gene repertoire for utilization of a large variety of carbohydrates (Wambui et al., 2020a, b, c, d) although yet, the metabolic activities occurring during spoilage have not been elucidated.

6.3.3 Microbial interactions leading to spoilage

As studies on the mechanisms underlying meat spoilage have progressed, it became clear that taking in situ microbial interactions into account is needed. Indeed, bacteria do not behave similarly when grown alone or in cocultures (Andreevskaya et al., 2018; Papadopoulou et al., 2020) showing the complexity of meat spoilage. Transcriptomic analyses have shown which genes were differentially expressed by *L. gelidum*, *L. piscium*, and *Paucilactobacillus oligofermentans*, and thus which functions varied, when these species were grown alone, or on two or three strain cocktails (Andreevskaya et al., 2018). In particular, carbohydrate metabolism, which participates to energy production for LAB but may also lead to the production of spoilage compounds in meat, appeared as being modulated in *L. gelidum*, depending on the presence of the two other bacteria (Andreevskaya et al., 2018). Such analyses performed by inoculating natural microbiota (Rouger et al., 2018) or strain cocktails (Chaillou et al., 2014; Kolbeck et al., 2021) in meat are still in their infancy, but these approaches should bring valuable information on meat spoilage through microbial ecology investigations.

6.4 Devices for bacterial spoilage monitoring

As a large variety of information is available on molecules causing meat spoilage, techniques to easily monitor the appearance of spoilage have been proposed. Those are based on different physicochemical devices and aim at detecting or measuring direct or indirect factors associated to meat spoilage. The main spoilage indicators used for these devices were pH, sulfurous molecules, or biogenic amines. As examples, colorimetric dyes used as pH indicators have been developed (Magnaghi et al., 2020; Chayavanich et al., 2020). Sensors for thiols or dimethyl sulfide were also designed (Chow, 2020; Magnaghi et al., 2020; Senapati and Sahu, 2020), and the biogenic amine histamine could also be detected through colorimetric sensors (Chow, 2020).

6.5 Major spoilage manifestations

The color of meat, especially for red meat, is the first appreciation criterion that can lead to reject the product. Most color defects result from chemical modifications in particular on myoglobin that can alter the color of the product. However, some bacterial species are well known to more specifically affect color, such as *Pseudomonas* species through the production of various pigments.

A second visual defect that can affect the consumer choice is the ropy appearance of meat products. In fact, it has been reported in the past that spoiling effect of some species stood in their ability to produce ropy slime. From a metabolic point of view, this ability relies on the production of polysaccharides in particular by LAB. However, despite some reported cases in the literature, this defect is nowadays little evidenced.

Odor and taste defects are by far the most prominent spoilage manifestations caused by microbial contaminants of meat. The odor is a complex association of molecules, association of which can be considered as pleasant or unpleasant. These are associated to metabolic activities of microorganisms leading to the production of volatile organic compounds. The main compounds that are produced by spoilage bacteria are alcohols, aldehydes, esters, ketones, sulfur compounds, and fatty acids. Many could be correlated to specific odors and to bacterial species producing them (for a review, see Casaburi et al., 2015). Thanks to the availability of many genome sequences, several metabolic pathways involved in the production of volatile organic compounds are known.

Biogenic amines are produced through enzymatic decarboxylation of amino acids. Whereas tyramine and histamine, issued respectively from tyrosine and histidine, are of safety matter, other biogenic amines result in food spoilage. In particular, putrescine and cadaverine, toxic only in large doses, are responsible for meat spoilage because of the putrefaction odor they cause. Their production during the storage of different meat products has been associated with bacterial development.

Blown-pack spoilage results from gas production, mostly CO_2, but can be accompanied by the appearance of off-odors. Such spoilage mostly concerns vacuum-packed meat, although it is also reported for meat products stored under modified atmosphere packaging. *Clostridium* species are the major species responsible for such spoilage.

6.6 Conclusion

Meat microbiology has been reinvestigated during the last few years thanks to new methods, and particularly direct DNA sequencing limiting the bias of cultural methods, although these Pasteurian approaches are still needed and fruitful. New species or species whose presence in meat had been underestimated were discovered. Genome sequencing of many bacterial species involved in meat spoilage has recently emerged. A deeper analysis of genome content

should bring new information on the metabolic functions that lead to spoilage by the production of unwanted molecules. DNA- and RNA-based methods enabled a better description of the microbial communities present in meat. Further analyses should help understanding how the multiple bacterial interactions may lead to so many manifestations of meat spoilage. This should also help in proposing adequate methods of processing or storage to improve meat safety. Microbial criteria for assessing shelf life might also be questioned, considering the presence of new, unknown, or unexpected species, as mentioned already by Jääskeläinen et al. (2016) and the necessity to study meat on a microbial ecology perspective.

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Chapter 7

The storage and preservation of meat: I—Thermal technologies

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7.1 Introduction

Storage of meat is a conventional practice necessary for distribution, retailing, keeping in the household, and often, palatability improvement of meat. Our ancestors would store hunted or domesticated meat in low humidity and low-temperature environments, such as natural caves, to prevent meat spoilage although they had no knowledge what was causing the problem. Later, as dwellings were built, cellars were constructed for food storage (Rixson, 2010). Ice, gathered from frozen ponds and lakes in winter, was used to keep cellar temperatures low (Leighton and Douglas, 1910). However, it was not until Louis Pasteur's discovery of microorganisms did food processors begin to invent technologies specifically targeting food spoilage causative agents.

It is well understood today that the single most important factor that limits the shelf life of fresh meat and often processed meat as well is microbial growth, although chemical reactions, such as lipid oxidation, also play a role in influencing the storage stability of meat. The stability of meat depends on the preservation technologies, both thermal and nonthermal. In all, they employ environmental conditions, which primarily discourage the growth of microorganisms. The methods are grouped into three broad categories based on control by temperature, by moisture, and more directly, by lethal agencies (bactericidal, bacteriostatic, fungicidal, and fungistatic). A particular method of preservation may involve the combination of several antimicrobial processes to form a "hurdle" control system against microbial proliferation (Leistner, 1995).

Temperature control to below or above the optimum range for microbial growth has a preventive action on microorganisms and is the most convenient, effective, and so-called "green" strategy for meat and meat product preservation. Hence, meat may be preserved by refrigeration that discourages the growth of spoilage and potentially pathogenic organisms. On the other hand, relatively mild heat treatments, such as pasteurization, can be applied to inactivate most spoilage microorganisms and destruct non-spore-formation pathogens without

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causing significant losses of organoleptic properties and nutrients of meat. To achieve total lethality, sterilization thermal processing is employed to destroy all vegetative cells, including pathogenic bacteria, allowing cooked meat products to achieve a high level of safety and stability at room temperature (Holdsworth, 1985). Conventional thermal processes to inactivate microorganisms include heating by steam and hot water; baking, roasting, and broiling in a heated chamber; moist cooking (boiling, stewing, braising, etc.). New heating technologies have also been introduced as potential heating methods for meat, for example, ohmic heating, dielectric heating, and radiant heating.

This chapter focuses on the principles of thermal technologies of meat preservation and highlights the most important aspects as related to their applications. The emphasis is on fresh red meat (beef, pork, and lamb). Physical, chemical, and biochemical reactions that contribute to meat quality changes during chill and frozen storage are briefly discussed; many related and more specific postmortem biochemical changes are covered in separate chapters, for example, Chapter 5 (conversion of muscle to meat) and Chapter 12 (meat tenderness).

7.2 Chilling

7.2.1 Chilling processes

Rapid cooling of beef, pork, and sheep carcasses to less than 10°C immediately postmortem is essential to meat quality preservation as well as microbiological safety. The achievement of rapid cooling requires a high air speed in the chill rooms or large air circulation volumes (e.g., 60–100 changes of air/h). Although a higher air velocity will tend to cause a greater weight loss, it permits the use of a high relative humidity. At the beginning of chilling, the air temperature can be as low as −10°C for pigs and sheep and air speeds as high as 180 m/min (600 ft./min). For beef carcasses, air speeds of 120 m/min (400 ft./min) and an air temperature of −1°C may be appropriate. Once the difference in temperature between the meat surface and the air becomes small, the air speed must be reduced to avoid desiccation. Air, supersaturated with water vapor and moving at a high speed, has been used to minimize evaporation from hot sides while providing a high capacity for heat removal. Although there are undoubted advantages to be gained from fast chilling, the application of ultrarapid chilling, for example, air at −30°C and 4 m/s, can cause "cold shortening" and toughening of pork (Van der Wal et al., 1995). Spray chilling the surfaces of pig carcasses has been reported to enhance the oxygenation of myoglobin without any increase in metmyoglobin (Feldhusen et al., 1995). In a comparison with air chilling, Greer and Jones (1997) showed that spray chilling of beef carcasses with water mist at 1°C, in four cycles during the first 4–16 h, significantly reduced carcass shrinkage by 0.08 g/100 g per hour. This could mean a very significant saving for a large meat-packing company.

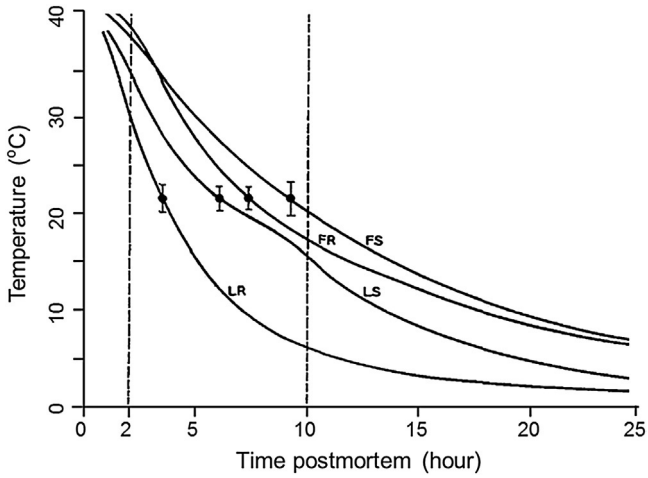

FIG. 7.1 Cooling curves of *Longissimus* muscle during chilling of beef. F and L: sides of fat and lean carcasses, respectively. R and S: rapid (2°C air moving at 90 m/min) and slow (9°C nonforced air) chilling conditions, respectively. Vertical bars: standard deviations when mean group temperatures attained 20°C (Lochner et al., 1980).

Depending on the size of the carcass, the attainment of a center temperature of 4–7°C may take up to 24–48 h. Hence, for a heavy, well-finished beef carcass with a thick subcutaneous layer of fat, the rate of cooling can be significantly slower than a light carcass, for example, grass-fed lean beef (Fig. 7.1). However, controlled and deliberated slow cooling, referred to as conditioning, may be advantageous with respect to the quality of meat. This is because a great deal of biochemical and physiological processes occur during the first 48 h postmortem that ultimately dictate the quality of meat (color, drip loss, etc.). For example, slow reductions of carcass temperature will allow muscle endogenous proteases more time to degrade myofibrils for improved tenderness (Goll et al., 1983). Muscles that are exposed to surface and thin in cross section tend to be susceptible to cold shortening. However, if the temperature of these muscles is allowed to be maintained at above 12°C within 10 h, cold shortening can be alleviated. Such practice, in principle, can be feasible; however, caution must be taken because if the chilling process is too slow, and the carcass temperature remains high for an extended period, the muscle becomes susceptible to the development of pale, soft, and exudative (PSE) condition (Lesiow and Xiong, 2013; Savell et al., 2005).

7.2.2 Chilling-induced fiber shortening and prevention

When muscle is subjected to chilling temperatures before the onset of rigor mortis, super contraction of fibers occurs due to excessive interaction between

actin and myosin in the presence of abundant ATP, resulting in a substantial reduction in longitudinal length of the muscle; hence, an extreme meat toughness. This phenomenon, known as "cold shortening," is common for hot-boned meat, especially for red muscles. A related but not similar condition is thaw rigor that also results from excessive cross-linking of myosin and actin when frozen prerigor muscle is thawed. In general, if the temperature of muscle is reduced to below10–15°C while it is still in the early prerigor condition (pH about 6.0–6.4), there is a tendency for shortening and, thereby, toughness on subsequent cooking (Locker and Hagyard, 1963). The intensity of cold shortening is correlated with the proportion of "red" fibers in the muscle. Bendall (1975) confirmed that "red" muscles were susceptible to cold shortening, whereas those with less myoglobin were much less so.

A widely accepted theory for muscle cold shortening is that at temperatures less than 15°C, calcium release from sarcoplasmic reticulum (SR) is stimulated, whereby the contractile actomyosin ATPase is much enhanced (Newbold and Scopes, 1967). Moreover, at low temperatures, the ATPase that is responsible for calcium update by SR has a reduced activity, which contributes to the accumulation of Ca^{2+} in the cytosol that triggers a cascade of reactions leading to actomyosin formation. Because mitochondria are also involved in calcium release, it is not surprising that red muscle fibers are more prone to cold shortening than white muscle fibers (Buege and Marsh, 1975).

When prerigor muscle remains attached to the bones through tendon, fiber contraction is restricted. Therefore, hot boning is highly undesirable and should be avoided unless the warm muscle is immediately utilized for processing, for example, the production of whole-hog sausage. Although the attachment of muscle to the carcass skeleton imparts physical constraints to myofibril contraction, therefore greatly minimizes cold shortening, fiber shortening still occurs in thin carcasses such as those from grass-fed cattle when chilled rapidly. In contrast, cattle that are finished on grain produce a thick layer of subcutaneous fat that serves as a heat insulate and prevents rapid muscle chilling. In this case, cold shortening tends to be much less appreciable.

A variety of technologies have been developed to prevent muscle cold shortening during chill storage: tempering, special postures of carcass hanging, electrical stimulation (ES), and others. Cold shortening can be avoided by cooling muscle swiftly to about 15°C and holding it at this temperature to allow the onset of rigor mortis. The temperature can then be lowered as quickly as is compatible with minimal surface desiccation. Although not all the muscles of carcasses, even if their temperature falls below about 15°C while in the early prerigor condition, are free of shortening, their attachment on the skeleton would restrain them sufficiently to prevent it. Of course, tempering requires a strict sanitation practice to prevent microbial growth.

For posture positioning, the conventional act involves carcass suspension by the Achilles tendon. Because not all muscles are equally stretched, certain muscles are more liable than others to shorten. Hence, by altering the posture

of the suspended carcass in various ways, the pattern of muscles that shorten may be changed. For example, pelvic hanging of beef and lamb carcasses (field stretching) can prevent shortening and improve tenderness in muscles, which are normally sensitive to cold stimulus (Ahnström et al., 2012; Bouton et al., 1974). A further improved method of altered posture is to sever the thoracic vertebrae between the 12th and 13th ribs and cut through the ischium on the pelvic bone and then hang the carcasses (Claus et al., 1997). This latter practice allows the stretching of certain muscles that are otherwise untouched for significant tenderness improvement. All these altered carcass postures, however, while permitting control of cold shortening during fast chill of muscles early prerigor, could be inconvenient in practice.

A more popular method to minimize cold shortening was devised by meat researchers in New Zealand decades ago: electrical stimulation, which has become a commercial-scale operation as a way to mitigate cold shortening, thereby improving meat tenderness (Adeyemi and Sazili, 2014). In ES, the carcass is stimulated via the nervous pathways in the immediate postmortem period using either a low (< 100 V) or a high (500–1000 V) voltage. Although low voltages are intrinsically safer in operation, they are less consistent in effect than high voltages (Bendall, 1980). The optimum pulse rate is between 15 and 25 pulses per second (pps). Higher frequencies tend to be relatively ineffective since they fall within the latency period of the muscles concerned. The optimum pulse width is about 20–40 ms. Shorter widths may fail to activate all the muscle fibers. High voltages are effective in accelerating postmortem glycolysis when applied for 1.5–2 min, whereas longer times (~ 4 min) are required with voltages of the order of 100 V. The response of beef carcasses to ES falls off quickly after about 50 min postmortem and that of lamb carcasses even sooner. It is thus desirable to apply the current within about 30 min of slaughter. The effect of ES on meat tenderness enhancement is indicated in Fig. 7.2.

The vast acceleration of postmortem glycolysis caused by ES signifies a concomitantly high rate of ATP breakdown, which, in turn, reflects marked activation of the contractile actomyosin ATPase by released Ca^{2+} ions. The latter also enhances the titer of phosphorylase a, which is an additional factor accounting for the increased rate of postmortem glycolysis (Newbold and Small, 1985). The current during pulse ES is short-lived; when it is discontinued, the ATP level is still relatively high, and the temperature has fallen little from its in vivo value. In these circumstances, the SR can presumably recapture Ca^{2+} ions readily, thus suppressing ATPase activity while the ATP level remains sufficient to effect muscular relaxation and the restoration of resting sarcomere length. Due to the depletion of glucose and glycogen while the carcass is still warm, cold shortening would not occur because there is little amount of ATP left to initiate fiber contraction when the muscle goes through the chilling period. Without ES, the low temperature prevents effective operation of the ATP-fueled Ca^{2+} pumps of the sarcotubular system, and stimulation of ATP breakdown is thus not inhibited, leading to cold shortening. Thaw rigor development is also avoided for

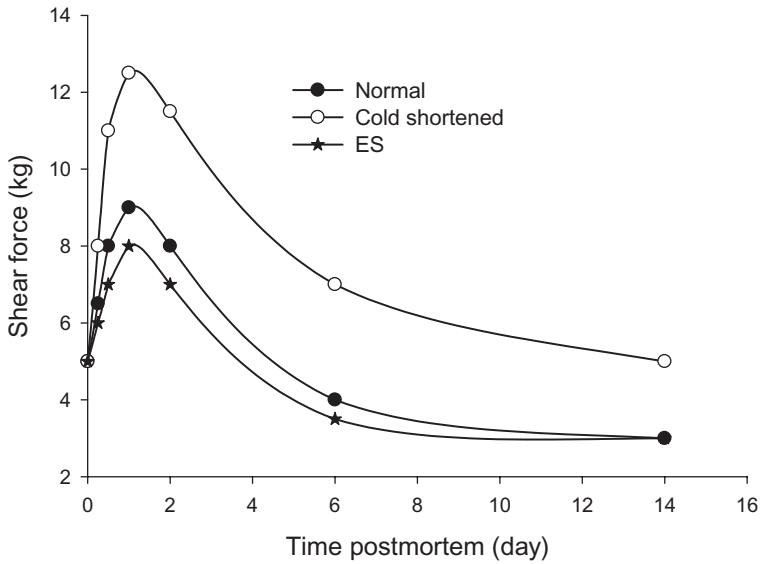

FIG. 7.2 Simulated toughness changes (Warner-Bratzler shear force) in beef *Longissimus* muscle during chill storage at 2–4°C. Normal: grain-finished; Cold shortened: grass-fed; ES: grass-fed and electrically stimulated.

muscle after ES for the same reason, namely the depletion of glycogen, thus, unavailability of energy sources for ATP production.

Factors other than the inhibition of fiber cold shortening have been put forth to explain the meat tenderizing effect by ES in chilled meat. It is not clear whether physical disruption of muscle fibers is involved. Marsh et al. (1981) found no histological evidence of tissue disruption in muscles, which had been electrically stimulated by current of frequency 2 Hz, whereas Takahashi et al. (1987) observed both severe contraction and breaking of sarcomeres when the current frequency was 50–60 Hz. Yet, it is generally agreed that activation of endogenous proteases by ES plays an important role in improved meat tenderization. Dutson (1977) and Savell et al. (1977) postulated that the release of Ca^{2+} from the sarcotubular system on ES may enhance proteolysis by calpains. Indeed, it has been shown that the activity of μ-calpain is enhanced by ES (Hwang et al., 2003). The acceleration of such proteolytic activity while the carcass temperature is still high (near in vivo) could contribute to the observed tenderness in muscles, which have been stimulated electrically in the immediate postmortem period. As the pH falls further, lysosomal membranes are damaged, which may promote the activity of liberated cathepsins for additional meat tenderness improvements.

Apart from the avoidance of toughening, ES has been associated with improved flavor and enhanced brightness of the surface red color on meat cuts (Adeyemi and Sazili, 2014). The latter effect possibly arises because the pro-

cess depletes the metabolites of surviving oxidative pathways in the muscle, or because the fast fall in pH causes the muscle proteins from treated carcasses to approach their isoelectric point much sooner, thereby "opening up" the structure and easing oxygenation of myoglobin. ES of prerigor carcasses may, in theory, cause exudative meat through the acceleration of postmortem glycolysis and therefore the attainment of acid pH values while the temperature is still relatively high. However, there is a general absence of marked exudation in electrically stimulated bovine muscle, which remains to be explained.

It should be noted that ES is particularly suited for lean carcasses, such as those from grass-fed beef, due to their susceptibility to cold shortening. For meat from grain-finished and fattened cattle that are less susceptible to cold shortening, ES does not seem to offer obvious advantages. This explains why in the United States, where heifer and steers are usually finished on a high-energy grain feedlot, ES is not commonly applied in the slaughter plant.

7.2.3 Meat tenderness changes during chill storage

The greatest benefit of postmortem chill storage (aging) of carcasses or deboned wholesale cuts is tenderness improvement due to myofibril degradation and, to a lesser extent, improved solubility of collagen, which are caused by muscle endogenous proteases (see Chapter 12). For beef, significant tenderness improvements can be achieved within the first 2 days postmortem, but generally it requires more than 2 weeks for the tenderness to reach the desirable level of consumer acceptance, i.e., about 3 kg of Warner-Bratzler shear force (Taylor et al., 1995). Because primal cuts are usually vacuum-packaged and boxed for wholesale distribution and subsequent inventory storage at the retail store, the time elapsed before the meat is put on retail display usually has passed 2 weeks. The fabrication of carcasses for boxed beef has largely replaced the traditional dry aging of beef and lamb carcasses. Nonetheless, the "dry aging" practice, i.e., chill storage of beef and lamb carcasses or primary cuts beyond the normal aging period up to 4 weeks (or longer), has gained renewed attention recently. Aside from additional tenderization achieved in some cases, there is a consistent flavor improvement in dry-aged cuts (Jose et al., 2020; Kim et al., 2016). Both volatile and nonvolatile compounds, attributed to lipid oxidation and accumulation of amino acids, short peptides, and nucleotides, are the main factors responsible for the enhanced palatability.

At least four groups of proteolytic enzymes are implicated in tenderness improvement of aged meat, namely calpains, cathepsins, caspases, and proteasome (Kemp et al., 2010). μ-Calpain and m-calpain are isomers requiring micromolar (μM) and millimolar (mM) range calcium concentrations for maximal activity (Edmunds et al., 1991). Since the calcium concentration in cytosol is in the μM range, μ-calpain seems to play a major role in the degradation of myofibrillar proteins and hence, meat tenderization postmortem. Calpains are able to degrade titin, nebulin, desmin, tropomyosin, troponin-T, and C-proteins, but do not affect

myosin and actin (Goll et al., 1983). Cleavages of these proteins lead to the disruption of the *Z*-disks. Because calpains can reproduce all the proteolytic changes in meat under normal aging conditions, it is believed that the calpain protease system plays a dominant role in meat tenderization (Koohmaraie et al., 1986). Calpains are susceptible to autolysis and are regulated by their endogenous inhibitor calpastatin. Moreover, the low-pH condition (pH 5.5–5.6) in postrigor muscle tissue would also limit the enzyme activity. These two factors explain why even undergoing a lengthy aging period, meat rarely becomes mushy.

Cathepsins are a group of acidic lysosomal proteases believed to be involved in the postmortem tenderization of meat as well. Five of the catheptic endopeptidases have been extensively studied, i.e., cathepsins B, D, E, H, and L (Ouali et al., 1987). These proteases are capable of degrading most of the same substrates affected by calpain and additionally, are effective against myosin and actin. However, the role of cathepsins in meat postmortem aging is subject to debate because cathepsins in intact muscle tissue are confined within the lysosomal apparatus, i.e., not in direct contact with myofibrils as do calpains. Moreover, these proteases have a very low pH requirement for optimal activity. In fact, cathepsins have a high affinity for myosin and actin, neither of which shows a degradation pattern in muscle that undergoes normal aging (Asghar and Bhatti, 1987). Nevertheless, it is generally accepted that during prolonged aging, lysosomal membrane is disrupted, and the released cathepsins will diffuse to the inter-myofilamental space to initiate protein degradation (Moeller et al., 1977).

The "multicatalytic proteinase complex" (MCP) is another possible enzyme system involved in postmortem changes in muscle structure. The 20S proteasome is the catalytic core of the enzyme complexes that have been studied for its potential role in meat tenderness. According to Robert et al. (1999), bovine proteasome is capable of degrading myofibrillar proteins, including nebulin, myosin, actin, and tropomyosin. Such proteolytic changes induced by 20S proteasome have been linked to improved tenderness of meat (Thomas et al., 2004). However, the degradation pattern of myofibrillar proteins by 20S proteasome does not mimic the degradation pattern observed in postmortem muscle, suggesting that MCO may not have a significant role in meat aging.

Another concept that has been introduced to explain meat tenderization is "programmed cell death" (Earnshaw et al., 1999; Herrera-Mendez et al., 2006). A large group of cysteine peptidases, known as "caspases," which are involved in the apoptosis and elimination of dead cells, appear to participate in muscle fiber disruption through degrading myofibrillar proteins. Caspases involved in apoptosis are subdivided into initiator caspases (e.g., caspases 8, 9, 10, and 12) and effector caspases (e.g., caspases 3, 6, and 7), depending on their location on the cell death pathway (Earnshaw et al., 1999). Caspases remain active in postmortem muscle, and their role in meat tenderization is attributed to their ability to inactivate calpastatin, thereby promoting the activity of calpains (Kemp and Parr, 2012). However, the exact role and biochemical mechanism of caspases in meat aging and tenderization are not fully elucidated.

7.2.4 Oxidative changes in chilled meat

During postmortem aging, proteolytic degradation of muscle fibers into short peptides, nucleotides, free amino acids, and various other nitrogen-containing compounds, contributes to meat flavor enhancement. On the other hand, chemical and biochemical reactions, particularly lipid oxidation and also protein oxidation, could cause flavor deteriorations due to the generation of secondary lipid oxidation products and sulfur compounds on the exposed surfaces of carcasses and meat cuts (Martinaud et al., 1997; Spanier et al., 1997). Of course, for meat that is chilled in a vacuum-sealed package, oxidation would have a negligible role in meat flavor change.

Deterioration in meat lipids may be due to direct chemical action or through the intermediary activity of enzymes (either indigenous or derived from microorganisms). In general, direct chemical deterioration is not so important in fresh meat carcasses but can be a part of quality loss when deboned meat is subjected to extended storage. Two types of deterioration occur: hydrolysis and oxidation. Lipolytic enzymes split fatty acids from triacyglycerols; with phospholipids that are abundant in membrane, inorganic phosphate is produced in addition to the release of free fatty acids, which are predominantly unsaturated. The fatty acids liberated in meat are generally not so offensive as compared with those produced in milk (short-chain fatty acids). On the other hand, due to the susceptibility of unsaturated fatty acids to radicals, the higher their amount, the more oxidative the muscle, hence, the more off-flavors (Min and Ahn, 2005). The rate of oxidation of intramuscular fat tends to be higher in nonruminants than in ruminants (e.g., poultry meat and pork in comparison with beef and mutton), in the less improved breeds, in muscles with relatively low contents of intramuscular fat, in the lumbar region of *Longissimus* muscle in the pig compared with the thoracic region (the reverse being true in beef animals), in animals on a low plane of nutrition, and in animals receiving large proportions of unsaturated fat in their diet, particularly in nonruminants (Lawrie, 1992; Love and Pearson, 1971; Rhee et al., 1996).

A considerable number of such differences may operate simultaneously. The relative tendency of pork muscles to become rancid and discolored exemplifies this complexity. Porcine *psoas* muscle has a higher proportion of polyunsaturated fatty acids (PUFAs), especially in the phospholipid fraction, than *Longissimus* muscle (Owen et al., 1975), and more prooxidative heme protein. Yet, during prolonged frozen storage (e.g., at −10°C), minced porcine *Longissimus* muscle undergoes oxidative rancidity and concomitant metmyoglobin formation, to a markedly greater extent than *psoas*. This anomalous behavior appears to be related to the higher ultimate pH of the latter (Owen et al., 1975). At high pH, the activity of the cytochrome system of enzymes is much enhanced, and this increases their metmyoglobin-reducing activity (Faustman and Cassens, 1990). Moreover, such enzymes are found at higher concentration in *psoas*. In porcine *psoas* muscle, therefore, the relatively high ultimate pH,

by minimizing prooxidant conditions, more than offsets the inherently greater tendency of its lipids to oxidize. On the other hand, for beef, the ultimate pH of both *psoas* and *Longissimus* muscles is generally normal, and this potentiates the effect of the higher proportion of PUFAs in the former (Rhee et al., 1988). Clearly, multiple factors must be known before accurate prediction of the behavior of a given muscle can be made.

The prooxidant effect of heme compounds in fat oxidation is reciprocal since unsaturated fatty acids accelerate the oxidation of myoglobin (see Chapter 11). As myoglobin and fats are brought into intimate contact with one another in meats, their coupled reaction will contribute to rancidity and discoloration simultaneously (Faustman et al., 2010). Secondary lipid oxidation products, such as malonaldehyde and 4-hydroxynonenal, can bind to nucleophilic histidine residues in myoglobin causing meat discoloration during chill storage. Suman et al. (2014) reported that the number of histidine residues adducted by reactive aldehydes was greater in beef myoglobin than in pork myoglobin. This seems to contribution to the notion that metmyoglobin formation induced by oxidized lipids is more relevantly important for color instability in beef than in pork. During cooking, both heme-bound and nonheme iron accelerate lipid oxidation. Nevertheless, it should be pointed out that the behavior of heme pigments and unsaturated fats, when in juxtaposition, is not fully understood. Kendrick and Watts (1969) had long postulated that at low lipid: heme ratios, heme compounds can stabilize peroxides or free radicals and exert an antioxidant effect.

7.2.5 Superchilling at subfreezing point

Superchilling, also known as "supercooling," "deep-chilling," "subzero-chilling," and "controlled freezing-point chilling," offers an alternative and promising technique for preservation of fresh meat. When a small part of the water content (usually on the surface of meat) is frozen while the main part remains superchilled and unfrozen, the term "partial freezing" is also used to describe a process (Magnussen et al., 2008). During superchilling, the temperature of a food product is generally lowered to 0.5–2.8°C below the initial freezing point (Duun and Rustad, 2007). The ice formed on the surface will absorb heat from the interior and reach an equilibrium eventually. This allows the product to obtain a uniform temperature at which it is maintained during storage and distribution (Magnussen et al., 2008). Superchilling gives fresh meat an internal ice reservoir so that there is no need for external ice around the product during transportation or storage. The superchilling temperature is achieved by means of ice slurries or in a superchilled chamber without ice. At superchilling temperatures, microbial activity is reduced to almost zero, and enzyme activity is substantially retarded as well; hence, biological spoilage is minimized.

Superchilling also includes chilling below the freezing point of pure water in a temperature zone where ice crystals do not form. Relying on the osmotic pressure, this type of superchilling allows the depression of the freezing point

of muscle tissue to as low as −0.7°C, thereby avoiding physical damage of the muscle cells due to ice crystal formation. For such superchilling to be successful, heat exchange must be precisely controlled to ensure very minimal temperature fluctuations (generally ±0.1°C) as compared with conventional refrigerated storage (±1°C).

Superchilling is widely used for commercial seafood (Beaufort et al., 2009; Olafsdottir et al., 2006). It has been applied to commercial chicken process as well in which chicken carcasses are initially chilled in cold water and then further chilled by cold air in an air freezer operating at −15°C for approximately 30 min. After packaging, they are again placed in an air freezer to achieve the required meat temperature, and the carcasses are then stored and distributed at −1°C to −2°C. There is now increasing interest in superchilling application for beef, pork, and other red meat species (Schubring, 2009). Superchilling of vacuum-packaged pork roasts at −2.0°C has been shown to improve the shelf life compared with traditional chill storage at 3.5°C (Duun et al., 2008). Superchilled pork roasts maintained a good sensory quality and low microbiological counts throughout the entire 16 weeks of storage, while the shelf life of conventionally chilled samples was just 14 days. Moreover, drip loss in superchilled samples was lower and showed less variation than in regularly chilled control meat. More research is required to help understand other aspects of the superchilling effects on meat. For example, superchilling has been found to promote the release of the proteolytic enzymes cathepsins B and L from lysosomes, causing an acceleration of fish muscle degradation (Bahuaud et al., 2008). Furthermore, Duun et al. (2008) noted that myofibrillar proteins denatured more easily during superchill storage than during conventionally chill storage of both salmon and cod fillets.

7.2.6 Chill storage in modified atmosphere environments

When animal carcasses are ready to leave the slaughter plant within a few days postmortem, the meat is fabricated into primal cuts that are typically vacuum-packaged and then shipped in boxes. Retail stores would receive boxed meat from the distributor within about 2 weeks after carcass fabrication. At the retail outlet or a distribution center, primal cuts are further cut into portion sizes (chops and steaks), which are subsequently placed either onto polystyrene foam trays and then overwrapped with air-permeable polyvinyl chloride (PVC), or onto trays that are filled with a mixture of gases having a specific composition then sealed under air-impermeable polypropylene-polyethylene film. The latter packaging system, known as "modified atmosphere packaging" or MAP, usually utilizes a gas mixture with a high percentage of oxygen (e.g., 75%–80% O_2) to ensure the saturation of the bright-red pigment oxymyoglobin and an elevated carbon dioxide concentration (e.g., 20%–25% CO_2) to inhibit aerobic microorganisms such as *Pseudomonas* and *Lactobacillus* (McMillin et al., 1999). Although the partial pressure of O_2 in MAP will slightly decrease and that of CO_2 will increase

during chill storage due to the respiration of mitochondria, the gas composition during storage requires no adjustment because the changes are relatively small. The MAP system as a "case-ready" convenient module has been successfully employed in retail outlets in the United States and EU since the 1980s.

The greatest advantage of MAP over the traditional PVC packaging (21% O_2/78% N_2/0.04% CO_2) is microbial growth retardation, hence, an extended shelf life, i.e., 14–21 days for MAP versus 4–5 days for PVC. While both packaging systems allow the initial bloom color of meat and lipid oxidation occurs at a similar rate (Ordonez and Ledward, 1977), the inhibition of aerobic bacteria in MAP leads to a remarkably long-term surface red color (a*) stability, which has been demonstrated across all red meat species, i.e., beef (McMillin et al., 1999), lamb (Fernandes et al., 2014), and pork (Delles and Xiong, 2014) during retail display. However, MAP tends to promote muscle protein oxidation during chill storage and increases drip loss (Lund et al., 2007).

7.3 Freezing

7.3.1 Freezing processes

Large-scale preservation of meat by freezing can be dated back to later 1800s, when the first frozen beef and mutton arrived in the United Kingdom from Australia. At that time, freezing offered a means of preserving during the long voyages of a surplus of meat animals in the southern hemisphere, especially in New Zealand and Australia, to Europe (Critchell and Raymond, 1912). Today, free trading between countries within the same continent (e.g., the European Union and the North American Free Trade Agreement) and between countries from different continents has entailed much greater application of freezing technology for transporting meat across borders. With proper packaging, the commonly used storage temperature (−18°C) for meat exportation will maintain acceptable quality while preventing microbial spoilage for beef, pork, and lamb for at least 1 year.

Freezing can be achieved with blast air, still air, or a super-cold plate. The rate of freezing has a critical impact on the quality of thawed meat. To protect quality characteristics, "freeze fast, thaw slow" is a widely recommended practice whenever possible. The rate of freezing is dependent not only on the bulk of the meat and its thermal properties (e.g., specific heat and thermal conductivity), but also on the temperature of the refrigerating environment and the method of applying the refrigeration. With smaller cuts of meat, the nature of the wrapping material used also affects the rate of freezing. The low conductivity of fat at both ambient and freezing temperatures and the greatly increased conductivity of meat when frozen are apparent. Such data enable accurate cooling and freezing rates to be calculated for commercial operations.

Before freezing, carcasses are chilled for 1–3 days at about 1°C to allow cooling, and the initial refrigerated storage would prevent thaw rigor. The briefly aged carcasses will then be fabricated into quarters or smaller primal

and wholesale cuts, which are subsequently frozen in a freezer at −10°C or a lower temperature. Hot meat after electrical stimulation sometimes is directly placed into a blast tunnel freezer without prior chilling, and the procedure has been used for lamb. Weight losses caused by evaporation during initial chilling and subsequent freezing and storage in the freezer will always occur. The temperature of freezing affects the weight loss, for example, weight losses by evaporation at −30°C are reportedly about 20% of those at −10°C (James, 1999). Moreover, wastage at the latter temperature increases the need to trim discolored surfaces. Packing in polythene reduces evaporation at −10°C to about the same level as that of meat at −30°C without such packaging. When unprotected meat surfaces are blast frozen, there is considerable freezer burn (the whitish or amber-colored patches seen on the surface of frozen meats). Freezer burn is caused by the sublimation of ice crystals into the atmosphere of the cold room, thus creating small air pockets on the meat, which scatter incident light. This happens because the ambient water vapor pressure is much less than the water vapor pressure above the meat surface.

7.3.2 Effects of freezing on muscular tissue

The advantages of storage of meat below the freezing point to prolong the microbiological shelf life tend to be offset by the exudation of fluid (drip) upon thawing, which is a primary adverse effect of the preservation method. Proteins, peptides, amino acids, lactic acid, purines, vitamins of the B complex, and various salts are among the many constituents of drip fluid. Other potential negative impacts of freezing include protein denaturation, lipid and protein oxidation, and discoloration, which contribute directly or indirectly to drip loss upon thawing (Leygonie et al., 2012). Because of the freeze-induced muscle fiber structural disarray, increases in meat tenderness have been observed in some cases (Lagerstedt et al., 2008; Vieira et al., 2009). The release of endogenous proteases, particularly lysosomal enzymes, due to ice crystal damage is thought to further contribute to the improved meat tenderness. However, where protein denaturation and resulting myofibrillar aggregation are extensive and the water-holding capacity is severely compromised, there is always an increased toughness for thawed and cooked meat.

The amount of drip loss is determined by two types of factors. In one category are the factors that determine the extent to which the fluid, once formed, will in fact drain from the meat. Among these are the size and shape of meat pieces (in particular the ratio of cut surface to volume), the orientation of cut surface with respect to muscle fiber axis, the prevalence of large blood vessels, and the relative tendency for evaporation or condensation to occur in the thawing chamber. Factors of this type are of greater importance with beef than with pork or lamb, because with the former more cutting is required to produce an easily handled quantity. The ultimate pH of muscle has a profound influence on the drip, showing an inverse relationship between them. However, even with

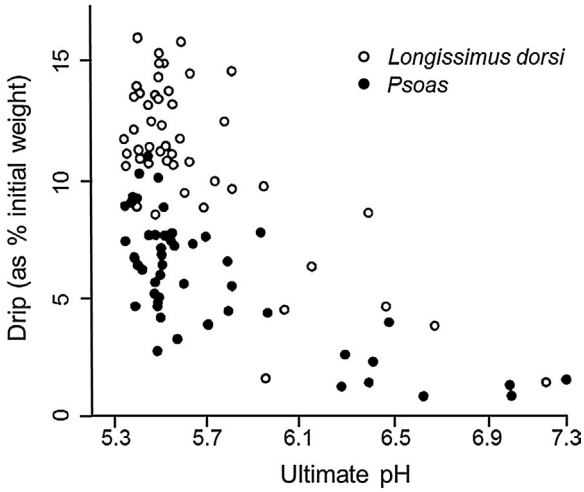

FIG. 7.3 The relationship between the ultimate pH in *Longissimus* and *Psoas* muscles and the extent of drip on thawing of the beef (Lawrie, 1959).

the same rate of postmortem pH fall and at the same ultimate pH, drip from the *Longissimus dorsi* is generally greater than that from *psoas* (Fig. 7.3). Clearly, different muscles have different intrinsic susceptibilities to damage during freezing and thawing.

Factors in the second category are much more fundamental. They are concerned with the nature of the freezing process in muscular tissue and with the water-holding capacity of the muscle proteins, thus determining the volume of the fluid, which forms at thawing. In general, the proportion of the total water in muscle that freezes increases rapidly at first as the temperature is lowered further below the freezing point; then more slowly, approaching an asymptote of about 98.2% at −20°C (Moran, 1930). Because not all the water in muscle freezes, the latent heat is lower than would be anticipated. The non-frozen portion increases as the fat content of the muscles increases (Fleming, 1969). However, as well as the extent, the rate at which the temperature of the meat falls is a most important consideration: the time taken to pass from 0°C to −5°C is usually regarded as an indicator of the speed of freezing. The fastest times so far obtained are of the order of 1 s. These have been achieved by placing a single muscle fiber in isopentane at −150°C. At such rapid rates, water freezes between the actual filaments of myosin and actin in aggregates so small that they do not distort the structure, even at the level of observation possible with the electron microscope (Menz and Luyet, 1961). These minute aggregates still appear to be crystalline and not amorphous and vitreous. As the time to freeze increases, structural damage to the muscle increases.

The sarcolemma is still undamaged when the freezing time has been extended from 1 s to 5 min, although with the latter time there would be consider-

able distortion of the myofibrils inside the muscle fiber (Menz and Luyet, 1961). As the time to freeze increases beyond about 5 min, damage to the sarcolemma proceeds through a series of maxima and minima corresponding to different kinds of ice formation, beginning within and eventually outside the fiber. With those freezing times where there is little damage to the sarcolemma, the muscle can be thawed with little formation of drip irrespective of ultimate pH because the water (converted from ice) is completely reincorporated by the proteins. It must be pointed out that fast rates of freezing are not always associated with less drip than slow rates. Only when the rate of freezing encourages the formation of intracellular rather than extracellular ice crystals will it be beneficial (Añón and Calvelo, 1980; Bevilacqua et al., 1979). Of course, the thawing condition is a confounding factor for the freezing effect; a slow rate of thawing is generally found beneficial. Gonzalez-Sanguinetti et al. (1985) reported that a decrease in thawing time (time elapsed from −5°C to −1°C) resulted in a decrease in exudate of beef. This was attributed to the melting of ice in the extracellular spaces, which resulted in the influx of water into the intracellular spaces and its subsequent reabsorption by the dehydrated fibers.

The relationship between the temperature of freezing (which is approximately inverse to the rates of freezing) and ice crystal formation in beef *Longissimus* muscle has been widely studied. As demonstrated by Rahelić et al. (1985), whereas water formed ice only intercellularly at −10°C and −33°C, and only intracellularly at −78°C and below, ice formed both inter- and intracellularly at −22°C, when the greatest structural damage was observed (Fig. 7.4). At this temperature, the solubility of the myofibrillar protein was also found to be the least (Petrović et al., 1993). Ice crystals formed within the I-band, but not within the A-band at −22°C, possibly because the water-holding capacity of the actin filaments is weaker than that of myosin filaments and the difference can be elicited in this temperature range (Rahelić et al., 1985).

Because of the large size of meat cuts, the commercially feasible rates of freezing are much too slow to produce predominantly intracellular ice forma-

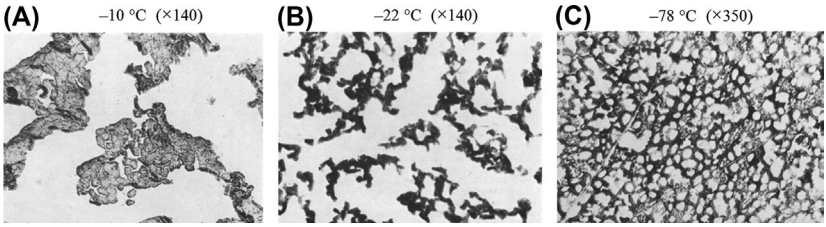

FIG. 7.4 Ice crystal formation in beef Longissimus muscle as influenced by the rate (time of freezing) and temperature of freezing. (A) Slow rate (−10°C in 798 min, intercellular crystals); (B) intermediate rate (−22°C in 226 min, inter- and intracellular crystals); (C) Fast rate (−78°C in 22 min, intracellular crystals) (Rahelić et al., 1985).

tion. With slow rates of freezing, ice crystals tend to form first outside the fiber, since the extracellular osmotic pressure is less than that within the muscle cell. As extracellular ice formation proceeds, the remaining unfrozen extracellular fluid increases in ionic strength and draws water osmotically from the supercooled interior of the muscle cell. This freezes onto the existing ice crystals, causing them to grow, thus distorting and damaging the fibers (Love, 1968). Moreover, the high ionic strength denatures some of the muscle proteins, and this factor, quite apart from the translocation of water, largely accounts for the loss of water-holding capacity of the muscle proteins and for the failure of the fibers to reabsorb, on thawing, all the water removed by freezing, manifested as drip (Xiong, 1997). Protein damage is generally a function of time and temperature of freezing. In general, the extent of denaturation of both sarcoplasmic and myofibrillar proteins increases with the length of frozen storage. Despite the adverse effect on water holding within meat, sensory juiciness seems to be less influenced by the process of frozen storage (Lagerstedt et al., 2008), highlighting the unreliability to predict organoleptic perception from a single instrumental test.

In addition to physical damage by ice crystals and destabilization by concentrated solutes, muscle proteins during meat frozen storage and thawing are susceptible to oxidation, which further contributes to drip loss and cooking loss in thawed meat (Utrera et al., 2014; Xia et al., 2010). Protein oxidation in frozen meat appears to begin in the unfrozen fraction of water where oxidative reactants may be concentrated, and it is usually coupled with lipid oxidation. However, differing from protein oxidation, lipid peroxidation that occurs on or close to surface of frozen meat requires no water. The secondary products generated from lipid peroxidation would partition at the fat/water interface to initiate protein oxidation leading to the denaturation of myosin and aggregation of myofibrils (Xiong, 1997). In fact, the increased oxidative stress in frozen meat has a downstream effect as thawed meat has a much shorter refrigerated shelf life when compared with previously nonfrozen meat due to accelerated lipid oxidation (Hansen et al., 2004). The freezing stress exacerbates lipid oxidation in post-thaw ground meat products during chilled retail display (Schilling et al., 2019).

7.4 Heating

7.4.1 General consideration

Heat treatments, through either pasteurization or sterilization, are historically a primary means to preserve meat and meat products. In the modern society, however, thermal treatments have become a secondary means to preserve a product's shelf life except for the parts of the world where energy supply and refrigeration systems are limited. In this context, thermal processing in the food industry today is used primarily for the manufacture of ready-to-eat (RTE) meat

or products, and the improved shelf life, of course, is an added benefit. Because meat that has undergone pasteurization heating, usually at a temperature less than 80°C, remains microbiologically susceptible, proper packaging and refrigeration conditions are required. On the other hand, meat products treated with sterilization temperatures (generally at above 121°C) are essentially free of pathogens as well as spoilage organisms, they are of a very long shelf life. Such products are stable at room temperature provided that the contents remain kept in tightly sealed containers, such as a laminated plastic or metal can.

Preservation of meat by thermal processing dates from the beginning of the 19th century when Appert (1918), while not aware of the true nature of the process involved, found that meat would remain edible if it was heated in a sealed container and kept so until the time of consumption. This method of preservation was later developed into the canning industry. Canned meat and meat products may be subjected to heat at two levels: pasteurization, which is designed to stop the growth of disease-generating microbes with minimum damage to meat; and sterilization, in which all or most bacteria are killed, but which alters the meat to a considerably greater degree. An important consideration in achieving sterility is the fact that certain microorganisms form spores, which may be heat-resistant. To destroy spores of certain thermophiles, an exceedingly high temperature, achieved only under pressure, would be required. The presence of nitrite additives, which are bacteriostatic, minimizes the chance for post-sterilization germination of spores. However, high-temperature processing usually compromises the organoleptic attributes of the commodity. In canning practice, "commercial sterility" is achieved by giving a degree of heat treatment sufficient to kill all vegetative bacterial cells. To avoid the germination of heat-resistant spores that might be present, it is essential to cool the cans rapidly after processing and to avoid storage at high ambient temperatures.

7.4.2 Pasteurization

For pasteurization heating, raw meat is packaged and then cooked in an oven, a temperature-humidity controlled chamber, or a hot water bath until the center temperature reaches about 70–80°C. Suitable packaging bags have been developed to withstand high temperatures, and the material consists of multilayer laminate structures formed by tubular extrusion. Common materials include nylon, polyethene, polypropelene, polyester, in different co-extrusion combinations. Cooked meat may be distributed and sold at the retail outlet while still in the original package, or after the original package is removed and then repackaged in a tray or box. Canning is also used to prepare pasteurized meats. In a typical operation, canned meat is cooked in a water bath at about 80C for several hours or steamed cooked for a short period of time. It is desirable to keep the difference between the cooking medium and the meat content to a minimum to minimize cooking loss and "jelly" formation. In the United States, all canned pasteurized meats must be nitrite-cured to comply with the federal regulation.

They must be labeled as "perishable-Keep Under Refrigeration." Thus, similar to all other types of pasteurized meats, canned meat products require refrigeration for distribution and storage.

In the modern day, pasteurization heating is rarely applied to unaltered raw meat for preservation purposes. Instead, meat is usually mixed with certain functional ingredients that are able to improve the palatability or impart microbial inhibition. Many ingredients act as multifunctional agents (organoleptic, product yield, preservation, etc.), e.g., sodium chloride, polyphosphate, and sodium lactate. In cured meat, nitrite imparts strong antimicrobial and antioxidant activities, in addition to its meat color-fixation effect (Cassens, 1995). Pasteurized cured meats are generally quite resistant to microbial spoilage. Perigo et al. (1967) conducted study for finding evidence that, during the pasteurizing process, nitrite reacts with some components of the medium to produce a substance that is strongly inhibitory to the growth of Clostridia. This may help to explain the fortunate, albeit unexpected, stability of this type of product anaerobically packaged. The efficacy of a given pasteurization treatment, at constant levels of salt and nitrite, in controlling the growth of *Clostridium botulinum*, varies between different portions of the pig carcass and between individual animals (Gibson et al., 1982). Breed appears to have no consistent effect. Because pasteurization temperatures are not adequate to destroy all microbes, it is important to ascertain that ingredients that may be used (e.g., spices, condiments, curing salts, sugar, milk powder, soy protein isolate, etc.) are sterile or almost sterile. On the other hand, some spices, because of their essential oil content, have bactericidal or bacteriostatic properties. For example, the antibacterial action of cloves is due to eugenol and that of mustard seed to allyl isothiocyanate (Brewer, 2011).

Resulting from thermal destruction of pathogens, meat and meat products that have received pasteurization process are safe to consume. These meats are therefore commonly referred to as RTE products. However, in recent years, the safety of RTE meats has been under scrutiny due to reported outbreaks associated with their consumption. The center of the issue is postprocess contamination, i.e., microbial contamination during repackaging of pasteurized meats. Tremendous efforts have been made by the food industry on developing hurdle technologies to minimize such postprocess contamination and growth of pathogens, concentrating on *L. monocytogenes* that can thrive in refrigerated meat and meat products. Post-package thermal pasteurization (steam or hot water) and the incorporation of bacteriostatic compounds, such as sodium diacetate and lactic acid, are the most common approaches to controlling L. *monocytogenes* in RTE products (Jiang and Xiong, 2015). The combination of re-pasteurization and natural antimicrobial additives has proven to be very effective in achieving a long shelf life, such as 3 months, in cooked meat.

7.4.3 Sterilization

The majority of canned meats are "commercially" sterilized, i.e., they are processed to the point at which all microorganisms and most of their spores have been killed. This permits more or less an indefinite storage life in the can, at ambient temperature, provided it is kept sealed. However, such product is markedly different from freshly cooked meat or pasteurized meat, exhibiting strong "cooked flavor" and substantially altered physical structures. In the early days of canning, meat products were heated in an open water bath; under this condition, the temperature of the cans failed to attain 100°C, and a long processing time was necessary to achieve commercial sterility. Increasing the boiling point of the water by adding salts such as calcium chloride made possible a great reduction in the processing time. By 1874, a controllable pressure steam retort had been invented; and between 1920 and 1930, information on the heat resistance of bacterial spores and on heat penetration into cans permitted the preparation of time-temperature processing schedules to control the canning process instead of relying on empiricism (Howard, 1949).

Although the pH is generally on the acid side of neutrality, meat is regarded as a low-acid food. Since the most lethal food-poisoning organism, *Clostridium botulinum*, has a lower limit of growth at pH 4.5, all foods such as meat, which support its growth, are given heat treatment sufficient to destroy it. At 100°C, the botulinum toxin is destroyed in 10 min. The presence of curing ingredients, notably sodium nitrite, in products such as canned hams makes them less liable to harbor *Cl. botulinum*. Because certain thermophilic bacteria capable of withstanding very severe heat treatment could be present, a degree of thermal processing that can seriously affect the meat and lower its nutritive value and flavor is required to achieve sterility. Therefore, sanitary measures to avoid initial contamination are essential to control these organisms.

Sterilized canned meats suffer considerable change in the process due to protein denaturation and aggregation by heat. The texture of canned meat after sterilization can be mushy, and marked deterioration in aesthetic appeal and eating quality often occurs. The color of canned meats will tend to resemble that of the cooked commodity, since the high temperatures will change the red pigment (myoglobin-Fe^{2+}) to its oxidized form, which appears brown (metmyoglobin-Fe^{3+}). Moreover, since meat (especially pork) contains appreciable quantities of thiamin (vitamin B1) and ascorbic acid (vitamin C) and they are destroyed by heat, the nutritive value of canned products will be more or less reduced. The loss of such labile nutrients will be exaggerated if the cans are subsequently stored for long periods at high ambient temperatures. Sterilized meat processing under exceeding high temperatures ($\geq$ 121°C) and pressure has a characteristic "overcooked" flavor resulting from chemical reactions of lipids and proteins. Nevertheless, sterilized meat is readily digestible, which may be a benefit for some consumers.

An alternative process to allow commercial sterility with minimal concomitant damage to the meat content had been developed in the 1980s that utilizes heat-sterilizable flexible bags (retort pouches) instead of cans. These are produced from multiple laminates, which are hermetically sealable. A typical 4-ply laminate for this purpose could consist of 12 μm polyester/12 μm Al foil/12 μm polyester/70 μm polyolefin (Paine and Paine, 2012). The retort pouch offers several advantages: the thermal process time tends to be significantly shorter than that for metal or glass containers (which minimizes loss of quality in the product); the shelf life is comparable to that of a frozen product without the need for a frozen chain for storage and distribution; there is less container/product interaction; and by permitting "boil-in-the-bag," the retort pouch speeds up preparation and serving (Paine and Paine, 2012).

7.5 Novel thermal procedures

7.5.1 Ohmic heating

Several emerging technologies for the thermal processing of meat (and other foods) have become available, which achieve the required degree of microbial destruction with minimum damage to the nutritive and organoleptic properties of the meat, for example, ohmic heating, dielectric heating, and radiant heating. In ohmic heating, an elevated temperature is developed by passing an electric current through the meat (which has a high resistance) (Fig. 7.5). The process permits continuous production, without heat transfer, and pasteurization or sterilization at relatively low temperatures. Hence, harm caused by the heat as in the conventional thermal treatments is minimized. This allows excellent retention of nutrients as well as maintenance of the organoleptic characteristics of meat producing high feeling of freshness and consumer satisfaction. Maximum electrical conductivity has been reported when beef fibers are aligned with the current flow, and the addition of sodium chloride and phosphate, which increase the electrical conductivity, results in an increase in the ohmic heating rates and quality of cooked meat (Kaur and Singh, 2016).

7.5.2 Dielectric heating

In dielectric heating, the energy of high-frequency alternating electromagnetic field is transferred to polar materials, such as water (dipoles) inside muscle tissue, to produce thermal energy. Dielectric heating includes both radio frequency (RF) and microwave (MW) heating. Modern MW ovens utilize electromagnetic waves with electric fields of much higher frequency and shorter wavelength than RF heaters. High-frequency radiowaves (1–100 MHz) cause oscillation of water molecules (dipoles) inside the meat and generate heat by friction. At even higher frequencies (300 MHz–300 GHz), i.e., microwave heating, the tempera-

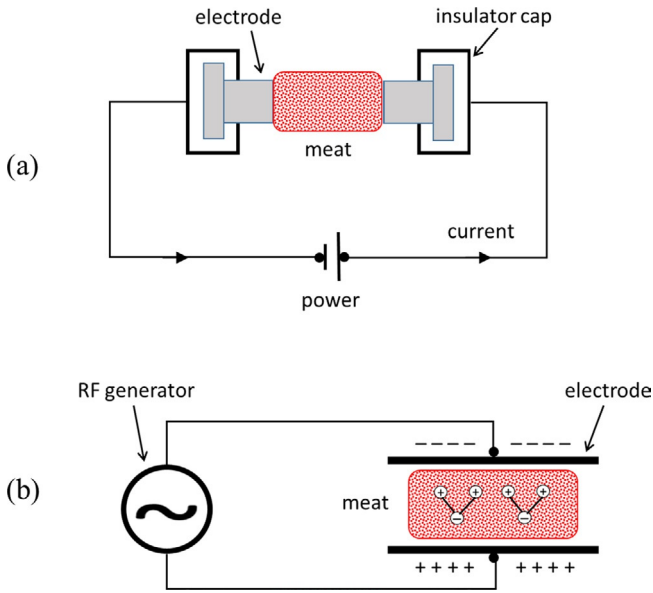

FIG. 7.5 Schematic diagrams of ohmic heating (A) and radio frequency heating (B) setup.

ture required to decrease microbial loads with minimum damage to the product can be achieved in minutes rather than hours.

Dielectric heating can be considered as a volumetric process allowing for efficient temperature rise (fast heating rates) by largely eliminating the temperature gradient. However, there are differences between RF and MW. As efficient as the energy transfer, for microwave heating, the penetration essentially stops where all the microwave energy has been converted to heat in the tissue. This can cause uneven heating inside the muscle tissue, e.g., a cold spot present inside. On the other hand, RF at intermediate frequencies has greater penetration over MW, shows greater promise than microwave systems as a method of rapid and uniform heating for meat.

Laycock et al. (2003) used RF to process several meat products, reporting a decreased cooking time (5.83, 13.5, and 13.25 min for ground beef, comminuted meat, and whole muscle, respectively, compared with 151, 130, and 109 min in water bath), lower juice losses, and acceptable water-holding capacity and texture. McKenna et al. (2006) compared the quality of leg and shoulder pork hams subjected to RF and steam heating. RF cooking of the hams resulted in a shorter cooking time and a greater cooking yield. However, RF-cooked hams had a lower water-holding capacity and were harder than steam-cooked hams. Moreover, RF-cooked whole muscle exhibited inferior color indicating that further research is required before the technology can be commercially implemented.

7.5.3 Radiant heating

This thermal process is perhaps not as suitable for meat preservation when compared with some other methods. However, by receiving infrared thermal energy, the temperature of meat surface will rise rapidly. The high surface temperature attained allows the surface of meat to be "grilled" to produce browning reaction. Radiant heating is restricted to thin pieces of meat of even cross section or to kill microorganisms on the surface of large meat products.

7.6 Future trends

Storage at refrigerated and subfreezing temperatures will continue to be the primary means to preserve the quality and extend the shelf life of raw and processed meat. To maximize the efficacy of thermal technologies, antimicrobial agents derived from natural sources as a hurdle factor will likely be used to complement the thermal effect. However, the meat industry must continue to be diligent in guarding the chemical safety of treated meat and meat products. Furthermore, with the advent of "superchilling" technology and the growing consumer demand for fresh food, storage of meat in a freezing-temperature chamber but without actually causing muscle tissue to be frozen will become an attractive and ultimately common means to preserve meat.

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Chapter 8

The storage and preservation of meat: II—Nonthermal technologies

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8.1 Introduction

This chapter covers three major nonthermal meat preservation technologies that can improve the safety, storage, and preservation of meat by killing pathogenic microorganisms or discouraging the growth of nonpathogenic microorganisms through the creation of unfavorable environments. Irradiation is the most efficient nonthermal technology that can eliminate pathogenic microorganisms and improve the safety of meat, but has some negative effects to the quality of meat products. In the irradiation part of the chapter, history, irradiation processes, principles, mode of actions, antimicrobial aspects, chemical and biochemical aspects of irradiation, which include lipid oxidation, color changes, off-odor production, and texture changes in meat by irradiation, organoleptic aspects and consumer acceptance of irradiated meat, and the detection methods of irradiated products, are discussed.

Ready-to-eat (RTE) meat products are traditionally among the largest category of foods treated by high-pressure processing (HPP). In HPP, packaged foods including meats are subjected to very high pressures to destroy pathogens and spoilage microorganisms while maintaining sensory attributes and nutritional quality. Depending on the pressure level, several types of damage can occur in microorganisms. The effectiveness of HPP in killing meat-borne microorganism is significantly affected by the characteristics of the meat products such as water activity and the amount of protein and fat. Also, substantial changes in meat protein structure, lipid oxidation, and color can occur depending on the level of pressure applied to meat products. In this respect, there is a crucial need for information on both the microbiological and quality characteristics of pressurized meat products if the meat industry were to make investments to use HPP on a routine basis.

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Freeze dehydration is an expensive process and not extensively used in commercial scale. In the freeze dehydration part of the chapter, histological aspects, physical and biochemical aspects, and organoleptic aspects to meat are discussed. The advantages and disadvantages or future perspectives of each technology are also briefly discussed.

8.2 Ionizing radiation

8.2.1 History of food irradiation

Although two patents were filed in 1905 and X-rays were applied to kill *Trichina* in pork in 1921, the concept of employing ionizing radiation to preserve food started in 1940. In the period 1954–64, the Quartermaster Corps of the U.S. Army carried out long-term studies on various meat products including ground beef and pork and bacon and concluded that irradiation could provide wholesome, economical, shelf-stable field rations. In the 1970s, the Food and Drug Administration (FDA) withheld approval for irradiated ham, and an international project on the future of irradiation was initiated. The National Aeronautics and Space Administration (NASA) has adopted irradiation process to sterilize meats for astronauts in space and used irradiated meats on the Apollo-Soyuz Test Project (ASTP) in 1975. In 1980, the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, and the World Health Organization (FAO/IAEA/WHO) stated that "irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological changes; hence toxicological testing of foods so treated is no longer required." More recently, WHO announced that food products irradiated above the 10 kGy ceiling are safe and wholesome and has recommended removing dose limit for irradiation (World Health Organization, 1999).

In the United States, potato was the first food product approved for irradiation in 1964 to inhibit sprouting during storage. However, the use of irradiation in meat was first approved for pork to control *Trichinella spiralis* in 1985, and then fresh and frozen poultry meat to control microorganisms in 1990. Chilled and frozen red meat, shell eggs, shellfish, and fresh produce were approved for irradiation in the late 1990s and 2000s, but cooked or processed food products are still waiting for approval. The approval date, dose, and purpose of food irradiation in the United States are listed in Table 8.1.

8.2.2 Irradiation process

Radiation energies are classified into three categories: electromagnetic radiation (γ-ray, X-ray), charged particle radiation (α-ray, β-ray, electron beam, photons), and uncharged particles (neutron). Among the ionizing radiation, only high-energy electrons from linear accelerators, X-rays produced by collision of the high-energy electrons with a metal (tungsten) target, and γ-rays from radioactive

TABLE 8.1 Approval of food irradiation in the United States.

Date	Products	Dose (kGy)	Purpose
1964, 65	Potatoes	0.05–0.15	Inhibit sprouting
1983	Spices and dry seasonings	<30	Disinfestation and decontamination
1985	Pork	0.3–1.0	Control of *Trichinella spiralis*
1985, 86	Dehydrated enzymes	<10	Control insects and microbes
1986	Fruits and vegetables	<1	Delay maturation and disinfection
1986	Herb, spices and seasonings	<30	Control of microorganisms
1990	Poultry, fresh and frozen	<3.0	Control of microorganisms
1995	Meat, frozen and packaged	>44	Sterilization only for NASA
1997, 99	Red meat, chilled	<4.5	Control of microorganisms
	Red meat, frozen	<7.5	
2000	Shell eggs	<3.0	Control of *S. Enteritidis*
2000	Sprouts	<8.0	Control of pathogens in seeds
2005	Fresh or frozen molluscan and other shellfish	<5.5	Control of Vibrio species and foodborne pathogens
2008	Iceberg lettuce and spinach	<4.0	Control of foodborne pathogens and extension of shelf life
2012	Uncooked meat, meat by-products, and certain meat food products	<4.5	Control of foodborne pathogens and extension of shelf life
2014	Chilled or frozen raw, cooked, or partially cooked crustaceans	<6.0	Control of foodborne pathogens and extension of shelf life

sources (e.g., Co^{60}) are useful in practice for treating foodstuffs (Brynjolfsson, 1989). The "ionizing" radiation has a strong enough power to eject electrons from the atoms/molecules of a material and produces ions and free radicals. Both γ-rays and accelerated electrons are used for food irradiation, but e-beam has advantages in terms of process control, energy efficiency, irradiation speed,

accuracy, and consumer acceptance compared with the γ-rays. The only disadvantage of electron beam is its limited penetration capability. Use of X-ray for food irradiation also has been tested, but its energy efficiency is < 30% of accelerated electrons (Olson, 1998). Regardless of radiation sources, the amount of ionizing energy absorbed in target materials is called "radiation absorbed dose." The unit (SI) measured for irradiation dose is Gray (Gy), which is equal to the absorption of energy equivalent to 1 J/kg of absorbing material (1 Gy = 1 J/kg).

The comparative characteristics of ionizing radiation sources used for food irradiation are shown in Table 8.2. Within broad limits, the important factor is the *total* dose received by the product. However, the effectiveness of irradiation varies depending on the type of radiation, the radiation intensity, and the targeted microbes (Kwon, 2010). Whatever the type of radiation used, the international standards for food irradiation do not allow > 10 MeV of energy to avoid an induced radioactivity that may arise in certain elements in foods.

The advantages of ionizing radiation for food preservation include their highly efficient inactivation of bacteria, the low total chemical changes they cause, and the appreciable thickness of material, which can be treated after packing in containers even those made of metal. Although irradiation is very effective in controlling pathogens, it can deplete antioxidants in the muscle, induce color change, produce off-odor volatiles, and negatively alter the sensory characteristics of meat products. Important chemical reactions, antimicrobial effects, impact to quality changes, and sensory characteristics and consumer acceptance of irradiated meat are discussed below.

8.2.3 Mode of action

Ionizing radiations produce ions and other chemically excited molecules in the exposed medium; but this is only the first of a series of chemical effects. Gamma-rays have high energy and do not ionize atoms directly but transfer

TABLE 8.2 The characteristics of ionizing radiation sources.

	Gamma ray	Electron beam	X-ray
Energy type	Electromagnetic	Charged particle	Electromagnetic
Energy volt (MeV)	1.17 + 1.33	~ 10	~ 5
Energy efficiency	Low (~ 30%)	High (~ 85%)	Low (~ 10%)
Penetration capability	Deep (60–80 cm)	Low (8–10 cm)	Deep
Source control	Continuous	Switch (on/off)	Switch (on/off)

Adopted from Kwon, J. H. 2010. Safety and Understanding of Irradiated Food. S. Y. Yoo, and K. W. Lee (Ed.). Korea Food Safety Research Institute. Seoul, Korea.

energy to secondary electrons, which then interact with other materials to form ions. When an accelerated electron enters materials, the energy can be transferred or absorbed by an electron of an atom in materials, which increases its energy level (excite) and ejects from orbit. The ejected electron, called "Compton electron," transfers its energy to a secondary electron and reduces the total energy of the Compton electron. The Compton electrons cause further excitation and ionization in the material (Compton effect), which continues until not enough energy is left to cause electrons to escape their orbitals. The first target of highly energized electrons is water molecule in biological substances such as meat. The dispersion of ions and free radicals is greater when water is present in free form than in bound form (dried products) or the crystalline form (frozen products) (Thakur and Singh, 1994). The hydroxyl radical (HO•), the primary radiolytic product of water, is a powerful oxidizing agent. Because the dispersion and capture of electrons are purely random, large molecules and compounds have a greater probability of being affected than smaller molecules (Diehl, 1995). Other radiolytic products of water also play important roles in chemical and biochemical reactions that influence the antimicrobial, quality, and sensory characteristics of meat. The radiolytic products produced from water by ionizing radiation are shown below:

$$\begin{aligned} &H_2O(\text{irradiation}) \rightarrow \cdot OH(\text{hydroxyl radical}) + \cdot H(\text{hydrogen radical}) \\ &\quad + H_2(\text{hydrogen}) + H_2O_2(\text{hydrogen peroxide}) + H_3O^+(\text{hydrated proton}) \\ &\quad + e^-_{aq}(\text{hydrated electron}) \end{aligned}$$

8.2.4 Antimicrobial aspects

Ionizing radiation destroys microorganisms by inactivating genetic materials in living cells either by the direct ionization, or excitation on DNA, or indirect effects through the production of radiolytic products that attack DNA indirectly (Smith and Pillai, 2004). The DNA bases are highly susceptible to ionizing radiation resulting in cleavage of phosphodiester bonds of DNA double helix. Hydroxyl radial (·OH) is known to have 90% damage rate to DNA molecules, and breaking bonds in the DNA cause the loss of a cell's ability to replicate and eventually lead to the death of the bacteria.

Irradiation damage to molecules is approximately proportional to their molecular weight. Thus, the very large molecules are particularly vulnerable. A sterilizing dose that reduces the numbers of *Cl. botulinum* by 10^{12} changes only about 0.2% of the proteins, 0.3% of the carbohydrates and 0.4% of the lipids in the irradiated product. Irradiation in the frozen state reduces all changes by 75%. Most of the meat and meat products require <10 kGy of irradiation to destroy pathogenic bacteria and parasites, but >10 kGy of irradiation is needed to eradicate microorganism except virus. Spore-forming bacteria and virus need higher dose of irradiation. The D-values of foodborne pathogens and spoilage bacteria are shown in Table 8.3.

TABLE 8.3 D_{10}**-values (kGy) of some foodborne pathogenic bacteria in meat products.**

Pathogen	D_{10} (kGy)	Medium	Reference
Aeromonas spp.	0.09	Chicken	Nagar and Bandekar (2011)
Bacillus cereus	0.59	Beef (marinated)	Jo et al. (2004)
Cl. sporogenes (spores)	1.98	Ham (uncured)	Silva et al. (2021)
	1.81	Ham (cured)	Silva et al. (2021)
Campylobacter jejuni	0.31	Chicken	Kudra et al. (2012)
Escherichia coli	0.24	Ground beef	Black and Jaczynski (2006)
	0.27	Chicken	Sommers et al. (2017)
Klebsiella pneumoniae	0.35	Chicken	Sommers et al. (2021)
Listeria monocytogenes	0.42	Ground pork	Mendonca et al. (2004)
	0.54	Ham	Lacroix (2017)
	0.61	Frankfurters	Sommers et al. (2016)
Salmonella enterica	0.47	Duck meat	An et al. (2018)
	0.64	Beef (marinated)	Jo et al. (2004)
Staphylococcus aureus	0.41	Chicken	Spoto et al. (2000)
	0.66	Beef (marinated)	Jo et al. (2004)
Staph. saprophyticus	0.64	Chicken	Sommers et al. (2017)
Yersinia enterocolitica	0.18	Ground pork	Bhaduri et al. (2014)

The susceptibility of bacteria to irradiation is influenced by irradiation dose, meat composition, physical conditions, temperature, and microbial factors. High-dose irradiation can reduce microbial populations effectively, but high-dose irradiation can have adverse effects on meat quality and the sensory attributes of meats (Zhao et al., 2018). The combined use of irradiation with other intervention technologies such as antimicrobial additives, heat, high hydrostatic pressure, etc., can increase the microbicidal efficacy of irradiation. Therefore, even with low doses of irradiation, the microbial safety of meat can be improved while the sensory attributes are maintained. The proteins and antioxidants in meat such as carnosine and tocopherol show protective effects to microorganisms because of their free radical scavenging activities (Diehl, 1995). The moisture content and water activity have a drastic impact on the microbicidal

efficacy of irradiation because hydroxyl radicals are mainly produced from the free water molecules (Thakur and Singh, 1994). Dried and frozen meat requires higher dose of irradiation than fresh meat because the formation of hydroxyl radicals from ice is much lower than free water, and ice impedes the migration of free radicals to other parts of the frozen product (Taub et al., 1979). The large populations of microorganisms reduce the microbicidal effect of irradiation at a given dose, and the sensitivity of microorganisms to irradiation varies among microbial types. Simpler life forms exhibit a higher resistance to irradiation than more complex life forms: viruses are more resistant to radiation than spores, which in turn show a higher radiation resistance than vegetative bacterial cells. Spore-forming bacteria show a greater radiation resistance than non-spore formers, while Gram-positive bacteria are more resistant to ionizing radiation than Gram-negatives. Bacterial cells in exponential phase are more sensitive to irradiation than those in lag or stationary phase. However, bacteria that have adapted to certain environmental stress or starved show a greater radiation resistance than those in stationary phase (Mendonca et al., 2004). The storage life of ground beef could be extended by 4–15 days at 4C with 1–5 kGy irradiation that results in a floral shift from Gram-negative bacilli to Gram-positive cocci as the dose of irradiation increases. Despite the general susceptibility of the cold-tolerant microorganisms to pasteurizing doses of irradiation, certain more resistant species are bound to survive even in small numbers. Such microorganisms spoil meat by producing a sour odor rather than the stale, musty odor from the pseudomonads, which grow in stored, nonirradiated chilled meat. The extension of storage life of some meat products by low-dose irradiation is shown in Table 8.4.

TABLE 8.4 Shelf life of meat products with or without irradiation.

Meat products	Dose (kGy)	Untreated shelf life (d)	Irradiated shelf life (d)
Beef top round	2	8–11	28
Beef burgers	1.54	8–10	26–28
Beef cuts under vacuum	2	NA	70
Corned beef	4	14–21	35
Whole and minced lamb	2.5	7	28–35

Adopted from Andrews, L. S., M. Ahmedna, R. M. Grodner, J. A. Liuzzo, P. S. Murano, E. A. Murano, R. M. Rao, S. Shane, and P. W. Wilson. 1998. Food preservation using ionizing radiation. Rev. Environ. Contam. Toxicol. 154:1–53.

8.2.5 Chemical and biochemical aspects

The main goal of irradiating meat is eliminating pathogens and improving the safety and storage stability of meat. However, the chemical and biochemical reactions of free radicals with meat components accelerate lipid oxidation, produce a characteristic odor, and alter meat color that significantly impact consumer acceptance. Consumers associate the brown/gray color in irradiated raw beef with old or low-quality meat, and off-odor and off-flavor with undesirable chemical reactions. Thus, understanding the chemical and biochemical reactions taking place in meat by irradiation and developing methods that can minimize or prevent such quality changes are important to improve consumer acceptance and the implementation of irradiation technology by industry.

8.2.5.1 Lipid oxidation

Polyunsaturated fatty acids (PUFAs) in meat can be easily oxidized under aerobic conditions. Irradiation can accelerate lipid oxidation in meat because irradiation can produce hydroxyl radicals, which can initiate lipid oxidation in meat. Irradiation-induced lipid oxidation in meat is dose-dependent, especuially under aerobic conditions (Ahn et al., 1998). In the absence of oxygen, however, irradiation has only marginal effect on the development of lipid oxidation in meat during storage. During post-irradiation storage, hydrogen peroxide produced from water, particularly in the presence of oxygen, gradually disappears while other meat constituents are oxidized. Irradiation has little effect on lipid oxidation of frozen meat even under aerobic conditions (Nam et al., 2002b) because the amount of free water that can be involved in the production of radiolytic compounds is small. Lipid oxidation produces various volatile compounds including hydrocarbons, alcohols, ketones, and aldehydes from fatty acids. However, aldehydes contribute the most to oxidation flavor and rancidity in cooked meat, and the amount of hexanal is highly correlated to the degree of oxidation in meat.

8.2.5.2 Off-odor production

All irradiated meat produces characteristic, readily detectable irradiation odor regardless of the degree of lipid oxidation. The irradiation odor was characterized as "metallic," "sulfide," "wet dog," "bloody and sweet," or "barbecued corn-like" and was distinctly different from oxidation odor. Earlier, radiolytic products of water-soluble sulfur compounds (methyl mercaptan and H_2S), radiolytic products of fat (hydrocarbons), and lipid oxidation products (carbonyls) were considered as the major volatiles responsible for the irradiation odor. Further studies, however, indicated that sulfur compounds (hydrogen sulfide, sulfur dioxide, mercaptomethane, dimethyl sulfide, methyl thioacetate, dimethyl disulfide, and trimethyl sulfide) were mainly responsible for the irradiation off-odor (Jo and Ahn, 2000; Fan et al., 2002). Many volatiles such as 2-methyl butanal, 3-methyl butanal, 1-hexene, 1-heptene, 1-octene, 1-nonene,

and oct-1-en-3-one were newly produced or greatly increased in meat by irradiation, but their contribution to irradiation odor was small compared with those of sulfur compounds. Because the threshold of sulfur compounds was much lower than other volatile compounds, the odor intensity was much stronger and stringent even at very low levels of sulfur compounds. The volatile profiles and sensory characteristics clearly indicated that volatiles from lipid oxidation contributed only a small part of irradiated off-odor in meat, which supported the concept that the chemical changes by irradiation were different from those of warmed-over flavor (Lee and Ahn, 2003). The perception of odor from samples containing sulfur volatiles changed greatly depending upon their composition and amounts present in the sample. Sensory panelists confirmed that all irradiated liposomes containing "sulfur amino acids" produced similar odor characteristics to irradiated meat, indicating that sulfur amino acids are mainly responsible for irradiation odor (Ahn, 2002; Ahn and Lee, 2002).

The sources and mechanisms of volatile production in meat by irradiation indicated that the majority of volatiles were produced through the radiolytic degradation of amino acid side chains. However, the volatile compounds produced in irradiated meat were not only the primary products of radiolytic degradation, but also the products of extensive chemical reactions including deamination, Strecker degradation, dehydrogenation, isomerization, cyclic reaction, and decarboxylation of the radiolytic products (Ahn et al., 2016a,b). Among the amino acids, the side chains of aliphatic, aliphatic hydroxyl, and sulfur-containing amino acids were highly susceptible to radiolytic attack. Aliphatic amino acids such as isoleucine, leucine, and valine produced branched-chain aldehydes such as 2-methyl butanal, 3-methyl butanal, and 2-methyl propanal by radiolytic deamination and decarboxylation, and serine produced acetaldehyde through the radiolytic deamination and decarboxylation α-carbon to generate ethen-1-ol, and then the Strecker degradation forms acetaldehyde (Ahn et al., 2016a). The majority of the volatiles from sulfur amino acids were through the direct radiolytic products of the side chains, but Strecker degradation and deamination, decarboxylation, hydrogenation, and oxidation of the primary radiolytic products were also involved (Fig. 8.1). The sulfur volatiles from cysteine and methionine produced odor characteristics similar to that of the irradiated meat, but the amounts of sulfur volatiles from methionine were far greater than that of cysteine (Ahn et al., 2016b; Jia et al., 2021a). In addition to proteins and amino acids, fatty acids are also involved in volatile production in meat by irradiations. Hydrocarbons are the major compounds formed from fatty acids by free-radical reactions. The amount of hydrocarbons produced were affected by irradiation dose, temperature, oxygen, and fatty acid composition of meat (Miyahara et al., 2002). The radiolytic degradation of fatty acid increases as the irradiation dose increases, but PUFAs are more susceptible to radiolysis than monounsaturated or saturated fatty acids.

The volatility of aroma compounds from food matrices depends on the vapor-liquid partitioning of volatile compounds. The release of polar compounds

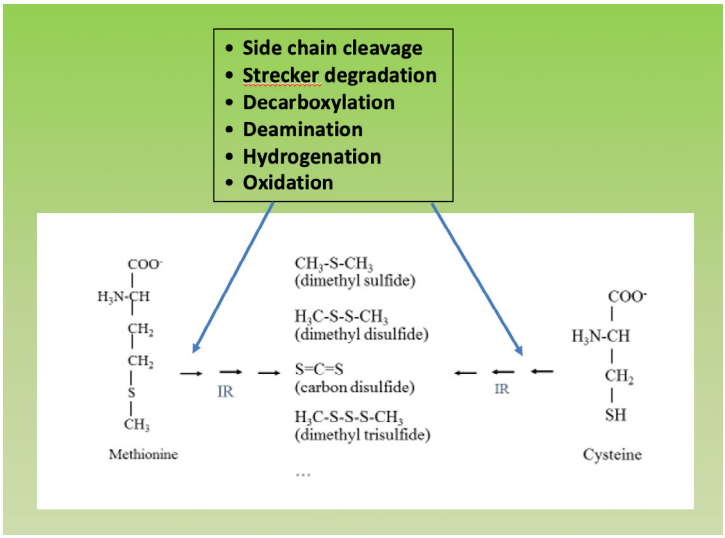

FIG. 8.1 Production of volatiles from sulfur amino acids by irradiation. *Adapted from Ahn, D. U., E. J. Lee, X. Feng, W. Zhang, J. H. Lee, C. Jo, and K. C. Nam. 2016b. Mechanisms of volatile production from sulfur-containing amino acids by irradiation. Radiat. Phys. Chem. 119, 80–84.*

such as aldehydes, ketones, and alcohols is greatly influenced by water, but nonpolar hydrocarbons are not affected. Thus, the interactions of volatile compounds with food components (e.g., carbohydrates, lipids, and proteins) and the physicochemical conditions of foods that determine the conformation of proteins affect the release of volatile compounds in foods (Godshall, 1997; Lubbers et al., 1998). This indicated that the relative amounts and the composition of volatile compounds released from meat systems could be significantly different from those in the aqueous system, and the amount of volatiles released from oil emulsion correlated negatively with the fat content (Jo and Ahn, 1999).

8.2.5.3 Color changes

Myoglobin, the main heme pigment of meat, exists in three different forms (met-, reduced-, and oxymyoglobin) with different proportions. Depending on the state of heme iron, the color expression and intensity are significantly different. Gas compounds such as O_2, CO, S, or NO can make the sixth ligand of heme ring only when the chemical states of heme iron are in reduced form. During the conversion of muscle to meat, all the oxygen inside the muscle is used up due to various enzyme activities. Thus, the myoglobin in the middle of meat block is usually in the reduced form and weakly binds with water molecule or stabilized by the distal histidine of globin. The color of such pigment is purple and is called deoxymyoglobin or reduced myoglobin. Upon exposure to high oxygen partial pressure conditions, oxygen binds to the sixth ligand of heme and produces bright red color. Discoloration of fresh meat to brown color

is mainly caused by oxidation of myoglobin to metmyoglobin when oxygen partial pressure is low. The oxidized brown color can be turned into bright red if the meat has a strong enough reducing power to convert the metmyoglobin to reduced myoglobin, and oxygen is bound to the reduced myoglobin.

The color changes in irradiated raw meat vary significantly depending on irradiation dose, animal species, muscle type (pigment content), and packaging type. The color of white meat (e.g., chicken breasts and pork loin) becomes pink or red when irradiated both in vacuum- and aerobically packaged conditions, and the increased redness was irradiation dose-dependent (Millar et al., 1995). Sensory panelists preferred the red color of the irradiated raw turkey meats to nonirradiated ones because irradiated meat looked fresh. However, the increased redness in irradiated light meats can be a problem if the red color persists in the meat after cooking. In red meat, the color changes are different from light meats: irradiation of raw beef under aerobic conditions produces unattractive brownish-gray color. The color changes of beef irradiated under vacuum-packaging conditions, however, were not always the same: some reported significant decrease in redness and development of brown discoloration while others found increased redness. An objectionable red color was observed when precooked chicken, turkey, and pork sausages were irradiated, and the increased redness was greater inside than on the surface, but the pink color inside the aerobically packaged irradiated cooked meat changed to brown or yellow after storage because of pigment oxidation (Nam and Ahn, 2002b, 2003b). The red pigment formed in irradiated raw and cooked light meats was characterized as carbon monoxide-myoglobin (CO-Mb) (Nam and Ahn, 2002a,b). For the pink color formation in light meats by irradiation, three essential factors, which include production of CO, generation of reducing conditions, and CO-Mb ligand formation, are essential. Furuta et al. (1982) found that significant amounts of carbon monoxide were produced from meat by irradiation. Among the meat components, glycine, asparagine, glutamine, pyruvate, glyceraldehydes, α-ketoglutarate, and phospholipids were the primary sources for CO production by irradiation, and the amount of CO produced was closely related to the structure of component molecules (Lee and Ahn, 2004). In addition to the production of CO, the heme iron in meat should be in ferrous state to form CO-myoglobin because CO can bind to myoglobin only when it is in reduced form. Irradiation generates reducing conditions in both aerobically and vacuum-packaged raw and cooked meat because the hydrated electrons (e_{aq}^-) can act as a powerful reducing agent (Swallow 1984). With the formation of CO-Mb, the intensity of red color increased greatly. The irradiation-induced red pigments (CO-myoglobin) was very stable during storage under vacuum conditions, but the CO-Mb receives continuous challenge by oxygen during storage under aerobic conditions. In cooked meat, both undenatured and denatured heme pigments are involved in CO-myoglobin formations. In aerobically and vacuum-packaged turkey breast, the a^* values were positively correlated with the irradiation dose and the amount of CO gas produced (Nam and Ahn, 2003b).

The mechanisms of color change in irradiated beef, especially under aerobic conditions, are different from those of the light meats: In the absence of oxygen, a reducing environment is established because of the hydrated electron and hydrogen radicals, and thus, the heme pigments in red meat are in ferrous form and color is red (Satterlee et al., 1971). In the presence of oxygen, however, strong oxidizing agents (superoxide and hydroperoxyl radicals) are formed from the reactions of O_2 and e_{aq}^- and O_2 and $^{\bullet}H$, respectively (Giddings, 1977). Therefore, irradiation of red meat under aerobic conditions favors ferric Mb (brown color). CO-myoglobin also can be formed in red meat by irradiation, but is only a small proportion of the total heme pigment because the amount of heme pigments in beef is about 10–100 times greater than that of the light meats. Although the color intensity of CO-myoglobin is stronger than other forms of myoglobin, the contribution of CO-Mb to overall beef color is much smaller than that in the light meat. Therefore, the color of aerobically packaged irradiated red meat is mainly determined by ferric myoglobin. Irradiation of red meat under vacuum conditions or addition of ascorbic acid to aerobically packaged meat creates reducing environments and can prevent brown color development in ground beef (Nam and Ahn, 2003a). A green pigment can be formed in meat by irradiation because hydrosulfide produced from glutathione or sulfur-containing amino acids can bind to heme pigments. Thiols are particularly susceptible to attack by free radicals and produce hydrogen sulfide. The greenish brown color was problematic when ground beef was irradiated under aerobic conditions, but anaerobic conditions protected the beef from discoloration. When vacuum-packaged irradiated beef was exposed to aerobic conditions in the middle of storage, the color bloomed to vivid fresh red color and was maintained during the remaining aerobic storage (Nam et al., 2004).

8.2.5.4 Texture change

Irradiation changes the texture of frozen, raw, precooked, and processed meat products, but the changes are minor (Zhu et al., 2004). The textural changes by irradiation are mainly due to protein denaturation and the reduction of water-holding capacity (WHC) (Kanatt et al., 2015). Irradiated chicken breasts had higher cooking loss and shear force than the nonirradiated ones. The mechanism for irradiation-induced water loss could be caused by the damage to the integrity of membrane structure of muscle fibers and the denaturation of muscle proteins. Shrinkage in sarcomere width (myofiber diameter) and disruption of myofibrils in meat were also noticed by irradiation, but the texture attributes of chicken breasts were not influenced by low-dose irradiation (1.0 and 1.8 kGy) (Lewis et al., 2002).

8.2.6 Organoleptic aspects of irradiated meat

Various organoleptic changes arise in irradiated meat depending on the dose, packaging, irradiation conditions, and storage. Odor and flavor can be adversely affected mainly due to the production of sulfur volatiles and aldehydes through radiolysis of amino acid side chains and oxidation of fatty acids, color by the production of CO-myoglobin, metmyoglobin, and sulphmyoglobin, and texture

by the changes in water-binding capacity and protein oxidation. Irradiation of frozen or dried meat can greatly reduce the radiochemical changes, but requires much higher dose to accomplish the same antimicrobial effect. Meat of high ultimate pH (DFD meat) appears to resist the changes that produce sulfur-containing volatiles upon irradiation (Nam et al., 2002a).

The possibility of minimizing quality changes in meat using protective additives such as antioxidants has been considered on the assumption that they would react with the activated molecules and free radicals produced and thus prevent them from attacking the organic molecules of the meat (Arshad et al., 2019). Other approaches such as packaging, masking agents, reducing agents, and in-package odor scavengers have been employed to reduce irradiation off-odor and color changes meat. The addition of antioxidants was effective in reducing lipid oxidation and color changes of irradiated meat. Vitamin E and phenolic compounds interrupt autoxidation of lipids either by donating hydrogen atom or quenching free radicals produced by irradiation (Nam and Ahn, 2003a). Addition of ascorbic acid to ground beef at 500–1000 ppm level was effective in maintaining the redness by lowering oxidation-reduction potential (ORP, generating reducing conditions) of irradiated ground beef, and the effect of ascorbic acid was stronger in "long-term-aged" than in "pre-aged" irradiated ground beef. The lowered ORP values by ascorbic acid maintained heme pigments in ferrous status and stabilized the color of irradiated ground beef (Nam and Ahn, 2003a). Dietary antioxidant treatments reduced the extent of lipid oxidation in meat during storage, but their effects differ among muscles types.

Packaging plays an important role on the color and odor of irradiated meat: Under vacuum-packaging conditions, lipid oxidation and color changes can be prevented. However, sulfur volatiles generated by irradiation are staying inside the packaging bag during storage and negatively affect the acceptance of irradiated meat. An appropriate combination of aerobic and vacuum packaging was effective in minimizing both off-odor volatiles and lipid oxidation in irradiated raw turkey breast during the storage. Nam and Ahn (2003b) developed a packaging method called "double packaging" in which meat pieces were individually packaged in oxygen-permeable bags first, and then a few of them were put in a larger oxygen-impermeable bag, vacuum-packaged, and then stored. The outer vacuum bag was removed 1–2 days before use. Double packaging was effective in reducing both lipid oxidation and the amount of sulfur volatile compounds. The combination of antioxidant additives with double packaging was more effective in controlling lipid oxidation, off-odor, and color changes than double packaging alone in irradiated meat. The beneficial effects of double packaging and antioxidant combinations on volatiles were more clearly shown in irradiated cooked than irradiated raw meat (Nam and Ahn, 2003b). The double-packaged irradiated turkey meat with antioxidants had only about 5%–7% of the irradiated vacuum-packaged cooked meat without antioxidants, and the production of aldehydes in irradiated was also prevented almost completely (Table 8.5). The combined use of double packaging and ascorbic acid was also very effective in maintaining the bright-red color of irradiated ground beef (Nam et al., 2004).

TABLE 8.5 Sulfur volatiles and TBARS values of irradiated turkey breast meat treated by different packaging and antioxidants combinations.

			Irradiated			
		Nonirradiated		Double pkg[1]		
	Vacuum pkg	Vacuum pkg	Aerobic pkg	None	S+E[2]	G+E[3]
Sulfur compounds	(Total ion counts×104)					
Dimethyl sulfide	1304^{b}	1990^{a}	140^{d}	831^{c}	676^{c}	546^{c}
Carbon disulfide	258^{b}	306^{a}	0^{c}	0^{c}	0^{c}	0^{c}
Dimethyl disulfide	0^{b}	$22{,}702^{a}$	0^{b}	32^{b}	0^{b}	43^{b}
Dimethyl trisulfide	0^{b}	554^{a}	0^{b}	0^{b}	0^{b}	0^{b}
Meat	TBARS (mg MDA/kg meat)					
0 d raw	0.66^{by}	0.84^{ay}	0.91^{ay}	0.83^{ay}	0.42^{dy}	0.55^{c}
10 d raw	0.72^{cy}	0.84^{cy}	2.18^{ax}	1.61^{by}	0.53^{cx}	0.53^{c}
Cooked	1.12^{dx}	1.67^{cx}	2.37^{ax}	2.09^{bx}	0.54^{ex}	0.64^{e}

[1]Vacuum packaged for 7 d and then aerobically packaged for 3 d; [2]Sesamol (100 ppm) and α-tocopherol (100 ppm) added; [3]Gallic acid (100 ppm) and α-tocopherol (100 ppm) added.
[a–c]Different letters within a row are significantly different ($P<0.05$); $n=4$.
Adopted from Nam, K. C., and D. U. Ahn. 2003c. Use of double-packaging and antioxidant combinations to improve color, lipid oxidation, and volatiles of irradiated raw and cooked Turkey breast patties. Poult. Sci. 82(5):850–857.

The addition of antimicrobial agents such as lactate, acetate, sorbate, benzoate salts showed synergistic effects with irradiation in killing microorganisms in meat, but their effects on meat quality were not consistent. This suggests that combined use of antimicrobial agents with irradiation can improve the safety of meat products without significant impact on meat quality, except that addition of benzoate salt significantly increased the content of benzene in the volatiles of irradiated RTE turkey ham and breast rolls (Zhu et al., 2004).

8.2.7 Consumer acceptance of irradiated meat

Consumers easily distinguish odor differences between the nonirradiated and irradiated meat. However, consumers could not detect the aroma difference between nonirradiated and irradiated raw and cooked meat after 3 days of storage under aerobic packaging conditions. This happened because sulfur compounds responsible for irradiation off-odor are highly volatile and disappear during storage under aerobic packaging conditions (Jia et al., 2021a; Lee and Ahn, 2004). They reported that antioxidants had no significant effect on the off-odor intensity of irradiated turkey meat in the consumer acceptance test but prevented lipid oxidation. Therefore, the combined use of aerobic packaging and antioxidants is recommended to improve consumer acceptance of irradiated poultry meat (Lee et al., 2003). A large proportion of consumers believed that irradiated foods pose a health risk and viewed food irradiation as moderately or highly risky. However, the acceptance of irradiated food was closely related to consumers' knowledge about food irradiation (Lusk et al., 1999). The more they know about the benefits of food irradiation, the higher positive attitudes toward irradiation, indicating the importance of consumer education. Several studies noticed that positive attitudes toward irradiation were increasing through education, but the effects of positive and negative information about irradiation on consumer response were different: a favorable description of irradiation increased consumer acceptance of irradiated products while an unfavorable description decreased it. When both positive and negative descriptions about irradiation were provided, the negative description dominated regardless of the source of negative information (Fox et al., 2002).

8.2.8 Detection

Analytical detection of irradiated foods and the proper control of irradiation at all levels are critical to facilitate international trade and to enhance consumer confidence and choice and the safety of irradiated foods. An ideal detection method should determine products specific to radiation effects or products that are proportional to the dose regardless of processing parameters and storage conditions. Also, the method should be simple, accurate, easy, rapid, and inexpensive. The detection of irradiated foods is mainly based on the radiolytic production of volatile and nonvolatile compounds from food components such as lipids, amino

acids, and proteins; the modification of DNA, carbohydrates, and proteins; and the formation of free radicals (Chauhan et al., 2009). Since a major feature of ionizing radiation is the negligible chemical change produced in the target, the detection methods should be very sensitive. As indicated earlier, ionization involves the production of free radicals, which are usually short-lived. These persist, however, when produced in hard material such as bone and can be detected by electron spin resonance (Raffi and Stocker, 1996). Radiolytic products from DNA (e.g., *cis*-Thymidine glycol) can be detected immunologically at a level as low as 10^{-15} mol. Dihydrothymidine is produced under anoxic conditions by the interaction of water-derived radicals and thymidine. It is a highly specific index of radiation treatment. Phenylalanine in proteins is converted to *o*-, *m*-, and *p*-tyrosine by irradiation. Since only *p*-tyrosine occurs naturally, the titer of the *o*- and *m*-tyrosine can be used as a measure of the irradiation received by the product (Delincee and Ehlermann, 1989). Radiolysis of the saturated triglycerides of foods produces 2-alkylcyclobutanones and can be used as markers for irradiation (Ndiaye et al., 1999). Irradiation produced carbon monoxide from biological components such as amino acids, lipids, and phospholipids and thus, was suggested as an irradiation marker (Lee and Ahn, 2004). Some hydrocarbons such as C16:2, C17:1, C14:1, and C15:0 and dimethyl disulfide were detected only in irradiated meat, indicating that these compounds can be used as potential markers for irradiated meat products (Kwon et al., 2012). However, all the suggested methods have merits and limitations. So, no single method can satisfy all the requirements and can be applied to all food systems because foods vary in their chemical, physical, and quality attributes. Selection of a suitable detection method generally depends on type of food, irradiation dose used, degree of precision required, and cost.

8.2.9 Future roles for irradiation in the preservation of foods

Food irradiation has a 70-year history of scientific research and testing, including toxicological and microbiological evaluation as well as testing for wholesomeness, and has been approved in about 60 countries, and approximately 71 commercial irradiation facilities are operating in the world (Kume et al., 2009). Therefore, irradiation may play a key role in improving the safety and preservation of foods in the future. However, current use of irradiation in foods is minimal except for certain food items such as herbs, spices, and tropical fruits for export. Most of the irradiation studies so far are done with raw meat because irradiation is not permitted for the meats with additives, further processed, or precooked RTE meat products. Therefore, more work should be done to prevent or minimize flavor, color, and taste changes in the further processed and precooked RTE irradiated meat products (Feng et al., 2017). Currently, little information on the mechanisms of taste/flavor changes in irradiated cooked meat is available. The effects of spices and additives on the taste/flavor of irradiated processed meat products and their roles on the antimicrobial efficiency of irradiation also should be studied.

8.3 High pressure

8.3.1 History of high-pressure processing of foods

The potential of high pressure to preserve food by destroying foodborne microorganisms was first demonstrated by H. Royer in 1895, but it was not until the early1980s that this technology was widely studied in food systems (Rivalain et al., 2010). Interest in HPP of foods sharply increased in the late 1980s, and the first commercialization of pressure-treated product (fruit jam) occurred in the Japanese food market in 1990. Since that time a variety of high-pressure-treated food products including guacamole, salsas, seafood, fruit juices, and RTE meats have become commercially available in the United States. Table 8.6 shows a list of some food companies and their pressure-treated meat products that are commercially available.

8.3.2 High-pressure processing

In HPP, foods are exposed to pressure ranging from 100 to 800 MPa for varying lengths of time depending on the objective of pressure treatment. Pressure vessels are designed to withstand very high pressures, and the useful life of a pressure vessel depends on the number or calculated pressure cycles it can endure for safe operation. For packaged foods, as much air as possible is removed during packaging of the food to allow the maximum amounts to fill the pressure vessel for each pressure cycle. Air removal also ensures that the applied pressure and resulting compression will not be wasted on air in the packages. Packages of food are loaded into a perforated container or directly into the pressure vessel. Water or a mixture of glycol and water is added to the pressure vessel to occupy all the space around the packaged food and serves as the fluid for transmitting the pressure. During pressure treatment, the temperature can be set between 0°C and 100°C, and the exposure times at selected pressures can range from a millisecond to more than 20 min.

High pressure applied to any food product results in an instantaneous transmission of pressure throughout the product irrespective of size, shape, and chemical composition. Pressures used for pasteurizing foods have little or no effect on covalent bonds, and thus, little chemical changes take place unless some other treatment such as heat is used in conjunction with high pressure. A combination of high pressure and heat can accelerate the rate of inactivation of microorganisms and enzymes. During HPP, the temperature of the pressurized food product increases due to the work of compression. This unavoidable thermodynamic effect is referred to as adiabatic heating that can increase temperature by 3°C for every 100 MPa increase in pressure. The time required for the pressure of the treated product to increase from atmospheric pressure to the desired treatment pressure is called the "pressure-come up time" (Farkas and Hoover, 2000). The main factors that determine the pressure come-up time are the target pressure, the volume of the pressure vessel, and the horsepower of the pump intensifier used for the high-pressure equipment (Balasubramaniam et al., 2008).

TABLE 8.6 Examples of national and international food companies that use HPP technology for treating meat products.

Country	Company	Meat product(s)	Reference (Website)
United States	Hormel Foods	Sliced deli meats, RTE meats	http://www.hormelfoods.com/
Italy	Ferrarini	Dried-cured meats	http://www.ferrarini.it/
Spain	Espuña	Sliced ham and tapas	http://www.espuna.es
United States	Kraft Foods[a]	Hot dogs	http://www.kraft.com
Romania	Chris Tim	Fermented sausages	http://en.cristim.ro/cris-tim-group/about-us/innovation
Netherlands	Zwaneberg	Filet American, steak tartar	http://www.zwanenberg.nl/en/about-us/innovation
United States	Cargill	Hamburger	http://www.cargill.com/products/foodservice/beef/brands/ (Fressure)
Greece	Creta Farms	Sliced deli meats	http://www.cretafarms.gr/en/proioda-creta-farms/creta-farms-combi/?Brand=4
United States	Perdue Farms[a]	Poultry strips	https://www.perdue.com/products/
Japan	Itoham	Sliced deli meats, RTE meats	http://www.itoham.co.jp/
Canada	MapleLodge Farms	Sliced deli meats, RTE meats	http://www.pressureprotection.ca/
United States	Foster Farms	Sliced deli meats, RTE meats	http://www.fosterfarms.com/
Greece	Ifantis	Sliced processed meat	http://www.ifantis.gr/en/index.php/our-products/2

		(mortadella, ham, and salami)	uncategorized/59-freshpress
Germany	Abraham	Sliced deli meats, RTE meats	http://www.abraham.de/
United States	Tyson Food[a]	Oven roasted chicken	http://www.tyson.com/Home.aspx
United Kingdom	Deli 24	Meat and cheese snack products	http://www.deli24.co.uk/Applications/Meats/
United States	Columbus Foods	Deli meat and fermented meats	http://www.foodprocessing-technology.com/projects/columbus-foods/
Italy	Rovagnati	Dry-cured meats	http://www.rovagnati.it/
Spain	Campofrio[a]	Sliced ham, Serrano ham, chorizo	http://www.campofrio.es
Canada	Santa Maria Foods[a]	Sliced dry cured meat	http://www.sharemastro.com

[a] *Does not advertise the use of HPP on the website.*

As pressure applied to the food product increases, the product decreases in volume. When the target pressure is attained, no more energy is added to the process. The pressurized product is held at a specified pressure for a period of time to obtain the desired microbial inactivation then pressure is released. The pressure-holding time or dwell time is the time from the end on the compression to the beginning of decompression. During the pressure release (decompression), an equal expansion in volume of the food product occurs. Therefore, the packaging used for the pressure-treated product must be flexible enough to withstand up to a 15% decrease in volume and regain its original volume without losing the intactness of its seal or barrier properties (Farkas and Hoover, 2000).

8.3.3 Antimicrobial mode of action

The antimicrobial mode of action of any agent (physical or chemical) involves that agent's damage to a cellular structure or function that is crucial to the survival or growth of the target organism. Several sites in the microbial cell can be damaged by high pressure depending on the level of pressure applied. Protein synthesis is inhibited by 50 MPa, while partial denaturation of proteins occurs at 100 MPa treatment. The increase in pressure to 200 MPa causes damage to the bacterial cell membrane, and further increase in pressure to about 300 MPa causes irreparable damage to enzymes and proteins. Those cellular lesions including disruption of the cytoplasmic membrane (Bansal et al., 2019; Lee et al., 2020), leakage of cellular material can result in death of the microbial cell if those lesions cannot be repaired (Abe, 2007). Depending on the level of pressure applied to foodborne microorganisms, several cellular lesions such as disruption of cytoplasmic membrane, denaturation of membrane proteins, inactivation of key metabolic enzymes, and ribosomal damage can occur simultaneously. Thus, it is challenging to determine the nature of the primary pressure-induced lesions (Hsiao-Wen et al., 2014).

The cytoplasmic membrane is the primary cellular site for pressure-related damage in bacteria because membrane damage can result in leakage of cytoplasmic components and loss of homeostasis. In bacteria exposed to HPP, bacterial cells showed cellular enlargement, membrane damage or detachment, DNA and ribosome damages, protein denaturation, and loss of intracellular contents (Manas and Mackey, 2004; Prieto-Calvo et al., 2014). After pressure treatment, the ribosomes of microorganisms can regain functionality under optimum condition, but cell viability can continue to decrease, indicating that the death of pressure-damaged cells is also related to other factors (Alpas et al., 2003).

8.3.4 Antimicrobial, chemical, and biochemical aspects

8.3.4.1 Antimicrobial aspect

The usefulness of HPP as a food preservation technology is based on destruction of foodborne pathogens, spoilage organisms, and inactivation of certain

enzymes without negatively changing sensory attributes and nutritional quality (Farkas and Hoover, 2000). The efficacy of HPP in destroying foodborne microorganisms is predicated on the level of applied pressure and the pressure-holding time. Microbial resistance to HPP is substantially variable and is highly influenced by microbial type and characteristics of the food matrix. However, depending on the extent of injury and certain intrinsic factors of the food product such as pH, water activity, ionic strength, and presence of antimicrobials, the injured organism may or may not be able to repair the pressure-induced injury. Inability of the organism to repair its injury will result in cell death.

The lethal effect of HPP against microorganisms is more pronounced in organisms with more cellular complexity compared with relatively simple organisms. Inactivation of microorganisms is influenced by several factors including Gram type, microbial physiological state, and species or strain differences within a species. HPP parameters (pressure temperature and time) are also important in the pressure-induced destruction of microorganisms. It is well accepted that pressure and temperature function synergistically in the destruction of vegetative bacterial cells (Heinz and Buckow, 2010). Generally, most foodborne bacteria exhibit the strongest tolerance to high pressure with the temperature range of 20°C and 30°C with lower temperatures contributing to decreased microbial tolerance. The application of high pressure in combination with elevated temperatures (>50°C) has also been proposed to circumvent the problem of barotolerant strains.

The application of high pressure to meat products is to improve their microbial safety without negatively affecting their desirable quality characteristics. The effectiveness of high pressure in killing meat-borne microorganisms can be markedly affected by the characteristics of the meat products (Table 8.7). Meat is a very nutritious food product, and nutrient-rich environments increase microbial resistance to high pressure (Tassou et al., 2007). Bacterial vegetative cells are more resistant to high pressure than yeast and molds. Most foodborne parasites are easily killed by pressures in the range of 200–300 MPa.

8.3.4.2 Microbial inactivation as affected by meat product characteristics

Meat product characteristics such as low water activity (a_w), and content of protein and fat alter the antimicrobial effectiveness of high pressure against meat-borne microorganisms. Low a_w increases the resistance of microorganisms to the killing effects of high pressure (Jofre et al., 2009; Simonin et al., 2012), but is inhibitory to microbial survival during storage of the pressure-treated product. It is likely that the progressive loss of viability of pathogens in stored pressure-treated meat products with low a_w is due to the death of sublethally injured survivors. While lowering the a_w of any food product can increase the pressure resistance of microorganisms, the type of solute used to lower the a_w can also influence the extent of microbial resistance to high pressure. At the same a_w values, sucrose solution confers greater barotolerance on *L. monocytogenes* compared with sodium

TABLE 8.7 Destruction of selected meat-borne vegetative bacteria by HPP with various pressure, time, and temperature combinations.

Microorganism	Meat or meat product	Pressure treatment	Reduction (log CFU/g) reference	Reference
Campylobacter jejuni	Chicken meat	300 MPa, 3 min, 30°C	3.0	Liu et al. (2012)
Campylobacter jejuni	Pork	300 MPa, 10 min, 25°C	6.0	Wackerbarth et al. (2009)
Campylobacter jejuni	Poultry meat	375 MPa, 10 min, 25°C	6.0	Solomon and Hoover (2004)
Escherichia coli	Genoa salami	600 MPa, 12 min, 25°C	4.7–5.8	Porto-Fett et al. (2010)
Escherichia coli	Ground beef	600 MPa, 3 min, 20°C	1.0	Li et al. (2020)
E. coli O157:H7	Ground beef patties	400 MPa, 12 min, 20°C	2.45	Morales et al. (2009)
E. coli O157:H7	Ground beef	450 MPa, 15 min, 20°C	6.9	Hsu et al. (2015)
E. coli FDA 5187	Ground beef	400 MPa, 20 min, 30°C	1.0	Baccus-Taylor et al. (2015)
Staphylococcus aureus	Dry cured ham	600 MPa, 31 min, 6°C	0.5	Jofre et al. (2009)
Staphylococcus aureus	Fermented sausage	400 MPa, 10 min, 17°C	None	Ananou et al. (2010)
Staph. condimenti	Chicken meat	600 MPa, 40 min, 40°C	>6.0	Liu et al. (2012)
Staphylococcus sciuri	Chicken meat	600 MPa, 40 min, 40°C	>6.0	Liu et al. (2012)
Lactobacillus viridescens	Ham	500 MPa, 5 min, 20°C	4.0	Park et al. (2001)

Lactobacillus sakei	Cooked ham	500 MPa, 10 min, 40°C	4.0	Hugas et al. (2002)
Yersinia enterocolitica	Pork	300 MPa, 10 min, 25C	6.0	Wackerbarth et al. (2009)
Listeria monocytogenes	Fermented sausage	400 MPa, 10 min, 17°C	None	Ananou et al. (2010)
Listeria monocytogenes	Turkey breast meat	500 MPa, 1 min, 20°C	0.9	Chen (2007)
Listeria monocytogenes	Ham	500 MPa, 3 min, 5°C	2.5	Teixeira et al. (2018)
Listeria monocytogenes	Dry-cured ham	450 MPa, 5 min, 11°C	0.32	Bover-Cid et al. (2011)
Listeria monocytogenes	Fermented sausage	400 MPa, 17 min, 10°C	0.6	Jofre et al. (2009)
Salmonella enterica	Raw chicken meat	500 MPa, 10 min, 20°C	7.0	Argyri et al. (2018)
Salmonella enterica	Raw minced poultry	450 MPa, 5 min, 15°C	3.0	Kruk et al. (2011)
Salmonella enterica	Raw minced poultry	450 MPa, 2 min, 20°C	3.3	Escriu and Mor-Mur (2009)
Salmonella enterica	Low acid fermented sausage	400 MPa, 10 min, 17°C	3.0	Jofre et al. (2009)

chloride solution (Koseki and Yamamoto, 2007). Based on this important observation, caution should be exercised in predicting microbial tolerance to high pressure based on a_w alone without considering the type of water-binding solute added to the meat product. Food components such as protein, fat, minerals, and sugars are known to influence the extent of microbial resistance to high pressure (Molina-Hoppner et al., 2004). However, the influence of major nutrients (proteins, carbohydrates, and fats) on the barotolerance of microorganisms has not always been consistent (Li et al., 2020).

8.3.5 Chemical and biochemical aspects

8.3.5.1 Effect of HPP on meat proteins

The application of HPP to meat products can produce varying amounts of modification in protein structure. From a mechanistic perspective, pressure causes unfolding of the structure of proteins, which can refold after a release of pressure. This unfolding and refolding phenomenon of pressure-treated protein can result in denaturation and alterations in electrostatic interactions of the native protein. While pressure-induced denaturation of proteins is a major catalyst of microbial inactivation, irreversible changes in muscle proteins are initiated at the level of high pressure required for microbial inactivation (Bajovic et al., 2012).

Various bonds and interactions that contribute to the native structure of meat proteins are all affected (to varying degrees) by HPP. Covalent bonds are the most resistant to breakage by high pressure due to their low compressibility. Salt bridges and hydrophobic interactions are easily disrupted by HPP, while hydrogen bonds seem to get stronger under pressure. Hydrophobic interactions, which are crucial to maintaining the quaternary structure of proteins, are sensitive to pressure. At pressures >200 MPa, substantial alterations in protein tertiary structure can be observed, whereas >700 MPa is required to change the secondary structure of proteins (Rastogi et al., 2007). At pressures up to about 300 MPa, muscle proteins including myofibrillar proteins tend to merely unfold; however, at pressures exceeding this level, muscle proteins exhibit more denaturation, gel formation, and agglomeration. These pressure-induced characteristics indicate the potential for use of selected HPP treatments in meat product development scenarios where improved gel structure and WHC can be obtained by application of certain pressure levels (Sun and Holley, 2010). For example, gelation characteristics of chicken myofibrillar proteins were significantly improved by moderate pressure (200 MPa) and low levels of added calcium chloride ($CaCl_2$), and the WHC of the myofibrillar protein-$CaCl_2$ gel was maximum following HPP treatment (Wang et al., 2020). Jia et al. (2021b) reported less drip loss in HPP-treated pork after its storage (−80°C) for 84 days.

Application of high pressure to meat has a marked effect on the actin-myosin complex of muscle filaments. In fact, pressure causes structural changes in muscle filaments that are likely associated with increased ATPase activity (at 30 MPa) and increase of soluble extracts from the myofibrils exposed to pressures greater than

150 MPa (Nishiwaki et al., 1996). The application of pressure (300 MPa) to post rigor beef muscle resulted in damage to muscle filaments in 5 min and that the z-line could not be seen in the pressurized meat.

8.3.5.2 Effect of HPP on lipid oxidation of meats

Hydrophobic interactions are easily disrupted by high pressure, and thus, lipid systems are the most pressure-sensitive biological substances (Rivalain et al., 2010). In fact, for every 100 MPa increase in pressure, the melting temperature (T_m) of triglycerides increases by approximately 10°C; therefore, lipids that exist as a liquid at ambient temperature will crystallize when subjected to high pressure.

The HPP treatment of fresh pork at < 300 MPa showed a negligible effect on lipid oxidation, but pressure levels higher than > 300 MPa accelerated lipid oxidation in pork, poultry, and beef (Kruk et al., 2011). Accelerated lipid oxidation triggered by high-pressure treatment occurs far more often during storage than immediately after the pressurization process of the meat (Orlien et al., 2000). However, handling history and variations in quality in raw meat may differentially affect the development of lipid oxidation following pressure treatment of meats (Tuboly et al., 2003). The pressure level required to trigger lipid oxidation in meat is dependent on the animal species from which the meat is derived. In turkey meat, lipid oxidation is accelerated if that product is treated at a pressure of less than 400 MPa for a relatively long time (30 min), while no lipid oxidation is detected in chicken meat until the application of 500 MPa. Increasing the pressure to 800 MPa resulted in oxidation that was equivalent to heating of the chicken meat at 80°C (Omana et al., 2011). High-pressure processing also accelerates oxidation in RTE meat products. Compared with ground cooked (50°C for 30 min) chicken thigh meat, pressure-treated cooked chicken exhibited more oxidation during subsequent refrigerated storage. Significant increases in TBA values and lipid-derived aldehydes were obtained from dry-cured ham that was pressurized irrespective of treatment time (Cava et al., 2009). High pressure has little effect on lipid oxidation below 300 MPa, but can have a significant effect at higher applied pressures.

8.3.5.3 Effects of HPP on color of meats

HPP significantly changes fresh meat color depending on the content of water and the a_w of the meat product (Ferrini et al., 2012). A significant increase in lightness (L^* color values) and a decrease in redness (a^* values) were observed when ground beef was subjected to pressures ranging from 200 to 500 MPa. This poses serious challenges in the marketing of this meat because consumers expect to see a bright red color in fresh beef. The pressure-induced color changes in fresh beef are caused by the protein coagulation and loss of protein solubility that alter the structure of proteins and the surface properties of meat. The redness ($a*$ values) of raw beef muscle increased with the increase of pressure from 50 MPa up to 350 MPa because of the activation of enzyme system involved in metmyoglobin reduction at the range.

The fat content of beef patties and pork sausages has been cited as a factor governing the extent of pressure-induced color changes in these meat products. High fat content (20% to 25%) has a strong association with pressure-induced color changes in raw meat especially for increased lightness (L^* values). In spite of the fact that high-pressure treatments caused visible changes in color of fresh meat, the color difference was markedly reduced following cooking of the meat (Mor-Mur and Yuste, 2003). Generally, high pressure does not alter the color of cured meats to the extent as observed with fresh meat. At pressures above 200 MPa, dry-cured ham exhibited a decrease in redness (Cava et al., 2009). The pressure-induced color changes were evident just after pressure treatment, but were not observed after 60 and 90 days of storage. An explanation for the relative color stability in pressure-treated cured meat products is the resistance of nitrosylmyoglobin pigment to oxidation (Pietrzak et al., 2007).

8.3.6 Future prospects of HPP

The technology of HPP has a good potential for producing safe meat products with extended microbial shelf life without the addition of food preservatives. Due to consumer demand for foods with "clean labels," the market for pressure-processed RTE meats is likely to exhibit robust growth in the future. The continued success in applying HPP to meat products will depend on support of research that focuses more on the influence on various levels of high pressure on meat quality characteristics so as to optimize the use of this versatile nonthermal technology in the production of safe and wholesome meat products.

8.4 Freeze dehydration

The removal of moisture from meat not only prevents the growth of microorganisms, but also eliminates them. The physicochemical characteristics of freeze-dried meat are different from those of fresh meat, but those differences become less apparent upon subsequent cooking. In freeze drying, frozen material is subjected to a pressure below the triple point and heated to cause ice sublimation to vapor (Fig. 8.2). Freeze-drying process can be divided into freezing (solidification), primary drying (ice sublimation), and secondary drying (desorption of unfrozen water) steps. In the first step (freezing), the liquid suspension is cooled and ice crystals of the pure water are formed. This increases the concentration of the remaining liquid (bound water). In the second step (primary drying), frozen material is subjected to a pressure below the triple point (at 0°C, pressure: 610 Pa) and heated to cause ice sublimation to vapor (Rahman and Rerera, 2007). The sublimation of ice crystals from the frozen product leaves porous plugs in the product. In the final step (secondary drying), the unfrozen water remaining (bound water) in products is removed by raising the temperature of shelves in chambers (Abdelwahed et al., 2006).

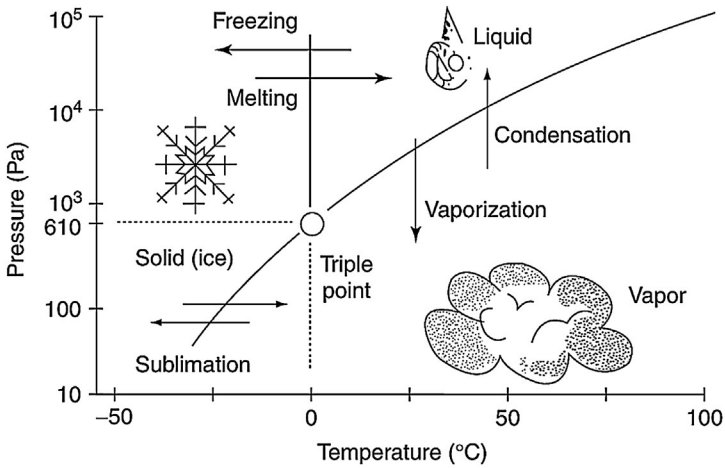

FIG. 8.2 Schematic diagram of the different states of water showing triple point. *Adapted from Nijhuis, H., E. Torringa, H. Luyten, F. René, P. Jones, T. Funebo, and T. Ohlsson. 1996. Research needs and opportunities in the dry conservation of fruits and vegetables. Drying Technol. 14(6), 1429–1457.*

The possibility of removing water from meat by sublimation from the frozen state rather than by evaporation of liquid had been apparent for some years. However, no satisfactory way of applying the process to production line was initially foreseen. In the 1950s, a large-scale process for freeze drying was first developed in the United Kingdom. Since it incorporated plates to enhance heat exchange during the initial phase of sublimation and supply heat to aid drying during the second phase, the process was called accelerated freeze drying (AFD). The low operating temperatures and speed of the process, minimal translocation of salts, and the honeycomb texture created by the direct sublimation of ice from the minute interstices of the tissues, freeze drying process caused little damage to the meat proteins.

8.4.1 Histological aspects

The evaporation of water from the fluid phase involves the movement of salts toward the surface of the meat and causes distortion of the muscle. However, sublimation of water vapor occurs directly from the nucleus of ice during freeze drying, and any distortion is limited to the areas around the nuclei (Babić et al., 2009). If operated at a low plate temperature, the freeze drying process causes relatively little gross histological change (Fig. 8.3) (Doty et al., 1953; Messina et al., 2016). The degree of distortion obviously depends on the size and number of the ice nuclei, which, in turn, is a function of the rate of freezing. The smaller the size of ice crystal nucleus, the less histological damage and finer honeycomb of air spaces are left after sublimation. With a sufficient speed of freezing, ice crystals will form inside the myofibrils between the molecules of actin and

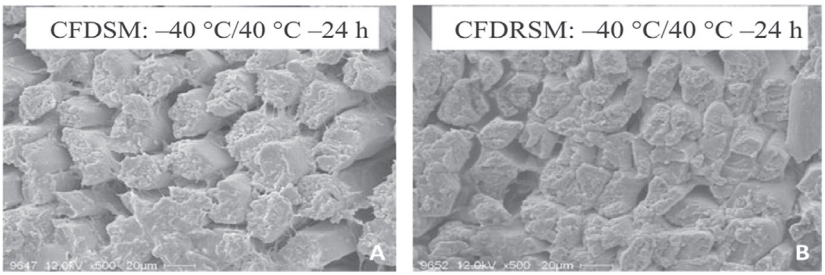

FIG. 8.3 Cross sections (×500) of freeze dried (A) and reconstituted (B) cooked *Semimembranosus* muscle. Freeze drying cycle was set as −40°C for 24 h and dried at 40°C for 24 h under a chamber pressure of 0.346 Pa. CFDSM: Cooked freeze-dried *Semimembranosus* muscle; CFDRSM: Cooked freeze-dried rehydrated *Semimembranosus* muscle. *Adopted from Messina, V., F. Pieniazek, and A. Sancho. 2016. Effect of different freeze-drying cycle in Semimembranous and Gluteus Medius bovine muscles: changes on microstructure, colour, texture and physicochemical parameters. Int. J. Food Sci. Technol. 51(5), 1268–1275.*

myosin, causing no alteration in structure, even at the level of observation given by the electron microscope (Menz and Luyet, 1961).

8.4.2 Physical and biochemical aspects

The difference between dehydrated and fresh meat can be minimized if the water removed from the former can be incorporated again on rehydration. The degree of reincorporation depends on the surviving WHC of the muscle. Under the commercial AFD conditions (plate temperature at 50–70°C), some loss of WHC is common because the high plate temperature causes partial denaturation of sarcoplasmic as well as myofibrillar proteins (Fig. 8.4). This lowers the solubility of the myofibrillar proteins, alters the actomyosin complex, and causes woodiness in texture. When the plate temperature is about 20–30°C, the AFD

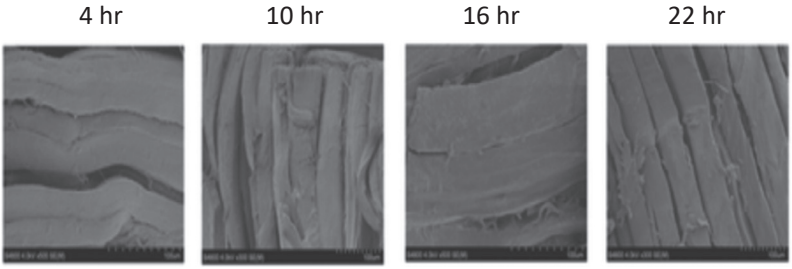

FIG. 8.4 SEM of shrimp dried by vacuum-freeze drying for 4, 10, 16, and 22 h. Primary drying was performed under the conditions of a shelf temperature at −35°C and a vacuum at 10 Pa for 3 h, followed by secondary drying at 50°C for 19 h under 10 Pa pressure. *Adapted from Ling, J., X. Xuan, N. Yu, Y. Cui, H. Shang, X. Liao, X. Lin, J. Yu, and D. Liu. 2020. High pressure-assisted vacuum-freeze drying: a novel, efficient way to accelerate moisture migration in shrimp processing. J. Food Sci. 85(4), 1167–1176.*

process does not affect the extractability of the myofibrillar proteins much. However, even under optimum operating conditions, excessive woodiness and difficulty of reconstitution can be encountered. Unlike the heating involved in hot air dehydration, freeze dehydration will not change the isoelectric point of muscle if operated under optimum conditions. However, this loss of WHC is not due to the freezing aspect of the freeze-drying process, but the plate temperature during the final phase of drying is implicated. Although the latter has little effect on the total moisture content after reconstitution, it affects the amount of moisture, which is firmly bound. Proteins may survive freeze dehydration in a substantially unaltered condition. The ATPase activity of actomyosin in rehydrated meat still maintains 80% of its initial value, and the muscle fibers still contract on the addition of ATP. On subsequent storage, however, calcium-binding power is lost due to a structural deterioration of the sarcoplasmic reticulum membranes.

8.4.3 Organoleptic aspects

In general, L^*, a^*, and b^* values of freeze-dried cooked meat after rehydration were closer to those of freshly cooked meat when samples were thinner. The meat processed by accelerated freeze drying retains much of the bright color of fresh meat, but shows lower tenderness and juiciness (Babić et al., 2009). Rehydration of freeze-dried meat with aqueous solutions of tenderizing enzymes, such as papain, helps to offset the somewhat adverse effect of freeze drying on tenderness. In the United States, freeze-dried steaks are now available in a moisture-proof pack with a compartment containing dried proteolytic enzymes, the latter being added to the reconstitution water on rehydration. From the nutritional point of view, freeze drying does not alter the biological value of the meat proteins a lot even though about 30% of the thiamin in the meat can be lost during freeze drying (Labuza and Tannenbaum, 1972). Lipid oxidation is a common chemical reaction to dried muscle products with high polyunsaturated fatty acid profiles. However, addition of natural antioxidants (e.g., phenolics) can improve their oxidative stabilities (Xie et al., 2019). Apart from its value under emergency conditions, freeze dehydration can provide palatable and nourishing meat, which will resist spoilage for a considerable period without refrigeration in remote areas or in the case of natural catastrophes (earthquakes, floods, etc.). Because of its lightness and high protein content, it is valuable in space flights. Although relatively high costs tend to depress the development of freeze drying, there has been renewed interest in the process (e.g., the production of freeze-dried beef for the Japanese market by Australia).

8.4.4 Future perspectives of freeze dehydration

Freeze-dehydration process takes a long time and uses additional energy to run the compressor and refrigeration units. This makes the process very expensive

for commercial use. However, a number of novel drying techniques such as microwave drying, dielectric drying, microwave-augmented freeze drying, centrifugal fluidized-bed drying, ball drying, ultrasonic drying, and high pressure in combination with freeze dehydration have been suggested to increase the drying rate. Based on the type of product to be dried, the final moisture of the finished product, the heat sensitivity of products, and the cost of processing, different combinations could be selected for a particular process. Also, combining freeze dehydration with ozone or other sterilization and packaging strategies can create products with longer shelf life than using it alone. It is also probable that other novel drying techniques can be combined with freeze dehydration and produce muscle proteins with specialized purposes (e.g., food additives in formulated foods or beverages) (Chen et al., 2017).

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Chapter 9

The storage and preservation of meat. III—Meat processing

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9.1 Introduction

The empirical observation that salting would preserve meat without refrigeration was made several thousands of years ago. Furthermore, the efficacy of drying had been also empirically recognized in meat preservation since very early times. There are many historical references about meat dehydration, and in fact, a variety of dried and cured meats were available by 2000 BCE as reported in Sumerian tablets written in cuneiform language. Traditional meat processing was reported to start in eastern and southwestern China by 770 BCE during the Zhou dynasty (Chen et al., 2015) and also in Europe where many references to dry-cured hams and fermented sausages are known from the ancient Romans and Greeks (Toldrá, 2002).

The production and consumption of dry meats expanded in areas where natural drying and ripening could be achieved, thanks to mild and sunny climates such as the Mediterranean Sea surrounded by Southern European and North African and Middle East countries (Toldrá and Hui, 2015). Water losses in such dry meats could reach up to 32%–34% in weight. On the other hand, smoking was a preservation way for cold areas such as northern countries where the climate did not allow a natural drying.

The term cured meat is widely used and covers a large number of meat products worldwide, but its meaning may vary depending on the kind of product and country of origin. The traditional use of the term curing refers to the use of a curing salt or cure consisting of sodium chloride with nitrate and/or nitrite that contributes to develop a characteristic cured pink/reddish color and flavor in the meat (Pegg and Honikel, 2015). Depending on how this cure is applied, cured meat products can be classified (see Fig. 9.1) as (1) dry-cured if the cure is applied dry to the surface of the meat or mixed with the mince and (2) wet- or pickle-cured when the cure is injected into the meat as a brine (Toldrá, 2002). Dry-cured meat products are aged/ripened and dried for several weeks,

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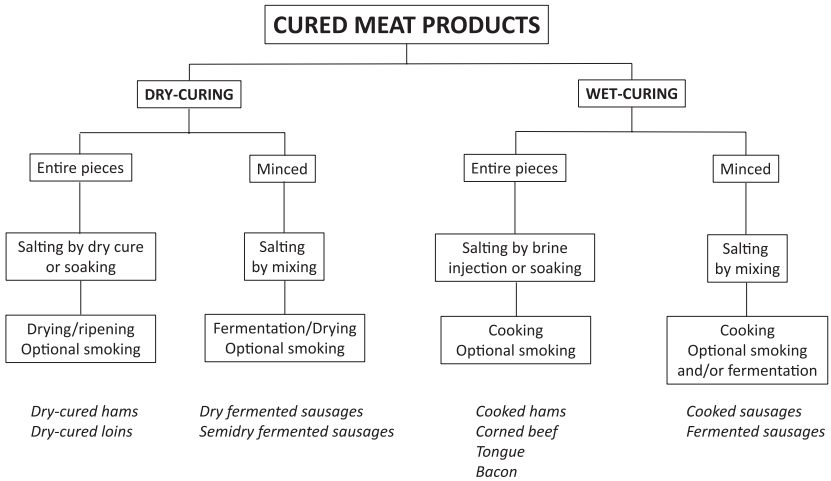

FIG. 9.1 Classification of dry-cured and cooked meat products. *(Adapted from Flores, J., Toldrá, F., 1993. Curing: processes and applications. In: Macrae, R., Robinson, R., Sadle, M., Fullerlove, G. (Eds.), Encyclopedia of Food Science, Food Technology and Nutrition, Academic Press, London, UK, pp. 1277–1282.)*

months, or even years. Wet-cured meat products are usually cooked. Both types of products can be optionally smoked. An exception is bacon that is dried for short time, cooked, and/or smoked. Representative typical meat products for each type of processing are shown in Fig. 9.1.

This chapter presents the most used meat processing technologies involving curing, fermentation, dehydration, and smoking followed by a brief description of the production processes for a wide range of meat products such as dry-cured ham, fermented sausages, and cooked meats.

9.2 Curing

Preservation was originally effected by sprinkling salt onto the meat surfaces. In due course, the meat was placed in brine, but both dry salt curing and tank curing have been practiced until the present time. Vascular pumping, or multiple injections of salt solution, is now employed to hasten curing. Granulated salt was formerly called "corn" and accounts for the term corned beef. It is of interest to note that the crystalline form and size of the salt used may affect its preservative properties. Smoking over a wood fire was originally employed to enhance the preservative action of curing, but the smoke flavor is now used mainly because people like the flavor of the smoked product.

As time has passed, cured meats are valued for their organoleptic quality per se being used in curing mixtures to develop and fix the color of meat, to inhibit microbial growth, or to develop characteristic flavor (Sindelar and Milkowski, 2012). However, in recent decades, there has been a tendency to lower the concentration of the curing ingredients, and this has made these mildly cured or

semipreserved products more liable to spoilage and reintroduced the need for some degree of refrigeration. Recognition of the value of sodium nitrate in producing an attractive color may well have been due to adventitious impurities in the sodium chloride employed. At the end of the 19th century, it had become recognized that meat-curing brines contained nitrite, that this was the color-fixing agent, and that the nitrite was produced by a reduction of nitrate (Cassens, 1997).

The part of the pig's carcass, which was most difficult to cure, was the top of the hind limb where the depth of meat was greatest. Consequently, prolonged curing was frequently required, sometimes up to 80 days. Perhaps for this reason, the methods employed for curing this area are particularly diversified. Where it is cut off from the side and separately cured, it is referred to as ham. The names of the available types in the United Kingdom—e.g., Yorkshire, Suffolk, Cumberland, Bradenham, Belfast—indicate more or less subtle variations in the process that includes cooking. Where the area concerned is cured on the side, it is referred to as gammon. Various types of dry-cured hams, which are eaten raw without cooking, are available in many countries, for example, the Spanish Iberian and Serrano hams, Italian Parma ham in Europe, and the Chinese Jinhua and Xuanwei hams. During the long drying period, there is considerable proteolysis and lipolysis that confer intense and distinctive flavors on the products (Toldrá, 2002). Other types of hams, such as the American Country-style and German Westphalia hams, are submitted to short drying processes and are smoked and may be cooked.

9.2.1 Salting

When meat is initially placed in curing brine, the exterior muscles will be exposed to a much higher concentration of salt than that established when equilibrium between meat and brine is subsequently attained. On the other hand, interior muscle locations will be subjected to a slow increase in salt concentration from physiological to equilibrium level. Since the behavior of meat in curing brines would thus be expected to vary according to the relative position of its constituent muscles to the brine, Knight and Parsons (1988) undertook a detailed histological and biochemical study of the process. When isolated myofibrils were exposed to 1 M sodium chloride, they swelled maximally, and A-band protein (mainly myosin) was extracted from them. Further increase in concentration to 5 M was associated with inhibition of swelling and protein extractability. Knight and Parsons (1988) also found that when myofibrils were exposed to solutions of increasing molarity in 1 M steps, the extractability of protein was more complete than when the muscles were exposed to 5 M sodium chloride. On the other hand, less protein was extracted from the A-band by 1 M sodium chloride if the myofibrils had first been subjected to 5 M brine, possibly because the latter had caused some denaturation of the myosin.

Salt concentrations of the brine (and the time of contact with the meat) and the microscopic structure of the musculature are relevant factors for salt penetration during curing, but there are other factors. For instance, an increased temperature increases the velocity of penetration and diffusion, but this obviously requires the strictest hygiene since it increases the risk of microbial spoilage. Thus, if curing salts are added early postmortem, before the carcass has completely chilled, they diffuse more rapidly and cured color develops faster. Moreover, the yield of raw product is improved and cooking loss is less. The penetration of salt into pork, which has been frozen and thawed, is about 20% greater than into fresh meat because muscle structure is affected by freezing and thawing. This is why time of contact with dry salt for dry-cured hams is reduced to about 20% (Toldrá and Aristoy, 2010).

Curing brines containing phosphates (especially polyphosphates) have been used to enhance the water-binding capacity of bacon and cooked hams. In some cases, the effect may be due to an elevated pH, but pyrophosphate is said to have a specific effect because it resembles ATP and interacts with actomyosin and by sequestering calcium ions.

There is a current trend toward the reduction in the amount of salt used for meat processing. This is due to the current dietary guidelines and demands of health authorities to reduce the intake of sodium because of its negative effects on the cardiovascular health. There are different strategies to reduce the amount of salt used. The most simple is the direct reduction in the amount of added salt. Another option is the partial replacement of sodium chloride by other chloride salts such as potassium chloride, calcium chloride, and magnesium chloride although the replacement rates are limited due to the bitterness when potassium is in excess or metallic aftertastes associated with calcium and magnesium (Toldrá and Barat, 2018).

9.2.2 Chemistry of nitrate and nitrite

Nitrite and nitrate are employed in cured meat products in the form of their sodium and potassium salts. Their safety used as food additives was evaluated by the European Food safety Authority (EFSA, 2017a,b). The term nitrite is generically referring to both the anion NO_2^- and the nitrous acid HNO_2. Nitrite is rapidly dissolved in the meat moisture due to its good solubility at the usual pH around 5.7, and about 99% exists as the anion NO_2^-. The small quantity of undissociated HNO_2 is in equilibrium with the generated nitrous anhydride (N_2O_3), which again is in equilibrium with the two oxides, NO and NO_2. Nitric oxide is very reactive and can react with other substances and/or meat ingredients. When exposed to air, nitric oxide (NO) is oxidized to NO_2, and this explains the antioxidant activity of nitrite in meat batters (Honikel, 2010). NO_2 can form nitrite and also nitrate explaining the presence of nitrate in those meat products that were added only nitrite. Incorporation of ascorbic acid, ascorbate, or erythorbate in the curing formulations at about ~500 mg/kg in the meat bat-

ter facilitates and assures the conversion of nitrite to nitric oxide. Ascorbate and erythorbate are able to sequester oxygen acting as antioxidant, thus not only retarding the oxidation of NO to NO_2 as well as the formation of nitrate but also reducing the formation of *N*-nitrosamines (Pegg and Honikel, 2015). The reactions are as follows:

$$2HNO_2 \leftrightarrows N_2O_3 + H_2O$$

$$N_2O_3 \leftrightarrows NO + NO_2$$

$$NO + ½O_2 \rightarrow NO_2$$

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$

So, the overall reaction if oxygen is available is: $2HNO_2 + ½\ O_2 \rightarrow HNO_2 + HNO_3$

The residual amount of nitrite is considerably lower compared with the amount of nitrite initially added to the product, making its control more difficult. About 10%–20% of the added nitrite could be analytically detected in cured meat immediately after processing (Cassens, 1997). The amount of added nitrite disappears once in the cured meat with the following distribution (Sebranek et al., 1973): losses with protein 20%–30%, with myoglobin 5%–15%, as nitrate 1%–10%, as nitrite 5%–20%, as nitrogen gas 1%–5%, with sulfhydryl groups 5%–15%, and with lipid 1%–5%. The generation of nitrogen gas is produced through the Van Slyke reaction (Martin, 2001). Although reduction of nitrite to nitric oxide can be effected either by bacteria or by the muscles' own enzyme system, the reduction of nitrate can be effected only by the former, hence the need for careful control of dry-cured ham salt, and bacon and cooked ham curing brines to ensure that the necessary microbial reduction of nitrate will occur.

9.2.3 Antimicrobial effect

During storage, cured meats deteriorate in the first instance because of discoloration, secondly because of oxidative rancidity in the fat, and thirdly on account of microbial changes—the latter gaining somewhat greater importance since the advent of prepackaged methods of sale.

Nitrite alone or in combination with other salts can inhibit the growth of several aerobic and anaerobic microorganisms. In fact, nitrite is well known to control botulism and suppress the outgrowth of *Clostridium botulinum* spores in cured meat products and can also contribute to control, although not suppress, the growth of several other pathogens such as *Listeria monocytogenes* under certain conditions (i.e., refrigeration) but not all (Sebranek et al., 2012). It can also control somehow *Bacillus cereus, Staphylococcus aureus*, and *Clostridium perfringens* (Alahakoon et al., 2015). However, nitrite is not effective in controlling Gram-negative enteric pathogens in commercially prepared foods.

The storage life of cured meats is enhanced by the specific antimicrobial action of nitrite in the curing brines; however, the increased use of prepackaging has introduced new potential hazards of spoilage from microbial action. Thus, while the procedure obviously lowers the risk of contamination of the product after wrapping, it increases the possibility of contamination during preparation, even though performed in white clean rooms, especially as large areas of cut surface are frequently exposed. The relatively high salt content of cured meat products, and its own halophilic microflora, tends to discourage the growth of the kinds of microorganisms likely to be introduced during handling even though the growth of *L. monocytogenes* is a certain risk. This hazard may increase with cooked cured products, and especially, in semipreserved items, the nature and number of introduced contaminants might have a pronounced effect on the organoleptic behavior of the product and, if they happened to be pathogens, on its safety.

Because vacuum packaging helps to prevent the oxidation of fat and pigments in cured meats, it is frequently employed.

9.2.4 Antioxidant effect

Salt has an accelerating effect on the oxidation of fat. As a result, cured meats are more liable than fresh to spoil through oxidative rancidity in the fat. The process of curing reduces the resistance of pork fat to oxidation to a much greater extent than would be expected if the direct influence of temperature was the sole factor involved. For packaging, as already indicated, gas packs employing nitrogen, or vacuum packs, are effective. Nevertheless, nitrite per se has an antioxidant effect, inhibiting fat oxidation in cooked cured meats that appears to be due to its chelation of nonheme iron and to the effect of the cooked pigment in blocking the prooxidant action of heme iron (Morrisey and Tichvayana, 1985). Smoking decreases oxidative rancidity, partly on account of the phenolic antioxidants it contains.

9.2.5 Color development

There has been much research on the development of color due to nitrite, both desirable and undesirable during processing. The attractive red pink color of cured meats before cooking is essentially that of nitrosomyoglobin (nitric oxide myoglobin). Haldane (1901) demonstrated the reaction of nitrite with myoglobin to form nitrosomyoglobin and the bright red pink color typical of cured meat products (see Chapter 11). In vitro nitric oxide can combine directly with myoglobin, even though the sequence of events in cured meats is more complicated. Nitrite firstly reacts with myoglobin to produce only metmyoglobin. The rate of formation of nitrosomyoglobin is proportional to the concentration of nitrite up to the point where the nitrite to metmyoglobin ratio is 5:l. Beyond this point, nitrite appears to be inhibitory, and this may explain why the conversion of myo-

globin to the cured meat pigment is frequently incomplete despite apparently much more than adequate nitrite concentrations. Detailed studies have elucidated the details of nitrosomyoglobin formation (Pegg and Honikel, 2015). The mechanism is as follows: (1) nitrite oxidizes myoglobin to metmyoglobin; (2) nitrite also oxidizes ferrocytochrome *e* to nitrosoferricytochrome *e* (catalyzed by cytochrome oxidase); (3) the nitroso group is transferred from nitrosoferricytochrome *e* to the metmyoglobin by NADH-cytochrome *e* reductase action, forming nitrosometmyoglobin; and (4) the nitrosometmyoglobin is reduced to nitrosomyoglobin by enzyme systems of the muscle mitochondria (even in the presence of nitrite concentrations causing rapid oxidation of oxymyoglobin). Nitrosometmyoglobin also autoreduces to nitrosomyoglobin under anaerobic conditions, but aerobically it breaks down to give metmyoglobin. Nitrite also reacts with tryptophan residues in nonheme proteins, and these are capable of transferring nitrite to metmyoglobin to form nitrosometmyoglobin, which, as previously indicated, can be reduced to nitrosomyoglobin.

In the case of cooked meat products, the globin portion of nitrosomyoglobin is denatured by heating, but the nitric oxide porphyrin rings system remains intact, forming the stable pink "cooked" pigment nitrosylhemochromogen, which is a pentacoordinated ferrous heme, where nitric oxide is the fifth ligand and which is not bound to the protein (Killday et al., 1988).

Nitrosomyoglobin and the cooked pink pigment nitrosylhemochromogen are much more susceptible to light and ultraviolet radiation than myoglobin. The pigments of cured meats may fade in 1 h under display lighting conditions. However, the color of fresh meats may last over 3 days, but they get oxidized under ultraviolet radiation. Since light accelerates oxidative changes only in the presence of oxygen, however, vacuum packaging, or packaging under nitrogen, can eliminate the effect although, of course, adding to the cost of the product. Occasionally, a particularly swift fading of the red pigment of cured meat is observed. In such cases, the labile form may be nitric oxide metmyoglobin and not nitrosomyoglobin, since in vitro the former is very easily dissociated by oxygen forming brown metmyoglobin. The oxidation of the cured meats pigments is also very rapid when oxygen is present. Unlike myoglobin itself, where the rate of oxidation is maximal at 4 mm oxygen partial pressure, the rate of nitrosomyoglobin oxidation increases directly with increasing oxygen tension. The only practical and effective antioxidant so far extensively used is ascorbic acid, either incorporated in the curing brine or sprayed onto the surface of the product after maturing.

9.2.6 Formation of nitrosamines

The possibility that carcinogenic nitrosamines may be produced from nitrite in curing processes was raised in the late 1960s (Lijinsky and Epstein, 1970). *N*-Nitrosamines can be formed in cured meat products through the reaction of a secondary amine with nitrite at high temperatures and a sufficiently low pH.

Only secondary amines can react, and therefore, they must be present. It must be taken into account that primary amines are immediately degraded into alcohol and nitrogen and that tertiary amines cannot react with nitrite at all (Pegg and Honikel, 2015). The nitrosation reactions involve the generation of nitrous anhydride (N_2O_3) from nitrite (NO_2^-) under acid conditions, which is then converted into NO and NO_2. NO^+ is formed in the presence of a transition metal ion such as Fe^{+3} and can react with an unprotonated secondary amine through a nucleophilic substitution reaction to form *N*-nitrosamines as follows (Pegg and Honikel, 2015):

$$2NO_2^- + 2H^+ \leftrightarrows N_2O_3 + H_2O$$

$$N_2O_3 \leftrightarrows NO + NO_2$$

$$NO + Fe^{+3} \rightarrow NO^+ + Fe^{+2}$$

$$R_2NH + NO^+ \rightarrow R_2N - N = O + H^+$$

Of course, the amount of generated nitrosamines is affected by the amount of residual nitrite, low pH, and intensity of heating (i.e., frying, grilling, baking), among other promoting factors (Herrmann et al., 2015a). However, it is very difficult to give a general behavior because nitrosation reactions are influenced by many variables including the presence of sulfhydryl compounds, certain phenols, and tannins in the meat product that inhibit more or less the formation of nitrosamines, ascorbic acid being the most important (Pegg and Shahidi, 2000). In fact, the incorporation of ascorbic acid, ascorbate, or erythorbate in the curing formulations at about 500 mg/kg has been a common practice in the industry because they contribute to reduce the formation of nitrosamines. Nitrite is reduced to NO, and the ascorbate is oxidized to dehydroascorbate; nevertheless, there have been vigorous efforts to lower the residual nitrite contents in cured meat products.

Nitrosamines may be volatile and nonvolatile. Among the volatile nitrosamines, *N*-nitrosodimethylamine (NDMA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine appear to be the nitrosamines more frequently found and reported in meat products (Flores et al., 2019). NPYR and NDMA are mostly detected in bacon after frying even though almost no nitrosamines have been reported in raw bacon before frying. NPIP may be formed from spices such as black pepper because it may contain NPIP precursors such as pyrroperine, pyrrolidine, piperine, and piperidine (De Mey et al., 2017). Volatile nitrosamines were analyzed in 70 products of the Danish market and 20 products of the Belgian market, and the mean levels of the amounts of individual nitrosamines were generally reported to be lower than 0.8 μg/kg (Herrmann et al., 2015b). *N*-nitrosamines were also detected in about 50% of 101 fermented sausages sampled from the Belgian market, but in any

case, the total amount of *N*-nitrosamines remained below 5.5 μg/kg (De Mey et al., 2014).

Nonvolatile nitrosamines (NVNA) are primarily *N*-nitrosamino acids, which occur in processed meat products at significantly higher levels than the volatile nitrosamines (Crews, 2010). In fact, when analyzing products from the Danish and Belgian markets, the mean levels of the individual NVNA were nearly 118 μg/kg even though higher levels may be found in particular types of hams or sausages (Herrmann et al., 2014). The most frequently detected NVNAs are *N*-nitrosothiazolidine-4-carboxylic acid (NTCA) and *N*-nitroso-2-methyl-thiazolidine-4-carboxylic acid (Herrmann et al., 2015b). It must be pointed out that NTCA and *N*-nitrosoproline have been related to the intensity of heating in both drying and frying (Herrmann et al., 2015a).

9.2.7 Production of meat products without nitrite

There is a trend toward the production of natural or clean-label meat products with no addition of either nitrite or nitrate. In this way, the negative perception of nitrite and the risks for nitrosamines formation are avoided, and it is considered as an attractive practice by producers and consumers even though there might be a risk regarding safety aspects and the presence of unknown residues in the proposed alternative (Rivera et al., 2019). They are termed as uncured and manufactured in the United States (USDA, 2020) according to the Code of Federal Regulations Title 9, CFR 317.7 and 319.2. However, some products were manufactured with sea salt or vegetable sources high in nitrate content. For instance, celery may contain up to 1500–2700 ppm of nitrate (Pegg and Honikel, 2015). Nitrate may constitute a source of nitrite in the presence of microorganisms with nitrate-reducing activity. First, natural curing processes included the use of a concentrated vegetable extract from celery containing a high nitrate concentration of about 3% and a purified strain capable of converting nitrate into nitrite. The process had to be adapted to include an incubation step for the formation of nitrite by the culture (Sebranek et al., 2012). This practice has some benefits such as eliminating E numbers and therefore, obtaining clean label meat products although the risk for N-nitrosamines generation remains because some nitrite might still be present in the meat product (Flores and Toldrá, 2021). However, in the European Union, the use of extracts is considered as a source of the additives and must be declared as such.

Other alternative technologies based on thermal and nonthermal treatments, especially targeted against *L. monocytogenes* and *C. botulinum*, have been studied to replace nitrite. These technologies, assayed either alone or in combination, include high-frequency heating, high-pressure processing, and addition of antimicrobials such as bacteriocins, plant extracts, and organic acids (Sebranek et al., 2012). Natural antimicrobials and antioxidants have been identified in herbs and spices, especially in their essential oils (Gassara et al., 2016; Oswell et al., 2018). Meat isolate *Staphylococcus sciuri* I20-1 is used to inhibit food-

borne pathogens in view of its application as a functional starter culture for the production of clean-label fermented meats. This strain showed the ability to produce a heat-stable antibacterial compound of a proteinaceous nature, a kind of bacteriocin-like substance, with a specific inhibitory spectrum against strains of *S. aureus* and both vegetative cells and spores of *C. botulinum*. However, the safety hazard remained because a subpopulation of resistant *S. aureus* against such bacteriocin was reported as well as a latent lack of persistence of the producer strain in the product and a potentially insufficient in situ production (Sánchez Mainar et al., 2016).

Regarding the development of the traditional cured meat color, this may be a challenge difficult to achieve. However, a study revealed that two strains of *Lactobacillus fermentum* were able to generate nitric oxide and produce an adequate color in sausages without any addition of nitrate or nitrite (Moller et al., 2003).

9.3 Fermentation

Meat fermentation was based for centuries on the development of desirable indigenous flora, but the composition of such flora changed quite frequently due to many variables involved (site of production, raw materials, operators, etc.) and in most cases lacked consistent quality. Back-slopping, a technique consisting of the addition of small amounts of previously fermented meat having good sensory properties, was a common practice although with heterogeneous quality (Toldrá, 2006a). Since the past decades, fermented meats are generally produced using microbial starters.

The microbiota of fermented meats has been identified through molecular techniques based on DNA finding, and the most relevant are *Lactobacillus sakei* and *Lactobacillus curvatus* among lactic acid bacteria (LAB), *Staphylococcus xylosus* among coagulase-negative staphylococci (CNS), and *Debaryomyces hansenii* among yeasts (Alessandria et al., 2015). LAB is the most usual microorganism in meat fermentation. *L. sakei* and *L. curvatus* grown at mild temperatures are used in European sausages where mild fermentation temperatures (20–30°C) are typically used while *Lactobacillus plantarum* and *Pediococcus acidilactici* are used in the United States because they grow well at higher temperatures (30–35°C) (Toldrá, 2006b). *Staphylococcus* is characterized by exopeptidase and lipolytic activity that contribute to good flavor development. *D. hansenii* is the predominant yeast in fermented meats, and the molds *Penicillium nalgiovense* and *Penicillium chrysogenum* can grow on the outer surface of certain characteristic sausages (Toldrá, 2012a,b,c).

Starter cultures are carefully developed based on the typical cultures grown in traditional fermentation. Examples of typical starter cultures used for meat fermentation are shown in Table 9.1. Such cultures must tolerate high salt content, acid pH, and low water activity, typical of fermented sausages. They must grow well at fermentation temperatures (i.e., 18–25°C in Europe or 35–40°C in

TABLE 9.1 Main microorganisms used as starter cultures in meat fermentation.

Microorganism	Genera	Species
Lactic acid bacteria	*Lactobacillus*	*Lactobacillus sakei, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus rhamnosus*
Lactic acid bacteria	*Pediococcus*	*Pediococcus pentosaceus, Pediococcus acidilactici*
Coagulase-negative staphylococci	*Staphylococcus*	*Staphylococcus xylosus, Staphylococcus carnosus, Staphylococcus equoris*
Yeasts	*Debaryomices*	*Debaryomices hansenii*
	Candida	*Candida fumata*
Molds	*Penicillium*	*Penicillium nalgiovense, Penicillium chrysogenum*

Adapted from Hammes, W.P., Bantleou, A., Min, S., 1990. Lactic acid bacteria in meat fermentation. FEMS Microbiology Reviews 87, 165–174; Hammes, W.P., Knauf, H.J., 1994. Starters in the processing of meat products. Meat Science 36, 155–168; Demeyer, D.I., Leory, F., Toldrá, F., 2014. Fermentation. In: Jensen, W., Dikemann, M. (Eds.), Encyclopedia of Meat Sciences, second ed., vol. 2. Elsevier Science Ltd., London, UK, pp. 1–7; Cocconcelli, P.S., Fontana, C., 2015. Bacteria. In: Toldrá, F., Hui, Y.H., Astiasarán, I., Sebranek, J.G., Talon, R. (Eds.), Handbook of Fermented Meat and Poultry, second ed. Blackwell Publishing, Ames, Iowa, USA, pp. 117–128.

the United States) and must contain the adequate enzymes to obtain the desired organoleptic characteristics in the product. Of course, they must lack decarboxylases to avoid amines generation or oxidative enzymes to avoid oxidation. Most of the current fermented sausages are produced with starter cultures consisting of LAB alone or combined with CNS and yeasts or molds (Flores and Toldrá, 2011). The main enzymes and their microorganisms of origin are shown in Table 9.2.

During fermentation, carbohydrates are converted into lactic acid by LAB. A schematic representation of the different biochemical pathways is shown in Fig. 9.2. The ratio between the L and D enantiomers depends on the action of L and D lactate dehydrogenase, respectively, and the presence of lactate racemase (Demeyer et al., 2014). The pH drops in accordance with the amount of lactic acid generated that mainly depends on the type of species used as starter, the composition and content of carbohydrates, and the fermentation temperature. The pH decrease is partially counterbalanced by the salt-solubilized and partly hydrolyzed muscle proteins and by ammonia production, and in those cases with fungal growth, some lactic acid is consumed and the final pH might even increase (Demeyer et al., 2014).

TABLE 9.2 Main enzymes from microorganisms and its biochemical effects during meat fermentation and ripening.

Enzymes	Microorganisms of origin	Biochemical effects
Glucohydrolases	LAB	Lactic acid generation
Endopeptidases	LAB	Protein breakdown, peptides generation
Exopeptidases	LAB and CNS	Generation of free amino acids
Nitrate reductase	Yeasts and CNS	Nitrate reduction to nitrite
Lipases	Yeasts and CNS	Generation of free fatty acids
Catalase	Yeasts and CNS	Antioxidant
Superoxide dismutase	Yeasts and molds	Antioxidant
Transaminase	Yeasts and molds	Transformation of amino acids
Deaminase/deamidase	Yeasts and molds	Lactic acid consumption and ammonia generation

CNS, coagulase-negative staphylococci; *LAB*, Lactic acid bacteria.

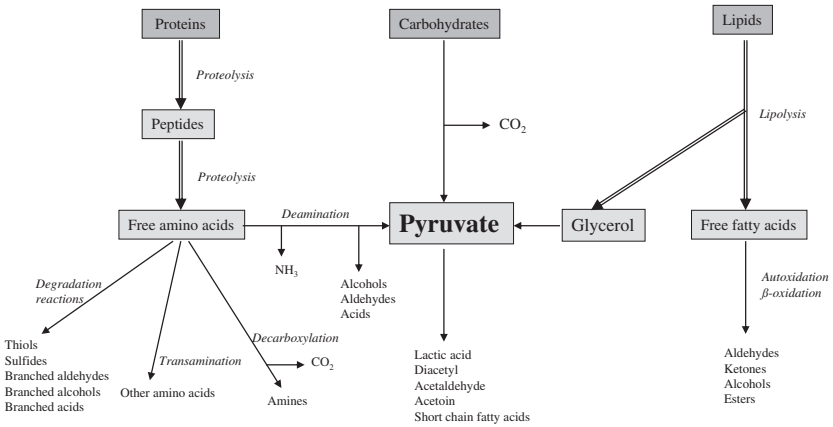

FIG. 9.2 Simplified overview of sausage metabolism. *Double lines* indicate pathways with a major contribution of muscle and adipose tissue enzymes. *(Reprinted from Demeyer, D.I., Leory, F., Toldrá, F., 2014. Fermentation. In: Jensen, W., Dikemann, M. (Eds.), Encyclopedia of Meat Sciences, second ed., vol. 2. Elsevier Science Ltd., London, UK, pp. 1–7, with permission from Elsevier.)*

Heterofermentative pathways may happen and produce secondary products such as acetic acid, acetoin, and others (Demeyer and Stahnke, 2002). The pH drop contributes to safety because it inhibits undesirable pathogens. There is also a joint action of bacterial and meat enzymes on sausage protein and lipid fractions whose changes represent another important transformation. Proteolysis

and lipolysis, especially the activity of muscle cathepsin D and lysosomal acid lipase, both active at acid pH, are also favored by mild pH drop. However, most enzymatic reactions involved in flavor generation are partially or even completely inhibited at pH below 5.0 (Toldrá, 2012a). In addition to pH, enzyme activities are influenced by other processing parameters such as the content of salt, nitrate and nitrite, and carbohydrates; the temperature of fermentation, drying, and ripening; the length of ripening time; changes in water activity; and the presence of spices and seasonings (Leroy et al., 2015).

9.4 Dehydration

The deprivation of available moisture can not only prevent the growth of the microorganisms found on meat but may also kill them. Water may be made unavailable by direct removal, as in dehydration and freeze dehydration, or by increasing the extracellular osmotic pressure, as in curing (see Chapter 8). Other recent innovations are based on vacuum or infrared-assisted hot air processing (Muga et al., 2021; Dinçer, 2021). With these processes the prevention of microbial change and the preservation of edibility involve the creation of a commodity that necessarily differs more from the fresh meat than does refrigerated meat, although on subsequent cooking these differences in nature are less apparent.

Early drying procedures, which are still continued in remote areas of the world, involved exposure of strips of lean meat to sunlight, as in the manufacture of pemmican by North American Indians, or a combination of light salting followed by air drying, as in the preparation of charqui (in South America), jerky (in North America), and biltong (in South Africa). Such products are considerably different from fresh meat, and to most they are lower in eating quality. Their preservation may be enhanced by fermentation via halophilic microorganisms. This way further developed toward the use of drying rooms, which had big doors and windows so that the air conditions within the room could be regulated by opening or closing the windows more or less according to the outside temperature and humidity. The degree of drying was evaluated by touching the product and eventually examining the color and shape (Zukal and Incze, 2010; Muga et al., 2020).

Other types of drying are those employed for dry-cured meats such as dry-cured ham and dry-fermented sausages. In these processes, the reduction in weight may reach up to 34% due to loss of water by evaporation. An example of moisture loss in the external and internal muscles of dry-cured ham is shown in Fig. 9.3. The internal part tends to retain more moisture (Toldrá, 2006c). The description of these processes is given later in this chapter.

The large-scale commercial production of dehydrated meat in a form, which, when cooked, was similar in nutritive value, and palatability to the fresh commodity resulted from research carried out during World War II. The surface-to-volume ratio in the minced meat was increased to achieve the necessary degree of moisture removal by the current of hot air used in the process. Initial products

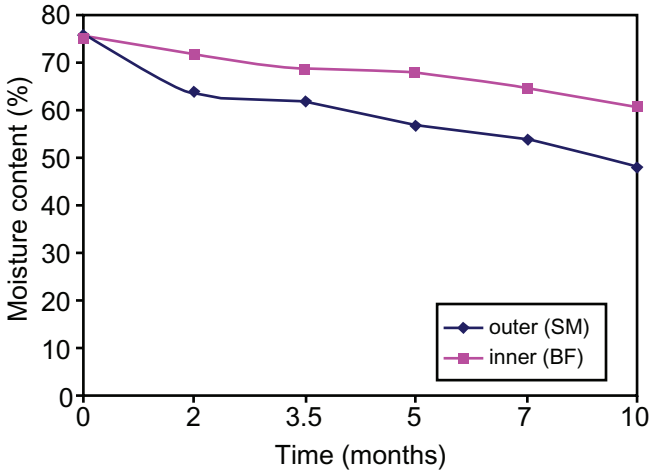

FIG. 9.3 Evolution of the moisture content in the external muscle *semimembranosus* and the internal muscle biceps femoris along the processing of dry-cured ham. *(Reprinted from Toldrá, F., 2006. The role of muscle enzymes in dry-cured meat products with different drying conditions. Trends in Food Science and Technology 17, 164–168, with permission from Elsevier.)*

were unacceptable due to rapid case hardening and difficulties in achieving further reductions in inner moisture. By first cooking the meat in slices, however, mincing it, and then drying it under carefully controlled conditions, with the temperature being kept below 70°C, a product could be prepared, which was almost indistinguishable in flavor and texture from raw minced meat, when comparisons of the fully cooked meats were made (Lawrie and Ledward, 2006). It was never issued as such to the domestic consumer, although it was utilized for certain manufactured meat products, but it proved most useful for the Armed Forces.

Today, the most common drying rooms are convective or air drying, which are fully automatized with computer that takes control of temperature, air flow rate and flow distribution, and relative humidity according to the moisture content in the product and its size, shape, and structure (Grau et al., 2015). Modern drying rooms allow a better drying control and are independent from the outside climatic conditions.

9.4.1 Changes during drying

The difference between dehydrated and fresh meat will obviously be minimized if the water removed from the former can be incorporated again on rehydration. The degree of reincorporation depends on the surviving water-holding capacity of the muscle in terms of both microscopic structure and the chemical state of the muscle proteins. When using hot-air drying procedures, the lack of

rehydratability is largely due to changes similar to those occurring during heat denaturation. The drying kinetics of fresh beef were determined for different temperatures, air velocities, relative humidity, and thicknesses of the product. The shape of the drying curves revealed two different periods, where the drying rate falls slowly and a second one with significant decrease in drying rate (Ahmat et al., 2015). The dehydration process can be optimized in meat by understanding how water is distributed within meat during dehydration. An effective and noninvasive way was achieved by obtaining hyperspectral images of beef slices, at six specific wavelengths in a pixel-wise manner, at different periods of the dehydration process (Wu et al., 2013).

Various physical factors determine the efficacy of a hot-air draught in dehydrating precooked minced meat. The temperature of drying is important. On the other hand, a relatively high fat content begins to retard the rate of drying, although it has little influence initially. This results in a serious increase in the time of dehydration if the fat content is above about 35% of the dry weight, and where it is above 40% of the dry weight, the spongy texture of the dry meat can no longer hold the molten fat, and it drips away. On the other hand, when the fat content is below about 35% of the dry weight, dehydrated meat of satisfactory water content can be obtained in a continuous hot-air drier and at a fixed drying time, despite considerable variability in the fat content. In general, however, a low-grade meat from a leaner type of carcass is preferred for the preparation of dehydrated meat in these circumstances (Lawrie and Ledward, 2006).

The degree of precooking is an important factor. If the meat is overcooked, its connective tissue framework will be changed to gelatin, and although it will give dry granules that reconstitute quickly, it will break down under compression. An undercooked meat, however, will have a slow drying rate and a slow rate of reconstitution, yielding a dry and brittle texture. Since the aqueous liquor exuding from the meat during the precooking period contains various soluble substances, it must be returned to the cooked meat before dehydration commences to retain the full meat flavor and nutritive value of the fresh commodity. Any fat rendered out on cooking may be returned to the meat according to the desired fat content.

For long-term storage, dehydrated meat must be compressed to exclude pockets of air or moisture and kept in an airtight and moisture-proof container, since its finely divided porous state makes it especially liable to attack by oxygen. Nonoxidative changes, whether enzymatic or chemical, are of secondary importance except at high storage temperatures. Thus, while restriction of oxygen will maintain the flavor of dehydrated meat for 12 months or longer at 15°C, nonoxidative deterioration may develop under nitrogen at 37°C. Generally, the moisture content of dehydrated meat is too low to permit bacterial growth, but if it rises above 10%, mold growth may occur after some weeks, generally *Penicillium* and *Aspergillus* spp. (Lawrie and Ledward, 2006).

9.5 Smoking

Smoke, generally produced by the slow combustion of sawdust derived from hardwoods (consisting of about 40%–60% cellulose, 20%–30% hemicellulose, and 20%–30% lignin), inhibits microbial growth, retards fat oxidation, and imparts flavor to cured meat. Traditionally, smoking was uncontrolled and consisted of burning the wood beneath the meat. The process can be more speedily carried out, and a product of consistent quality is produced, by controlled smoking in a kiln and by electrostatic deposition of wood smoke particles. Direct deposition of smoke particles makes a negligible contribution to the process: vapor absorption by surface and interstitial water is much more important. Today, the modern smokehouses are computer-controlled and take care of the temperature of smoke generation and its flow circulation as well as drying air. They also include the gear for cleaning and neutralization of the spent smoke (Sikorski and Kolakowski, 2010). Different types of smoking may be applied depending on the type of meat product (Sikorski and Sinkiewicz, 2015): (1) cold smoking, operating at 12–25°C applied from few hours to several days, is typically used for dry-fermented sausages and pork belly; (2) warm smoking, operating at 25–45°C for a few hours, is typically used in the manufacture of baked or scalded sausages, pork back fat, and hams; and (3) hot smoking, operating at 45–90°C for up to 12 h, is used in the manufacture of some assortments.

Part of the bactericidal action of smoke is due to various phenols as well as carbonyl compounds such as formaldehyde, but the composition of wood smoke is complex. Depending on the species of wood and the smoldering parameters, the smoke may contain different content of CO, CO_2, alcohols, carbonyl compounds, carboxylic acids, esters, hydrocarbons, nitrogen oxides, and phenols. The highest yield of smoke phenols, especially guaiacol and syringol and their respective derivatives, occurs at temperatures 400–600°C. The phenolic fractions contain guaiacol and its derivatives and many other compounds up to 240°C (Sikorski and Kolakowski, 2010).

Smoke may also contain up to 60 identified polycyclic aromatic hydrocarbons (PAHs), with 16 of them being PAHs with mutagenic and/or carcinogenic activity (see Chapter 18). The indicator for many years representing the other carcinogenic PAHs was benzo(*a*)pyrene (BaP), but it was not generally accepted as a good representative. The index PAH4, which is the sum of BaP, benzo(*a*) anthracene, benzo(b)fluoranthene, and chrysene, is considered a better indicator of the potential carcinogenicity of the smoke (EFSA, 2008). The European Commission Regulation 835/2011 (European Commission, 2011) set an upper limit for PAH4 of 12 ng/g and kept a limit of 2 ng/g for BaP.

In view of the risk hazard of carcinogenesis from smoked meat, there have been many attempts to produce carcinogen-free smoke, for example, by condensation, followed by fractional distillation and purification. The selected fraction is diluted with water in which the benzopyrenes are insoluble. The use of such liquid smokes has expanded, and liquid smokes are used worldwide.

They may be supplemented by the addition of specific phenolic substances having a fruity flavor and odor. The liquid smoke is used for smokeless smoking of meats, and the content of PAH4 can be effectively controlled (Sikorski and Sinkiewicz, 2015).

The flavor imparted by smoking varies according to the conditions used to produce the smoke. Moreover, the same smoke will produce different aromas with different meats. To some extent, therefore, the flavor of the smoked product depends on the reaction between the components of the smoke and the functional groups of the meat proteins. Thus phenols and polyphenols react with -SH groups and carbonyls with amino groups. The color imparted by smoking may be light yellow to dark brown, depending on the type of wood and conditions of smoking. It is also affected by heating and storage.

9.6 Processing technologies for cured meat products

It is not the purpose of this book to give detailed consideration to the nature of the various processed meats that are available in many countries. Nevertheless, there is much recent interest in relating the changes occurring during maturing (ripening) of such products. The development of proteomics techniques has revealed the large number of small peptides produced by proteolysis at various stages of ripening (Di Luccia et al., 2005), some of them with influence of flavor but other with interesting bioactivities such as antihypertensive, antioxidant, antidiabetic, or antimicrobial (Mora et al., 2013; Toldrá et al., 2020a). Such investigations can be expected to provide the knowledge necessary to deliberately enhance the expression of desired flavor components and/or the health benefits of the products by increasing the release of bioactive peptides (Toldrá and Reig, 2011).

9.6.1 Dry-cured ham

Dry-cured ham is a flavorful product that has been produced for centuries, not only in the Mediterranean area but also in China and other areas. These products have been, and still are, very important for local economy, culture, gastronomic heritage, and tradition (Toldrá, 2016). The basic processing is quite simple and is mostly based on salting, drying, and ripening where the product develops an intense and characteristic texture and flavor. Typical European hams are the Spanish jamón Ibérico and jamón Serrano; French jambon de Bayonne; Italian prosciutto di Parma, prosciutto San Daniele, and prosciutto Toscano; Belgian jambon d'Ardenne; Portuguese presunto; and Croatian Istrian dry-cured ham (Toldrá, 2014a). Most hams are protected with certifications such as the Denomination of Protected Origin, the Protected Geographical Indication, or the Traditional Speciality Guaranteed in the European Union that must accomplish specific regulations and are controlled by consortiums (Parolari, 1996). Typical country hams in the United States are produced in North Carolina,

Tennessee, Missouri, Kentucky, and Virginia. These dry-cured hams started in the United States with the settlers by 1600s and are smoked and cooked before consumption (Hanson et al., 2015). Dry-cured hams have been also produced in China for centuries, with Jinhua ham (produced in Jinhua), the Xuanwei ham (Yunnan), and Rugao ham (Jiangsu) being the most relevant (Zhou and Zhao, 2015). There are other types of dry-cured meats that are also very typical within Europe, based on other pieces such as loins or forelegs that can be even from other animal species (Toldrá, 2014a).

A schematic flowchart for the processing of dry-cured ham is shown in Fig. 9.4. The reception of the raw materials, which are the back legs of pig crossbreeds, is the first stage. Hams are examined, the pH is measured, and they are weighed and rubbed with the curing salt (mixture of sodium chloride and potassium nitrate) on the external surface, even though nitrate may not be added for certain types of hams. Next stage is salting where hams are completely covered with salt or with a given certain amount of salt proportional to the

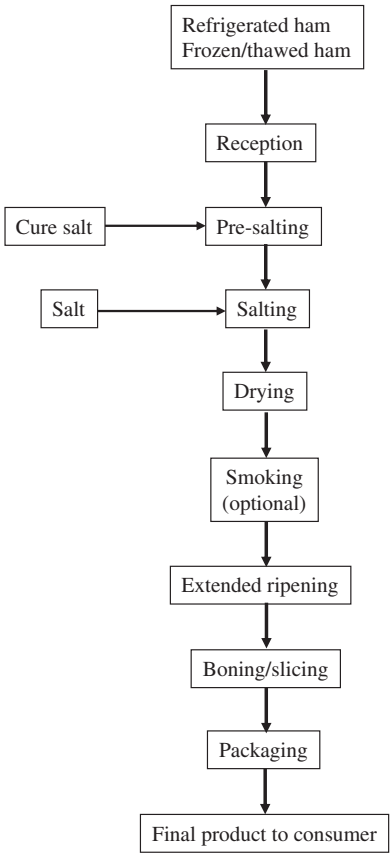

FIG. 9.4 Process flow diagram for the processing of dry-cured hams.

ham's weight. During the 11–15 days of salting, salt and curing agents penetrate into the hams and start diffusing. Once salting is finished, hams are washed out of the excess of salt and hanged for the postsalting period, also named as salt equalization because the main goal is for salt and curing agents to diffuse through the entire piece during the 2–3 months of this stage. Then, hams start the drying/ripening stage that may last up to 9–12 months, until hams reach a total weight loss of 32%–34%. Drying temperatures range from 16°C to 25°C depending on the processing time; in general, lower temperatures require longer times, and the relative humidity is kept between 65% and 80% depending on the drying degree (Toldrá, 2014b). Drying takes place in computer-controlled modern drying chambers where air speed, temperature, and relative humidity are checked periodically. The product thickness greatly affects drying time as well as the water profile inside the final product. So, the control of drying is critical because water has to diffuse from the inner part of the ham to the surface, and then it is evaporated to the chamber environment. To be efficient, both rates of diffusion and evaporation should proceed in a similar way. In case of an excess of evaporation when the relative humidity in the chamber has a lower value than normal, it may lead to excessive evaporation on the surface of the ham resulting in dehydration. This is a phenomenon known as hardening that gives a dry hard texture and dark color on the external area of the ham (Toldrá, 2006d). Hams are considered dried once they reach 32%–34% weight losses, and they may be sold for consumption or be further ripened for extended flavor development. Hams may be commercialized as an entire piece including the bone and foot, as a boned and molded block that facilitates its slicing, or sliced packaged under vacuum or modified atmosphere.

In case that hams are submitted to further ripening, they are covered with lard to avoid excessive dryness and subjected to ripening at mild temperatures, 10–15°C, for up to 18, 24, or even 36 months. In this way, hams develop an exquisite and intense flavor generated through further chemical and enzymatic reactions.

Smoking may be applied, but this is only usual in short processed hams (<3–4 months), typical in northern European hams and American country hams (Toldrá, 2012b).

Endogenous muscle enzymes are responsible for most of the biochemical changes in dry-cured hams (Toldrá, 2012c). The most important are proteolysis and lipolysis, responsible for the generation of compounds with direct influence on taste and aroma. Proteolysis consists of the enzymatic hydrolysis of major sarcoplasmic and myofibrillar proteins and generates a large amount of peptides and free amino acids (Toldrá, 2006b). Main muscle peptidases are cathepsins B, D, H, and L; calpains I and II; tripeptidylpeptidases I and II; dipeptidylpeptidases I, II, III, and IV; carboxypeptidases A and B; and alanyl, arginyl, leucyl, and methionyl aminopeptidases (Toldrá and Reig, 2015). The mode of action of all these peptidases is schematized in Fig. 9.5 where the generation of free amino acids and di- and tripeptides from both the amino and carboxy

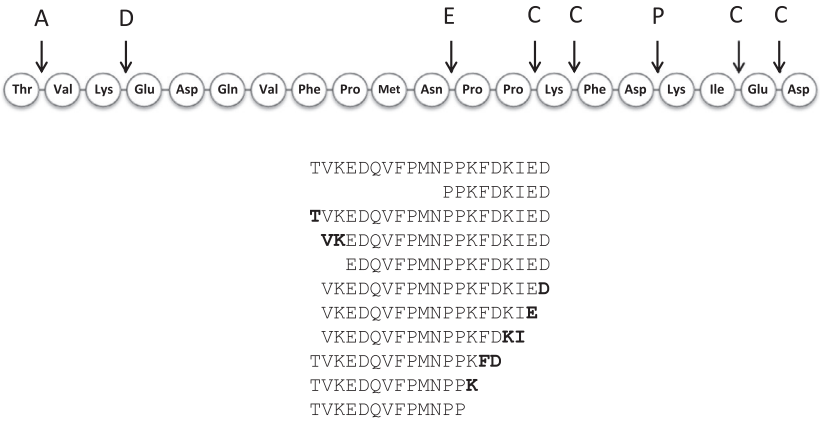

FIG. 9.5 Scheme of food protein hydrolysis and enzymes involved. The amino acids sequence is a fragment belonging to myosin heavy chain. Aminopeptidase (A), Dipeptidylpeptidase (D), Endopeptidase (E), Carboxypeptidase (C) and Peptidylpeptidase (P). *(Reprinted with permission from Toldrá, F., Gallego, M., Reig, M., Aristoy, M-C. and Mora, L. (2020) Recent progress in enzymatic release of food-derived peptides and assessment of bioactivity. Journal of Agricultural & Food Chemistry 68, 12,842–12,855. Copyright 2020. American Chemical Society.)*

terminals may be observed (Toldrá et al., 2020a). The released peptides have been studied with proteomic tools confirming that thousands of peptides are released during processing (Mora et al., 2013), some of them reported as bioactive peptides with antihypertensive and antioxidant activity (Mora et al., 2015; Toldrá et al., 2020b). The presence of peptides with antihypertensive activity was somehow confirmed in a clinical trial with human volunteers where their blood pressure was reported to be slightly reduced after regular consumption of dry-cured ham in spite of its high content in salt (Montoro-García et al., 2017). Lipolysis consists of the enzymatic generation of free fatty acids through the breakdown of triacylglycerols and phospholipids. Main lipolytic enzymes are lysosomal acid lipase and acid phospholipase that are located in the muscle, and neutral lipase located in the adipose tissue (Toldrá and Reig, 2015). Lipases show good stability during the process and also vary in activity depending on the type of breeding and age. Free fatty acids are released during the process up to 10 months in the muscle and up to 6 months in the adipose tissue (Toldrá, 2012c). The released fatty acids containing double bonds are susceptible to further oxidative reactions that will generate volatile compounds with particular aroma characteristics.

In general, muscle enzymes show good stability during the dry-curing process even though they are partially inhibited by salt. There is some variability in the activity of these endogenous muscle enzymes depending on the original crossbreeds and the age of the pigs (Toldrá and Reig, 2015).

9.6.2 Fermented sausages

Fermented sausages were already produced by Ancient Romans and Greeks. The origin of words such as sausage and salami may proceed from the Latin expressions "salsicia" and "salumen." A flowchart for the processing of fermented sausages is shown in Fig. 9.6. Raw materials include chilled pork and/or beef meats and porcine fats, which are chopped and submitted to comminution in a grinder. Once comminuted, the batter is added with salt, nitrite (and nitrate), carbohydrates, microbial starters, spices, sodium ascorbate, and optionally, other nonmeat. The batter is fully homogenized under vacuum for a few minutes. Then, the batter is stuffed under vacuum into casings with both extremes clipped. The casing may be either natural, collagen-based, or synthetic. Once stuffed, sausages are placed in racks, hanging in either natural (traditional sausages) or air-conditioned drying chambers. Sausages start to ferment, when microorganisms start growing and developing. Relevant biochemical changes take place, especially focused on the enzymatic breakdown of carbohydrates, proteins, and lipids. Physical changes also happen, mainly acid gelation of meat proteins and drying. Temperature, relative humidity, and air speed are controlled for an adequate microbial growth and enzyme action.

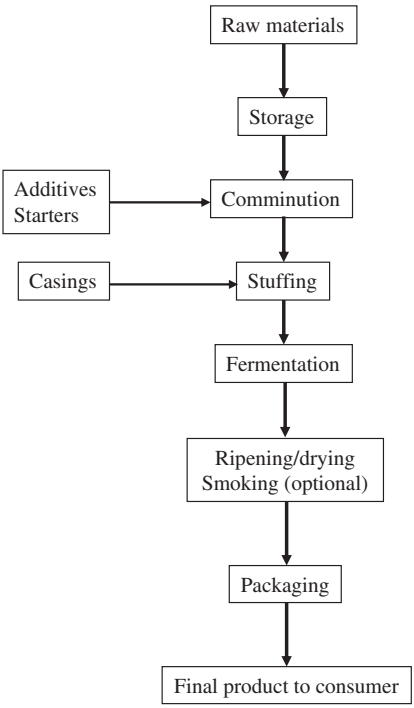

FIG. 9.6 Process flow diagram for the processing of dry-fermented sausages.

The extent of fermentation and drying depends on the location and climate. European dry-fermented sausages are consumed raw, without further heating while heating is a general practice in the United States for all types of sausages (Maddock, 2015). Chinese-style fermented sausages are semidry and also are consumed after heating (Chen et al., 2015). In the United States, sausages are fermented at relatively high temperatures followed by a mild heating process, as a kind of pasteurization, instead of drying, to kill any *Trichinella*. Thus, starters such as *L. plantarum* or *P. acidilactici,* which grow well at those temperatures, are typically used. The drying period tends to be omitted, as in the "summer sausage," elaborated in the United States and fermented at 38°C.

In Europe, different technologies may be found, and *L. sakei* or *L. curvatus*, which grows well at mild temperatures, is the LAB most often used as starter cultures (Toldrá, 2012a). There is a historical trend toward short-processed (up to 3 weeks), usually at temperatures >20°C, smoked sausages in cold and humid countries, as in northern Europe. Shelf life and safety are mainly due to the fast drop to acid pH (<5.0 after 3 days) and to smoking rather than to drying (Demeyer and Stahnke, 2002). On the other hand, Mediterranean sausages are heavily seasoned and seldom smoked; fermentation takes place at milder temperatures (<24°C), followed by mild drying conditions for a longer time, usually several weeks or months. The pH drop rate is slower and ends at higher pH (>5 after 3 days). Shelf life is mostly due to drying and reduced water activity (Demeyer et al., 2014). A comparison of main parameters distinguishing Northern and Mediterranean sausages is shown in Table 9.3. The time required for the fermentation stage is a function of the temperature and type of microorganisms used as starter. The length of the ripening/drying period may vary from 7 to 90 days or even longer, depending on factors such as the kind of product, diameter, dryness degree, fat content, and desired flavor profile and intensity. The progress in drying reduces the water content, up to 20% weight loss in semidry-fermented sausages and 30% in dry-fermented sausages.

Enzymes from both muscle and microbial origin are involved in reactions related to the development of color, texture, and flavor. An intense proteolysis happens during fermentation and ripening, resulting in the accumulation of polypeptides that are further hydrolyzed to small peptides by muscle and microbial peptidylpeptidases and to free amino acids by muscle and microbial aminopeptidases (Toldrá, 1998). Free amino acids are relevant not only for taste but also for aroma because they can generate volatile compounds through Strecker degradations and Maillard reactions. Amino acids can be deaminated or deamidated into ammonia by deaminases and deamidases, respectively, from yeasts and molds. On the other hand, lipids also experience an intense lipolysis resulting in the generation of free fatty acids (between 0.5% and 7%) through the enzymatic hydrolysis of triacylglycerols and phospholipids (Toldrá and Flores, 1998). Proteolysis and lipolysis are more extensive in longer ripened sausages.

Catalases are mainly present in microorganisms such as *Staphylococcus*; they are responsible for peroxide reduction and thus contribute to color and

TABLE 9.3 End products of metabolism in northern and mediterranean types of sausages, produced in Belgium[a].

	Northern-type sausage (NS)		Mediterranean-type sausage (MS)	
Dry matter (DM) (%)	57 (4)		67[b] (3)	
pH	4.8 (1)		5.5[b] (2)	
% in DM				
Crude protein	31 (7)		28[b] (9)	
Crude fat	61 (7)		81 (8)	
NaCl	5.3 (12)		6.1 (11)	
mmol per 100 g DM				
Lactate	21 (12)		17[b] (17)	
Acetate	1.0 (14)		0.86 (19)	
Sugars	0.56 (23)		0.40[b] (18)	
mg N per g N				
Peptide α-NH_2-N	30 (13)		27[b] (16)	
Free α-NH_2-N	23 (10)		37[b] (13)	
Ammonia-N	3 (22)		10[b] (18)	
μg BSAeq per mgCP[c]				
Myosin (200 kDa)	18 (30)		25[b] (12)	
HMM (150 kDa)	24(21)		24 (8)	

Continued

TABLE 9.3 End products of metabolism in northern and mediterranean types of sausages, produced in Belgiuma—cont'd

	Northern-type sausage (NS)		Mediterranean-type sausage (MS)	
Actin (46 kDa)	35 (14)		39 (10)	
38 kDa	15 (7)		15 (7)	
mg per g TFA[d]				
Free fatty acids	27 (4)		37[b] (11)	
Aroma compounds[e]	(1)	(2)	(1)	(2)
Hexanal	123	2836	227	11,568
3-Methyl butanal	44	856	32	941
3-Methyl butanol	355	580	315	1015
2-Methyl butanal	7	198	7	225
2-Methyl butanol	22	0	47	0
Ethyl esters	221	0	82	625
Diacetyl + acetoin	3052	311	681	167

[a] *Mean value of 25 determinations per type (five batches and five sausages per batch); values in parentheses are coefficient of variation, i.e., standard deviation as percentage of mean value.*
[b] *Significant difference ($P<0.05$) between mean values for NS and MS.*
[c] *BSAeq, bovine serum albumin equivalents determined by semiquantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CP, crude protein.*
[d] *TFA, total fatty acids.*
[e] *Aroma compounds are given as (1) nmol 4-methyl-2-pentanone per kg obtained from headspace analysis and (2) ng per kg obtained after steam distillation.*
Reproduced from Demeyer, D.I., Leroy, F., Toldrá, F., 2014. Fermentation. In: Jensen, W., Dikeman, M. (Eds.), Encyclopedia of Meat Sciences, second ed., vol 2. Elsevier Science Ltd., London, UK, pp. 1–7, with permission from Elsevier.

flavor stabilization. Nitrate reductase, also present in these microorganisms, is also important for reducing nitrate to nitrite in slow-ripened sausages with an initial addition of nitrate (Flores and Toldrá, 2011).

9.6.3 Cooked ham

Cooked ham, also known as gammon in the United States, is the piece of pork deriving from the hind legs of swine, which is preserved through processes such as cooking and, optionally, smoking. Cooked ham is a widely consumed product worldwide. For instance, it may reach up to 26% of the delicatessen products sold in Europe, with France, Spain, and Italy being the major consumers (Casiraghi et al., 2007). Cooked hams may receive the name of the region where they are manufactured such as the Italian prosciutto cotto or French Jambon de Bourgogne or French Jambon de Reims. A schematic flowchart for the processing of cooked ham is shown in Fig. 9.7.

Hams may be preserved through refrigeration or kept under frozen storage and thawed just before processing. They are controlled for hygienic conditions

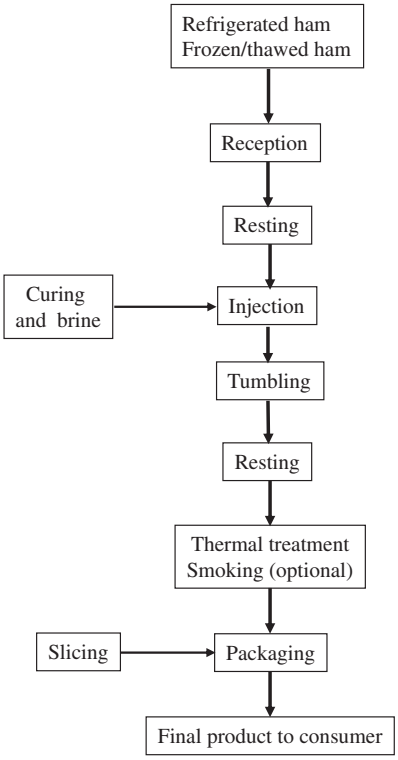

FIG. 9.7 Process flow diagram for the processing of cooked hams.

and pH because those with low pH values, such as pale, soft, and exudative (PSE) hams, have a low water-holding capacity that will give higher cooking losses and drier hams (see Chapter 14). Once into the process, hams are brine injected through a multineedle system to solubilize proteins and favor the binding of muscles, obtaining a better yield. Sodium or potassium nitrite is added to the brine for preservation and for pink color generation through the formation of nitrosylhemocrome. The brine may also contain some carbohydrates (sucrose, dextrose, or corn syrup) to impart a pleasant mild taste. Sodium ascorbate or sodium erythorbate may also be added to assure the disappearance of nitrite, which is reduced to nitric oxide, and avoid the generation of nitrosamines. Low-quality hams may include phosphates, polyphosphates, or pyrophosphates that are added to the brine to increase the water-holding capacity and thus retain more injected water (Toldrá and Reig, 2007).

The use of "tumbling" or massaging of hams in rotating drums is a common practice to assure a correct distribution of curing salts. Tumbling of hams consists of the slow movement of hams into rotary tumblers with inner baffles operating for a few hours under vacuum to avoid air bubbles and further undesired oxidations. This practice can shorten the curing period by facilitating the distribution of curing salts. It involves massaging the pieces of meat against one another in the presence of about 0.6% of their weight of salt. This draws out salt-soluble proteins (mainly actomyosin) to the meat surface and enhances the overall water-holding capacity when these gels are heated. The increase in water-holding capacity observed is related to the concomitant binding of brine (Dolata et al., 2004). If tumbling is carried out for more than 12 h, however, the binding strength of the ham tends to decline. The procedure has been increasingly applied to the production of ham (from the major muscles of the hindquarter) during the past decades. Most recently, tumblers that operate under vacuum have been used, whereby subsequent cooking losses are virtually eliminated (partly because dissolved air is minimized), and this makes it possible to produce hams with a greater brine content than in traditionally produced hams (Toldrá et al., 2010). The low fat content of the muscles now used for ham production also enhances the brine content of the muscular tissue.

Once curing salts have been distributed uniformly, hams are then canned in metallic molds that will give the final shape to the hams or packaged in special plastic bags (zero water loss) and are submitted to cooking under a strict control of time and temperature to inactivate pathogens and spoilage microorganisms. The cooking process, which can be considered as a kind of pasteurization, is applied so that the time/temperature achieved in all parts of the ham is equivalent to 2 min at 72°C. In fact, the actual temperature achieved in the center of the ham may vary between 68°C and 75°C. Heating is transferred from the heating medium to the ham surface by convection and from the ham surface to the inner parts by conduction. There are three different types of cooking procedures that may be applied. Heating at a fixed temperature was used in the past but is now less used. The other two ways that are currently used consist of either heating

until reaching a fixed temperature inside the ham (usually 68°C) or heating by stages (step-by-step increase in the temperature, <25–30°C each step). Once cooking is finished, hams must be cooled down to <5°C, performed by immersion or showering with cold water. This must be performed rapidly (<10 h) because of the risk for microbial growth, especially from 50°C down to 12°C (Toldrá and Reig, 2016). Vacuum cooling reduced significantly the cooling rate (Desmond et al., 2000).

The final quality of cooked ham is affected by the raw material used, the composition and quantity of the brine injected, the rate and extent of tumbling or massaging, and the cooking time and temperature (Delahunty et al., 1997). Other parameters also affect the quality of cooked ham. For instance, a higher content of noncollagen muscle protein was appreciated as a better quality by consumers (Válková et al., 2007). The sensory characteristics of hams are developed during cooking as a consequence of multiple enzymatic and chemical reactions taking place. The characteristic pink color is due to the nitrosylhemochromogen pigment. A final smoked flavor may be achieved if hams are submitted to smoking, which is optional. Hams are then packaged either as a full piece to retailers or as ham slices packaged under vacuum or modified atmosphere that are more convenient for commercial distribution. The storage temperature must be kept under refrigeration because the quality deteriorates rapidly at storage temperatures exceeding 4°C (Ran et al., 2021).

9.6.4 Bacon: Wiltshire cure and modern bacon production

The Wiltshire method consists of an old, traditional method of curing, whereby the pork leg is immersed for several days in brine that gives a product with high quality (Delahunty et al., 1997). After cooling the carcass, the sides are trimmed. This involves removal of the *psoas* muscle, the scapula, and the aitch bone (bones of pelvis). The trimmed sides are next chilled to the temperature of the curing cellar (3–7°C) where curing takes place in four stages: (1) brine (pickle) is pumped into the sides, (2) the sides are either sprinkled with dry salt or placed in a tank of brine, (3) the sides are removed and stacked for some time in a maturing cellar (at 3–7°C), and (4) the sides may be smoked.

The concentration of sodium chloride in the injected brine (pump pickle) is about 25%–30%. It also contains 2.5%–4% of potassium or sodium nitrate and in some cases 0.5%–1% sugar. About 18–25 injections are required to obtain an approximate uniform distribution, most being given to the gammon region. Injection was manual being replaced in modern Wiltshire by multineedle injection. The "shoulder pockets" (scapula cavities) are filled with solid salt, and the sides are placed in curing tanks. The total amount of brine injected is about 5% of the weight of the side. In a large tank the sides are stacked and lightly covered with sodium chloride and potassium nitrate in the ratio 10:l. They are battened down, brine is run in, and the sides remain submerged in it for 4–5 days. The composition of this brine (tank pickle) is between 20% and 28% with respect to

sodium chloride and 3%–4% with respect to potassium nitrate when first prepared, but before it can be used in curing, it must be seeded with specific, salt-tolerant microorganisms that will be responsible for nitrate reduction.

After removal from the brine tanks, the sides are stacked in cellars for 7–14 days or longer. During this period, known as maturation, the sodium chloride, the nitrate, and the nitrite become more evenly distributed throughout the musculature, and the typical color and flavor of bacon develop.

The bacon thus produced may be consumed unsmoked (green), but probably the greater proportion is smoked for 2–3 days for adding flavor, but also for giving a preservative action and also delay rancidity in the fat on the surfaces of the bacon sides. If the temperature is allowed to rise too high during smoking, on the other hand, deep-seated bacterial growth may be encouraged.

Bacon can be also produced from pork belly. A development in the preparation of bacon was slice curing. In this process, slices of pork muscle 2–8 mm thick are passed for 2–15 min through a brine containing 8%–10% sodium chloride and 0.02% sodium nitrite. Maturation occurs over a few hours. The process gives a uniform product in less than a day instead of the 10–21 days of the traditional Wiltshire process. Endeavors have been made to avoid the remote possibility of excess production of nitrite from nitrate in the traditional Wiltshire cure, by omitting the use of nitrate in the pickle and adding only about 200 ppm of nitrite (Lawrie and Ledward, 2006). Such bacon is said to develop the flavor associated with the traditional cure provided it is permitted to mature for a reasonable period of time (e.g., upward of 2 weeks).

Hot curing has generally not been applied to entire sides, as in the Wiltshire cure, because of chilling difficulties, but it can be applied readily in a modified Wiltshire process in which immersion in brine is replaced by dry salting (Taylor et al., 1980), and sides are chilled quickly by being hung individually. Acceptable bacon can be produced in this way within 5 days of slaughter (Taylor et al., 1982).

The modern bacon production uses freshly prepared brines and curing performed either by immersion or in bag. In the immersion curing, the sides are injected with the brine with a target weight gain near 10%. The pieces are then stacked in the tanks where they are fully covered with fresh brine up to 3 days. Taint arises less frequently with tank curing, because a high concentration of salt builds up quickly and discourages bacterial growth, even if the pH is high. Once finished, the sides are removed and stacked for maturation to get the surface dry, and typical color and flavor developed. In the bag curing, the sides are deboned, injected, and placed within an impermeable bag, which is vacuum-sealed and left for a minimum of 2 days for curing salts equilibration and color and flavor development. The final salt content is 3% (Sheard, 2010). Most bacon is sold sliced either in vacuum packages or in modified atmosphere packing. Once opened, bacon must be kept under refrigeration and consumed within 3–4 days.

When comparing the cured meat products from three halothane genotypes (NN, Nn, and nn), Fisher et al. (2000) demonstrated that there was a progressive

lowering of bacon yield, and an increase in cooking loss, as the gene signifying halothane sensitivity (namely nn) increased. The net increase in yield was 3% for PSE meat in comparison to 10% for normal pork. Nevertheless, in curing meat from PSE pork, the incorporation of porcine collagen enhances the water-holding capacity of the product (Schilling et al., 2003). Bacon is typically smoked, and this has an impact on its flavor (Deng et al., 2021). So, the profile of volatile aroma compounds may be influenced by the type of wood species or the type of smoking method (wood, liquid, and paper smoking) used (Guo et al., 2020).

9.7 Conclusions and future trends

There are different manufacturing technologies available for the production of cured meat products, depending on the raw materials and the processing conditions. Furthermore, there are also variations in the production and consumption of cured meat products throughout the world because of the strong influence of local traditions, climate, and culture.

A clear trend for the future is the reduction in the use of nitrite as a preservative. The risk for nitrosamine generation is promoting research in many laboratories to find alternative preservation ways for cured meats even though the development of the traditional cured meat color may be a challenge difficult to achieve.

There are some relevant trends related with sustainability. Drying is energy-consuming, and the meat industry has a significant potential for reducing the energy use. So, there is a need for the development of cost- and energy-efficient drying processes for cured meat processes, also in the management and reutilization of salt. Meat industry generates large amounts of by-products that need to be disposed. New disposal alternatives are investigated looking for new applications of by-products as well as to increase its profitability by using them as raw materials for the production of added-value products.

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Chapter 10

The storage and preservation of meat: Storage and packaging

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10.1 Introduction

Fresh muscle-based foods are highly perishable food products. This is largely due to water activity (a_w) and availability of specific nutrients, which bacteria, yeasts, and molds require for growth (Jay et al., 2005). There are a number of traditional intervention systems employed to counter the destructive roles played by such microorganisms, such as chilling, freezing, drying, salting, curing, smoking, use of chemical preservatives, irradiation, and so on (see Chapters 7–9). However, it is the application of appropriate packaging materials and systems that permits and strengthens these preservative approaches, thereby maximizing their effects over longer storage periods, which is a requirement for modern-day retailing of such food products. In particular, the use of modified atmosphere packaging (MAP), including several vacuum packaging (VP) approaches, and the gradual uptake of smart, active-based packaging materials have allowed for greater and more successful control of microbial spoilage, as well as oxidatively driven chemical spoilage, in muscle-based product quality over longer shelf life periods.

Spoilage of fresh chilled foods involves a number of complex processes and brings about chemical, physical, biochemical, and biological changes (see Chapter 6). These changes can often interact, and consequently, a change in one single property can affect all of the others (Walker, 1992). The main objective in chilling fresh meats is to decrease the rate of microbiologically associated enzymatic activity. In fact, as the temperature decreases the microbial lag phase extends and growth rate increases (Walker, 1992). It has been documented that there are three classes of substrates utilized by microbial association with fresh meats, namely nitrogen energy sources such as proteins and amino acids, different compounds that contribute in the glycolytic pathway (including lactate, glucose, and glucogen), and metabolic products (such as lactate and pyruvate)

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(Nychas et al., 2008). It is important to understand the chemistry of meat spoilage before intervention systems can be put in place.

Lactic acid bacteria along with *Pseudomonas* sp., *Shewanella putrefaciens*, and *Brochothrix thermosphacta* have been shown to be the main spoilage bacteria of fresh meat stored at low temperatures, under vacuum, gas-flushed MAP, or aerobic conditions, possessing a low or high pH (Garcia-Lopez et al., 1998). *Pseudomonas* sp. thrive in aerobic conditions, while lactic acid bacteria, *Clostridium* spp., *Bacillus* sp., *Shewanella putrefaciens*, and *Brochothrix* sp. thrive under anaerobic conditions (see Chapter 6). It is important to note that the type of spoilage is directly related to the microbial flora present initially on the meat, as well as that contributed by the environmental conditions within which the muscle food resides (Nychas et al., 1998).

The objective of this chapter is to provide a brief introduction to fresh meat preservation systems, including the employment of novel chilling regimes and packaging systems in an attempt to reduce raw meat and muscle food spoilage, primarily by microbiological means.

10.2 Impact of microbiology on fresh meat quality attributes

The quality of fresh meat is the most important factor in a consumer's decision to purchase that product. Due to the differences in consumer preferences, fresh meat quality can be difficult to define. Fresh meat quality is correlated to muscle fiber characteristics affected by a variety of factors, such as preslaughter animal stress, muscle structure, chemical environment, processing, product handling, changes in muscle tissues postmortem, microbiological load, and population present in meat, etc. (Joo et al., 2013; Lee et al., 2010).

Fresh meat quality is defined scientifically by factors including water-holding capacity, nutrient availability, colorants, tenderness, flavor, spoilage, etc. How consumers perceive quality depends very much on the human senses of appearance, odor, taste, and texture (Joo et al., 2013).

For fresh muscle, particularly red meat, color can be considered the most important quality trait since it is the sole sensory-based quality attribute available to the consumer at the point of purchase by which they can judge product quality. The color of meat is dependent on the amount of myoglobin present in the muscle, with myoglobin levels being affected by a number of factors, including diet, exercise, environmental factors, and genetic factors. Color stability is dependent on the rate of oxymyoglobin oxidation (Faustman et al., 2010). The color, odor, and texture of beef are also directly affected by the oxidation of the polyunsaturated fatty acids in fresh meats (Kanner, 1994).

With regard to fresh red meat, tenderness is the most important factor when a product is eaten. Meat tenderness is directly related to muscle fibers and the extent of proteolysis and lipid oxidation that has taken place in the meat (Lee et al., 2010).

During the microbial spoilage of fresh meats, new products are created by microorganisms present on meat surfaces (Jay et al., 2005). The spoilage biota present catalyzes energy sources in the form of lactic acid, glucose, specific amino acids, water-soluble proteins, and urea (Nychas et al., 2008). This spoilage produces odorous compounds such as ammonia, H_2S, amines, and indole (see Chapter 6). It has been highlighted that the breakdown of glucose is the main causative agent for off-odor development during the storage of meat, mainly due to the presence of lactic acid bacteria, which produce organic acids from fermentation reactions (Jay et al., 2005; Nychas et al., 1998).

10.3 Common technologies used to preserve fresh meat products and assist in a combined manner to extend product shelf life

10.3.1 Chilling

To be correctly preserved, fresh meat products must be stored below optimum temperatures that support microbial growth (see Chapter 7). When meat products are stored at low temperatures, microbiological activity is only depressed, but not prevented, with activity recommencing once optimum growth temperatures are reestablished (Berk, 2013; Beaufort et al., 2009).

Many factors affect the rate at which heat will be extracted during chilling. Factors such as the shape and size of the packaged entity, the temperature and speed of the air in the chill, the attributes of the product itself (water content, weight/density, initial food temperature, specific heat capacity, and latent heat content) will all impact on the rate of chilling (Heap, 1992).

10.3.2 Superchilling

The rate at which beef is chilled is critical, too fast or slow may result in poor meat quality. The low temperatures that are obtained through superchilling or very fast chilling (VFC) bring about substantial releases of calcium from the sarcoplasmic reticulum to the myofibrils (Jaime et al., 2012). It has been suggested that an early supply of free calcium, along with high muscle pH, can result in an increased activation of calpains, thereby bringing about intense tenderization, which could overcome the toughness that is caused by cold shortening. This was demonstrated by Honikel (1998), who found an acceptable tenderness for VFC beef after 7 days when compared with a 14-day conventional chilling regime.

When a food is chilled below its initial freezing point, often 1–2°C below the actual freezing point, the product is then described as being superchilled (Magnussen et al., 2008). There have been numerous and conflicting definitions of superchilling; for instance, Beaufort et al. (2009) described superchilling as just below the initial freezing temperature, while Ando et al. (2004) defined superchilling as the temperature zone below 0°C where ice crystals are not produced. Combining the effect of low temperatures and the conversion of some of

the water present into ice makes a product less accessible to natural deteriorative processes (Kaale et al., 2011).

Superchilling inhibits or terminates most microbial growth, thereby increasing the shelf life of fresh meat by 1.4–4.0 times that of other chilling methods (Magnussen et al., 2008). During superchilling, ice is formed on the meat surface, which absorbs heat internally from the product until equilibrium is reached; consequently, an internal ice reservoir is reached preventing the need for external ice around the product during storage or transportation (Dunn and Rustard, 2007).

How superchilling effects the quality of a product largely depends on the species, processing, storage, and muscle type being utilized. For VFC to be successful with beef carcasses, the meat must be removed as soon as possible from the carcass (thereby employing hot boning) and reduced to a suitable size for a rapid temperature fall to be successful (Taylor et al., 1998). Superchilling has demonstrated numerous advantages on food quality (Hansen et al., 2009), with the majority of benefits being highlighted for seafood products. For example, as far back as 1969, Carlson (1969) showed that the shelf life of fish could be increased from 21 to 35 days when the temperature was reduced from −1 to −3°C. Hansen et al. (2009) demonstrated that superchilling slowed down biochemical quality degradation of salmon fillets and showed, when compared with frozen stored product, that protein denaturation and structural damage were less in superchilled product. As a consequence, fast chilling techniques can be considered particularly useful in the control of microbial spoilage of food products.

10.3.3 Use of chemical preservatives

For millennia, humans have used a wide range of naturally occurring substances derived from organic and inorganic sources for addition to foodstuffs in an attempt to make them safer to eat and to allow consumption of these food products over a longer period of time, thereby making the effort of collecting, catching, or harvesting the initial raw food materials more worthwhile. Initial food additives would have been crude in form and would have constituted the use of roots, bark, leaves, stems, ground mineral powders, and crudely extracted salts. As time passed and trade routes opened up around the globe, desirable and more specifically refined forms of preservatives were employed for food usage owing to their abilities of being capable of naturally coloring or flavoring foods. Herbs and spices became extremely popular for food usage for all of the reasons suggested, and their use as preservatives for centuries led to the development of ethnic dishes, which are still in existence today. For the past 200 years, major efforts have gone into trying to understand how and why such materials have the ability to preserve foods. From such research, thousands of substances have been characterized based on their preservative action, some of which have biotic effects and controlled food spoilage through the control of biological spoilage entities (in particular, microorganisms) and others which have abiotic effects

and control the physical and chemical spoilage of foods (in particular, oxidative reactions). Owing to technologies that have enhanced substance identification and chemistries, which have allowed for maximal extraction and purification of such substances, commercial industries can now, not alone, sell purified extracts, but can also synthetically manufacture the same preservative substances or offer completely new derivatives or variants. For example, it is possible to get a rosemary extract for food usage, that is, polar or nonpolar, any color, any flavor intensity, and action specific (antioxidant or antimicrobial or both). Natural compounds, such as essential oils, phytochemical extracts, lactic acid, chitosan, nisin, and lysozyme, to mention but a few, can all be used in meat preservation and can prolong its shelf life (Zhou et al., 2010). The move at retail and commercial levels is to deviate away from the use of synthetic preservatives to those that have a greater acceptance and more natural appeal with consumers.

What is really interesting about chemical preservatives is their ability, in many cases, to work synergistically with certain packaging systems to maintain product quality for longer, thereby extending shelf life. For example, Kahraman et al. (2015) studied the effect of rosemary (*Rosmarinus officinalis* L.) essential oil (REO) and MAP on the survival of certain pathogens (*Salmonella typhimurium* and *Listeria monocytogenes*) in poultry fillets and on meat quality over a refrigerated storage period of 7 days. Addition of 0.2% REO to poultry fillets improved product color, reduced lipid oxidation, and reduced microbial counts. A nonexhaustive listing of natural active agent examples applied to meat-based products is provided in Table 10.1.

More information can be found in other reviews (Lucera et al., 2012; Tiwari et al., 2009). In fact, natural preservatives can be used alone or in combination with other novel preservation technologies to facilitate the replacement of traditional approaches (Tiwari et al., 2009).

The permitted use of preservatives in food products, including muscle-based foods, is subject to legislative approval. While the use of synthetic food additives and preservatives is suitable for food preservation, for example, in the European Union (EU) currently, naturally sourced antimicrobials have more potential for commercial usage due to the limited health risks associated with their usage (Table 10.2).

10.3.4 Ionizing radiation

Since the initial discovery that radionucleotides were capable of destroying organisms through DNA damage brought about by high-energy emitted particles and X-rays, ionizing radiation had been studied as a means of controlling and harnessing its potential for a multitude of applications, including its use in maintaining food product quality and extending shelf life (see Chapter 8). Due to the high penetrating capabilities of X-rays and particles, microorganisms present on food surfaces within food packaging systems are destroyed

TABLE 10.1 Relevant examples of natural active agents applied to meat-based products (Lucera et al., 2012).

Products and storage conditions	Natural compounds	Main results
Fresh minced beef patties packaged under MAP	Thymol (250, 500, 750 mg/kg)	Better effects on product quality were obtained for sample with increased amount of thymol, under MAP conditions (shelf life about 7 days)
Minced beef mixed with soy protein stored at 4°C	Sage essential oil (0.1%, 0.3%, and 0.5%)	The highest concentration of essential oil controlled development of main microorganisms
Meat balls stored at 10°C	0.2% of cranberry, rosemary, and lovage extracts	Rosemary extract was the most effective on product shelf life (13.3 days)
Sausages stored at 4°C under vacuum conditions	Sodium lactate (0%, 0.6%, 1.2%, 1.8%) as alternative to nitrite	Sodium lactate improved the microbiological quality, extended shelf life, and exhibited a better antimicrobial effect than nitrite
Fresh sausage	Oregano and marjoram essential oil	Addition of oregano and marjoram essential oil exerted a bacteriostatic effect
Broiler chicken wings stored at 4°C	Dipping treatment for 10 min with chlorine dioxide, lactic acid, and fumaric acid	Samples treated with lactic acid alone showed the most effective reduction on *Escherichia coli* and mesophilic bacteria
Fresh chicken meat stored under MAP at 4°C	Treatments with nisin and EDTA, alone or in combination	Chicken was better preserved under treatments with 500 IU/g of nisin and 50 mM of EDTA, even up to 24 days
Fresh beef	Organic acids (citric, lactic, acetic, and tartaric)	Organic acids promoted a significant shelf life extension
Fresh chicken sausage stored at 4°C	Rosemary or Chinese mahogany (500, 1000, 1500 ppm)	Chinese mahogany and rosemary improved meat quality
Turkey bologna stored at 4°C	Coating with gelatin containing Nisaplin and Guardian	Both Nisaplin film and Guardian film effectively inhibited *Listeria monocytogenes*
Meat pieces	Combined application of oregano essential oil and acetic acid	Combination of essential oils and organic acids inhibited microbial growth and proliferation of pathogens such as *Staphylococcus aureus*

EDTA, ethylenediaminetetraacetic acid; *MAP*, modified atmosphere packaging.

TABLE 10.2 Legislatively approved food additives in the European Union.

Additive	Code assigned by European legislative authority
Acetic acid	E260
Benzoic acid	E210
Butylated hydroxyanisole	E320
Carvacrol	
EDTA	
Citric	E330
Ethanol	E1510
Lactic acid	E270
Lauric acid	
Sodium benzoate	E211
Sorbic acid	E200

EDTA, ethylenediaminetetraacetic acid.

(Lawrie and Ledward, 2006). This method is more efficient than thermal treatments due to the low energy needed to reduce microbial counts (Aymerich et al., 2008). The application of irradiation dosage on food composition and quality has been widely studied. Graham et al. (1998), demonstrated that if radiation dosage level is correctly applied, then even one of the most sensitive nutrients, namely thiamine, would not be affected during irradiation of muscle foods. In the United Kingdom, food regulations (1990) allow for a maximum irradiation dosage (e.g., 7 kGy for poultry), and all irradiated foods are required to have a label indicating that they have received such treatment. Irradiation technology has been accepted in 50 countries (Aymerich et al., 2008; Zhou et al., 2010). The radionuclides approved for food irradiation include 137Cs and 60Co (Zhou et al., 2010). It is fair to say, however, that consumer resistance to its use in foods generally, especially within the EU, continues to dampen the widespread usage of this preservation technique.

10.3.5 Other potential and developing technologies for raw meat and muscle-based products

As time has progressed, the distinction between raw or fresh muscle foods and those that are deemed to be processed has blurred, especially among consumers. Fresh and raw meat and muscle-based products are presented to consumers in

raw sauces, marinades, stir-fry, or seasoned with herbs and spices. Consequently, as these chilled products need to be thermally processed for consumption, it is probably fair to say that thermal treatment of muscle food products is the single most important processing factor that distinguishes between raw and processed meat and muscle-based products.

While old and well-established preservation techniques have been described in this chapter previously, it is worth mentioning that relatively new processing technologies, with preservation-linked capabilities, have evolved over the past two decades in particular. Technologies, such as high-pressure processing, ultrasound application, and cold-plasma treatment, may offer acceptable and wide-scale commercial application for the shelf life extension of raw meat or fresh muscle-based food products. However, for the purposes of this chapter, they will not be discussed further.

Irrespective of the processing technologies employed to extend product shelf life, no technology can significantly impact on the storage life of food products unless accompanied by adequate packaging materials and systems. Therefore, it can be categorically claimed that of all the preservation approaches utilized to maintain the quality of muscle-based food products for the purposes of extending shelf life, packaging is paramount.

10.3.6 Packaging technologies employed for raw meat and fresh muscle-based food products

Fresh meat and muscle-based food products are generally packaged using a select number of packaging materials (usually laminate or coextruded polymeric-based films and/or trays) and packaging systems, which are based on either removing gases by creation of some form of vacuum or creating packaging systems by introducing set gas mixes (MAP) or allowing slow gas permeation through the packaging materials over time (overwrapping). The following section deals with these materials and systems in more detail including several novel packaging machines applied for each section.

10.3.7 Vacuum packaging

VP is a process of removing the gases (with particular emphasis on the removal of oxygen) from within the packaging environment, thereby creating an anaerobic environment and subsequently inhibiting the growth of spoilage bacteria and prolonging product shelf life. Meat products are usually vacuum-packed in a heat-shrinkable plastic pouch, coupled with a low gas permeability, and then heat-sealed to ensure product containment. An example of vacuum-packaged beef is shown in Fig. 10.1. Close contact between the product surface and the packaging material is essential to obtain effective VP (Gill and Molin, 1991). The color of pigmented meats retains the dark purplish/brown color of freshly slaughtered muscle and which represents muscle pigment in the myoglobin

FIG. 10.1 Example of vacuum-packed beef.

state. While this has always been relayed to be a negative owing to consumers not liking this form of meat color, certain retailing organizations have bucked this widely accepted view by successfully retailing red meat subprimal cuts in essentially vacuum pack formats.

As a vacuum-packaged product is exposed to heat during the heat-sealing process, possible activation of psychrophilic and psychrotolerant spores occurs, which in turn can lead to early onset of blown pack spoilage (BPS) (Bell et al., 2001). Due to the fact that bacteria such as *Clostridium* are strictly anaerobic, VP offers an ideal environment for growth and spoilage. However, the true benefit of using VP is that due to the oxygenless environment that it creates, rapid aerobic meat spoilers such as *Pseudomonas* species are controlled and product shelf life is greatly extended. Spoilage of red meat that has been vacuum-packaged usually develops over several weeks and is generally brought about by the presence of *Brochothrix thermosphacta* or the presence of lactic acid bacteria. This may represent a concern in particular when shipping meat over long distances (Adam et al., 2013).

There is different equipment applied for VP depending on production volumes. For small production, VP chamber machine is usually used (Fig. 10.2).

Firstly, a product is placed inside VP laminate or co-extruded bags (usually pouches), which are placed in a vacuum chamber and facing toward the vacuum pump. After closing the lid on the chamber (second picture on a figure from the left), air is evacuated, thus creating a vacuum inside the pouch and chamber. Then, sealing the lips on the edges of the machine seals the closing edge of the pouch and the chamber opens. Sealing temperature and dwell time can be adjusted for different packaging materials manually, as can the level of vacuum applied. It has to be noted that some machines have the option of gas mixture injection for bulk gas flushing or “mother packaging” of food products (Kennedy et al., 2005).

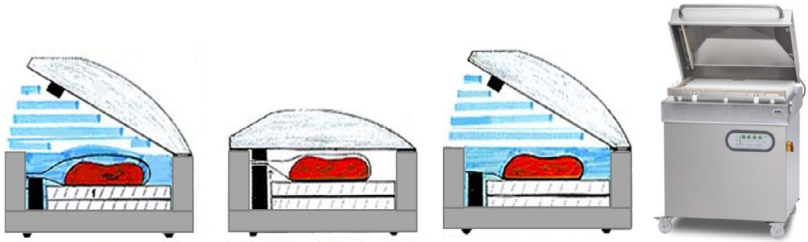

FIG. 10.2 Schematic representation of VP of meat cuts in pouch bags on manual chamber machine and actual VP machine.

FIG. 10.3 In-line K7 conveyor vacuum packaging chamber machine from VC999.

When commercial production volumes increase, VP machines are designed to operate at higher speeds and can be fitted with conveyance systems (Fig. 10.3).

Rotary VP machines consist of several chambers, which work simultaneously for high-volume meat packaging and rotary arrangements of the chambers economize on space, thereby allowing installation of other machines and conveyance systems about them (Fig. 10.4).

10.3.8 Heat-shrink wrapping

The advantage of employing heat-shrink wrapping, which is a form of VP, is that it is a quicker process than VP and usually employs the use of low-cost films. While not used extensively for meat products and never for meat products that require long shelf lives, this packaging approach is employed for meat transportation. Heat-shrink packaging draws the packaging tightly around the meat, reduces pack corners that can get caught in conveyor belts, improves the barrier properties of the packaging materials employed (which must be orientated on manufacture), improves appearance, and reduces drip loss from the product. An example of heat-shrink packaging is shown in Fig. 10.5.

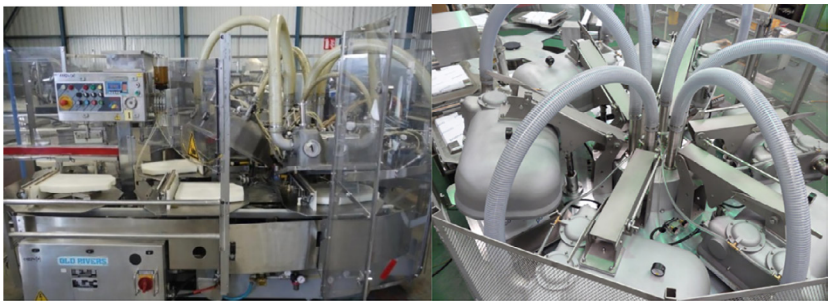

FIG. 10.4 Cryovac (United States) octopus 8620 rotary VP machine and a top view of such machine.

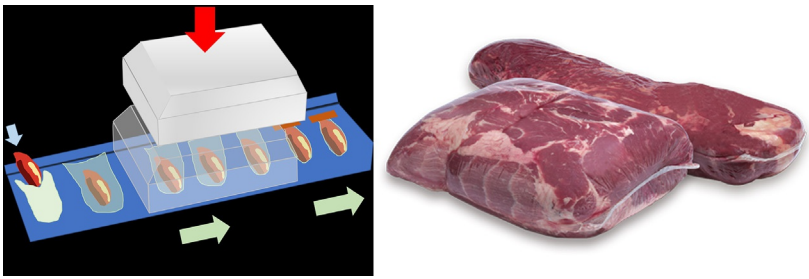

FIG. 10.5 Vacuum heat-shrinking process and packed meat. *(Photos taken from the websource: cryovac.com.)*

Just as heat sealing for VP was linked to the initiation of BPS, it has been suggested that the short-term heat treatment necessary to carry out the heat shrinkage of the plastic film to the product can activate *Clostridia* spores that may be present on the product, subsequently resulting in actively metabolizing vegetative cells, thereby producing the phenomenon of BPS. It has been observed that meats inoculated with *Clostridia* spores and which were vacuum-packed and subsequently heat-shrunk (a typical commercial application) demonstrated rapid onset of BPS compared with nonheat-treated samples (Bell et al., 2001). In another study conducted by Moschonas et al. (2011), short heat-shrinking treatments and chill storage temperatures were investigated to determine their effects on the onset time of BPS. Beef steaks were inoculated with 10^3 CFU/cm^2 spore suspensions of five different gas-producing *Clostridia*. These samples were vacuum-packed and subjected to different heat treatments, 50°C/15 s, 70°C/10 s, 90°C/3 s, and no heat application. Samples were then stored at −1.5°C, +1°C, or +4°C and examined daily to determine the onset time for BPS. The results showed for each of the strains that pack treatment and storage temperature had significant effects on BPS onset time. The results showed that the industry could reduce the risk of BPS by avoiding the higher-temperature treatments (90°C/3 s or 70°C/10 s) and by storing vacuum-packed meats at lower temperatures (−1.5°C).

FIG. 10.6 FlOW-VAC heat shrinking vacuum machine.

Bell et al. (2001) conducted a trial to determine if high-temperature treatments that are applied in heat shrinking of vacuum packs affected the onset of *Clostridia* BPS. Packs containing beef striploins were inoculated with *Clostridium estertheticum* NCIMB 12,511 and five other psychrotolerant clostridial isolates. Some packs were subjected to heat shrinkage and stored at −1.5°C, +1°C, or +4°C, while others underwent no heat treatment. During this study, it was shown that postpackaging, heat shrinkage accelerated BPS with *Clostridium* spp.

A heat-shrinking packaging process applies a heating temperature, which causes the packaging material to shrink within seconds of exposure (Fig. 10.6). Flow-wrapping processes form bags or pouches from sheet material fed and formed from rolls/reels/webs of material (on the left). This process can be adapted to different products, meat cuts, pack formats, etc., with online monitoring of the packaging process. These systems can also be fitted with gas flushing capabilities and are often referred to as form, fill, and sealing systems, which can be horizontal or vertical depending on product demands. Therefore, vacuum packaging or gas-flushed MAP can be achieved using this technology.

10.3.9 Vacuum skin packaging

In VP applications, air pockets and wrinkles in the packaging containing exudate from the product are susceptible to bacterial growth and are therefore undesirable. For vacuum skin packaging (VSP) applications, meat cuts are placed on high gas barrier trays typically comprised of polystyrene or polypropylene (PP) base materials and vacuum-sealing barrier films are then heat shrunk to conform to the shape of the product (Belcher, 2006).

The use of VSP technologies reduces the presence of air packets and wrinkles, minimizes the risk of bacterial growth, and prolongs product shelf life.

FIG. 10.7 Variety of packed products, which can be formed using a vacuum skin packaging machine.

The reduced levels of oxygen in VSP compared with standard VP minimize oxidative reactions and reduce aerobic microbial growth. In VSP, the upper film cover of a product is heated, making the film shrink tightly around the product (Vázquez et al., 2004). As in the case of VP and heat-shrinking applications, heat-sealing operations and heat-shrink processes may generate sufficient heat to partially create conditions that encourage anaerobic microbial spoilage and spore germination.

In a study performed by Lagerstedt et al. (2011), beef quality traits were assessed comparing VSP skin packaging with VP and high-oxygen (80%) MAP. Beef samples were cut and aged in vacuum packs for 7 days. Samples were then cut into steaks and frozen, stored in skin packs, vacuum packs or MAP, and stored for an additional 7 or 17 days. The results showed that samples stored in VSP had lower purge losses and higher sensory scores compared with MAP samples.

Different packaging formats can be achieved by employing a vacuum skin packaging machine, including vacuum skin protruding, hybrid flat 3D, protruding modified atmosphere packaging (MAP), which are shown in Fig. 10.7.

10.3.10 Modified atmosphere packaging

MAP of meat has continued to be one of the most reliable retailing packaging methods used to increase product shelf life of commodities such as burgers, meat medallions, sausages, minced meat, and steaks (Fig. 10.8) and as a consumer-friendly presentation format. For both comminuted and noncomminuted products, MAP has been the packaging format of choice as it has allowed products to be presented with various appearances and in a manner that prevented meat products from having a squashed appearance. However, VSP is

FIG. 10.8 Minced meat in modified atmosphere packaging.

now challenging MAP for all manner of meat product types and formats owing to the manner in which this technology has evolved in recent years.

MAP preserves food by manipulating the gaseous environment immediately around the product. The gaseous environment typically has a different gaseous composition from that of normal atmospheric air. Depending on the product being packaged, a suitable gas mixture must be selected for that product based on its chemical composition and on the spoilage factors that need to be controlled and which are unique to that product. In general, the gaseous environment created is based on the manipulation of gases, which make up the air that we breathe, namely carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2). The reason that preservative control of foods can be achieved through the use of MAP is simply because of the inherent properties associated with these gases.

CO_2 is used due to its selective bacteriostatic properties, which prevent or delay the growth of fast spoiling aerobic bacteria such as *Pseudomonas* spp., which dislikes the presence of this gas, even at low concentrations (Hintlian and Hotckiss, 1987; Cooksey, 2014; Vermeulen et al., 2013). However, for MAP of meat and most other foods, a starting concentration of around 20% CO_2 is required in all MAP food packs (Stiles, 1990). It is important to note that CO_2 does not affect the growth of all microorganisms in this way. For example, lactic acid bacteria thrive in the presence of CO_2 and low O_2. Absorption of CO_2 largely depends on the fat and water content of the product being packaged and the temperature at which the product is being held (Jakobsen and Bertelsen, 2000, 2004). Generally, the higher the fat and water content of a product and the lower the holding temperature, the greater will be the absorption of CO_2 into the product. Excessive CO_2 absorption into a product such as meat can cause moisture loss, negatively impact on texture, and result in product discoloration. O_2 is used to maintain the fresh red color of meat; however, it must be noted that while O_2 is delivering this commercially desired effect, it is also driving negative oxidative reactions leading to lipid oxidation, protein oxidation, microbial growth, and vitamin degradation. N_2 is an inert gas, and its main functions are to displace oxygen (for reasons provided above) and to counterbalance CO_2 levels in packs to keep the shape of the package by preventing pack collapse (which can be caused when excessive levels of CO_2 are absorbed by the meat product,

thereby creating an internal vacuum, which leads to pack implosion). Other gases can be used for a variety of functions, for example, carbon monoxide is used to maintain the red color in meats and argon has been used in place of N_2 in meat packs (Day, 1992; Hunt et al., 2008).

In the following sections, different barrier materials and technologies will be discussed for gas-flushed MAP packaging in order that protective gas contents are maintained inside the pack. Special software can be used for the selection of packaging material for the appropriate gas content and packaged product volume (Mahajan et al., 2007). Regarding packaging machines for gas flushing MAP, there are numerous machine types on the market, and a small typical example of such a machine is shown in Fig. 10.9. This example consists of a four-tray chamber and a roll of lidding film, which seals each product-filled tray after closing the chamber. Prior to sealing the lidding material to the tray, the chamber, and consequently, the trays are flushed with a protective gas mixture.

For gas flushing MAP of high volumes of product, automatic machines are used commercially and as shown in Fig. 10.10 (a two-tray seal station) and Fig. 10.11 (a five-tray seal station). Different parameters can be adjusted on these much larger-scale machines, such as seal temperature, operational speed, packaging material flexibility in terms of material type and format, gas mixture control and delivery systems, etc.

FIG. 10.9 Manually operated gas flushing MAP for a four-tray sealing machine.

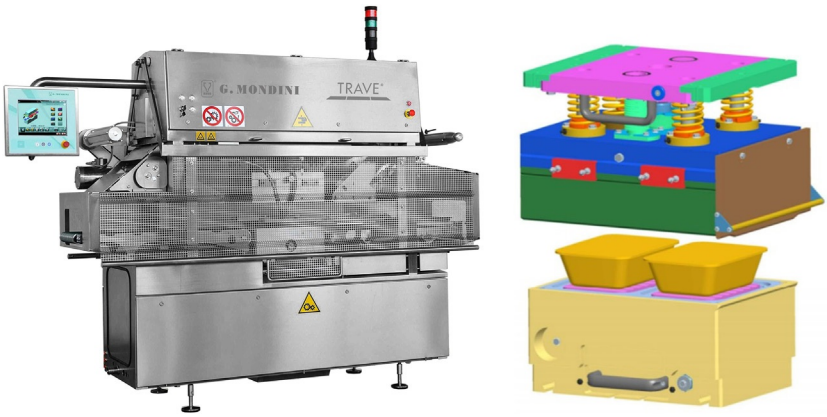

FIG. 10.10 Tray Sealer Trave-340 (Mondini) and two-tray seal station.

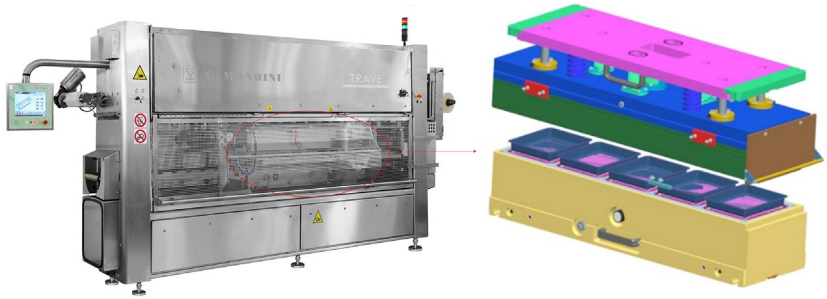

FIG. 10.11 Tray sealer Trave 1000 (Mondini, Italy) and five-tray seal station.

10.4 Packaging materials used for fresh meat and muscle-based products

10.4.1 Barrier materials, test methods

Since the development of plastics around the middle of the last century, commercial food packaging materials and packaging formats have evolved enormously. These developments along with improvement in chilling technologies and revolutionary developments in distribution chains have meant that fresh foods, such as meat, could be distributed over greater distances owing to extensions in product shelf life. Prior to plastics development, products such as meat would have to be processed in some way and subsequently packaged in some form of metal (usually steel-based) packaging for the purposes of distribution over long distances.

The development of plastics offered the food industry, the retailing sector, and the consumer many advantages. These materials were light in weight, clear

in appearance, easy to handle, tough, strong, impermeable to water or gases or both, capable of being colored and printed, heat sealable in cases, and could be shaped or formed giving rise to the term, which is still used today for these materials, namely thermoplastics. Later, it was discovered that the properties of individual plastics could be taken advantage of in a more significant way by combining or joining different plastics together in laminated constructions or in co-extrusion process.

The most commonly used plastics in the meat industry in recent times are polyethylene (various types based on molecular weight), PP, ethylene vinyl acetate, polyvinyl chloride, polyvinylidene chloride (PVDC), polyamide (PA), polyethylene terephthalate, polystyrene, ethylene vinyl alcohol (EVOH), and polyvinyl alcohol. These plastics have been typically used in laminate constructions for film or tray applications, and careful consideration is undertaken when deciding to combine films and trays so that each is compatible and will deliver adequate product containment. Since this chapter was last presented, some major changes have taken place with regard to plastics used by the food industry. Environmental issues around the recyclability of plastics have led to question marks being raised about the continued short and long-term use of plastics such as PVC, PVdC, PS, and others. While no official legislative bans exist with respect to such plastic use, many meat and food industries have moved away from using some plastics on recyclability and environmental grounds. This move has seen some meat companies adopt single plastic sources in order to make recycling of postconsumer waste materials possible. This is a situation that will need to be monitored as it evolves.

When a fresh meat product is packaged, a unique ecobiological system is created. The characteristics of the meat product respond to the environmental conditions presented, especially in relation to gaseous environment and holding temperature. The meat microflora, which predominantly resides on the product surface, reacts to whatever environment that is presented. Typically, if excess oxygen is present within the pack and temperature abuse occurs, then microbial growth and product spoilage quickly proliferate. Additionally, negative biochemical and chemical processes will progress within the meat product under these same conditions. Therefore, it is imperative to control storage temperature and to ensure that the gas barrier properties of the packaging materials employed are high and will prevent excessive entry of oxygen into the meat package from the external atmosphere surrounding the container.

Barrier properties for meat packaging materials can be expressed as permeation or transmission rate. Oxygen transmission rate (OTR) is the volume of oxygen, which penetrates through one square meter of a film over the period of a day under standard temperature and pressure ($mL/m^2/24\,h$ at STP). Equally, water vapor transmission rate (WVTR) is the mass of water vapor, which penetrates through one square meter of a film over the period of a day under standard temperature and pressure ($g/m^2/24\,h$ at STP).

10.4.2 Testing methods for gas barrier evaluation of films

There are several standard testing methods for evaluation of the barrier properties of films. The most useful standard for determination of O_2 transmission of packaging materials is ASTM D 3985, which employs a special O_2 sensor. One of the most useful pieces of equipment for standardization is the MOCON Ox/Tran module (MOCON company, United States) (Fig. 10.12A). The film sample is installed between two cells into which two gases are presented. From one side, a mixture of N_2 with 2% H_2 is supplied. From the other side, a single source of high-purity oxygen is supplied (Fig. 10.12B). The side into which O_2 flows is pressurized, thereby forcing O_2 through the film or packaging material. Oxygen that emerges on the other side of the film, is directed to the sensor for O_2 evaluation (Fig. 10.12B). Oxygen entering at the nickel cadmium anode reacts with the cathode material to produce cadmium hydroxide, which the sensor can then detect as an electrical signal, and thus, a graph of OTR can be displayed.

The same mechanism operates for WVTR measurement, but where water vapor is applied instead of O_2 and only pure N_2 gas is used as the carrier gas (ASTM—F1249 standard for WVTR—Current MOCON PERMATRAN-W method).

Barrier properties of polymeric materials are impacted on by the environment within which the materials are held and are affected by factors, such as temperature, relative humidity, material type, and material thickness. The OTR and WVTR of different packaging materials, along with the optimized O_2 barrier values required for meat packaging, are shown in Fig. 10.13.

For example, PA is a widely used polymer in the meat industry. One of the many reasons that it is used in meat packaging is due to its oxygen barrier properties (OTR of 10–50 cc/m^2/24 h at STP). Co-extrusion or lamination of PA with a suitable polyolefin for pack sealability gives a wide variety of meat packaging applications, such as barrier trays, VSP, or lidding films for MAP. The barrier properties of different plastic materials used for meat packaging are shown in Table 10.3 (Kenneth, 2008).

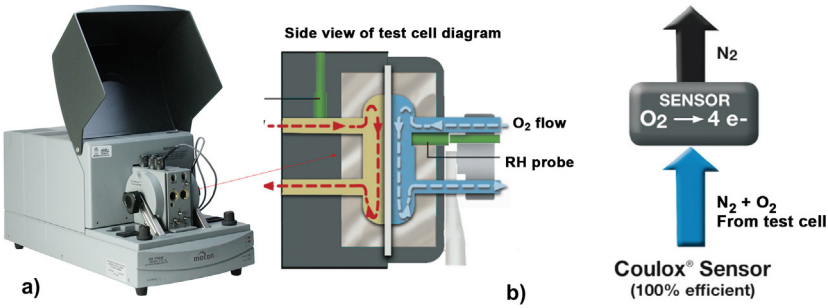

FIG. 10.12 (A) MOCON Ox-Tran module; (B) MOCON cell permeation concept.

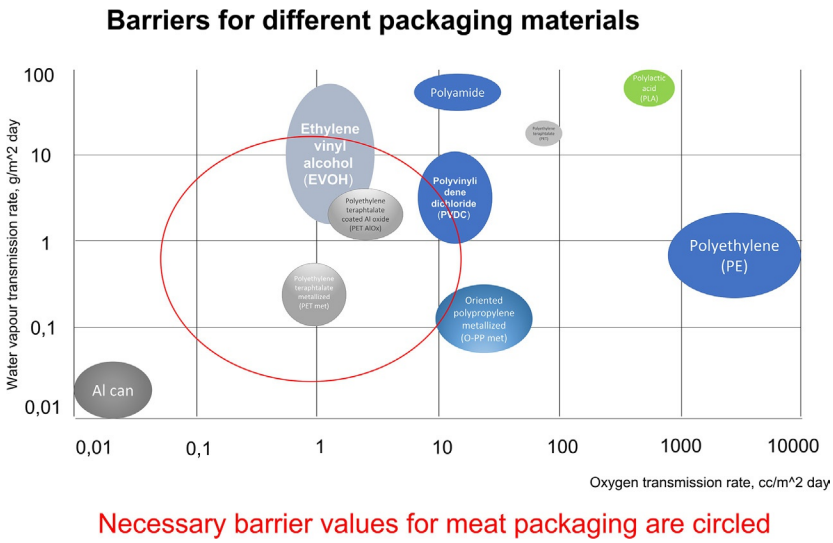

FIG. 10.13 Oxygen transmission rate (OTR) and water vapor transmission rate (WVTR) of different packaging materials. *EVOH*, ethylene vinyl alcohol; *PA*, polyamide; *PET*, polyethylene terephthalate; *PLA*, polylactic acid; *PP-O*, oriented polypropylene; *PVDC*, polyvinylidene chloride.

Film properties can vary depending on types of materials and brands used. When the distribution cold chain is long, the moderate barrier properties of 50 mL/m^2/24 h at STP of PA are not sufficient enough for long storage time of meat products. This situation can be improved through the orientation (controlled stretching) of the polymer at the point of manufacture (PA becoming OPA). This process improves numerous physical properties associated with the polymer, but particularly, barrier properties. If this still does not improve the permeation situation enough, then another polymer such as EVOH copolymer could be applied or laminated between the PA and polyolefin layers (with an OTR below 5 mL/m^2/24 h at STP depending on the film thickness employed). EVOH is comprised of a polyethylene component, which possesses thermoplastic hydrophobic properties and provides it with very good processing capabilities during cast and blown extrusion and a gas barrier component, which is soluble in water (Fig. 10.14).

Due to the presence of OH groups in EVOH, this polymer can quickly interact with water in conditions of high humidity, and thus, why the EVOH layer must be sandwiched between PA and a polyolefin as described above. EVOH can be added in plastic trays or flexible films in co-extrusion processes for the purposes of prolonging the shelf life of fresh meat. For example, minced meat can be stored for 1 week in tray with polyethylene terephthalate (PET)/EVOH/polyethylene (PE) (302 μm) (high barrier structure) with high barrier lid film PP/Polyurethane/PE/EVOH/PE (58 μm in thickness) at 2°C under MAP conditions according to EVOH supplier Kuraray, Japan (www.eval.eu).

TABLE 10.3 Properties of major packaging resins for meat and poultry.

Packaging resin	WVTR (g/m^2 day)	OTR (cc/m^2 day)	Tear strength (g/mL)	Transmission of light (%)	Heat-seal temperature (°C)	Note
Polyvinyl chloride	1.5–5	8–25	400–700	90	135–170	Moisture impermeable, resistant to chemicals
Polyvinylidene chloride	0.5–1	2–4	10–19	90	120–150	Vapor barrier, high hardness, abrasion resistant
Polypropylene	5–12	2000–4500	340	80	93–150	Clear, readily processed
High-density polyethylene	7–10	1600–2000	200–350	–	135–155	Used for structure
Low density polyethylene	10–20	6500–8500	150–900	65	120–177	Lidding films: high-strength, low-cost sealant
Ethylene vinyl alcohol	1000	0.5	400–600	90	177–205	Oxygen barrier
Polyamide	300–400	50–75	15–30	88	120–177	High heat and abrasion resistance; clear, easily thermoformed, printable
Polyethylene terephthalate	15–20	100–150	20–100	88	135–177	Abrasion and chemical resistance

OTR, oxygen transmission rate; *WVTR*, water vapor transmission rate.
Data based on 1 mil. films (Kenneth, W.M., 2008. Review. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Sci. 80, 43–65.).

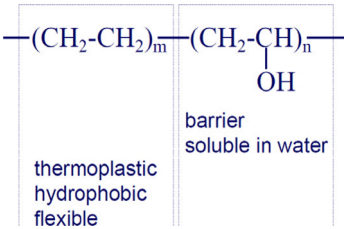

FIG. 10.14 A typical ethylene vinyl alcohol formula.

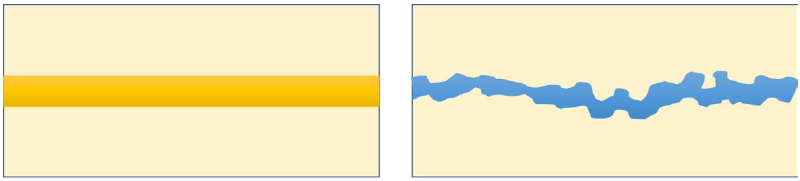

FIG. 10.15 Cross section schemes of multilayer films after heat shrinking. *(www.eval.eu)*

Owing to the ease at which it can be processed, EVOH can be used in heat-shrinkable barrier packaging materials in VP applications. The compared cross sections schemes of heat-shrunken PE/EVOH with PVDC-based films are shown in Fig. 10.15.

As it can be seen from Fig. 10.15, EVOH film layer has much lower shrink tension compared with the PVDC film, which means that film adheres firmly to the fresh meat product without squeezing the product so tightly that it forces unsightly meat fluids into the edges of the package.

10.4.3 Nanotechnologies in barrier packaging: Nanocoatings and nanofillers

As outlined, EVOH is an excellent gas barrier material; however, the presence of OH groups in its composition makes it prone to moisture damage when placed in environments of high humidity, thereby causing it to lose its gas barrier properties. However, this problem was recently overcome by Nippon Gohsei company (another supplier of EVOH material based in Japan) through the application of nanotechnology.

Nanotechnology is described as a process of fabrication, characterization, and/or manipulation of materials, devices, or structures that have at least one dimension and are approximately 1–100 nm in length (Duncan, 2011). Nanoscale particles, macromolecules, and other nano-objects have significantly different chemical and physical properties to that of macroscale materials, despite possessing the same composition (Duncan, 2011). There are different nanotechnologies currently used in the packaging industry, such as nanolayered co-extrusion processes, nanocoatings, and nanoparticle addition into films or

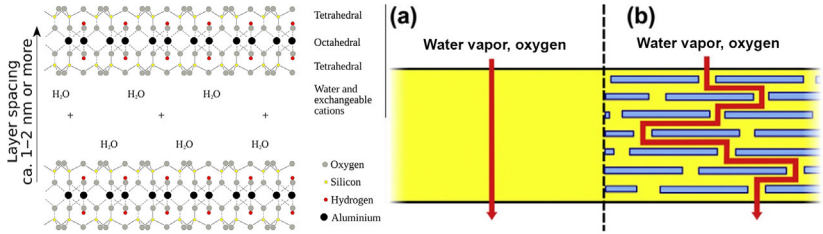

FIG. 10.16 Structure of montmorillonite clay (top): (A) pathway of O_2 and water vapor through film without nanoclays on the left and (B) with the addition of exfoliated nanoclays in polymer matrix forming a labyrinth for water and O_2 molecules on the right (Arora and Padua, 2010; Duncan, 2011).

lacquers. Nanotechnologies are or can be employed in packaging materials to create new properties or augment existing ones.

The most commonly used nanoparticles in the food packaging industry are nanoclays, employed in the form of montmorillonite (MMT) (Pereira de Abreu et al., 2010), Fig. 10.16A.

MMT clays consist of nanometer-scale platelets of magnesium aluminum silicate. Their dimensions are 1 nm in thickness and 100–500 nm in diameter (Arora and Padua, 2010). When they are exfoliated in a polymer matrix, they create a labyrinth structure, which creates hurdles and reduces the penetrating ability of gas molecules through packaging materials, thereby increasing the barrier properties of these materials (Fig. 10.16B). The tortuous pathway increases the mean gas diffusion length through the packaging and consequently increases the shelf life of spoilable foods (Duncan, 2011).

Addition of MMT composites into EVOH copolymer increases its OTR properties. Sornol NC7003 (Nippon Gohsei, Japan) EVOH with MMTs of O2BlockBarriertechnology from Nanobiomatters Industries S.L. (Spain) notably improves the barrier properties over conventional EVOH, especially at high humidity as presented by fresh meat products (Nanobiomatters). Nanoclays can be added to a polymer-like PA, thereby increasing barrier properties. The OTR property comparison provided for PA-6 with PA-6 containing MMTs (nano-PA 6) is shown in Fig. 10.17. The addition of MMTs to the polymer improved OTR properties by up to 62% and was demonstrated that it could be used successfully for military meat applications.

Alternative cost-effective nanotechnological processes, such as nanocoatings, have been shown to be capable of increasing the barrier properties of conventional packaging materials by coating them with aluminum or silica oxides (Alcan coating technology, Amcor). The company Applied Materials (United States) has developed in-line processes and equipment for oxidation coating of AlO_x or SiO_x onto PET, BOPP, oriented polypropylene, and oriented polylactic acid materials.

Nanooxide-coated films have high barrier properties to gases and water vapors. For example, an OTR of 110 cc/m^2/24 h at STP for plain PET can be decreased to a mere 2 cc/m^2/24 h at STP following nanocoating with AlO_x. Additionally, the application of special barrier lacquers can produce a film OTR

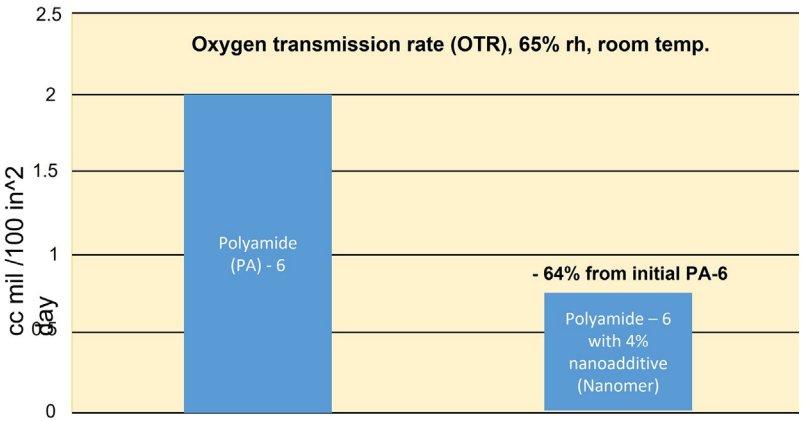

FIG. 10.17 Comparison of oxygen transmission rate (OTR) properties of polyamide (PA)-6 and PA-6 with montmorillonites (Nanocor, United States).

of 0.01 mL/m^2/24 h at STP, thereby making the material comparable to metal foils but possessing the important feature of transparency for fresh food product application. Oxide coatings are environmentally friendly, and coated materials can be recycled. Laminated, nanocoated films containing oriented polyolefins can be used: as cover films through which product can be seen, in pouches for retort application or VP, and for lidding films for MAP applications. Several companies sell AlO_x PET films on the market: Ceramis from Amcor (United States), Barrialox from Torayfilms (Japan), CeramAlOx from Ultimetfilms (United Kingdom), NANALOX from Danaflex Nano (Russia). AlO_x- or SiO_x-coated PET is cheaper than PA films and possesses higher barrier properties but has low crack resistance. Consequently, the application of PET nanocoated films for VSP of meat would be problematic in this regard as the bending and shrinking process of the materials around the meat product would potentially induce cracking. However, research is ongoing to assess the effectiveness of oxide coatings in polymeric materials such as biaxially orientated PP and PA, the latter of which would be of more interest to the fresh meat industry. EVOH and nanoadditive-containing films can also be used as an alternative to this issue or the application of special lacquers containing MMTs. For example, InMat's trade name lacquer Nanolok PT ADV-7 reduces both material and process costs when compared with the lowest-cost transparent high O_2 barrier material, EVOH. Coating thicknesses in the range of 0.5–0.8 μm provide better O_2 barrier properties than 10–20 μm of EVOH and provide much better oxygen and moisture barriers when compared with PVDC-coated PET. These transparent coatings can be applied using commercial roll coating equipment while providing performance competitive with more expensive vacuum and plasma-deposited coatings (Inmat, United States).

There are different common structures that can be used for meat packaging (Table 10.4).

TABLE 10.4 Examples of different packaging material structures, which are appropriate for fresh meat applications and which may contain nanotechnologies.

Packaging type	Structure	Advantage
Vacuum	PE/PA	Low price
	PE/EVOH/PE/PA	Middle price, high barrier, good puncture resistance
	PE/EVOH nanoclay/PE/PA	Superior barrier, long shelf life, good puncture resistance
	PE/PA nanoclay	High barrier, good puncture resistance
	PET AlO_x, SiO_x/PE	High barrier comparable to PE/EVOH/PE/PA, lower price comparable to PE/PA, more sustainable
	PET AlO*x* PE/EVOH nanoclay/PE/PA	Extremely high barrier, very long shelf life
MAP	Lid film	Low cost, good puncture resistance
	PE/PA	
	PE/EVOH/PE/PA	High barrier
	PE/EVOH nanoclay/PE/PA	Superior barrier, prolonged shelf life
	PET AlO*x*, SiO*x*/PE	Low cost, high barrier, sustainable
	Tray structure	
	PET/EVOH/PE PE/tie/PA/EVOH/PA/tie/PE PA/EVOH/tie/PE	
Skin packaging	Tray structure	Low price
	Styrene or PP Vacuum shrinking film	
	PP/EVOH/PP	
Vacuum heat shrinking	PE/EVOH/PE	Better barrier after shrinking, lower shrink tension
	PVDC	Can be used without co-extrusion with PE
	Ethyl vinyl acetate/PVDC/ethyl vinyl acetate	High puncture resistance

10.4.4 Antimicrobial packaging

The use of antimicrobials in packaging films, in an attempt to make them antimicrobial in nature, has generated great interest in their capacity to enhance shelf life and increase product safety (Cruz-Romero et al., 2013). To date, numerous categories of antimicrobial films have been developed by researchers and companies using a host of application techniques. Antimicrobials may be directly incorporated into the packaging film, incorporated by means of a sachet or label that is attached to the packaging or coated directly onto the packaging surface by using inherently antimicrobial polymers or coatings that exhibit film-forming properties, and through the use of bioactive, contact-friendly coatings, which can be applied directly onto the food surface (Coma, 2008).

Antimicrobial packaging is a form of smart packaging, with the materials employed exhibiting antimicrobial effects either by nonmigratory, immobilized direct meat contact or via slow migration of antimicrobials onto the meat surface (direct contact) or through release of antimicrobials into the package environment (indirect contact). Antimicrobials can be introduced into the meat packaging system through the employment of sachets, labels, coatings, etc. Numerous companies produce antimicrobial additives for the packaging industry, including incorporation into plastic-based packaging materials via extrusion processes. Biomaster (Addmaster) and POLYBATCH (A. Schulman) are antimicrobials or antimicrobial masterbatches, which actively interrupt cell function and inhibit cellular growth. MCX 122,009 (RTP Co) is a 25% silver-based master batch for PE and PP film manufacture, and many others are available on the market. Reviews concerning antimicrobial packaging have been presented by Appendini and Hotchkiss (2002), Suppakul et al. (2003), Quintavalla and Vicini (2002), and Singh et al. (2016), and these reviews make reference to the use of antimicrobially active packaging materials in meat industry. A number of antimicrobials used in packaging are shown (Table 10.5).

Silver has been used since ancient times for reducing bacteria in drinking water and to make milk last longer. Nanoclays and zeolites containing silver ions and silver nanoparticles are currently used in packaging materials as additives, which can be added during film extrusion as a master batch or colloid solution. Different trade names for silver additives for packaging applications include AgION, Apacider, Bactekiller, Bactiblock, Biomaster, IonPure, IrgaGuard, Novaron, Surfacine, and Zeomic.

In terms of biodegradable materials, third-generation, packaging and coatings are now being investigated for high-volume production. Recently, different biodegradable coatings possessing antimicrobials were developed by Clarke et al. (2017) for prolonging the shelf life stability of fresh beef. These authors incorporated bitter orange extract or sodium octanoate into edible beef gelatin films coated onto a plasma-treated PE/PA laminate, and these coatings showed

TABLE 10.5 Antimicrobial compounds used in smart packaging applications.

Class	Example
Alcohol	Ethanol
Bacteriocins	Bavarcin, lacticin, nicin
Chelators	Citrate, EDTA, lactoferin, polyphosphate
Enzymes	Glucose oxidase, lactoperoxidase
Fatty acids	Lauric acid
Natural phenols	Catechin
Organic acids	Benzoic acid, citric acid
Organic acid salts	Potassium sorbate, sodium octanoate
Natural extracts	Bitter oranges extract
Polysaccharides	Chitosan, lysozyme
Metal oxides	ZnO, TiO, CuO
Silver-based compounds	$AgSiO_2$, Ag colloidal, Ag nanoparticles

EDTA, ethylenediaminetetraacetic acid.

a wide range of antimicrobial activity, thus prolonging vacuum-packaged beef shelf-life for greater than 14 days compared with control samples stored at 4°C. Transparent films made of pullulan, another edible material produced by the fungus *Aureobasidium pullulans*, and the addition of silver and zinc oxide nanoparticles, oregano, or REO were effective against pathogenic microorganisms, such as *Staphylococcus aureus*, *L. monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* in plate overlay assays (Morsy et al., 2014). Such films have a very good appearance and can be used as antimicrobial packaging for meat and poultry. Different reviews devoted to antimicrobial edible films and coatings for meat and meat product preservation have been presented by Cagri et al. (2004) and Sánchez-Ortega et al. (2014), among others. More examples of antimicrobials used in polymer matrices for different meat products are shown in Table 10.6 (Papadochristopoulos et al., 2021).

Antimicrobial packaging has been shown to increase the shelf life of meat products either by direct antimicrobial action or through modification of barrier film properties, thereby reducing oxygen penetration through packaging materials and subsequently, depriving microbes availability to the gas. The choice of appropriate antimicrobial packaging materials is dependent on legislative issues, local market demands, packaging materials and systems available, economic gain (which needs to be assessed across the entire distribution chain), and company marketing vision and philosophy for new products.

TABLE 10.6 Antimicrobial packaging with essential oil and plant extracts or their components for meat products (Papadochristopoulos et al., 2021).

Antimicrobial agent—Concentration applied	Food product	Packaging material	Microorganisms targeted	Indicative reduction (Log_{10} CFU/g, Log_{10} CFU/mL, Log_{10} CFU/cm^2)
Angelica Root—0.1% *v/w*	Pork	Polyethylene (PE) film	Total counts	2.34
			Brochothrix thermosphacta	1.49
			Carnobacterium spp.	1.04
			Enterobacteriaceae	1.95
			Staphylococcus sp.	1.17
			Pseudomonas sp.	0.89
			Enterococcus sp.	0.81
Chrysanthemum—1.5% *w/v*	Beef	Chitosan/nanofibers film	*Listeria monocytogenes*	1.2–1.5
Cinnamon—10% *w/w*	Pork	Polylactic acid (PLA)/b-cyclodextrin nanofilm	Total viable counts	4.5
5 mg/mL	Chicken	Sodium alginate (NaAlg) coating	Total viable counts	1.4
			Psychrotrophic bacteria	1
			Pseudomonas spp.	0.8
			Enterobacteriaceae	0.6
			Lactic acid bacteria	1.5
			Yeasts and molds	0.5
			L. monocytogenes	1

Continued

TABLE 10.6 Antimicrobial packaging with essential oil and plant extracts or their components for meat products (Papadochristopoulos et al., 2021).—cont'd

Antimicrobial agent—Concentration applied	Food product	Packaging material	Microorganisms targeted	Indicative reduction (Log_{10} CFU/g, Log_{10} CFU/mL, Log_{10} CFU/cm^2)
Clove—0.1% *v/w*	Pork	Polyethylene (PE) film	Total counts	2.49
			Brochothrix thermosphacta	2.17
			Carnobacterium spp.	1.62
			Enterobacteriaceae	2.37
			Staphylococcus sp.	1.73
			Pseudomonas sp.	1.34
			Enterococcus sp.	1.26
3%	Ground chicken meat	Carboxymethyl cellulose-polyvinyl alcohol (CMC-PVOH) film	Total viable counts	3
1, 2 mg/g	Beef	Chitosan-myristic acid nanogel coating	*Salmonella enterica* ser Enteritidis	0.3–0.8
0.5 g in 9×5 cm film	Ground chicken	Linear-low density polyethylene (LLDPE) film	*Salmonella typhimurium*	7
			L. monocytogenes	6
Coriander—0.5%, 1%	Chicken	Sodium alginate (NaAlg)-glycerol coating	Mesophilic bacteria	1–1.5
			Psychrotrophic bacteria	0.8–1.2
			Lactic acid bacteria	1.3–2.3
			Coliforms	1.4–2.4
			Staphylococcus aureus	1.2–2.1
			Molds and yeasts	1.1–1.5

Cumin—Black zira—0.5%, 1% *v/v*	Ground beef	Polylactic acid (PLA)-nanocellulose (NC) film	Total viable bacteria	0.7
			Lactic acid bacteria	0.1–0.2
			Enterobacteriaceae	0.5–0.8
			Psychrotrophic bacteria	0.2–0.3
			Staphylococcus aureus	0.6–1.2
			Pseudomonas spp.	0.1–0.3
0.5%, 1%, 2% *v/v*	Chicken	Chitosan coating	Mesophilic bacteria	0.45–0.59
			Psychrotrophic bacteria	0
			Lactic acid bacteria	0
			Enterobacteriaceae	0
			Yeasts and molds	0
Eucalyptus—0.5%, 1%, 2% *v/v*	Chicken	Chitosan coating	Mesophilic bacteria	0–0.69
			Psychrotrophic bacteria	1.11–1.41
			Lactic acid bacteria	0
			Enterobacteriaceae	0
			Yeasts and molds	0
Garlic—2%, 4%, 6%, 8% *w/w*	Ready-to-Eat (RTE) beef loaf	Low density polyethylene (LDPE) film	*L. monocytogenes*	0.2–0.5
			Escherichia coli	0–0.2
			Brochothrix thermosphacta	0–0.2
Horseradish, mustard—0.6, 1.2 μg/h release rate	Chicken	Glass vial inside high density polyethylene (HDPE) film	*Salmonella enterica* ser Typhimurium	1–1.2
			L. monocytogenes	0.1–0.6
			Total aerobic bacteria	0.5–1

Continued

TABLE 10.6 Antimicrobial packaging with essential oil and plant extracts or their components for meat products (Papadochristopoulos et al., 2021).—cont'd

Antimicrobial agent—Concentration applied	Food product	Packaging material	Microorganisms targeted	Indicative reduction (Log_{10} CFU/g, Log_{10} CFU/mL, Log_{10} CFU/cm^2)
20%, 28%, 40%, 52%, 58% *v/w*	Cooked chicken	Cellulose film with carbon nanotube (CNT)	*Salmonella* choleraesuis	2.5–8
			Psychrotrophic bacteria	1.5–8
			Mesophilic bacteria	0.7–8
Lemongrass—2%	Pork sausage	Polylactic acid (PLA) film	*L. monocytogenes*	1.47
Nutmeg—0.5%, 1%, 1.5%, 2%	Beef	Sage seed mucilage coating	Total viable count	3–8
			Psychrotrophic bacteria	1.8–5.5
			Escherichia coli	0.5–2
			Staphylococcus aureus	1–3
			Yeasts and molds	1–5
Oregano—1%, 2%, 3% *v/v*	Beef	Soy protein coating	*Escherichia coli* O157:H7	1.2–1.83
			L. monocytogenes	0.9–1.9
			Staphylococcus aureus	1.8–2.86
5% *v/v*	Ground beef patties	Soy protein film	Total viable count	0
			Lactic acid bacteria	0
			Staphylococcus spp.	0
			Pseudomonas spp.	0.6
			Coliforms	0.5

Pepper—2% *w/w*	Chicken	Carboxymethyl cellulose (CMC) coating	Mesophilic bacteria	1.5–2.2
			Psychrotrophic bacteria	1.5–2
Peppermint—0.5%, 1% *v/v*	Ground beef	Polylactic acid (PLA)- nanocellulose (NC) film	Total viable bacteria	0.7
			Lactic acid bacteria	0–0.4
			Enterobacteriaceae	0.6–0.8
			Psychrotrophic bacteria	0.4
			Staphylococcus aureus	0.6–0.8
			Pseudomonas spp.	0.1–0.4
Rosemary—4% *w/w*	Chicken	3 layers film (paper-metallic layer- high density polyethylene (HDPE))	Mesophilic bacteria	0.3
			Enterobacteriaceae	0
			Pseudomonas spp.	0.24
			Brochothrix thermosphacta	0.37
5 mg/mL	Chicken	Sodium alginate (NaAlg) coating	Total viable counts	1.6
			Psychrotrophic bacteria	1.6
			Pseudomonas spp.	1.3
			Enterobacteriaceae	1.6
			Lactic acid bacteria	1.7
			Yeasts and molds	0.8
			L. monocytogenes	1.5

Continued

TABLE 10.6 Antimicrobial packaging with essential oil and plant extracts or their components for meat products (Papadochristopoulos et al., 2021).—cont'd

Antimicrobial agent—Concentration applied	Food product	Packaging material	Microorganisms targeted	Indicative reduction (Log_{10} CFU/g, Log_{10} CFU/mL, Log_{10} CFU/cm^2)
0.1% *v/w*	Pork	Polyethylene (PE) film	Total counts	2.53
			Brochothrix thermosphacta	1.95
			Carnobacterium spp.	1.5
			Enterobacteriaceae	2.48
			Staphylococcus sp.	1.39
			Pseudomonas sp.	1.08
			Enterococcus sp.	0.98
4% *w/w*	Beef	3 layers film (paper-metallic layer- high density polyethylene (HDPE))	Psychrotrophic bacteria	0–0.7
			Brochothrix thermosphacta	0–1.5
			Pseudomonas spp.	0–0.8
			Enterobacteriaceae	0–0.8
Thyme—1%, 2%, 3% *v/v*	Beef	Soy protein coating	*Escherichia coli* O157:H7	1.2–1.8
			L. monocytogenes	1–1.97
			Staphylococcus aureus	1.6–2.6

5% *v/v*	Ground beef patties	Soy protein film	Total viable count	0
			Lactic acid bacteria	0
			Staphylococcus spp.	0.2
			Pseudomonas spp.	0.9
			Coliforms	0.6
1%	Sausage	Chitosan coating	Yeasts and molds	0.6–1.5
Turmeric—2% *w/w*	Chicken	Carboxymethyl cellulose (CMC) coating	Mesophilic bacteria	2.2–3
			Psychrotrophic bacteria	3.5

10.5 Smart and intelligent packaging systems

As discussed in Section 10.1, many packaging formats and materials are available for commercial meat production. Utilization of different injected gas mixtures or vacuum maintains necessary internal package environments for better food storage once barrier materials deliver adequate containment and consequently, preservation. To enhance shelf life stability of products, antimicrobial systems can be used additionally to barrier materials to further delay microbial growth via the slow release of a migrative substance onto the meat surface.

Product shelf life is dependent upon the microbial and chemical stability of packaged foods, and the primary processing strategy in preservation terms for meat products is the appropriate application of packaging and chilling systems. However, challenges posed through the movement of meat packages through the chill-chain test the consistency of temperature control and the integrity and physical performance of packaging materials. This is particularly so when distribution chill-chains become longer and more complex owing to greater distances between markets. Consequently, special additional quality controls measures are required, and these must operate within the chill environment and be supported by the conventional packaging systems and materials used. Such quality control measures involve using smart packaging materials, which have broadly fallen into two technological categories, namely active packaging and intelligent packaging. According to Kerry and Butler (2008) smart packaging "encompasses aspects of packaging design and the incorporation of mechanical, chemical, electrical and electronic forces, or combination of these, within the package."

Intelligent packaging according to Robertson (2006) is the packaging that includes an indicator that can be external or internal and which provides information about the quality of the food (Robertson, 2006; Kerry and Butler, 2008). Intelligent packaging systems can be applied in meat packages, which indicate the degree of spoilage that has taken place in a packaged product, can indicate if there was temperature abuse during delivery, can show freshness integrity, can indicate the presence of various gases, etc. Active packaging systems can be applied to meat packs in an attempt to modify conditions within the pack, thereby influencing product shelf life through direct microbial or chemical control, gas levels in packs, controlling temperature, etc., thereby doing more than simply indicating food pack issues.

There are different smart systems available on the market appropriate for fresh meat packaging utilization (Ozdemir and Floros, 2004; Hogan and Kerry, 2008; Dobrucka and Cierpiszewski, 2014). One of the simplest smart systems is sachet as for antimicrobials. Also sachets can also include gas-absorbing (scavengers) compounds, zeolites, for example, which absorbs off order or excess of oxygen inside packaging. At the same time, there are gas-emitting systems (CO_2 emitters) that can be applied for each MAP for very slow transmission of CO_2 molecules through barrier material by concentration gradient to the atmosphere (Arvanitoyannis, 2012; Realini and Marcos, 2014). Such sachets can be placed directly into packaging or be a part of the packaging material. Some com-

FIG. 10.18 Gas scavengers variety.

pounds as for antimicrobials systems can be incorporated in packaging material and can be activated by UV irradiation, for example, so-called smart or stimuli-responsive materials (more about responsive materials for food packaging can be found in Brockgreitens and Abbas, 2016). In Fig. 10.18, scavengers examples are represented . Such systems have been traditionally used as a sachet. Several commercial gas regulating smart packaging systems are represented in Table 10.7.

Not only O_2 and CO_2 gas absorbers exist on the market. For products with high-water-content moisture absorbers can prolong shelf life by absorbing the excess of water. Sirant Ltd. (United Kingdom) developed Dri-Fresh moisture absorbing pads for fresh meat, fish, and poultry. MacAirlaid Inc. produces MeatGuard absorbent pads having superabsorbent fibers. There are several examples of multiple functions absorbers and emitters represented in Table 10.8.

Intelligent packaging systems are more complex than active systems and include different types of chemical and electrosensors for the indication of temperature jumps, freshness by change of color of the sensor, and can include radio tags or QR codes for interaction with the smart phones or scanners. Intelligent packaging systems do not adapt or modify atmosphere inside pack, but indicate about changes of different parameters including gases, off order, temperature, and other parameters which are necessary to control (Rooney, 1995; Fitzgerald et al., 2001; Dobrucka and Cierpiszewski, 2014; Smiddy et al., 2001; Liu et al., 2015; Kelly et al., 2018).

It also allows packaging to communicate with different devices and even whole store signaling to the server about changes in the packaging, thus adding an additional layer of a quality control and thus reducing food-borne disease cases. Especially such indicators are useful for a long supply chain. The market of such indicators increases each year (Ghaani et al., 2016; Mohebi and Marquez, 2015; Biji et al., 2015).

TABLE 10.7 Commercial gas regulating smart packaging systems used for packaging of muscle based food products.

Type	Company	Commercial name	Function/form	Product examples
O_2 Absorber	Mitsubishi Gas Chemical Co., Japan	Ageless type ZP and ZPT	Iron powder oxidation	Dried meats, beef jerky, processed meats, fish, and seafood
		Ageless type SS	Fast reacting type, self-reacting	
		Ageless type GLS	Organic noniron type suitable for metal detectors	
		Ageless type FX-L	Water dependent (initiated) iron oxidation	
		Ageless OMAC	Iron-based film for aseptic and retort processing applications	
	Standa Industrues, France Distr. by Emco Packaging Systems, United Kingdom	ATCO	Iron powder oxidation, iron-based labels with	Meat, fish, poultry and seafood products
	Toppan Printing Co., Japan	Freshlizer	Iron powder oxidation sachet ascorbic acid oxidation	
	Chevron Phillips	OSP	Multilayer polymer with ethylene methyl acrylate, cyclohexane methyl acrylate	Wet and dry food
	Multisorb Technologies Inc., United States	FreshPax	Iron powder oxidation sachets	Sliced meats, smoked and cured meats
		FreshCard	Multifunctional O_2 absorbing cards	
		JerkyFresh	O_2 absorbing packets and strips	
		Fresh Max	O_2 absorbing adhesive labels	
	Desiccare, Inc., United States	O-BUSTER (imported from Taiwan)	Iron powder oxidation by absorbing packets and strips	Processed and dried meats
		IRON FREE	Noniron type	Processed and dried meats
	Pillsburry Co., United States	OxySorb	Multilayer polymer film	

	Sealed Air Corp. (Cryovac Div.), United States	Cryovac OS 2000	Multilayer polymer activated by ionizing radiation	Dried or smoked meat products, processed meats
	Plastipak Packaging Inc.	Diamond Clear	PET resins for containers and bottles	Tea, tomato products, vitamin enhanced products
	Albis Plastic, Germany	Shelfplus O_2, Shelfplus O_2 3200	Oxygen absorbing film and high barrier film	
	Clairant International, Switzerland	OXYGUARD	Sachets	Processed meat
	Amelco Dessicants Inc.	H Type, S Type, P Type	Sachets	Dried fruits and nuts, bakery products, snacks
	GCP Applied technologies	CELOX 210, 210B, 210W, 300, 2002, 2003	Oxygen scavenging closure sealant and masterbatch, non-PVC scavengers with polyolefins	PET and bottle applications
	Nutricepts, Inc.	OxyVac	Enzyme based oxygen scavenger	MAP foods with $a_w > 0.65$ and cheese products
	Drypak	DryPak Oxygen Absrober	Oxygen absorbing sachets	Dry and solid foods
	OhE Chemicals, Inc., Japan	TOMATSU	Active carbon and oxygen absorbing component contained in green tea. Suitable for metal detectors	Dried laver, sardines, ham, confectionery
		SEQUL	Iron-based scavenger	Confectioneries, dried fish
		CRISPER HF	Ethylene absorbing sachets	Apples, Japanese pears, persimmons, kiwi, broccoli, melon
CO_2 Absorbers	Standa Industrues, France and Emco Packaging Systems, United Kingdom	ATCO	Carbon dioxide absorber bags	Products with CO sensitivity

Based on Boz, Z., Welt, A.B., Brecht, K.J., Pelletier, W., McLamore, E., 2018. Review of challenges and advances in modification of food package headspace gases. J. Appl. Pack. Rese. 10 (1), 62–97.

TABLE 10.8 Commercial multifunction gas absorbers and emitters.

Freud Corporation, Japan	Negamold	Moisture dependent and self-reaction type oxygen absorbers, controlling the yeast and *Bacillus subtilis* by ethanol vapor	
Emco Packaging Systems, United Kingdom	OxyFresh	O_2 emitting with CO_2 scavenging technology	Superatmospheric O_2 packaging of fresh produce
Nutricepts, Inc.	OxyVac-S	Enzyme based O_2 scavenger and CO_2 emitter	Fresh whole, divided cooked muscle foods
Mitsubishi Gas Chemical Co., Japan	Ageless type GT	O_2 absorbing and CO_2 emitting sachets	Solution for packaging shrinking in several products e.g., fresh and processed meat, poultry, fish and fresh produce

Based on Boz, Z., Welt, A.B., Brecht, K.J., Pelletier, W., McLamore, E., 2018. Review of challenges and advances in modification of food package headspace gases. J. Appl. Pack. Rese. 10 (1), 62–97.

One of examples of the integrated freshness indicator is represented in Fig. 10.19 developed by Insignia Technologies.

Such sensing film is applicable in MAP packaging by gluing on the inner surface of the lidding film. After flushing of CO_2 during packaging process, the label becomes yellow and if CO_2 concertation reduces during time the label changes its color. SensorQ (Food Quality Sensor International Inc., United States) (Fig. 10.20) also presented in the form of a label shows the progress of the meat spoilage by changing its color from orange yellow to green.

Impak corporation developed Tell-Tab, which can be added inside MAP or vacuum packs and shows a tiny oxygen concentration changes up to 0.1% by changing color of the balls from pink to a blue (Fig. 10.21).

The presence of pathogenic bacteria can be observed not only by changing of oxygen or CO_2, but also directly as in medical research by the reaction based on antibody-antigen. Toxin GuardTM (Toxin Alert, Ontario, Canada) developed a visual sensor integrated into polymer packaging film, which can indicate the presence of pathogenic bacteria. Interesting technology was developed by SIRA Technologies, a freshness sensor in the form of a bar-code label called Food Sentinel Systems to detect pathogens in meat packaging. The same reaction of antigen-antibody when exposed to contaminants such as *Salmonella* spp., *Escherichia coli* 0157:H7, or *L. monocytogenes* covers necessary lines for the barcode making it unscannable on a retailer site on a till indicating that product was spoiled and thus cannot be sold (Fig. 10.22).

FIG. 10.19 Insignia Technologies freshness label.

FIG. 10.20 SensorQ sensing label.

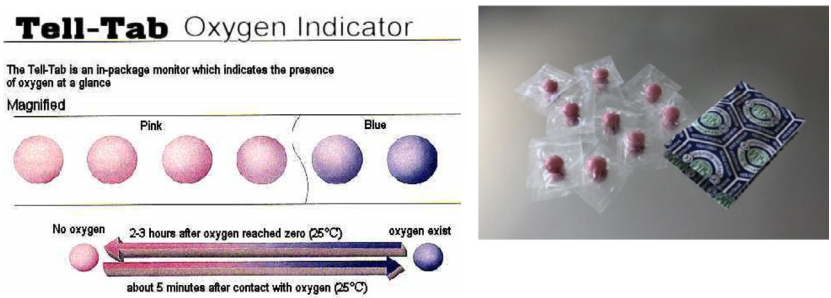

FIG. 10.21 Oxygen indicator from Impack. Color changing depending on oxygen is shown, and the tablets are on the right.

FIG. 10.22 Food Sentinel Systems barcode (Sira). (A) Ready to use; (B) product expired and code is unscannable.

There are more complex systems appearing on the market where not only chemical, but electrical sensing systems are applied and connected to a radio antenna, which allows communication of an intelligent label with a smart phone by NFC (Fig. 10.23). Such sensors can include electrical moisture or gas sensor with even small display where different packaging parameters can be indicated.

Innoscentia AB, a Swedish developer of dynamic sensor labels, developed a real-time quality indicator, which can show not only product freshness, but also communicate with the smartphone (Fig. 10.24).

As it is known, meat microflora quickly grow upon temperature increase. During transportation, storage temperature can change especially when there is a hot weather outside a van's freezer. When products are delivered, they should be promptly placed on the products shelf equipped with a chiller. During van unloading, temperature can jump up due to outside temperature difference causing microflora growth reducing product shelf life. This change should be detected and controlled.

Time Temperature Indicators (TTI) can help with this issue indicating temperature jumps by color changes of unreversible thermochromic printed inks printed on the label or by indicating real temperature on a small display integrated into the packaging or a box. It is also possible to transfer temperature line data from the sensor by NFC to a smartphone. The OnVu label has a blue color print in the center, which changes color to gray if the storage temperature was no appropriate (Fig. 10.25).

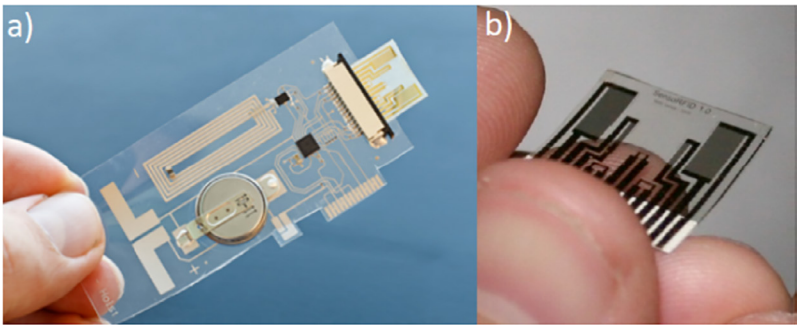

FIG. 10.23 (A) Photograph of an "Intelligent Label" with the battery; (B) Photograph of sensors, which can be integrated in any packaging (Smits et al., 2012).

FIG. 10.24 Innoscentia AB freshness label.

FIG. 10.25 OnVu label on MAP packaging with chicken breast.

Thermochromic inks also can be printed on the packaging in any form with different color shifts depending on the necessary temperature indication. The 3M company (United States) developed MonitorMark TTI, which can show continuous temperature changes during 5 weeks (Fig. 10.26). Another indicator was developed by American Thermal Instruments, (United States) indicating temperature in small periods of time (Fig. 10.27). The display can indicate shipment status and gives a clear, readable notification detailing whether the shipment is okay or if a temperature excursion has been reached. It shows time and

FIG. 10.26 MonitorMark TTI from 3M company.

FIG. 10.27 TTI from American Thermal LLC. *www.americanthermal.com*.

surrounding temperature (accuracy tolerance ±0.5C), visual signal Go/No Go on LCD Screen. The indicator can easyly be adhered to any lagre packaging or a cointainer and can be activated at point from start of meat products delivery (American Thermal Instruments).

There are other different examples of TTIs available on the market represented in Table 10.9. Wang et al. (2015) represented different systems for TTI including chemical reactions.

The company ThinFilms (Norway) represented electrical-based TTI (Fig. 10.28). Such TTI was printed on the polymer film substrate by conductive inks with the laminated display indicating real temperature. Such sensor can be integrated in any packaging. Especially it is required for large boxes with smaller packs inside. The Blulog company developed NFC data logger, which gathers temperature data during time and can signal about temperature jump by a diode (https://venturebeat.com).

Internet of things has recently started coverage of all things we communicate with. Food packaging is not an exception. Intelligent packaging systems integrated with NFC or RFID tags can communicate via radio signal with the server signaling about outdated products and different parameters changes inside packaging. EVRYTHNG (United States) company developed a cloud service for a retail site for real-time data of management interface Fig. 10.29.

TABLE 10.9 Different market available TTIs.

Principle	Category	Storage	Activation	Potential drawbacks	Commercial cases
Polymerization-based TTI	Chemical	LT	RT	T C	HEATmarker/ Fresh-Check
Photochromic-based TTI	Chemical	RT	Light	I	OnVu
Redox reaction-based	Chemical	Oxygen free	Oxygen	I	–
Diffusion-based TTI	Physical	LT	M	I C	Monitor Mark/Tempix
Nanoparticle-based TTI	Physical	LT	M	T C	–
Acid-base reaction-based TTI	Enzymatic	LT	M	I	CheckPoint

LT, low temperature; *RT*, room temperature; *M*, mixture; *T*, toxicity; *I*, inaccuracy; *C*, cost.
Data from (Wang et al., 2015).

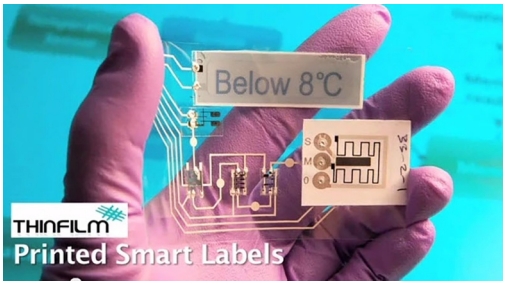

FIG. 10.28 SmartLabel from ThinFilms (Norway); integrated screen shows temperature. *https://venturebeat.com/2012/12/19/thin-film-electronics-demonstrates-a-way-to-make-disposable-interactive-smart-labels-that-replace-bar-codes/*.

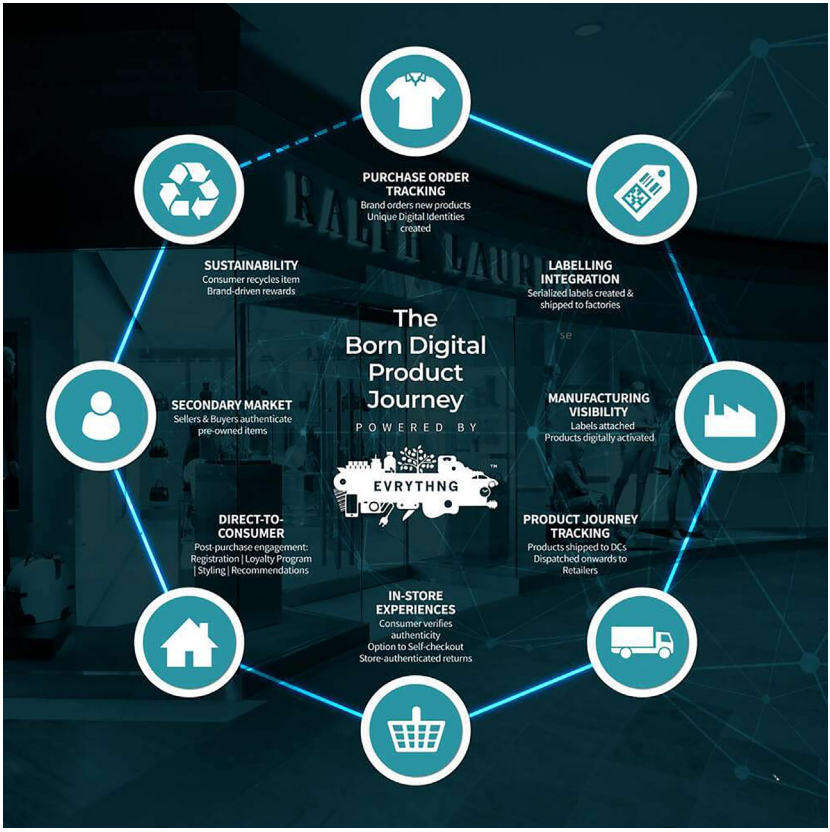

FIG. 10.29 Elisa company cloud service scheme. Gathering information from a product on a store front and analyzing the information for product management. *https://evrythng.com*.

This is the developing area in food packaging interactive systems, and there are several other services available on the market for the Internet of things connected to packaging.

10.6 Conclusions

Numerous techniques that have been used since ancient times for meat preservation, and which still persist today in modified formats, have been augmented by the development of many new techniques, which assist in prolonging the shelf life of meat products from enhanced chilling systems to advanced ionizing radiation and plasma treatment. Over a shorter timeframe, similar developments have occurred for packaging technologies from chemical modifications of basic food packaging polymers and laminate constructions to nanotechnological applications and the application of sensing technologies and the Internet of Things in meat packaging for prolonged product storage, visual quality control using a wider range of modified packaging systems. The availability of all of these technologies has created many opportunities for meat companies to develop new markets, which heretofore would have been seen to be challenging owing to greater logistical demands in transporting and storage of meat, issues of time and product shelf life, climatic and general environmental issues presented by market region, and operating cold chain, how and where the meat products were to be sold, inherent market values, and linked product price category.

From a European perspective, the development of the meat industry is greatly hampered in terms of employing many new technological developments in the area of preservation, particularly with respect to new packaging materials and systems. Until the meat industry, like many other segments of the food industry, receives clear guidelines on the use of smart packaging, materials and systems from EU legislators and furthermore are encouraged to become more innovative through the uptake of smart packaging technologies developed within the EU, then the meat industry will not be in a position to take full advantage of new market opportunities outside of the EU, many of which are more receptive to these technologies than the European market.

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http://www.nanocor.com/tech_papers/antec-nanocor-tie%20lan-5-07.pdf.
http://www.nippon-gohsei.com/soarnol.
http://www.torayfilms.eu/toray-article/nouveaux-films-pet-haute-barriere-alox/?lang=en.
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https://www.astm.org/Standards/D3985.htm.
https://www.astm.org/Standards/F1249.htm.

Chapter 11

The eating quality of meat: I Color

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11.1 Introduction

Sensory aspects of a food are important for evaluating its suitability for consumption, and in particular, the appearance of meat is especially critical to the consumer's perception of quality. Slight differences in color that deviate from the consumer-defined ideal can lead to substantial losses in retail value or premature disposal of a valuable and safe nutrient source, which contributes to food waste (Maia Research Analysis, 2020). Commercial production of plant-based meat analogs have recognized the criticality of color to the perceived quality of meat, and one company has developed an analog product that contains the red heme protein, soy leghemoglobin, from bioengineered yeast (Watson, 2019).

Meat's appearance is a function of physical (Purslow et al., 2020) and chemical (Ramanathan et al., 2020a) factors. For example, the amount of moisture on a meat surface will influence the degree to which light is reflected and the gloss and/or lightness perceived by an observer. The protein myoglobin is the heme-containing macromolecule that is substantively responsible for meat color, and its chemistry is the focus of this chapter. Hemoglobin and cytochromes are related heme proteins that can also contribute to pigmentation, but their concentrations in meat are generally negligible, and we do not consider them significant to this overview of meat color. Cell-based meat has attracted significant interest (Faustman et al., 2020), and heme proteins have been recognized for their ability to provide appropriate color, and for myoglobin, specifically, to enhance the proliferation and metabolic activity of satellite cells (Simsa et al., 2019).

In its simplest presentation, there are two critical properties of myoglobin that influence its impact on meat color—concentration and its redox stability. The remaining portion of this chapter focuses on the variety of environmental and physiological factors, both antemortem and postmortem, that affect

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myoglobin concentration and redox stability, and how these relate to the perception of meat color.

11.2 Myoglobin concentration

Myofibers form the basic structural unit of muscle; they are multinucleated and packed with contractile (i.e., myofibrillar) proteins. These proteins require energy for contraction, and this energy (i.e., ATP) can be generated by aerobic and/or anaerobic means. Muscles located within animals each have their own specific function and differ markedly relative to the energy metabolism of their constituent myofibers. Fast-twitch myofibers rely primarily on glycolysis to generate ATP, while energy for slow-twitch myofibers is obtained through aerobic means. Myoglobin is an iron-containing protein in the muscle that functions to store and deliver oxygen required for aerobic generation of energy. Thus, muscles that are composed of a majority of slow-twitch myofibers contain greater concentrations of myoglobin and are often referred to as "red" muscles (i.e., dark meat). Alternatively, "white" muscles (i.e., white meat) contain more fast-twitch fibers and much less myoglobin.

The concentration of myoglobin in postmortem muscle reflects the muscle's need for oxygen storage/delivery in vivo, a function of selection pressures, and fundamental biology of the animal. As such, myoglobin concentration will vary based on muscle type, species, animal age, diet, and/or environmental challenges (Table 11.1). For example, the blue whale must store significant quantities of oxygen for its underwater pursuit of food. Myoglobin concentration is much greater in the muscle of this marine mammal than in terrestrial animals. Wild birds generally contain greater concentrations of myoglobin in their flight muscles than domesticated meat birds; the latter generally having been selected for a more sedentary existence (Pages and Planas, 1983; Table 11.1). Pigs are considered to provide more white meat and beef cattle more red meat, and relative to each other, their muscle myoglobin concentrations are lesser and greater, respectively (Lawrie, 1966). Differences have also been reported for the same muscle among different pig breeds (Newcom et al., 2004). The concentration of myoglobin in the muscle also increases with animal age (Kagen and Linder, 1968; Nishida and Nishida, 1985; Table 11.1), and sausage makers may include meat from older animals for the purpose of increasing pigmentation.

The concentration of myoglobin in different muscles within the same animal carcass can vary dramatically (Table 11.1) and is influenced by both genetics and environment (Cross et al., 2018). White chicken breast meat contains little to no myoglobin, while leg meat is relatively rich in the heme protein (Nishida and Nishida, 1985; Table 11.1). McKenna et al. (2005) reported the myoglobin concentrations for 19 different muscles in beef; values for four of the most commercially significant ones are presented in Table 11.1. Additionally, for a particular muscle within a species, the concentration of myoglobin is influenced by animal genetics. For instance, myoglobin content in beef *longissimus thoracis*

TABLE 11.1 Myoglobin concentrations in muscles of different animals.

Reference	Species	Age	Muscle	Conc
Lawrie (1966)	Rabbit	n.d.	n.d.	0.20%
	Sheep	n.d.	n.d.	0.25%
	Pig	n.d.	n.d.	0.06%
	Ox	n.d.	n.d.	0.50%
	Blue Whale	n.d.	n.d.	0.91%
Yu et al. (2017)	Pig	98 days	Longissimus dorsi	0.19 mg/g
		161 days	Longissimus dorsi	0.28 mg/g
Viana et al. (2017)	Chicken	n.d. (market age; not specific about the days)	Pectoralis major (organic)	0.96 mg/g
			Pectoralis major (nonorganic)	0.79 mg/g
Pages and Planas (1983)	Domestic chicken	n.d.	Pectoralis	1.0 mg/g
	Seagull	n.d.	Pectoralis	5.5 mg/g
Kagen and Linder (1968)	Duck	2-day posthatch	Pectoral	0.3 mg/g
		8-day posthatch	Pectoral	0.2 mg/g
		Adult	Pectoral	2.2 mg/g
Nishida and Nishida (1985)	Chicken	0 day (hatch)	Leg muscle	2.7 mg/100 g
		4 days	Leg muscle	4.0 mg/100 g
		6 weeks	Leg muscle	32.7 mg/100 g
		12 weeks	Leg muscle	86.1 mg/100 g
		24 weeks	Leg muscle	142.0 mg/100 g
Nishida and Nishida (1985)	Domestic chicken	n.d.	Breast Pectoralis profundus	0 mg/100 g
			Leg Semimembranosus	65.5 mg/100 g
McKenna et al. (2005)	Beef cattle	n.d.	Semitendinosus	3.60[a] mg/g
			Psoas major	4.10[b] mg/g
			Longissimus lumborum	4.62[c] mg/g
			Biceps femoris	5.41[d] mg/g
McKeith et al. (2016)	Beef cattle	n.d.	Longissimus lumborum	4.36 mg/g

[a, b, c, d] *Values with different superscripts are different (P < .05). n.d., not described.*

is heavily influenced by breed (King et al., 2010), with Limousin demonstrating lesser myoglobin concentrations than Angus and Simmental breeds.

The diet that animals are fed can also influence the muscle concentration of myoglobin, and this is most readily observed in veal meat. Husbandry practices associated with production of traditional "white" or milk-fed veal have involved the restriction of iron in the diet of calves up through approximately 4 months of age. Animals that receive greater concentrations of iron, a critical molecular component of myoglobin, accumulate greater amounts of the protein pigment in their muscle. MacDougall et al. (1973) studied the effect of dietary iron (basal, 10 μg Fe/g dry matter feed; 40 μg Fe/g dry matter feed; 100 μg Fe/g dry matter feed) on myoglobin concentration of several commercially valuable muscles. A dose-dependent positive correlation between dietary iron and muscle myoglobin concentration was clearly demonstrated. In some markets, there have been attempts to market "red" veal derived from young bovine animals that were not fed an iron-restricted diet (Faustman et al., 1992). Agboola et al. (1988) reported that inclusion of monosodium phosphate and α-tocopherol in milk-replacer diets of veal calves led to lesser myoglobin concentrations (1.53 mg/g) than for control animals (1.86 mg/g).

11.3 Myoglobin structure

Myoglobin has two major components to its structure, an apoprotein and heme. Similar to other proteins, myoglobin's apoprotein is defined by primary, secondary, and tertiary structure. Myoglobins of meat-producing animals (livestock and poultry) typically contain 153 amino acids (i.e., primary structure), and the ordered sequence of these is highly conserved among different species (Fig. 11.1). Myoglobin is also characterized by substantial α-helical secondary structure, which confers relatively significant stability to the protein. The folding of primary and secondary structures in myoglobin results in a globular tertiary (three-dimensional) structure. The tertiary structure of myoglobin can be disrupted by the application of physicochemical forces including heat, acid, or high pressure, and this leads to denaturation of the protein and color change. This is most typically observed in heat-induced denaturation of myoglobin, which occurs during cooking. Myoglobin does not denature abruptly at a single temperature but rather loses its tertiary structure over a narrow temperature range defined by the meat environment in which it is located. Consumers routinely use this color change to assess cooking doneness from a rare (red internal color) to well-done (brown/gray appearance).

The heme portion of myoglobin is responsible for the protein's red color in meat/muscle. In the living animal, the heme group binds oxygen and facilitates myoglobin's role in storing and transporting oxygen in muscle. In the postmortem condition, myoglobin continues to bind oxygen because heme chemistry remains active. Heme is an iron-containing protoporphyrin ring (Fig. 11.2) and located within the tertiary structure of myoglobin (Fig. 11.3). Iron may exist

```
                    Position
Species             1      10         20         30         40         50
Sperm whale         VLSEGEWQLV LHVWAKVEAD VAGHGQDILI RLFKSHPETL EKFDRFKHLK
African elephant    GLSDGEWELV LKTWGKVEAD IPGHGEFVLV RLFTGHPETL EKFDKFKHLK
Horse               GLSDGEWQQV LNVWGKVEAD IAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Beef                GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Water buffalo       GLSDGEWQLV LNAWGKVETD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Sheep               GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Goat                GLSDGEWTLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Red deer            GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
White-tailed deer   GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Pig                 GLSDGEWQLV LNVWGKVEAD VAGHGQEVLI RLFKGHPETL EKFDKFKHLK
Rabbit              GLSDAEWQLV LNVWGKVEAD LAGHGQEVLI RLFHTHPETL EKFDKFKHLK
Chicken             GLSDQEWQQV LTIWGKVEAD IAGHGHEVLM RLFHDHPETL DRFDKFKGLK
Emu                 GLSDQEWQHV LTIWGKVESD LAGHGHEILM RLFHDLPETL DRFERFKGLT
Ostrich             GLSDQEWQQV LTIWGKVESD IAGHGHAILM RLFQDHPETL DRFEKFKGLT
Barn owl            GLSDQEWQQV LTIWGKVESD LPGHGHAVLI RLFQDHPETL DRFEKFKGLK
Mallard duck        GLSDQEWQQV LTIWGKVEAD LAGHGHAVLM RLFQDHPETL DRFEKFKGLK
Emperor penguin     GLNDQEWQQV LTMWGKVESD LAGHGHAVLM RLFQDHPETM DRFDKFRGLK
                        *  **   **     *  *    **  *    ***     ** *   *

                    Position
Species             51     60         70         80         90         100
Sperm whale         TEAEMKASED LKKHGVTVLT ALGAILKKKG HHEAELKPLA QSHATKHKIP
African elephant    TEGEMKASED LKKQGVTVLT ALGGILKKKG HHEAEIQPLA QSHATKHKIP
Horse               TEAEMKASED LKKHGTVVLT ALGGILKKKG HHEAELKPLA QSHATKHKIP
Beef                TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Water buffalo       TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Sheep               TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Goat                TGAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Red deer            TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
White-tailed deer   TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Pig                 SEDEMKASED LKKHGNTVLT ALGGILKKKG HHEAELTPLA QSHATKHKIP
Rabbit              SEDEMKASED LKKHGNTVLT ALGAILKKKG HHEAEIKPLA QSHATKHKIP
Chicken             TPDQMKGSED LKKHGATVLT QLGKILKQKG NHESELKPLA QTHATKHKIP
Emu                 TPDQMKASEE LKKHGVTVLT QLGKILKLKG KHEAELKPLA QTHATKHKIP
Ostrich             TPDQMKASED LKKHGVTVLT QLGKILKQKG KHEAELKPLA QTHATKHKIP
Barn owl            TPDQMKGSED LKKHGVTVLT QLGKILKQKG NHESELKPLA QTHATKHKIP
Mallard duck        TPDQMKGSED LKKHGVTVLT QLGKILKQKG NHEAELKPLA QTHATKHKIP
Emperor penguin     TPDQMRGSED MKKHGVTVLT QLGKILKQKG HHESELKPLA QTHATKHRVP
                     ***            *     *  *   *   *    * *   **  *

                    Position
Species             101     110        120        130        140        150
Sperm whale         IKYLEFISEA IIHVLHSRHP GDFGADAQGA MNKALELFRK DIAAKYKELG YQG
African elephant    IKYLEFISDA IIHVLQSKHP AEFGADAQAA MKKALELFRN DIAAKYKELG FQG
Horse               IKYLEFISDA IIHVLHSKHP GDFGADAQGA MTKALELFRN DIAAKYKELG FQG
Beef                VKYLEFISDA IIHVLHAKHP SDFGADAQAA MSKALELFRN DMAAQYKVLG FHG
Water buffalo       VKYLEFISDA IIHVLHDKHP SDFGADAQAA MSKALELFRN EMAAQYKVLG FHG
Sheep               VKYLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFRN DMAAQYKVLG FQG
Goat                VKYLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFRN DMAAQYKVLG FQG
Red deer            VKYLEFISDA IIHVLHAKHP SNFGADAQGA MSKALELFRN DMAAQYKVLG FQG
White-tailed deer   VKYLEFISDA IIHVLHAKHP SNFGADAQGA MSKALELFRN DMAAQYKVLG FQG
Pig                 VKYLEFISEA IIQVLQSKHP GDFGADAQGA MSKALELFRN DMAAKYKELG FQG
Rabbit              VKYLEFISEA IIHVLHSKHP GDFGADAQAA MSKALELFRN DIAAQYKELG FQG
Chicken             VKYLEFISEV IIKVIAEKHA ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Emu                 VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Ostrich             VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Barn owl            VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Mallard duck        VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Emperor penguin     VKYLEFICEA IMKVIAEKHS ADFGANCQAA MKKALELFRH DMASRYKEFG FQG
                            **   * ***  * *     * *   *            **  **   *
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FIG. 11.1 Primary structure of myoglobin from different species. *Indicates the residues that differ between avian and mammalian myoglobins.

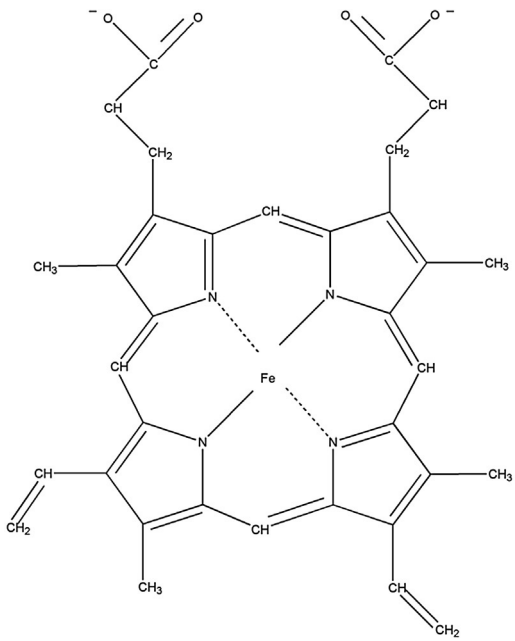

FIG. 11.2 Planar view of the heme group of myoglobin. The iron can be in a ferrous (Fe^{2+}) or ferric (Fe^{3+}) form. *(Reproduced from Suman, S.P., Joseph, P., 2014. Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, second ed., vol. 3. Elsevier, Oxford, pp. 244e251, (Chapter 84), with permission of Elsevier Limited, Oxford, United Kingdom.)*

in a reduced ferrous, 2+, or oxidized, ferric, 3+ state. Myoglobin is capable of binding oxygen only in the ferrous state; changes between these two redox states are critically important because they are accompanied by color changes in fresh meat.

Heme's functionality is determined by the redox chemistry of its iron atom. Heme iron has six coordination sites that permit its interaction within its surrounding environment. Four of these six secure iron within the planar structure of heme via protoporphyrin nitrogen atoms (Fig. 11.2). A fifth coordination site anchors the heme molecule, through noncovalent means, to the apoprotein. The sixth coordination site is available to bind a ligand; in meat these can include oxygen, water, nitric oxide, or carbon monoxide. The type of ligand that is ultimately bound depends on the redox state of the heme iron and the relative abundance of a given ligand in the vicinity of the myoglobin molecule. For example, in meat, myoglobin's heme iron can bind oxygen only in a ferrous state; oxidation of heme iron to the ferric state will result in the binding of water. Some ligands bind more tightly than others, and this is true for carbon monoxide, which binds more tightly than oxygen.

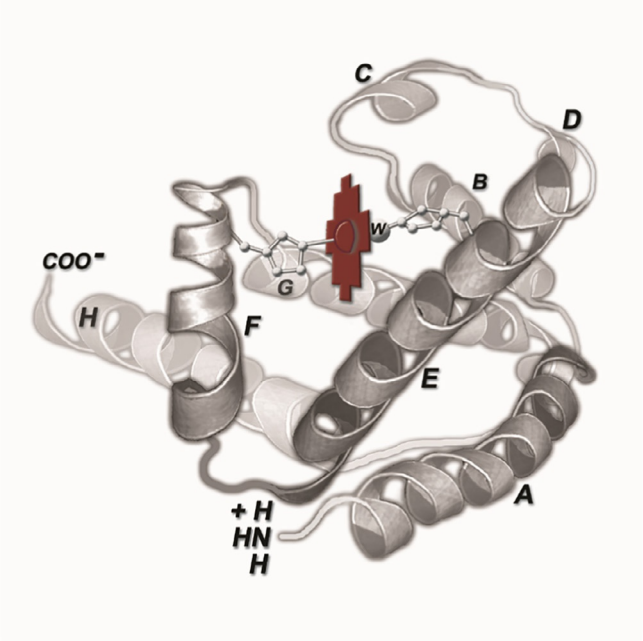

FIG. 11.3 The myoglobin molecule consisting of heme attached to globin. A–H indicate the eight helical segments of the globin moiety. The heme group is located in a hydrophobic cleft, where only small ligands such as oxygen and carbon monoxide have ready access. Owing to the hydrophobic environment, water (W) has limited access to the heme group. *(Reproduced from Suman, S.P., Joseph, P., 2014. Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, second ed., vol. 3. Elsevier, Oxford, pp. 244e251, (Chapter 84), with permission of Elsevier Limited, Oxford, United Kingdom.)*

In the living animal, this can have serious health consequences that lead to carbon monoxide poisoning (hemoglobin in the blood is a heme protein as well, and its ability to deliver oxygen to bodily tissues is prevented when carbon monoxide binds its heme groups). The principles of the interactions between myoglobin and ligands have been applied by the meat industry in different packaging technologies (such as aerobic packaging, modified atmosphere packaging, vacuum packaging) in an effort to maintain desired color of fresh meats (Eilert, 2005; McMillin, 2008).

11.4 Color phenomena in fresh meat

11.4.1 Myoglobin oxidation and reduction

In fresh meat subsurfaces, myoglobin is generally present in a ferrous nonoxygenated form. This is referred to as deoxymyoglobin (DeoxyMb) and is purplish red in appearance. As meat is cut and exposed to air, atmospheric oxygen will

bind heme iron to form ferrous red oxymyoglobin (OxyMb); this process is referred to as "blooming." Eventual oxidation of heme iron to a ferric state will lead to the dissociation of oxygen and subsequent binding of water by heme iron to form ferric brown metmyoglobin (MetMb).

Carbon monoxide can bind to ferrous myoglobin (COMb) and produce a red color that is nearly identical to that of OxyMb. This may occur through purposeful intent as has been implemented in the fish industry (Kristinsson et al., 2006) or unintentionally in gas-fired oven cooking of processed meat products where inefficiencies present within the heating elements can lead to some small production of carbon monoxide (Cornforth et al., 1998). The interconversions of myoglobin redox forms in packaged fresh meats upon exposure to ligands are presented in Fig. 11.4.

It is important to note that the conversion of myoglobin from a ferric to ferrous state, known as metmyoglobin reduction, can occur in meat (Bekhit et al., 2005). Metmyoglobin reduction is dependent on reducing equivalents provided by metabolites in the muscle sarcoplasm and occurs until these are exhausted. Thus, early in the display of meat, the actual color observed is believed to be the net result of concurrent oxidation and reduction processes. Oxidation processes ultimately prevail, and the net browning of fresh meat observed in retail settings is inevitable.

A summary of the different redox/ligand combinations that can occur in myoglobin and affect fresh meat color is presented in Table 11.2. Additional information on the protein's spectral characteristics as they affect estimates of myoglobin concentration is presented in Section 11.8.

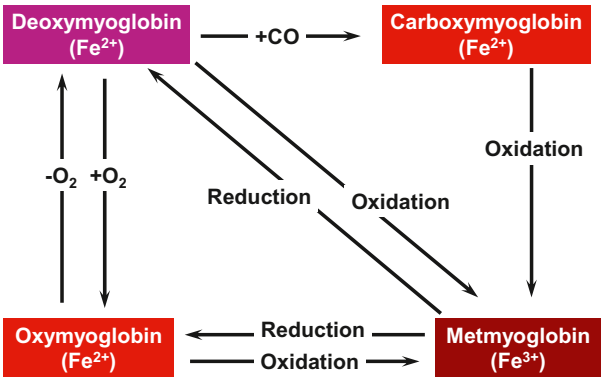

FIG. 11.4 Interconversions of myoglobin redox forms in packaged fresh meats. *(Reproduced from Suman, S.P., Joseph, P., 2014. Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, second ed., vol. 3. Elsevier, Oxford, pp. 244e251, (Chapter 84), with permission of Elsevier Limited, Oxford, United Kingdom.)*

TABLE 11.2 Chemistry of major pigments in fresh meats.

Pigment	Source species	Color	Formation	Oxidation state of heme iron	Status of globin	Absorption maximum (nm)			Reference
						Soret	Alpha	Beta	
Deoxymyoglobin	Horse	Purplish-red	Deoxygenation of oxymyoglobin; reduction of metmyoglobin	Fe^{2+}	Native	439	555	NA	Broumand et al. (1958)
Oxymyoglobin	Horse	Cherry-red	Oxygenation of deoxymyoglobin	Fe^{2+}	Native	420	582	544	Bowen (1949)
Metmyoglobin	Horse	Brown	Oxidation of oxymyoglobin and deoxymyoglobin	Fe^{3+}	Native	409	630	500	Bowen (1949)
Carboxymyoglobin	Horse	Cherry-red	Binding of CO with deoxymyoglobin	Fe^{2+}	Native	NA	581	543	Suman et al. (2006)
Cyano-metmyoglobin	Pig	Brown	Addition of cyanide to myoglobin	Fe^{3+}	Native	NA	NA	540	Warriss (1979)
Cytochrome c	Horse	Red	NA	Fe^{2+}	Native	415	550	521	Girard et al. (1990)
Sulfmyoglobin	NA	Green	Reaction of hydrogen sulfide with myoglobin	Fe^{2+}	Native	420	617	NA	Nicol et al. (1970)
Metsulfmyoglobin	NA	Red	Oxidation of sulfmyoglobin	Fe^{3+}	Native	405	715	595	Nicholls (1961)

Continued

TABLE 11.2 Chemistry of major pigments in fresh meats—cont'd

Pigment	Source species	Color	Formation	Oxidation state of heme iron	Status of globin	Absorption maximum (nm)			Reference
						Soret	Alpha	Beta	
Acid ferrimyoglobin peroxide	NA	Green	Reaction of hydrogen peroxide with metmyoglobin at acidic conditions (pH 4.5); distal histidine is oxidized	Fe^{3+}	Native	NA	NA	589	Fox et al. (1974)
Ferrimyoglobin peroxide	NA	Red	Reaction of hydrogen peroxide with metmyoglobin at alkali conditions (pH 8)	Fe^{3+}	Native	NA	NA	547	Fox et al. (1974)
Ferrocholemyoglobin	NA	Green	Irreversible oxidation of heme in myoglobin with ring opened	Fe^{3+}	Native	NA	635	NA	Nicol et al. (1970)

NA, not available.

Reproduced from Suman, S.P., Joseph, P., 2014. Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, vol. 3. second ed. Elsevier, Oxford, pp. 244–251 (Chapter 84), with permission of Elsevier Limited, Oxford, United Kingdom.

11.4.2 Factors endogenous to meat that affect myoglobin redox stability

As noted earlier, muscle is composed of different metabolic fiber types, and the biochemistry of these continues to influence chemistry/biochemistry in the postmortem condition. Mitochondrial-based oxygen consumption can be significant in meat cuts containing muscles that are primarily oxidative in their metabolism, particularly in the postmortem prerigor and early postrigor timeframes. Active oxygen consumption by mitochondria decreases the relative oxygen partial pressure (pO_2) in meat, and there is a fascinating relationship between myoglobin redox status and pO_2 (Fig. 11.5). At pO_2 values of 0 mmHg or greater than 100 mmHg, ferrous forms of myoglobin (DeoxyMb and OxyMb, respectively) predominate. However, at a pO_2 value of approximately 4 mmHg, MetMb predominates. From a packaging perspective, the implication is that meat must be packaged in anoxic or oxygen-saturated conditions to avoid low pO_2-induced oxidation to MetMb and undesirable browning.

The pO_2 effect can be observed directly when a freshly sliced and bloomed steak is cut vertically (i.e., perpendicular to the original cut surface of the steak). The side profile of the cut perpendicular face will display an oxygenated OxyMb layer at the top, with a thin brownish MetMb line just beneath and a thicker dark purplish red DeoxyMb layer below that (at least until atmospheric oxygen binds to the DeoxyMb). These result from oxygen diffusion that occurs from the steak surface (prior to the perpendicular cut) and the resulting gradient of pO_2 that is a function of oxygen penetration and which is readily visualized.

Lipid oxidation is a process in which unsaturated fatty acids in triacylglycerols or membrane phospholipids are attacked by oxygen. This leads to breakdown of the fatty acid into smaller molecular fragments that generally

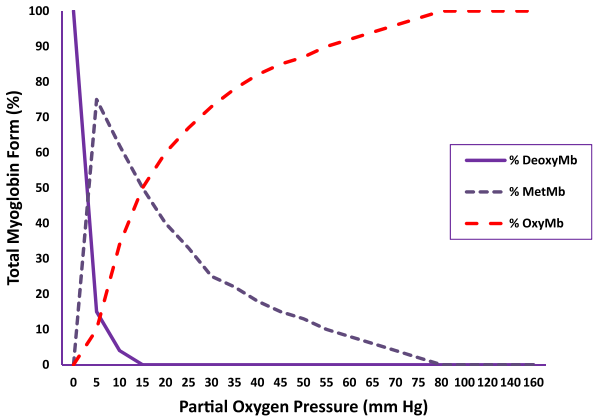

FIG. 11.5 Relationship between oxygen partial pressure and myoglobin redox status. *(From Forrest, Aberle, Hedrick, Judge, Merkel, 1975. Principles of Meat Science. ISBN:0-7167-0743-8; Hedrick, H.B., Aberle, E.D., Forrest, J.C., Judge, M.D., Merkel, R.A., 1994. Principles of Meat Science. third ed. Kendall Hunt Publ. Co., Dubuque, IA.)*

affect flavor and/or odor in a negative manner and that can hasten discoloration believed to occur through reaction with myoglobin (Faustman et al., 2010). Greater lipid oxidation will occur where concentrations of unsaturated fatty acids, catalysts (e.g., iron or copper ions), and oxygen are greatest. A variety of secondary products of lipid oxidation have been demonstrated, in vitro, to bind covalently to oxymyoglobin and hasten its oxidation to metmyoglobin, including 4-hydroxynonenal (HNE; Faustman et al., 2010). Evidence that this can occur in situ was recently presented through mass spectrometric identification of HNE-alkylated myoglobin in beef (Wang et al., 2021).

Interestingly, myoglobin has been implicated as a catalyst and/or facilitator of the lipid oxidation process. Both ferrous and ferric myoglobins have been shown to be pro-oxidative albeit under different conditions; activation of ferric to ferryl (Fe^{+4}) myoglobin in the presence of peroxides, particularly hydrogen peroxide, results in a strong initiator of lipid oxidation (Baron and Andersen, 2002).

Mitochondria remain functional for up to 60 days in postmortem muscles, and in addition to their role in affecting local pO_2, these subcellular organelles have also been implicated in metmyoglobin reduction, a process that can also play a critical role in fresh meat color stability (Egbert and Cornforth 1986). The muscle to meat conversion is accompanied by an accumulation of metabolites derived from the tricarboxylic acid cycle in postmortem muscles. Metabolites such as lactate, succinate, malate, and pyruvate are endogenous to skeletal muscles and are known to stabilize color of fresh meat through their interactions with mitochondria and enzyme systems leading to NADH replenishment and subsequent metmyoglobin reduction (Ramanathan and Mancini, 2018; Ramanathan et al., 2019).

The pH of postmortem muscle is lower than that in the corresponding muscle of the living animal, a result of postmortem, prerigor glycolysis. The redox stability of myoglobin is most stable at pH in vivo and decreases with decreasing pH (i.e., increasing acidity). Different muscles may have normal ultimate pH values that differ from each other in a consistent and relative manner (Hunt and Hedrick, 1977); a pH value of 5.6 is often communicated as an overall average for meat. Also, in some situations, the pH of postrigor meat may be at a value significantly greater than normal (e.g., in dark, firm, dry meat; DFD). In these situations, the redox stability of myoglobin may be affected. The acidification of processed meats for preservation purposes will reduce myoglobin's redox stability. Lowered pH leads to more rapid oxidation of ferrous to ferric myoglobin; it also results in decreased water-binding ability of postmortem muscle, which leads to more surface moisture and greater light reflectance leading to a paler appearance.

Temperature, a major exogenous factor, may interact with pH to affect myoglobin stability. Under normal slaughter conditions, there is an average timeframe over which pH declines from its antemortem to ultimate postmortem value at rigor. In beef cattle and pigs, this may require 24 h and 12 h, respectively. Over the same time period, there is a decline in the temperature of

carcass muscles from 37°C to 4°C (as the carcass is fabricated and ultimately stored in a chiller). Circumstances that result in more rapid pH decline while muscle temperatures are relatively elevated, for a given time point postmortem (prerigor), will present conditions that are high temperature and low pH relative to the norm. This can affect the conformational stability of myoglobin and its redox stability. This is observed in pork muscles from animals suffering from porcine stress syndrome in which the appearance of pork will present with pale coloration. Rapid and effective chilling will slow biochemical (i.e., postmortem glycolysis) reactions in the carcass postmortem and allow a slower pH decline, which is less damaging to muscle proteins.

The application of high-throughput metabolomics and proteomics techniques has helped researchers to understand metabolite and protein changes in muscles related to meat color. Succinate and NADH are two important metabolites that can provide reducing equivalents necessary for metmyoglobin reduction (Ramanathan et al., 2010; Tang et al., 2005). The addition of succinate to isolated mitochondria and metmyoglobin increased metmyoglobin reduction in vitro. Similarly, incubating NADH with isolated mitochondria and metmyoglobin increased reduced myoglobin form by electron-transport-mediated and enzymatic metmyoglobin reduction. The role of succinate in meat color stability (related to metmyoglobin reduction) and muscle darkening (related to pH and oxygen consumption) was demonstrated utilizing gas chromatography-mass spectrometry-based nontargeted metabolomics. Results of this work revealed that color-stable *longissimus lumborum* muscle contained greater succinate concentrations on day 7 of display compared with color-labile *psoas major* muscles (Abraham et al., 2017). Succinate levels were greater in dark-cutting muscles than normal-pH muscles (Ramanathan et al., 2020b), suggesting succinate's role in oxygen consumption and muscle darkening. Fumaric acid, creatinine, and fructose can regenerate NADH, and the concentrations of these glycolytic/tricarboxylic acid cycle metabolites decreased with aging and display time (Mitacek et al., 2019). Muscle discoloration is muscle-specific, and muscles with a greater proportion of oxidative (red) fibers discolor more quickly than muscles with a greater proportion of glycolytic (white) fibers. Results from proteomics and metabolomics research indicated the greater presence of glycolytic proteins and metabolites in *longissimus lumborum* than *psoas major* muscles (Abraham et al., 2017; Joseph et al., 2012). The role of mitochondrial proteome in beef color biochemistry was also examined recently. Mitochondria isolated from beef *longissimus lumborum* and *psoas major* exhibited differences in proteome profile (Ramanathan et al., 2021); the differentially abundant mitochondrial proteins were enzymes, binding proteins, and proteins involved in biosynthesis, suggesting that mitochondrial proteome critically contributes to muscle-dependent beef color stability.

The interactions between sarcoplasmic proteins and myoglobin influence color stability of postmortem skeletal muscles (Ramanathan et al., 2020a). Differential abundance of antioxidant proteins, chaperones, and metabolic

enzymes has been attributed to the inter- and intramuscular variations in beef color (Joseph et al., 2012; Nair et al., 2016, 2018a,b). Antioxidant proteins and chaperones are more abundant in color-stable beef muscles (such as *longissimus lumborum*) than in their color-labile counterparts (such as *psoas major*) contributing to the improved color stability of the former. Interestingly, these differentially abundant sarcoplasmic proteins undergo muscle-specific changes during aging (Nair et al., 2018a,b).

The structure-function relationship of proteins is altered by posttranslational modifications, and these can occur antemortem or postmortem. Posttranslational modifications of myoglobin were suggested as potential factors compromising beef color stability and contributing to variations in fresh beef color observed between individual animals/carcasses (Canto et al., 2015). Results from a recent mass spectrometric study identified multiple posttranslational modifications (i.e., phosphorylation, methylation, carboxymethylation, acetylation, and HNE alkylation) in myoglobin isolated from postmortem beef *longissimus lumborum* during aging and observed that these modifications compromise myoglobin redox and color stabilities (Wang et al., 2021).

11.4.3 Factors exogenous to meat that affect myoglobin redox stability

The reactions of myoglobin are greatly influenced by temperature such that ferrous myoglobins oxidize to metmyoglobin more rapidly at higher display and storage temperatures. The colder the meat is, the less quickly myoglobin will oxidize.

Given that the conversion of ferrous myoglobins to MetMb is, in and of itself, a result of oxidation, and that lipid oxidation also enhances myoglobin oxidation, it is not surprising that antioxidant chemicals can improve myoglobin redox stability. A review of the effects of endogenous and exogenous antioxidants on meat color revealed that reducing agents (e.g., ascorbic acid) and plant-based phenolics delay both lipid and myoglobin oxidation (Faustman et al., 2010). Ascorbate is an effective water-soluble reductant long recognized for its ability to delay meat discoloration (Greene et al., 1971; Howes et al., 2019). The phenolic antioxidant that has demonstrated the most dramatic color-preserving effect in fresh meat, particularly beef, is α-tocopherol (Vitamin E). An inverse correlation between α-tocopherol concentration in muscle and MetMb formation exists until a threshold antioxidant concentration is reached beyond which there is no benefit to having more α-tocopherol present (Faustman et al., 1989). This threshold was also demonstrated for delaying lipid oxidation. It is important to note that dietary delivery of α-tocopherol to cattle was far superior for realizing this color-stabilizing effect than ingredient delivery of α-tocopherol to postmortem beef (Mitsumoto et al., 1993). Many studies have revealed that the positive effect of dietary α-tocopherol was sufficient to add 1–3 days to fresh beef shelf life. Feeding distiller's grains can

negatively impact color and oxidative stability in beef (Kinman et al., 2011; Leupp et al., 2009; Mello et al., 2012; Roeber et al., 2005), and supplementing vitamin E at 250 or 500 IU per animal per day can minimize the color-destabilizing effects of distiller's grains.

Bacterial contamination of fresh, unprocessed meat is inevitable. For meat that is displayed or stored aerobically, bacteria appear to enhance myoglobin oxidation until they reach a concentration of approximately 10^8 CFU/cm^2, a spoilage level. Below this level, some meat scientists believe that bacteria reduce redox stability by competing with ferrous myoglobin for oxygen and that at sufficient levels, they can lower the local pO_2 to a level that enhances MetMb formation (see pO_2 discussion above). When bacterial spoilage occurs, irreversible formation of a red color may occur that is believed to result from the binding of bacterial metabolites to myoglobin.

Fresh and cured meats are displayed in retail cases under lighting that is commonly incandescent or fluorescent-based. Light catalyzes MetMb formation and also plays a critical role in myoglobin photooxidation (Renerre and Labadie 1993). While both fresh and cured meats discolor following exposure to light, the effect of visible light is more pronounced on cured meat pigment than on fresh meat pigments. Incandescent and fluorescent lighting similarly induce fading of cured meat surfaces. Ultraviolet light will also enhance color fading but can also induce brown discoloration in fresh meat possibly through partial apoprotein denaturation. Interestingly, freezing offers no protection against light-induced discoloration.

It is well known that the type of lighting affects a consumer's perception of fresh meat color (Calkins et al., 1986), and lighting systems with a color temperature of 2900–3750 K are generally recommended for illuminating displays of fresh meat. Light-emitting diodes (LED) have been recently employed in food display areas. Recent studies comparing fluorescent and LED lighting indicated that fresh meat stored in LED lighting retained red color for a longer period than those under fluorescent display (Boyle, 2015).

The presence or absence of gases/ligands within the packaging environment of meat can significantly affect its color stability. In a practical sense, this is the principle behind fresh meat packaging. While oxygen-enriched packaging systems enhance the cherry-red color stability of fresh meat over storage in air, fading will still occur a few days later. On the other hand, fresh meats packaged in carbon monoxide modified atmospheres will retain their cherry-red color for more than 2 weeks. Complete exclusion of oxygen, carbon monoxide, or any other gases, is achieved through vacuum packaging, and this process can achieve substantial shelf life for fresh meat. However, the meat appears purplish-red due to the predominance of DeoxyMb, a color that consumers remain wary of (Fig. 11.4).

High hydrostatic pressure, at or above 100 MPa using liquids as pressure transmitter, has been employed as an effective nonthermal strategy to improve microbial safety of food products, including fresh meat (Bak et al., 2019). Exposure to high pressure (100–800 MPa) inactivates microorganisms and

thus, extends the shelf life of muscle foods (Cheftel and Culioli, 1997; Simonin et al., 2012). While this technology is effective in pathogen destruction, it is known to compromise meat color (Cheftel and Culioli, 1997; Simonin et al., 2012; Ma and Ledward, 2013). Discoloration in fresh meats subjected to high pressure is attributed to globin denaturation, heme release, and oxidation to metmyoglobin (Carlez et al., 1995). Exposure to high pressure leads to discoloration in fresh (Cheftel and Culioli, 1997; Jung et al. 2003; Bak et al., 2012) as well as dry-cured (Andres et al., 2006; Cava et al., 2009) meats. Interestingly, the color of dry-cured meats is less affected by high pressure than raw meat color, possibly due to the resistance of nitrosylmyoglobin pigment to oxidation (Pietrzak et al., 2007).

11.5 Color in cooked nitrite-cured and salted uncooked meats

The process of curing (also referred to as corning) refers to salt-based preservation of meat (see Chapter 9). Sodium chloride is most commonly used, but other salts may be employed as well. In particular, a significant proportion of cured meats include the addition of sodium nitrite; potassium nitrite may also be used but is much less commonly employed. The evolution of sodium nitrite use is believed to have started from its accidental inclusion as a minor contaminant in sodium chloride. As processors noted the advantages gained from such contaminated salts, often mined from certain geographical locations, research eventually uncovered the chemical nature of nitrite.

Sodium nitrite, $NaNO_2$, aids greatly in the preservation of cooked, cured meat. Its inclusion is critical for preventing the foodborne illness, botulism. Combined with myoglobin naturally present in the meat and under conditions of heat used in cooking, the classic pink color of cooked ham and bacon results. The addition of this additive also protects against off-flavor development, which typically occurs during storage of uncured cooked meat. Nitrite may be added as an additive in and of itself or delivered to processed meat products through the addition of other ingredients such as high nitrate-containing plant-based materials (e.g., vegetable extracts, impure mineral salts). Nitrate is readily converted to nitrite during meat processing.

The chemistry of sodium nitrite (and its intermediates) that occurs during cured meat production is complex. For the purposes of this chapter, a simple description of the major chemical changes is provided. The addition of nitrite to meat occurs prior to cooking through the application of a dry rub or brining. The nitrite dissociates from its sodium counter ion and is ultimately converted to nitric oxide within the meat product. During the cooking step, heat-induced denaturation of myoglobin occurs, and this results in the heme group being exposed as the tertiary structure of apomyoglobin is lost. Nitric oxide binds to the heme iron at its sixth and fifth ligand positions to form dinitrosylferrohemochrome, the pink pigment associated with cured, cooked meats. The chemical nature of various cured meat pigments is detailed in Table 11.3.

TABLE 11.3 Chemistry of major pigments in cured meats.

Pigment	Source and species	Color	Formation	Oxidation state of heme iron	Status of globin	Absorption maximum (nm)	Reference
Nitrosyl metmyoglobin	Pork	Brown	Reaction of nitric oxide with metmyoglobin	Fe^{3+}	Native	NA	Killday et al. (1988)
Nitrosyl myoglobin	Pork	Red	Reduction of nitrosyl metmyoglobin	Fe^{2+}	Native	NA	Killday et al. (1988)
Nitrosyl hemochrome	Cooked, cured pork	Pink	Head-induced denaturation of nitrosyl myoglobin	Fe^{2+}	Denatured	540	Hornsey (1956)
Nitrimetmyoglobin	Uncooked, cured meat	Green	Addition of excess nitrite	Fe^{3+}	Native	NA	Fox (1987)

NA, not available.

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As noted previously, the apoprotein of myoglobin stabilizes heme and provides partial protection against oxidation. The loss of the apoprotein tertiary structure predisposes heme to greater oxidative susceptibility. Heme iron is believed to catalyze formation of free radicals through a photooxidation process, and so cured meat that is displayed under light is often vacuum-packaged to minimize the opportunity for oxygen to participate in oxidation reactions that would lead to color fading.

Nonnitrite-based curing of uncooked, dried meat products is also undertaken particularly in southern Europe. In general, iron is tightly bound within the heme group, but in certain dry-cured meats that are aged for extended periods of time, it may be displaced. In prosciutto-type dry-cured hams, the extended salt curing process results in displacement of iron from myoglobin's heme group by zinc resulting in red Zn-protoporphyrin IX (Wakamatsu et al., 2004).

11.6 Cooked meat color

Cooking leads to heat-induced denaturation of myoglobin and exposes heme to the external environment (King and Whyte, 2006). Globin denaturation in MetMb results in formation of brown ferrihemochrome (also known as denatured globin hemichrome), which is the pigment responsible for the dull-brown internal color of cooked meats (note that brown color on the external surfaces of heated meats also involves pigments derived from the Maillard reaction, which is unrelated to myoglobin-based pigments).

Denaturation of globin in ferrous myoglobin forms generates pink/red ferrohemochrome (also known as denatured globin hemochrome), which is readily oxidized to brown ferrihemochrome. In contrast, heat-induced denaturation of carboxymyoglobin (in CO-treated meats) leads to formation of pink-red denatured globin CO hemochrome. The chemistry of pigments in cooked meats is detailed in Table 11.4.

Various factors influence the thermal stability of myoglobin and formation of internal cooked meat color (King and Whyte, 2006; Suman et al., 2016). The thermostability (resistance against heat-induced denaturation) is dependent on the protein's redox state; COMb and DeoxyMb have greater thermostability than MetMb and OxyMb (Sepe et al., 2005; Hunt et al., 1999). As a consequence, storing meats in high-oxygen modified atmosphere packaging (MAP), bulk packaging, and/or aerobic packaging often compromises myoglobin thermal stability and enhances the propensity for myoglobin to brown upon application of heat in cooking. However, storage in vacuum and CO MAP improves myoglobin thermal stability and helps cooked meat retain redness. The rate and extent of heat-induced denaturation in livestock and poultry myoglobins increase with temperature (Joseph et al., 2010), and numerous studies have reported lesser redness in meats cooked to greater relative internal temperatures than those at low temperatures. High meat pH (especially above 6.2) provides myoglobin with protection against denaturation when compared with meat of

TABLE 11.4 Chemistry of major pigments in cooked meats.

Pigment	Source species	Color	Formation	Oxidation state of heme iron	Status of globin	Reflectance minimum (nm)			Reference
						Soret	Alpha	Beta	
Denatured globin hemochrome	Cooked pork or beef	Pink or red	Heat-induced denaturation of ferrous myoglobin; reduction of globin hemichrome	Fe^{2+}	Denatured	424	530	558	Tappel (1957) and Ghorpade and Cornforth (1993)
Denatured globin hemichrome	Cooked pork	Brown, tan or gray	Heat-induced denaturation of metmyoglobin; oxidation of globin hemochrome	Fe^{3+}	Denatured	405	495	545	Tarladgis (1962)
Nicotinamide hemochrome	Turkey	Pink or red	Reaction of heat-denatured myoglobin with nicotinamide under reducing conditions	Fe^{2+}	Denatured	420	529	558	Tappel (1957) and Cornforth et al. (1986)
Denatured globin CO hemochrome	Cooked beef	Pink or red	Heat-induced denaturation of carboxymyoglobin	Fe^{2+}	Denatured	NA	542	571	Tappel (1957) and John et al. (2004)

NA, not available.

Reproduced from Suman, S.P., Joseph, P., 2014. Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, vol. 3. second ed. Elsevier, Oxford, pp. 244–251 (Chapter 84), with permission of Elsevier Limited, Oxford, United Kingdom.

normal pH (Trout, 1989). In contrast, low pH conditions can facilitate exposure of the heme group, via acid-induced unfolding of tertiary structure and even partial loss of helical structure, resulting in greater susceptibility of myoglobin to denature over a range of cooking temperatures (Janky and Froning, 1973). Within a species, meat pH is a muscle-specific trait; *psoas major* muscle exhibits greater pH than its *longissimus lumborum* counterpart in beef (Seyfert et al., 2006; Joseph et al., 2012) as well as lamb (Tschirhart-Hoelscher et al., 2006) carcasses. Ground beef patties prepared from *psoas major* muscle (pH ~5.7) retained more red color than the same products manufactured from longissimus muscle (pH ~5.45) when cooked to same internal temperature under identical conditions (Suman et al., 2004). Further studies in beef whole-muscle cuts also reported greater internal redness in psoas steaks than in longissimus ones when cooked to same internal temperature (Suman et al., 2009).

11.7 Anomalies in meat color

In addition to the colors normally observed in fresh and cooked meats, several anomalies (or color defects) can be observed under specific circumstances encountered in the preparation of meat and meat products. While some of these defects are cosmetic in nature, others are indicative of spoilage, or incomplete cooking, which can pose potential food safety risks.

11.7.1 Color defects in fresh meat

Physicochemical and biochemical interactions between myoglobin and chemical compounds, light, and other elements in muscle structure can sometime result in abnormal color/appearance of fresh meat.

Heat ring. This phenomenon occurs in the periphery of the cut longissimus muscle surface and appears as a dark ring; it is generally observed during carcass grading. Beef carcasses demonstrate this to a greater effect than other livestock, particularly in heavy carcasses, with a minimal subcutaneous fat layer. The lower fat cover provides less insulation, and it is believed that more rapid cooling facilitates the appearance of heat ring.

Greening. Green discoloration in fresh meat is, in general, due to formation of hydrogen sulfide or hydrogen peroxide; these two compounds are produced by bacteria and react with myoglobin. Sulfhydryl-producing bacterial species such as *Pseudomonas mephitica* can generate hydrogen sulfide in meats, whereas lactic acid bacteria generate hydrogen peroxide under aerobic conditions. The reaction of hydrogen sulfide with ferrous heme iron in myoglobin leads to formation of green sulfmyoglobin pigment. On the other hand, acid ferrimyoglobin peroxide (also known as hydroperoxymetmyoglobin) is the green pigment formed by the hydrogen-peroxide-induced oxidation of myoglobin under acidic conditions.

Iridescence. Iridescence is a physical phenomenon wherein shiny, rainbow-like colors are visible in raw, dry-cured or cooked meat surfaces (Mancini, 2007). Iridescence is not associated with myoglobin redox chemistry, but can be misinterpreted as a sign of spoilage or unwholesomeness. In cooked meat surfaces, the most common colors associated with iridescence are green, red, orange, and yellow. Diffraction of light as a result of the striated structure and fibrous nature of skeletal muscles is believed to cause iridescence in meats. This color problem is associated with the muscle microstructure; therefore, iridescence is seen only on surfaces of whole meat cuts, but not in ground meats.

Dark cutting. Dark cutting, also known as dark, firm, and dry (DFD) meat has an abnormally dark purplish-red lean. DFD is a problem in beef caused by glycogen depletion in animals prior to slaughter resulting abnormally high pH (even as high as pH 6.8) in postmortem muscle. The high pH condition results in greater water-binding capacity than meats of normal pH; the muscle appears dark because of bound (i.e., not exuded onto the surface) greater intracellular water, which reflects less light. Due to high intracellular water, the muscle will also appear firm and dry. The higher muscle pH provides some protection to myoglobin against denaturation, enhances aerobic cellular metabolism, and helps maintain heme iron in the reduced ferrous state.

Pale, soft, and exudative (*PSE*) *meat*. The PSE condition is a quality defect that originates during the postmortem period between slaughter and onset of rigor mortis. The rate at which rigor mortis is established will influence the relative temperature at which a given carcass pH value is recorded. PSE meat results from antemortem stress and certain carcass handling practices immediate postmortem, both of which lead to a condition of rapid buildup of lactic acid and onset of rigor mortis in carcass muscles after death. The quick onset of rigor mortis means that carcass temperatures have not cooled to the same degree as normal (i.e., non PSE) carcasses, and this in turn results in an elevated muscle temperature being coupled with the acidic pH associated with rigor onset. This high temperature/low pH (relative to normal) condition causes partial denaturation of protein structures that yield meat, which is soft and exudative. The damaged proteins are unable to bind water to the extent that of normal meat and the exuded moisture reflects light readily (Hughes et al., 2014) producing a pale appearance with reduced consumer acceptability. In addition, myoglobin (and other sarcoplasmic proteins) is partially denatured or adsorbed onto myofibrillar proteins, which further contributes to the pale appearance (Kauffman and Marsh, 1987; Liu et al., 2015). While color quality is a concern in PSE meat, the larger problem is its poor functionality (sensory, water-holding capacity). Historically, PSE has been observed predominantly in pork and poultry; however, the incidence of PSE-like conditions in beef has been increasingly reported (Aalhus et al., 1998; Warner et al., 2014). PSE-like conditions have been identified on the inside region of the two-toned semimembranosus beef muscle (Sammel et al., 2002a,b), and differential rates of

chilling (Sammel et al., 2002b) and potential variations in glycolysis (Nair et al., 2016, 2018b) in this large muscle have been offered as possible causes for these observations.

11.7.2 Color defects in cooked meats

At the point of consumption, consumers utilize the dull-brown color of cooked meat as an indicator of doneness and food safety. Deviations from this appearance, particularly due to pinkish colors, are often interpreted an indication of undercooking (King and Whyte, 2006; Suman et al., 2016). Anomalies exist that call each of these assumptions into question.

Premature browning in beef. Premature browning is a condition in cooked beef where myoglobin denaturation occurs at an internal temperature that is less than 71°C, the USDA-recommended temperature for destroying foodborne pathogens. Consumers generally assume that a brown internal color of cooked meats indicates safe preparation, and thus, premature browning poses a potential food safety risk rather than a cosmetic concern. The thermostability of myoglobin depends on its redox state and bound ligand, with COMb and DeoxyMb demonstrating greater thermostability than OxyMb and MetMb. Thus, cooked color is dictated by the predominant form of myoglobin present in the raw meat before cooking. From this standpoint, factors that favor oxidation of beef (e.g., ground beef) will predispose the meat to premature browning. In contrast, vacuum packaging, carbon-monoxide-modified atmosphere packaging, and antioxidants minimize the conditions for premature browning to occur.

Pink color defect in poultry. This defect is observed in fully cooked, uncured chicken, and turkey meats in which thoroughly cooked product interiors appear pink; this results in rejection of otherwise safe meats. The unique biochemistry of poultry myoglobins (i.e., greater molecular mass compared with the red meat counterparts) may contribute to increased thermostability resulting in incomplete myoglobin denaturation in cooked meats. In addition, the interactions of myoglobin with combustion products (CO, NO, NO_2) have been also reported as causative factors. Application of nonmeat ingredients such as nonfat dried milk, sodium citrate, calcium chloride, and sodium tripolyphosphate minimizes the occurrence of pinking (Sammel and Claus, 2003; Sammel et al., 2007), although the exact mechanisms for this effect has not been elucidated.

Persistent pinking in beef. Any abnormal pink appearance (known as persistent pinking) in cooked beef, when the consumers expect a product with a cooked appearance, is considered undesirable. Among the numerous factors that contribute to this defect, the most prominent ones are high meat pH (>6.0) and combustion products resulting from cooking equipment (CO, NO). High pH minimizes myoglobin denaturation, and grass-fed beef and beef from cattle exposed to preslaughter stress have been reported to exhibit average postmortem

muscle pH above 5.9 resulting in persistent pinking. Incorporation of lactic acid increases myoglobin denaturation and can be applied as a strategy to mitigate persistent pinking in beef.

11.8 Measuring meat color

The measurement of color is critical to studies concerned with this sensory aspect of meat (American Meat Science Association, 2012). The appearance of color can be based on quantification of various myoglobin forms, which permits assessment of chemical changes that affect color. Physical changes that affect perception of color/appearance (e.g., effects of surface moisture on light reflectance) are more effectively measured by tristimulus colorimetry (Loughrey, 2001) and sensory techniques involving human subjects.

Fresh meat color measurement generally involves the analysis of total myoglobin concentration and/or the relative proportions of one or more redox forms of myoglobin (i.e., Deoxy-, Oxy-, Met-; Faustman and Phillips, 2001; Krzywicki, 1979, 1982; Mancini et al., 2003). For whole slices of meat, this is generally accomplished by surface reflectance techniques using a spectrophotometer equipped with a diffuse integrating sphere. Ground meat may be measured in the same manner if only a surface analysis is desired, but more commonly is homogenized in an appropriate buffer and the filtered aqueous extract analyzed by spectrophotometric light absorption. DeoxyMb, OxyMb, and MetMb each have characteristic absorption behavior across the visible spectrum. Interestingly, at equal concentrations, they all absorb the same amount of light at 525 nm, and this wavelength is referred to as the isosbestic point for total myoglobin (i.e., all redox forms). Thus, methods for following color changes in meat attempt to quantify the relative proportions of the three redox forms by measuring light absorption at wavelength maxima specific for each form relating those values to the total concentration of myoglobin evaluated at 525 nm (Tang et al., 2004).

Strategies to estimate discoloration in meat surfaces and browning in myoglobin solutions were developed on the basis of the three myoglobin redox forms that are present in conventionally bloomed meats (i.e., DeoxyMb, OxyMb, and MetMb). With the approval to use CO in MAP systems for fresh meat in the United States in 2004, COMb became relevant to meat color and discoloration; browning index was developed as an estimate of browning or oxidation in meat extracts that potentially contained both OxyMb and COMb (Suman et al., 2006).

Cured and noncured cooked meat color can similarly be assessed by surface techniques. Cured meats are typically packaged in vacuum, and if surface measurements are made through the packaging film, then color plates used to standardize the evaluation instrument should also be covered in the same film. Pigment concentrations are obtained by extracting the heme complex from the meat product (Cornforth, 2001). The heme molecule is hydrophobic, and

acetone is commonly used to partition the heme complex away from the homogenized aqueous meat matrix for analysis.

Tristimulus colorimetry uses information obtained from objective analysis of light reflectance and attempts to correlate it with the perception of color by an observer (American Meat Science Association, 2012). This technique does not permit quantification of myoglobin per se, but rather measures the relative amount and type of color present. It can stand alone or be correlated with evaluations made by sensory panelists.

Various novel noninvasive technologies have been developed to evaluate surface color and subsurface myoglobin chemistry. Near-infrared tissue oximetry could be applied as a robust tool to evaluate subsurface myoglobin chemistry (Mohan et al., 2010). In addition, noninvasive reflectance spectrometry can be applied to determine myoglobin redox state in whole-muscle steaks and ground beef (Khatri et al., 2012; Bjelanovic et al., 2013).

11.9 Summary statement

The color of meat is critical to the consumer's perception of quality and directly affects the purchasing decision. While meat is an excellent source of nutrition, it will not be purchased or consumed if its appearance is not consistent with the norm expected by consumers. Myoglobin's role in meat color has been characterized extensively, and its chemistry/biochemistry continues in the postmortem muscle of meat-producing livestock. Postmortem skeletal muscle is not an inert tissue, and the continued interactions between myoglobin and other biomolecules in the complex muscle food matrix critically influence the appearance of meat during retailing as well as after cooking. Antemortem and postmortem practices can strongly affect the quantity and stability (redox and thermal) of myoglobin in meat. A fundamental understanding of myoglobin chemistry is necessary in order to optimize husbandry and process development for maintaining an acceptable appearance of meat.

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Chapter 12

The eating quality of meat: II—Tenderness

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12.1 Introduction

12.1.1 Definition and measurement

Tenderization is the generalized term for the process that leads to improvement in tenderness and in reality can only be measured *postrigor* in meat. A measure of tenderness is the subjective consumer or trained panelist appreciation of the meat, and a high score is desirable, and connective tissue and the amount of intramuscular fat can influence the score given. An objective measure of tenderness (or toughness) is the force required to shear a standardized piece of meat with low shear values being desirable (Hopkins et al., 2006). Of the eating quality traits, tenderness is affected by both production and processing factors to the largest degree (Young et al., 2005), and it is an important meat quality trait.

Methods to subjectively determine the tenderness of meat will be outlined elsewhere (see Chapter 15). Generally, tenderness is measured using objective mechanical measures, as these are cheaper, less time-consuming, and remove much of the subjective nature of sensory testing (Shorthose and Harris, 1991). The objective measurement of tenderness of cooked meat can be undertaken with a variety of devices as outlined by Purchas (2014). A device that shears a standardized piece of meat (shear force) is the most common (Purchas, 2014), but devices with a biting action are also used, such as the MIRINZ tenderometer (Hopkins et al., 2013), and other devices that compress the meat have also been used. To improve the precision of estimates of shear force and compression, recent work has focused on establishing the coefficient of variation and thus the optimum number of technical replicates (e.g., Holman et al., 2015), which must be measured for each sample. The state of connective tissue can also be determined by measuring adhesion values, but this is not a common measurement.

There are three main factors that impact on the tenderness of meat: (1) background toughness related to collagen content and inherent cross-links, (2) rate and extent of tenderization during aging, and (3) muscle contraction during the

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onset of rigor (Hopkins and Geesink, 2009). Two antagonistic processes (toughening and tenderization) take place during the *postmortem* (PM) storage period (Hopkins and Thompson, 2001a), and the toughening process can be minimized by limiting the extent of muscle shortening during rigor development (Hopkins and Thompson, 2001a). These factors are themselves affected by production traits, such as genotype, age, gender, and handling and can be manipulated to some effect by processing after slaughter, and the cooking method also impacts on final tenderness.

12.1.2 Effect of muscle type on tenderness

It has been known for a long time that there are distinct differences in tenderness between muscles within a carcass (see Table 12.1 and also Torrescano et al., 2003), and the biochemical reason for these differences has been well characterized (see Chapter 4). Also, differences have been shown within the same muscle. For example, in a study of the *biceps femoris*, *semitendinosus*, *semimembranosus*, and *adductor* muscles of beef, it was shown that intramuscular variation was greater than the variation between muscles (Reuter et al., 2002), with the *biceps femoris* exhibiting the greatest intramuscular variation. This adds to the complexity of comparing studies when examining the impact of various factors on tenderness (e.g., gender).

It has been suggested that systematic and significant differences in tenderness between locations within muscles could be exploited, with known regions of toughness being removed for manufactured products. This known variation particularly between muscles has been applied in the development of the Meat Standards Australia (MSA) grading system where the prediction model for the

TABLE 12.1 Tenderness values (Warner-Bratzler Peak Shear Force: *N*) of various muscles from beef animals of three ages.

Muscle	12 months	24 months	36 months
Semitendinosus	50.0	61.8	68.9
Biceps femoris	50.0	58.8	68.0
Vastus lateralis	44.8	51.7	58.6
Semimembranosus	54.3	58.7	63.0
Gluteus medius	45.6	48.6	51.7
Longissimus lumborum	46.8	49.6	52.5
Psoas major	33.9	35.4	36.9

Adapted from Shorthose, W.R., Harris, P.V., 1990. Effect of animal age on the tenderness of selected beef muscles. J. Food Sci. 55, 1–8, 14.

eating quality of beef (Thompson, 2002) is based on statistical models, where anatomical cut descriptions are replaced with determination of the eating quality of muscles. Thus, the same muscle in different carcasses can exhibit detectable differences in traits, such as tenderness, and the model with a range of inputs provides predicted eating quality values. In a related way the within cut variation, as a function of different muscles, was fundamental to the development of muscle profiling and the application of seam boning in the United States (Jones et al., 2005).

12.1.3 Preslaughter factors that impact on tenderness

There are a number of preslaughter factors that can impact on the final tenderness of meat, irrespective of what happens to the meat postslaughter. These factors include the genetics of the animal, which in this chapter is limited to genotype effects, the age and gender of the animal, and how they are handled from the farm to the abattoir. These factors are discussed below.

12.1.3.1 Genotype effects

The genetic makeup of animals impacts on a vast array of traits, and here these effects are constrained to the combination of genes expressed in different genotypes or breeds. The term genotype encompasses crossbred animals, which are a significant component of different production systems across species. This section does not include specific gene effects, which will be covered elsewhere (see Chapter 2).

Some studies have shown either no differences in objectively measured tenderness between sheep breeds and crossbreds (Hopkins et al., 2007a) or inconsistent differences that were not explained by variation in other traits that influence tenderness, such as pH, sarcomere length, carcass weight, or fat levels (Purchas et al., 2002).

No sire breed effects on taste panel-assessed tenderness were reported by Safari et al. (2001) in comparisons of Merino lambs and other breeds, including Texel × Merino or Poll Dorset × Merino. Hopkins et al. (2005) reported minimal differences in consumer-assessed tenderness between genotypes, except that the Merinos had lower sensory scores than Border Leicester × Merino lambs for two different muscles, which may have reflected a slower rate of pH decline in the Merino lambs. Work by Pannier et al. (2014) showed by contrast that male Terminal (meat breeds) sired lambs had lower tenderness scores (~5 points on a 0–100 scale) for the loin and topside compared with the male Maternal and Merino sired lambs that had similar scores. This effect could reflect the fact that the Terminal sires used by Pannier et al. (2014) had estimated breeding values (EBVs) that indicated that these sires were on average leaner (less fat) than their breed average. It is known that this can lead to a decline in tenderness (Hopkins et al., 2007b). Sire effects have also been clearly shown in cattle also, and the extent of these effects is dependent on the portion of the carcass under consid-

eration. This sire variation is exploited in the use of EBVs in breeding schemes. In more recent years, some of this variability has been linked to genes encoding for proteases such as μ-calpain (Page et al., 2004), and it has clearly been shown that if cattle possess two copies of favorable alleles for calpains, then sensory tenderness is improved (Robinson et al., 2012), with lesser effect for the forequarter oyster blade cut (based muscles such as the *infraspinatus*) than for the striploin (based on the *Longissimus et lumborum*).

The most significant impact on tenderness in cattle is the effect of *Bos indicus* genes, which have been clearly shown to decrease the tenderness and increase shear force of muscles such as the *longissimus* as shown in Table 12.2 for Nellore sired steers (Wheeler et al., 1996). There were minimal differences between the other sire breeds. The *B. indicus* effect is accounted for in the MSA grading scheme for beef (Thompson, 2002) in recognition of the effect on palatability, of which tenderness is one aspect, and the *B. indicus* "effect" has been identified for many years.

It is of interest that although double-muscled cattle, such as the Belgian Blue, have a coarser grain (texture), this does not lead to higher shear force, and this is because in these cattle there is less intramuscular connective tissue (IMCT) per unit area (Albrecht et al., 2006). Despite the fact that no marked differences between breeds have been found in the gross content of connective tissues, other factors such as the chemical nature of collagen (see Chapters 3 and 4) could be implicated in creating differences in the tenderness of their meat. In a

TABLE 12.2 Effect of sire breed on the shear force (*N*) and tenderness (sensory) of the *Longissimus* at a constant weight (324 kg) or fat thickness (12 mm).

	Carcass weight		Fat thickness	
Sire breed	Shear force	Tenderness	Shear force	Tenderness
Hereford	54.9	4.7	58.0	4.7
Charolais	59.9	4.4	54.8	4.4
Shorthorn	57.8	4.7	57.9	4.7
Galloway	57.2	4.8	57.3	4.8
Nellore	70.2	4.0	70.7	4.0
Piedmontese	52.5	5.0	45.5	5.1
Least significant difference	5.9	0.31	6.2	0.33

Adapted from Wheeler, T.L., Cundiff, L.V., Koch, R.M., Crouse, J.D., 1996. Characterization of biological types of cattle (Cycle IV): carcass traits and longissimus palatability. J. Anim. Sci. 74, 1023–1035.

study on young bulls of 15 different breeds Christensen et al. (2011) observed that total and insoluble collagen in the *longissimus* muscle was higher in dairy breeds than in meat breeds and that collagen characteristics showed correlations to raw meat texture measured by compression, but not with the shear force of cooked meat. Tenderness measured by sensory or shear force is a heritable trait (Wheeler et al., 1996), but it is notable that compression, which is an indicator of connective tissue, is less heritable (Thompson et al., 2006).

Although there have been some reports that there are breed effects on the tenderness of pig meat, these are inconsistent (Sosnicki, 2015), with no apparent advantages attributable to one particular breed, although due to the influence of intramuscular fat in the Duroc breed, some markets prefer the overall eating quality of meat from this breed (Sosnicki, 2015).

12.1.3.2 Age effects

In general, increasing age correlates with decreasing tenderness as shown in Table 12.1 for beef muscles. The decrease in tenderness appears to be less marked with beef from animals older than 18 months (although this is muscle dependent). In the study reported by Jeremiah et al. (1971), which examined five muscles from the hind leg of sheep (ewes and wethers) ranging in age from 74 to 665 days for shear force and tenderness, a positive correlation was shown between animal age and decreasing tenderness. Across five muscles the correlation was −0.46, and between shear force of the *m. semimembranosus* and animal age, it was 0.33. Hopkins et al. (2007a) reported much higher shear force values for the *m. semimembranosus* from sheep aged 14–20 months versus those aged 8–14 months. This outcome is consistent with expectations based on the data of Young et al. (1993), which demonstrated a reduction in collagen solubility as animal age increased and a commensurate increase in shear force. The effect has been shown also in other species such as Alpacas, with sensory tenderness of the *Longissimus et lumborum* showing a significant decrease between the ages of 18 and 36 months irrespective of gender (Smith et al., 2016). This effect was again clearly linked to a reduction in collagen solubility with a threefold reduction between 18 and 36 months of age. These reductions in solubility are due to increased cross-linking between collagen molecules. Bouton et al. (1978) combined shear force data for four leg muscles from sheep and examined the effect of age of the animal. It was found that shear force did increase with increasing animal age, and there were smaller differences between ages if the meat was cooked at 80°C as opposed to 60°C.

With increasing animal age, the proportion of salt- and acid-soluble collagens decreases in bovine muscle. The extent of intra- and intermolecular cross-linking between the polypeptide chains of collagen concomitantly increases (Carmichael and Lawrie, 1967). Further insight on the changing character of collagen with increasing animal age emerges from the data of Young et al. (1993). This shows that as animal age increases (Table 12.3), collagen solubility

TABLE 12.3 Collagen solubility and concentration in the *Biceps Femoris* from mixed sex sheep according to chronological age.

	Age at slaughter (days)				
	42	70	274	365	Standard error of the difference
Collagen solubility (%)	51.7*a*	45.5*b*	27.9*c*	27.7*c*	3.66
Collagen concentration (g/100 g) fresh weight	1.13*a*	1.08*ab*	0.92*b*	1.03*ab*	0.09

Means followed by a different letter (*a*, *b*, *c*) in a row are different at the $P = 0.05$ level.
Adapted from Young, O.A., Hogg, B.W., Mortimer, B.J., Waller, J.E., 1993. New Zealand J. Agric. Res. 36, 143.

decreases (cross-links become nonreducible), whereas collagen concentration remains relatively constant.

Young and Braggins (1993) endeavored to clarify the relative importance of the concentration and the solubility of collagen in determining the tenderness of sheep meat. They concluded that the former was the predominant determinant of eating quality, whereas solubility was more closely associated with the objective determination of shear force.

In situ examination of meat by ultraviolet optical probes has revealed an increase in the incidence of fluorescence peaks as the perimysium increases in beef animals between 12 and 17 months of age. A subsequent decrease in fluorescence of the meat of animals between 17 and 24 months reflects the separation of perimysial layers by muscular tissue, but the fluorescent peaks broaden as the perimysial layers thicken (Swatland, 1994).

As Bouton et al. (1978) have indicated, age/tenderness relationships reflect not only direct chronological changes in muscular and connective tissues, but also associated effects due to the increasing bulk and fatness of carcasses with age. These influence the differential effect of cooling conditions on the extent of "cold shortening" in specific muscles. Given the production systems for pork, age effects are less significant, and no difference in shear force of the *longissimus* was found in a study where pigs were reared to the slaughter weights of 116, 124, or 133 kg (Latorre et al., 2004).

12.1.3.3 Gender effects

The report of Johnson et al. (2005) showed that both the *M. longissimus* and *M. semimembranosus* from rams had significantly higher shear force values compared with ewes in animals 8 months or younger. Small differences were reported by Hopkins et al. (2007a) across a number of genotypes with wethers producing significantly tougher *m. longissimus* than ewe lambs over an age range of 4–22 months.

TABLE 12.4 Interaction between age of castration in cattle and gender for shear force (*N*).

Muscle	Age of castration (steers)			Bulls
	3 months	7 months	12 months	12 months
Longissimus lumborum	84.3	91.1	89.2	98.0
Gluteus medius	62.7	64.7	68.6	71.5
Semitendinosus	59.8	61.7	61.7	59.8
Psoas major	39.2	38.2	37.2	38.2

Adapted from Rodriguez, J., Unruh, J., Villarreal, M., Murillo, O., Rojas, S., Camacho, J., Jaeger, J., Reinhardt, C., 2014. Meat Sci. 96, 1340.

A number of studies reviewed by Field (1971) have shown that steer meat was more tender than that from bulls, although later studies have highlighted the interaction between gender and muscle type (Table 12.4) with bulls producing tougher *longissimus*, at 12 months of age than steers, but the effect was not translated to an effect on sensory tenderness as assessed by a trained panel, and there was no difference in shear force for other muscles due to gender. The difference in tenderness between muscles was thoroughly shown by Belew et al. (2003).

In pigs the meat from barrows (castrates) has often been reported to have a similar shear force to gilts (Leach et al., 1996; Latorre et al., 2004), with this flowing on to sensory tenderness also (Leach et al., 1996). Channon et al. (2004) showed that the *longissimus*, from entire (boars), had higher shear force values than that from female pigs after 2 days of aging, but if the meat was aged for 7 days, the differences disappeared, suggesting that degradation of myofibrillar proteins was slower in the *longissimus* from the entire pigs.

12.1.3.4 Handling effects

Livestock pass through a series of phases on their way from a farm to an abattoir. Commonly, these phases include (1) farm curfew without access to feed, (2) saleyards, and (3) abattoir with transportation required for the delivery of the animals to the latter two phases. Some animals are sold directly to abattoirs, thus avoiding the saleyard and dual transport journeys. All slaughter animals spend time in lairage (i.e., resting) at abattoirs, and this time period varies depending on processor numbers and the order of slaughter.

Animals that face stressful situations will have an altered metabolism dependent on the type of stress (Ferguson and Warner, 2008). This can lead to significant depletion of muscle glycogen, and if the level falls to 45–57 mmol/kg, then a "normal" ultimate pH will not be reached when the animal is slaughtered (Tarrant, 1989). This will lead to increases in toughness specifically up to about

pH 6.0 (Purchas et al., 1999). In pigs the use of electric prods and other factors such as stocking density has been shown to impact on the pH of pork, and often this will cause increased rates of glycolysis (Brandt and Aaslyng, 2015), which potentially leads to an increase in protein denaturation and reduced tenderization. Jacob et al. (2005) reported minimal impact on sheep meat-eating quality traits such as tenderness when sheep were held in lairage for up to 48 h. However, in a study with adult sheep held in lairage at an abattoir, Toohey and Hopkins (2006) showed that there was a significant interaction between lairage time and electrical stimulation, such that unstimulated meat was tougher (as measured by shear force) from animals held in lairage for 2 days compared with those held for 1 day of lairage, with no effect in stimulated meat. In balance, it seems the high pH effect due to preslaughter factors is the most important in cattle, in terms of subsequent tenderness.

12.1.4 Postslaughter factors

Within a given muscle, where amounts and type of connective tissue are constant, there can be considerable differences in tenderness caused by postslaughter conditions. The most immediate of these is PM glycolysis.

12.1.4.1 Postmortem glycolysis

After death, muscle filaments are in a continuous state of contraction and relaxation, and as glycolysis proceeds, glycogen levels drop, ATP is depleted, and the filaments enter *rigor* and a contracted state. This does not occur across all muscles simultaneously with a concomitant fall in pH, and Jeacocke (1984) showed for single fibers that there was a contracture as the final ATP disappeared (i.e., *rigor*), and each fiber had its own time course depending on initial glycogen. A small temperature-dependent degree of contracture occurs for each muscle fiber as it enters *rigor. Rigor* is a term applied to individual muscle fibers becoming depleted of ATP, whereas *rigor mortis* is a term that refers to the muscle stiffness that occurs after all muscle fibers enter *rigor* at which point tenderization is considered to commence (Devine and Graafhuis, 1995). The onset of the sequential progression into *rigor* for each muscle fiber can be tracked by measuring isometric tension and muscle shortening (Hertzman et al., 1993) where the development of isometric tension increases at higher temperatures (Hertzman et al., 1993). Above 10–15°C, the traces reveal a steady increase in tension *pre-rigor* and continuous shortening. Such traces can be interpreted as arising from a succession of individual muscle fibers, each exhausting their energy reserves (i.e., *rigor*) and each separately shortening (Jeacocke, 1984); these sum to create tension. Stiffness is a consequence of each single fiber going into full *rigor*, with irreversible cross-bridge formation of the contractile components, actin, and myosin, a reflection of myosin molecules being devoid of ATP leading to the formation of the actomyosin complex. With increasing numbers of fibers entering *rigor*, the stiffness increases and is significant when the muscle reaches

a pH of approximately 6.0. At some stage the bulk of stiff muscle prevents significant shortening of fibers not in *rigor*, from a cold contracture (if exposed to low temperatures). Given these processes, the rate of *rigor* onset has a real impact on subsequent tenderness.

The degree of shortening, or of tension development, during the onset of rigor mortis in muscle, which is free to shorten, is a direct function of temperature down to about 15°C (Locker and Hagyard, 1963). If such isolated muscle is exposed to temperatures lower than about 12°C at this time, there is again an increasing tendency to shorten—it being similar at 2°C as at 40°C—and this is associated with decreased tenderness on cooking (see Chapter 7). As the temperature falls below 12°C, a *prerigor* contracture (cold shortening) takes place until *rigor* is completed. This arises from increased cellular calcium from the sarcoplasmic reticulum with falling temperature that, in turn, activates actomyosin ATPase. The rise of calcium in the sarcoplasm under these conditions is due to the failure of the sarcoplasmic reticulum to sequester sarcoplasmic calcium (Jaime et al., 1992) and the release of calcium from mitochondria, when ATP concentration is not limiting (>3.5 μM), due to anoxic and cold conditions (below 15°C) causing inactivation of the ATP-driven calcium pump (Honikel and Hamm, 1978). On exposure of excised, *prerigor* muscle to temperatures, which cause cold shortening, the degree of toughness in the cooked meat increases as the degree of *prerigor* shortening increases from 20% to 40% of the initial length; thereafter, as the degree of shortening increases to 60%, toughness once more decreases (Marsh and Leet, 1966). Shortening up to 40% of initial length signifies a greater degree of cross-linking of actin and myosin and is reflected by a greater degree of toughness in the meat. Electron micrographs show that, in such muscles, the ends of the myosin filaments buckle against (or pierce) the *Z*-lines. The decrease in toughness observed in cooked muscles, which have shortened beyond 40% of their initial length, may signify disruption or tearing of the structure (Marsh and Leet, 1966). Indeed, electron micrographs have indicated that the phenomenon is probably due to the fracturing of certain sarcomeres by those that have severely shortened and the rupture of the Z-disks by proteins, such as myosin (Voyle, 1969). Later studies (Marsh et al., 1974) by electron microscope revealed that, with shortening greater than 50%, a series of nodes developed in the fiber in agreement with the findings of Voyle (1969). These were regions of supercontraction: between them there was fracturing of the fiber, which appeared sufficient to account for the decreased toughness. Supercontractions have been also observed in electrically stimulated muscle (Hwang et al., 2003).

Converse to the "cold-shortening" effect is what can be called heat toughening/shortening due to low pH and high temperature during *rigor* development. This effect has been reported in cattle (Warner et al., 2013) where it is defined as a pH below 6 with the temperature at 35°C or greater. Indeed, the speed of glycolysis under these conditions may lead to an exudative condition in beef, somewhat comparable to pale soft exudative (PSE) in the pig, particularly in the

deep hindquarter muscles and thus limit aging potential. This can be avoided by removal of the meat from the hot carcass and chilling, which could include very fast chilling (Jacob and Hopkins, 2014), but this is not applicable for all situations. Other potential strategies, such as immersion cooling, vascular flushing, or fat trimming, are discussed by Jacob and Hopkins (2014). Where the rate of pH fall is inordinately fast, as with the PSE condition in pigs, sarcoplasmic proteins are denatured and precipitate on to those of the myofibrils; and the latter are also denatured to some extent since they become less soluble, and the surface hydrophobicity is increased and the myofilament lattice spacing reduced in these circumstances (Liu et al., 2016).

Under conditions likely to lead to cold-induced shortening electrical stimulation (ES) has a role. ES involves passing an electric current through the body or carcass of freshly slaughtered animals. This electric current causes the muscles to contract increasing the rate of glycolysis resulting in an immediate fall in pH (ΔpH that ranges from 0.6 pH units at 35°C to 0.018 units at 15°C) (Hwang et al., 2003). In recent years, there has been development of new ES technology in Australia that has enabled retrofitting of units in established abattoirs. This technology applies electricity with short square pulses and operates at medium voltages overcoming the safety concerns of high-voltage systems (Devine et al., 2014).

The *extent* of PM glycolysis, apart from its rate, also has an effect on the tenderness of pork, beef, and lamb (Lewis Jr. et al., 1962; Bouton et al., 1973b; Watanabe et al., 1996), and it has been amply confirmed in beef (Purchas et al., 1999) and lamb (Devine et al., 1993) that tenderness is least at ultimate pH values between 5.8 and 6.2. As the ultimate pH increases from 5.5 to 6.0, tenderness appears to decrease; at ultimate pH levels above 6, however, tenderness increases once again (Fig. 12.1).

Watanabe and Devine (1996) subsequently showed that the breakdown of titin and nebulin is minimal at an ultimate pH in this region and concluded that one effect of ultimate pH on tenderness is exerted through its action on proteolytic enzymes. However, the study of Purchas et al. (1999) clearly showed that a combination of sarcomere length and proteolysis did not fully explain the curvilinear relationship. This conclusion was based on examining the relationship in unaged and aged beef meat.

Both shear force and adhesion, as respective measures of the contributions of myofibrils and connective tissue to toughness, decrease as the ultimate pH rises (Bouton et al., 1973a). In the region of pH 6.8, tenderness becomes excessive and is associated with a jelly-like consistency in the meat. The exact tenderness/pH relationship varies between different muscles. For example, a curvilinear relationship was reported for the *biceps femoris* and a linear one for the *semimembranosus* (Bouton et al., 1971), but either way tenderness improves above a pH of approximately 6.0. If *prerigor* meat is heated quickly enough, so that the enzymes effecting PM glycolysis are inactivated faster than the heat can accelerate their activity, a high pH will result. If it is of the order of 7, this would be expected

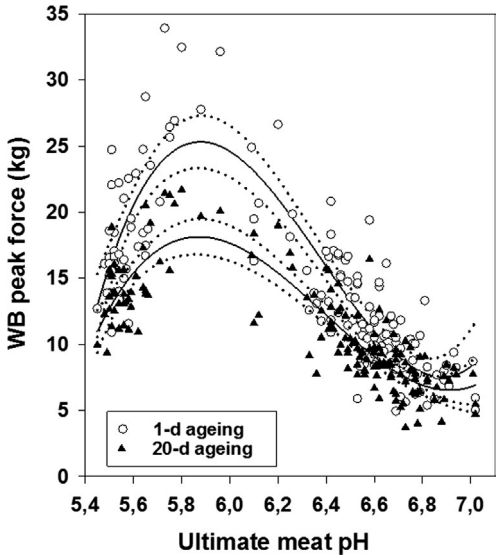

FIG. 12.1 Relationship between shear force and ultimate pH of bovine *longissimus et lumborum* muscles aged for 1 or 20 days. *WB*, Warner-Bratzler. *(From Purchas, R.W., Yan, X., Hartley, D.G., 1999. Meat Sci. 51, 135; reproduced by kind permission of Professor Roger Purchas and Elsevier Science Ltd.)*

to enhance tenderness (Bouton et al., 1971) to a much greater extent than the shortening of the excised muscle would diminish it (even though such shortening during cooking is especially severe with *prerigor* meat). It has been shown, in fact, that the relative tenderness of *prerigor* cooked meat is directly related to the level of pH, which has been attained at the moment of cooking (Miles and Lawrie, 1970). The enhanced tenderness may be a reflection of the greater water content and water-holding capacity of the muscle proteins (see Chapters 5 and 14) and of the increased filament spacing of the muscle fibers at high pH. Some of the tenderness of *prerigor* meat can be similarly explained. Changes in the distribution of water between the intracellular environment and extracellular spaces may also be a factor, additional to those of myofibrillar contraction and the nature and orientation of connective tissue in determining tenderness. The water-holding capacity of meat increases for at least 2 pH units on each side of the isoelectric point of the muscle proteins. It is evident that a high pH, at least within the physiological range, is associated with enhanced tenderness; it has now been shown that pH values on the acidic side of the isoelectric point are also associated with increased tenderness (Gault, 1985). Such pH values would not be encountered naturally, of course, but would occur during the manufacture of brine-treated products.

12.1.4.2 Muscle stretching

Since Locker (1960) discovered the relationship between sarcomere shortening and tenderness, many studies have investigated possible techniques to

TABLE 12.5 Warner-Bratzler shear values (*N*) for muscles measured at 2–3 days *Postmortem* obtained from sides of beef hung by either the Achilles tendon or aitchbone.

	Method of suspension	
Muscle	Achilles tendon	Aitch bone
Longissimus lumborum	107.9	55.9
Vastus lateralis	86.3	53.0
Semimembranosus	82.4	50.0
Gluteus medius	78.5	39.2
Biceps femoris	63.7	65.7
Infraspinatus	62.3	58.8
Semitendinosus	59.8	58.8
Psoas major	35.3	49.0

Adapted from Bouton, P.E., Fisher, A.L., Harris, P.V., Baxter, R.I., 1973c. J. Food Technol. 8, 39.

increase sarcomere length and hence improve meat tenderness. Of the methods, Tenderstretch is the oldest researched method and is achieved by suspending carcasses from the aitchbone (*obturator foramen*) in split carcasses or the pelvis in whole carcasses as they come off the slaughter chain. This places increased tension on major leg muscles and loin muscles, before they pass through rigor (Hopkins, 2014), and prevents the shortening of sarcomeres and thus reduces the overlap between actin and myosin. This technique has a dramatic effect on muscles, such as the *longissimus* and hind leg muscles, such as *vastus lateralis* and *semimembranosus* (Table 12.5) but increases the toughness of the *psoas major*, an effect observed across species (i.e., beef, sheep, and alpaca). If the hind leg is weighted at the same time, further increases in sarcomere length can be achieved in the *longissimus* (Hopkins et al., 2000), leading to a further reduction in toughness. This response could also be partly attributed to disruption of the I-band proteins.

The improvement in tenderness is so dramatic that the need for prolonged aging is virtually eliminated in a number of muscles; in addition, the variation in tenderness along the *longissimus* muscle is reduced. Commercial adoption of this technique has seen resurgence as processors have developed ways to handle and store tender-stretched carcasses, such as adopting methods to rehang carcasses from the Achilles tendon after attainment of rigor and streamlining the processing of carcass movement and boning. Fisher et al. (2000) demonstrated that pelvic suspension of pig carcasses markedly increased tenderness and enhanced the brine uptake and yield, of hams prepared from the *semimembranosus*

and *gluteobiceps* muscles, without adversely affecting the quality of those from *biceps femoris*. More recently, a process called "Tendercut" has been developed in the United States (Claus et al., 1997). This technique involves severing of bones and connective tissue to enable the weight of the carcass to stretch selected muscles prior to the onset of rigor while the carcass is still suspended by the Achilles tendon. It only affects tenderness in the loin region and thus has reduced applicability. Compared with Tenderstretching, the Tendercut process overcomes the need for additional chiller space and avoids the need to train boners on new cutting lines, but is much more difficult to do on a processing line.

It has been demonstrated that *prerigor* muscle excised from the carcass and held to prevent shortening (e.g., by wrapping; Devine et al., 2002) can provide a potentially economical way to speed up processing and at the same time minimize toughening. This concept was developed largely to prevent the *prerigor* excised muscles from contracting and to therefore mimic the skeletal restraint normally provided by the carcass. Hildrum et al. (2000) reported a significant improvement in tenderness for wrapped hot-boned beef *M. longissimus* after 2 and 9 days of aging. However, Hildrum et al. (2000) in the same study reported that for wrapped hot-boned beef *M. semimembranosus*, there was no significant improvement in meat tenderness observed, probably because of the inability to limit contraction in the much larger and odd-shaped *semimembranosus*. Such an approach does require chilling at a temperature that minimizes rigor shortening. Several different approaches have been developed in recent years.

The Pi-Vac Elasto Pack System, which was first patented in 2001, is a method of tightly wrapping hot-boned muscles in an elastic wrapping material *prerigor* to prevent shortening and toughening of the meat. The system uses a highly flexible packaging sleeve, which is expanded using a partial vacuum to allow the meat to be inserted. Once the vacuum is turned off, the flexible packaging retracts to its normal dimensions. This exerts longitudinal forces on the meat, preventing the contraction of the muscle. Almost all of the oxygen is also forced out of the packaging. The subsequent bound meat product has been labeled TenderBound. This technology can come in three different sizes, and some commercialization of this approach has occurred in Europe. There is limited published data on the performance of the Pi-Vac Elasto Pack System; however, one study using hot-boned beef (*M. longissimus*), which was excised 90 min PM, not only showed an increase in sarcomere length and meat tenderness compared with unrestrained muscle (O'Sullivan et al., 2003), but also reduced the variability in tenderness to provide a more consistent tenderness. An alternate approach to the Pi-Vac system is the SmartStretch technology, which was patented in 2010. The technology uses a flexible rubber sleeve, which is surrounded by four inflatable bladders that are housed within an airtight chamber, and a full description is provided by Taylor and Hopkins (2011).

Extensive research has been undertaken on SmartStretch with mixed results. In sheep a 24% increase in *M. semimembranosus* length resulted in shear force reductions of 46% at 0 day aging and 38% at 5 days aging, and this was matched

with a significant increase in sarcomere length (Toohey et al., 2012a). A further study examined the effect when the muscles were stretched as part of a whole sheep meat hind leg. A 14% increase in leg length resulted in a shear force reduction in the *m. semimembranosus* of just 16% and 18.4% in the *m. biceps femoris* at 0 day aging and no significant difference at 5 days aging (Toohey et al., 2012b). By contrast, these improvements could not be replicated when *longissimus* or *semimembranosus* from old cattle was subjected to SmartStretch, but a study using younger cattle (Taylor et al., 2012) with a dentition score of 2 or less showed significant improvements in sarcomere length and tenderness of hot-boned *M. gluteus medius* at 0 day of aging, but the effect was nullified with aging.

Although prevention of shortening is considered to improve tenderness by reducing the overlap of actin and myosin, in a converse way the increase in toughness due to shortening may not be solely explained by an increase in the interdigitation of myosin and actin filaments (Voyle, 1969). Indeed Bouton et al. (1973a), while confirming that shear values are highly dependent on the degree of myofibrillar contraction in muscles of normal ultimate pH, also noted that adhesion values (which reflect the state of the intrafibrillar connective tissue) are significantly increased in contracted fibers. As an indication that collagen may make a more positive contribution to the degree of toughness in shortened muscle, Rowe (1974) found evidence for alterations in the perimysial connective tissue, which paralleled the degree of shortening of the sarcomeres. As the muscle contracted, the loose configuration of the collagen changed to a well-defined lattice.

12.1.4.3 Aging

It has long been recognized that the tenderness of meat increases when it is aged (e.g., stored at chill temperatures for varying periods), and this is illustrated in Fig. 12.2 as an exponential relationship. It was suggested more than a century ago that tenderization is due to enzymatic activity, but the mechanism that drives this tenderization *postrigor* has demanded significant attention, and several explanations for this process have been proposed (see the next section). The major group of enzymes implicated in this process is the calpains (Hopkins and Geesink, 2009). It has long been clear that muscles contain proteolytic enzymes, which operate much more readily at higher temperatures, and that, in general, higher temperatures cause tenderization to occur in a faster time frame than at lower temperatures. Nevertheless, the *rate* of tenderization decreases gradually as holding temperatures rise from 40°C to 60°C. It then decreases sharply, ceasing altogether at 75°C (Davey and Gilbert, 1976). Proteolysis can continue even in shortened muscle without the meat becoming tender (Locker and Wild, 1984), illustrating the interaction with sarcomere length and demonstrating that ultimate tenderness levels are not determined by only one mechanism (Starkey et al., 2016).

The improvement in tenderness with aging is influenced by the muscle under consideration as shown by Stolowski et al. (2006). In some muscles, there

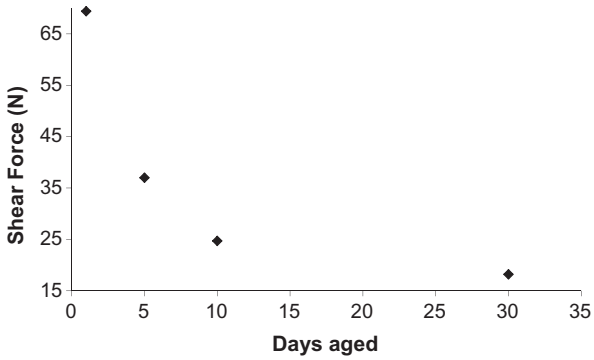

FIG. 12.2 Relationship between shear force and days aged of ovine *longissimus et lumborum* muscles aged for up to 30 days. *(Adapted from Pearce, K.L., Hopkins, D.L., Jacob, R.H., Williams, A., Pethick, D.W., Phillips, J.K., 2009. Meat Sci. 81, 188.)*

is minimal reduction in shear force with aging (e.g., *biceps femoris*: Stolowski et al., 2006), but in the *longissimus*, reductions over 20% in shear force have been reported with aging for up to 42 days in beef, with even greater reductions in lamb when aged for 30 days (Pearce et al., 2009). The rate of the aging effect differs between species, with alpaca *longissimus* showing an 8% reduction in shear force with aging from 5 to 10 days (Smith et al., 2015), in comparison, say, with lamb at 33% over the same period (Pearce et al., 2009). Monin and Ouali (1991) have extensively reviewed the reasons for differentiation between muscles in the rates and extents of aging, which they undergo PM (see Chapter 5). In general, connective tissue is stable PM although after extended periods of storage, IMCT does show signs of structural changes. In fact, Nishimura et al. (1998) described a method to examine the mechanical strength of IMCT and showed that after 10 days of aging, there was a decrease in strength of this tissue in beef meat.

Related to the degradation of myofibrillar proteins is an increase in the extractability of perimysial collagen, but it is not clear that this contributes to improvements in tenderness per se.

12.1.4.4 Proteolytic mechanisms

Proteolysis is the main factor that drives the tenderization during aging. After slaughter, some proteases continue to be active, and others become activated due to changing conditions in the postmortem muscle. Hence, postmortem proteolysis is a multienzymatic process (Ouali et al., 2013). As a result, muscle proteins are degraded, ultimately resulting in increased meat tenderness. The largest effect on meat tenderness comes from the degradation of myofibrillar proteins or other proteins associated with myofibrils. Protein degradation is overall not very extensive; however, some myofibrillar proteins are significantly degraded (Hopkins and Thompson, 2002a). The reactions involved are complex and not fully elucidated.

Myofibrils are weakened during postmortem storage, and they are therefore more easily broken into shorter segments upon mechanical homogenization of meat. The myofibril fragmentation index (MFI) has been developed as one measure of the weakening due to proteolysis (Møller et al., 1973) and has been used as a predictor of meat tenderness. Electron micrographs of myofibrils used in MFI assays have shown that the rupture due to shearing forces of homogenizer blades occurs in the I-band rather than at the *Z*-disks. Some early studies suggested that Z-disk degradation is one of the major factors that contributes to meat tenderization. However, the proteolytic degradation associated with tenderization is manifested as a change in the muscle ultrastructure, including breaks at the junction of the I-band and the Z-disk or just adjacent to the Z-disk at N2 lines (Taylor et al., 1995). Available evidence suggests that something besides or in addition to Z-disk degradation is involved in postmortem tenderization. Costameres are filamentous structures that link myofibrils to the sarcolemma and are involved in force transduction in muscle contraction. Hence, degradation of proteins within costameres may weaken the muscle structure. Costameres contain proteins such as vinculin, talin, desmin, and vimentin, and proteins that constitute the costameres are degraded early postmortem. Intermediate filaments are other filamentous structures, and they surround the Z-disks and link adjacent myofibrils at the area of the Z-disk. A main protein of the intermediate filaments is desmin, and intermediate filaments are degraded during cold storage, and their degradation has been linked to increased water holding of myofibrils (Zeng et al., 2017) and reduced drip loss of pork (Yang et al., 2021). In addition to costameres and intermediate filaments, degradation of a third cytoskeletal structure, the giant protein titin, has been associated with increased meat tenderness. Titin is anchored at its N-terminal end in the Z-disk and extends through the I-band into the A-band area where it reaches the M-line. Titin and nebulin are constituents of the N2 line, and both are susceptible to proteolytic degradation, and degradation of these proteins is believed to result in myofibril fragmentation and ultimately in meat tenderization (Huff Lonergan et al., 2010). Low-shear-force beef has been associated with degradation of the proteins titin, nebulin, filamin, desmin, and troponin-T (Huff-Lonergan et al., 1996), and results suggest that the calpain system plays a key role in the postmortem protein degradation (Hopkins and Thompson, 2002b).

Among proteases investigated for their possible role in postmortem proteolysis and meat tenderization, the calpain system is by far the most studied, followed by lysosomal enzymes (e.g., cathepsins), caspases, and the proteasome (also known as the multicatalytic proteinase complex). A significant number of studies have linked the calpain system to the tenderization process and considered it responsible for the majority of postmortem proteolysis and essential for softening of meat texture during storage (Bhat et al., 2018). The main arguments are that i) they degrade the same proteins that are degraded during postmortem aging, ii) they are able to produce similar degradation products, iii) that high calpastatin activity may inhibit tenderization, and iv) inhibtors of the calpains have

been shown to halt tenderization. Calpain-1 (μ-calpain), calpain-2 (m-calpain), and their endogenous inhibitor calpastatin are the primary candidates to be involved in the process. Calpain-1 and -2 are relatively specific for their substrates; they often cleave them at only a few sites and do not completely degrade the substrates to small peptides. Calpains have pH optimum around 7.5 and require Ca^{2+} to become active. Calpain-1 needs 3–50 μM Ca^{2+} for half-maximal activity, while calpain-2 needs 400–800 μM Ca^{2+} (Goll et al., 2003). Upon activation by Ca^{2+}, the catalytic subunit of calpains will undergo a conformational change and autolyze. As a result, the catalytic subunit of calpain-1 degrades from 80 to 76 kDa through a 78 kDa intermediate form, whereas the catalytic subunit of calpain-2 is reduced to 78 kDa. Calpastatin inhibits calpains by binding to the enzymes and thereby forming a calpain-calpastatin complex. The binding of calpastatin to calpain prevents proteolytic activity and occurs only after activation of calpain by Ca^{2+}. The calpains are at the time of slaughter mainly located in the sarcoplasm. The amount of calpain-1 in the sarcoplasm that can be extracted and subsequently activated by Ca^{2+} declines rapidly during postmortem storage (to around 20% after 24 h), whereas the corresponding calpain-2 activity declines more slowly. The decline in extractable activity from the sarcoplasm is related to the postmortem increase in free Ca^{2+} (Lyu and Ertbjerg, 2021). The decline has been ascribed to the following processes: (i) binding of calpain to calpastatin following activation by Ca^{2+}, (ii) association of calpain to myofibrils or other subcellular organelles following activation by Ca^{2+}, (iii) instability of autolyzed calpains resulting in a loss of activity. An increase in free Ca^{2+} in the sarcoplasm will activate calpain-1 early postmortem, but the level to fully activate calpain-2 is not reached even after prolonged cold storage of beef and lamb. In pork, calpain-2 is partly autolyzed during aging (Pomponio et al., 2008), suggesting that calpain-2 contributes to meat tenderization in some species. Autolysis of calpain-2 is accelerated by higher muscle temperatures early postmortem (Pomponio and Ertbjerg, 2012) and by the freezing-thawing process of pork (Zhang and Ertbjerg, 2018) and beef (Colle et al., 2018).

Other proteolytic systems than the calpain system contribute to meat tenderization. Early after death of the animal, the process of apoptosis (programmed cell death) is initiated in the muscles. Apoptosis (Kemp and Parr, 2012) and the function of the antiapoptotic heat shock proteins (Lomiwes et al., 2014) have been suggested to be associated with meat tenderization either directly or indirectly. Caspases are implicated in apoptosis and responsible for the majority of early proteolytic cleavage that leads to cell death. The initial action of caspases is then followed by contribution from other proteolytic systems. There is evidence of an interaction between the caspase and calpain protease systems, and the ability of caspases to replicate some of the postmortem proteolytic changes in myofibrils has been demonstrated, including degradation of desmin and troponin-I and -T (Kemp and Parr, 2012).

Cathepsins belong to a large family of *exo*- and *endo*-peptidases, and several of them have a pH optimum close to that of postmortem meat. Cathepsins are

in vivo located in lysosomes; however, during aging, they are gradually released to the sarcoplasm from where they may contribute to degradation of myofibrillar proteins later postmortem (Ertbjerg et al., 1999). Low muscle pH and high carcass temperature destabilize the integrity of the lysosomal membrane and accelerate the release of the encapsulated proteases. Cathepsin L has been reported to degrade structures in the *Z*-disc and hydrolyzes proteins such as titin, nebulin, α-actinin, myosin heavy chain, troponin-T, and troponin-I (Mikami et al., 1987). However, experiments using protease inhibitors have suggested that the cathepsins B and L are unlikely to play a major role in proteolysis and tenderization processes at least in the early postmortem period (Hopkins and Thompson, 2001b).

The proteasome is a multicatalytic protease complex (MCP) with high molecular weight and in skeletal muscle is involved in a large proportion of the in vivo protein degradation. MCP consists of two 19S regulatory entities attached to both ends of a 20S multicatalytic barrel-shaped structure housing the catalytic enzyme activities, and together they compose the 26S proteasome. The 26S proteasome degrades proteins ligated to several units of ubiquitin. The tagging of proteins to ubiquitin requires ATP; however, after depletion of ATP, the proteasome complex dissociates into the 20S and 19S components. Protein substrates of the dissociated 20S proteasome do not need to be tagged with ubiquitin, and hence, ATP is no longer required. The proteasome activity in bovine muscle declines slowly postmortem and has been shown to remain in substantial amounts after 7 days of cold storage (Lamare et al., 2002). An inhibitor of proteasome activity was able to prevent degradation of myofibrillar proteins such as nebulin, myosin light chain, and troponin-T (Houbak et al., 2008), and a postmortem sequential degradation of structural proteins was suggested, where calpain initiates the degradation and thereby allows the proteasome to act later.

In summary, current evidence suggests that early postmortem proteolysis is caused by calpain-1 and its interaction with caspases. After this stage, calpain-2 and other proteolytic systems intervene with contributions from cathepsins, 20S proteasomes, and possibly other proteolytic systems not yet investigated in detail.

12.1.4.5 Chemical intervention

Recognition that certain plants, fungi, and bacteria produced nontoxic proteolytic enzymes was followed by their incorporation into commercial meat tenderizers. These were first used as dips. As such, they were somewhat unsatisfactory since they over tenderized the surface, producing a mushy texture (and sometimes unusual flavor), and since they were unable to penetrate within the meat, left the interior unaffected. Different methods have been used to disperse solutions throughout meat, and the use of needling machines has been shown effective (Toohey et al., 2011) and the most practical. An alternative approach applied by Han et al. (2009) was to infuse carcasses *prerigor*, in this case with

a kiwifruit juice extract, thus avoiding the issues associated with infusing live animals, which is an impractical approach.

There are a number of plant-derived enzymes that have been shown to degrade meat protein; these include papain (from paw paw), bromelain (from pineapple), ficin (from fig), and actinidain (from kiwifruit), and then there are several enzymes derived from bacteria or fungi. The action of these has been extensively reviewed (Bekhit et al., 2014), and thus limited details will be provided here. The challenge with the utilization of these enzymes is to control the extent of their action, for enzymes such as papain can easily result in meat having a mushy texture as they can indiscriminately degrade collagen and myofibrillar proteins. To this effect, recent work has focused on characterizing the temperature and pH conditions required for these enzymes to exhibit optimal activity and identifying the individual proteins that are degraded (Ha et al., 2013). For example, Ha et al. (2013) reported that bacterial protease G and the fungal 31 K protease preparations were similar in their ability to hydrolyze collagen, whereas a lower specific activity was obtained for a fungal 60 K protease preparation, but at the same time the latter protease had a high specificity for collagen type 1. In contrast, protease G hydrolyzed most myofibrillar proteins in a nonspecific way, and papain degraded proteins, such as titin, nebulin, actin, and isomers of myosin, and this is the reason this enzyme has such a dramatic effect of meat structure and thus tenderness. Actinidain was found to be more effective at hydrolyzing beef myofibril proteins than zingibain (from ginger), which was more effective at hydrolyzing connective tissue proteins (Ha et al., 2012). There are now commercial plant-derived enzyme products available for industrial use that are based on actinidain (Toohey et al., 2011), and there is potential for combining proteases for targeting specific tenderizing applications. In a recent study using alpacas, the concept of combining injection of meat with actinidain, along with the application of both electrical stimulation and tenderstretching, was examined (Biffin et al., 2020). This showed in the *longissimus* aged for 5 days that electrical stimulation and tenderstretching combined reduced shear force by more than 18%, and when actinidain was injected into the meat, the reduction was more than 40%. Interestingly, the improvement as evident by a reduction in shear force from injection of actinidain did not translate when the meat was subjected to consumer testing, and it seems there was some interaction with the other treatments, which the consumers did not react positively too, given improvements in sensory traits in beef normally processed have been reported from the injection of actinidain (Lees et al., 2019).

Prerigor injection of high ionic solutions creates an atypical environment within the muscle, and this may in itself result in premature membrane rupturing and the release of endogenous enzymes. Isolating the mode of action of ion injection on tenderization has been a major challenge since ions such as Ca^{2+} can cause shortening when injected *prerigor* as well as activate endogenous enzymes. When meat is infused with calcium chloride solutions PM, enhanced tenderness has resulted, and this has been attributed to stimulation of calpains

during subsequent conditioning (Whipple et al., 1994), even when such infusion does not occur until 24 h PM (Boleman et al., 1995). A summary of the results of infusing meat with ions is provided by Hopkins and Bekhit (2014). Farouk et al. (1992) showed that a solution could be administered to lamb carcasses by vascular infusion at 10% by weight, and this changed the rate of glycolysis and the extent of muscle shortening. Further work (Farouk and Price, 1994) used the same infusion process and revealed that infusion of a solution containing maltose, glycerin, dextrose, and sodium and potassium tripolyphosphates lowered the temperature by ~2°C compared with noninfused carcasses during the early PM period (3 h), but also increased the rate of pH decline. Glycolysis was completed in 6 h compared with 12–24 h for the control, and so under rapid chilling conditions, this should provide protection against cold-induced shortening.

A number of organic acids such as acetic, citric, and lactic have been used to tenderize meat. Administration is often by marination, but this requires extended storage periods for penetration into the meat. A number of studies have investigated the efficacy of these acids after injection into meat. These acids cause a significant decrease in pH, creating an environment that is optimal for cathepsin activity, which is confirmed by the degradation of proteins such as myosin, a protein that is not significantly degraded under normal (control) PM conditions. Associated with the drop in pH is swelling of the meat. Another effect of this treatment is a weakening/solubilization of perimysial collagen and a lowering of the temperatures required for denaturation of connective tissue. Overall organic acids lead to an increase in tenderness (Table 12.6).

The impact of the infused compounds on meat quality will be greatly dependent on the PM time of the treatment (reflecting the pH and the temperature

TABLE 12.6 Sensory assessment (ease of first bite and chewiness; 1 = extremely tough to 10 = extremely tender) for beef *M. Pectoralis profundus* treated with 0.5 mol/L lactic acid at either 1 or 24 h *Postmortem* and aged for 2 or 14 days.

	Lactic acid (1 h)	Lactic acid (24 h)	Control	Aging period (days)
Ease of first bite	6.7*a*	7.4*a*	4.3*b*	2
Chewiness	6.0*a*	6.3*a*	5.3*a*	2
Ease of first bite	6.0*a*	6.4*a*	4.2*b*	14
Chewiness	5.7*a*	6.1*a*	4.4*b*	14

Means followed by a different letter (*a*, *b*) within a row are significantly different ($P<0.05$).
Adapted from Berge, P., Ertbjerg, P., Larsen, L.M., Astruc, T., Vignon, X., Mølle, A.J., 2001. Meat Sci. 57, 347.

of the meat), the concentration of the infused compounds (level of activation or modification), and the method of infusion (the distribution of the compounds in the meat).

12.2 Measuring tenderness

As outlined in the introduction, tenderness of meat can be assessed either subjectively using a trained sensory panel or with consumers. Scrutiny of the literature reveals a range of scales and sampling approaches that have been used for the former application. This approach has application if small differences in attributes between samples are to be detected (AMSA, 2016), but the responses may not reflect the wider consumer base. The focus on consumer panels has increased significantly with the development of MSA, which was developed by the Australian red meat industry to improve the eating quality consistency of beef (Polkinghorne et al., 2008) and sheep meat (Russell et al., 2005). This is because the current system is based on 1.2 million consumer taste tests by over 170,000 consumers (MLA, 2020) from 12 countries, which makes it the largest database in the world. The MSA system was designed to account for all factors that affect eating quality from the paddock to the plate, but was predicated on the use of consumers as the arbitrators of eating quality of which tenderness is an important trait.

There is no doubt this approach has to cope with wider variance because the panelists are not carefully trained, but the philosophy was to use the demographic that consumes the meat. Variables such as cooking method have been shown to impact on results (Thompson et al., 2005b) as have the age, gender, the number of adults in the household, and the degree of cooking (Thompson et al., 2005a). This methodology has been applied to other species, such as alpaca (Smith et al., 2015).

The objective measurement of tenderness is dealt with by Purchas (2014) as outlined in the introduction. Although testing samples fresh is deemed the most appropriate (AMSA, 2016), this is often impractical and because thawing will effectively allow meat to age, potentially at different rates depending on the thawing rate cooking from freezing overcomes these issues, and this approach has been applied for a large number of samples (Hopkins et al., 2015). Even with the application of the same protocols between laboratories, differences in absolute values will occur (Hopkins et al., 2010), and a recent publication has highlighted the range of protocols that have been used (Holman et al., 2016) and suggests a need for more standardization. This overview compiled details from articles ($n = 734$) published in peer-reviewed animal and food science journals and limited to only those testing the shear force of unprocessed and nonfabricated mammal meats. It was found that *M. longissimus* samples were the most tested (55.2%), followed by the *M. semimembranosus* (6.4%), and the most used tenderometer was that manufactured by Instron (31.2%), and the most popular blade was the Warner-Bratzler (WB) (68.8%) (Holman et al., 2016).

Round cores were the most popular for testing followed by cuboidal strips, but the round cores are not as suitable for testing of lamb meat, which has much smaller muscles, and in this case cuboidal strips are preferred (Hopkins et al., 2015). The slice shear force method developed for beef uses a flat blade different to the WB, and there is some evidence this has a stronger relationship with sensory panel tenderness ratings (Shackelford et al., 1999). Development of this relationship is important for it allows the significance of treatments within studies, which only measure shear force to be related to consumer levels of acceptability. Such thresholds have been proposed for lamb (Hopkins et al., 2006) and beef (Aalhus et al., 2004).

12.3 Conclusions and future trends

With improvements in the tracking of individual animals through the production process and integration of this with the use of known genetics, there is scope to improve the tenderness of meat especially from extensively raised animals, by providing informative feedback to producers, and in some countries, this is now a reality. Future developments to improve the tenderness of meat are likely to embrace combinations of methodologies postslaughter. For example, there is scope to combine electrical stimulation, stretching, and injection of muscles with proteolytic enzymes to improve the tenderness of meat, and there are commercial examples where this has been applied. Currently, there is some knowledge that must be obtained to maximize such an approach, particularly when the inherent differences in muscle are considered, but the accumulated knowledge about the causative enzymes driving proteolysis has laid a solid foundation for further improvements as the "biological variation" is manipulated.

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Chapter 13

The eating quality of meat: III—Flavor

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13.1 Aroma and taste compounds

Flavor is a complex sensation that involves the combination of olfactory, gustatory, and trigeminal sensations, so it is named as a multifaced sensory experienced when the food is placed in the oral cavity (Small, 2012). Many sensations have an impact on flavor, taste, smell, color, texture, sound, irritation, and temperature, but it has been proposed that the combination of taste and smell is unique. The existence of five basic tastes is well accepted: bitter, sour, sweet, salty, and umami. Umami, the fifth taste, is known as the sensation produced by glutamate that makes the food delicious although the presence of substances such as 5′-ribonucleotides enhances the response (Wu et al., 2021). In mammalians, the identification of the taste receptors for bitter, sweet, and umami has produced advancement in the understanding of taste evolution (Liman et al., 2014). On the other hand, sour and salty are considered mineral tastes as the stimulus is produced by a simple element being sour taste elicited by acidic pH and organic acids and salty taste by sodium concentrations. Anyway, the known taste receptors for bitter, sweet, and umami have been shown to be expressed in many cell types and not restricted to gustatory organs (Robino et al., 2021). In this way, these receptors will contribute to the ability to sense. In addition to the five tastes, a second category of flavor enhancers has been defined to describe palatability named with the term Kokumi that represents a delicious mouthfeel, continuity, and complexity (Kuroda and Miyamura, 2015). Moreover, a calcium-sensing receptor has been related to the perception of kokumi substances. Several studies are focused on determining the ability of the gustatory system to detect the presence of other compounds such as fats or trying to separate the gustatory effect of lipids from oral and odor modalities (Liu et al., 2016).

It has long been a matter of common observation that the organoleptically desirable taste and odor of meat develop on cooking. The taste of raw muscle is bland, being only slightly sweet, salty, sour, or bitter, according to its biochemical state and origin. The composition of the meat affects flavor perception as it

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contains molecules responsible for elucidating basic tastes but also will release flavor molecules during cooking and eating (Mottram, 1998). In this respect, the peptides and amino acid content, free fatty acids, nucleotides are per se contributing to the five tastes and act as flavor precursors during the biochemical reactions involved in cooking and meat processing. In addition, the degradation on heating of thiamine (vitamin B1), glycogen, sugars, and organic acids affects meat flavor.

Several L-amino acids contribute to sweet (Ser, Gln, Gly, Thr, Ala, Val, Met, Lys, Pro, Cys) and sour (Thr, Asp, Glu, Asn) tastes while generally hydrophobic amino acids contribute to bitterness (Tyr, Val, Met, Trp, Phe, Ile, Leu, His, Arg, Lys, Pro, Cys) (Kawai et al., 2012). Nevertheless, the important contribution of glutamate to umami taste has been widely studied in terms of the natural products responsible for its taste and the taste receptors involved (Nishimura et al., 2016; Wu et al., 2021). Not only L-glutamic acid, its monosodium salt, L-aspartic acid, succinic acid, tartaric acid, and 5′-ribonucleotides produce umami taste, several γ-glutamatyl peptides have also been recognized as umami molecules (Kuroda and Miyamura, 2015).

13.2 Volatile compounds generation reactions

It is well established that the flavor of cooked meat is affected by the major precursors, proteins, and lipids, although the volatile compounds formed during processing and cooking are essential for the meat aroma (Mottram, 1998). Several studies have reported the existence of thousands of volatile compounds in different meat species with a high number of compounds identified in beef (Resconi et al., 2012; Vasta et al., 2011; Frank et al., 2016b) and sheep and lamb meat (Vasta and Priolo, 2006; Resconi et al., 2013; Watkins et al., 2013; Du et al., 2020). In the case of pork meat, several studies were focused on cooked pork meat (Elmore et al., 2001; Meinert et al., 2007; Kosowska et al., 2018) while many of them deal with pork meat products (Thomas et al., 2013, 2014, 2015; Benet et al., 2016; Flores and Olivares, 2014; Ozkara et al., 2019). In addition, other studies are dealing with other meat species such as duck (Liu et al., 2019, 2020).

The main reactions involved in the development of cooked meat aroma are the lipid degradation (oxidative reactions), Maillard reactions, Strecker degradation, thiamine degradation, and carbohydrate degradation reactions. The relationships among these reactions are shown in Fig. 13.1. These reactions have been supported and extended by more specific chemical studies of heat-induced changes in amino acids, carbohydrates, and fats, both in isolation and in mixtures (Campo et al., 2003; Vermeulen et al., 2005).

13.2.1 Lipid degradation (oxidative reactions)

Fats or fat-soluble precursors were also shown to be implicated in accounting for species differences and in contributing generally to meat flavor. There are,

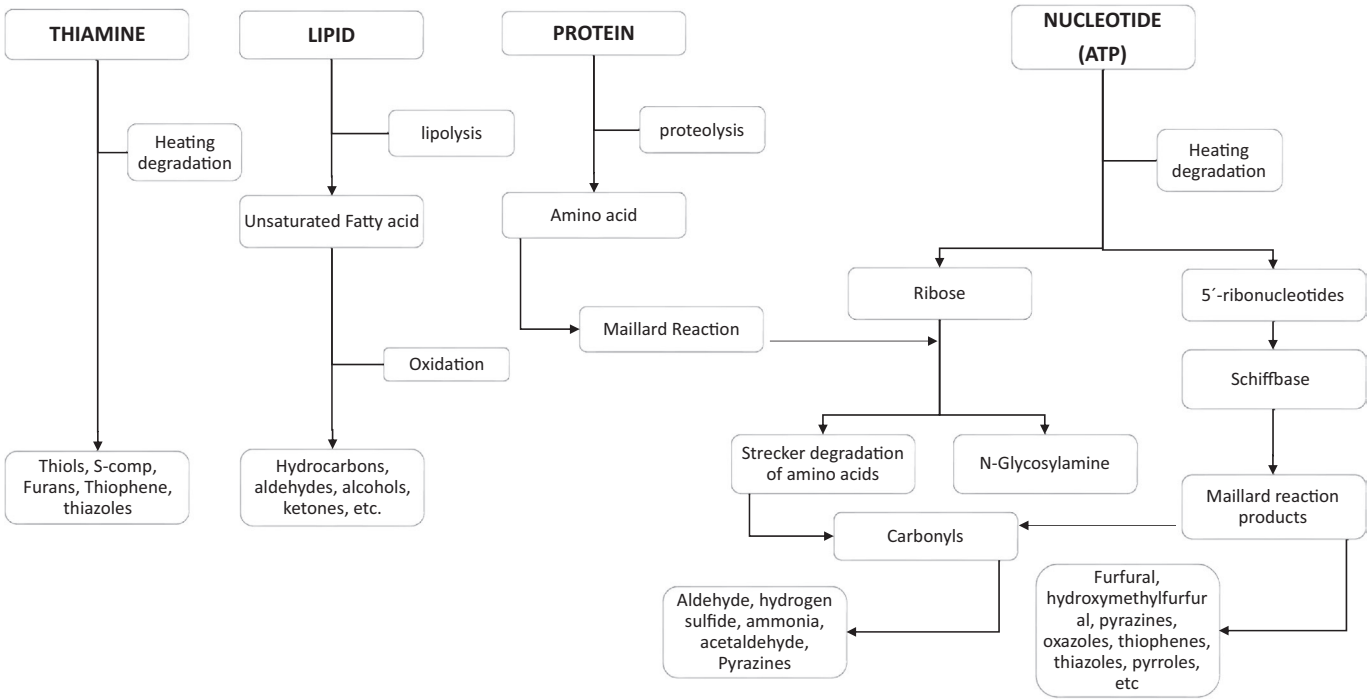

FIG. 13.1 Biochemical reactions involved in the development of cooked meat aroma.

of course, considerable differences between species in intramuscular fat (see Chapter 4). The oxidative reactions during cooking of unsaturated fatty acids produce the generation of a wide number of volatile compounds, aldehydes, ketones, alcohols, aliphatic hydrocarbons, acids, and esters (Mottram, 1998). The induced oxidation of unsaturated fatty acids is responsible for the cooked meat aroma, development of rancid notes during storage periods, and cured aroma of meat products (Toldrá and Flores, 2007). Those unsaturated fatty acids with more than two double bonds are rapidly oxidized acting as regulators of the meat shelf life (Wood et al., 2004). Thus, phospholipids with a higher polyunsaturated content than triacylglycerols are more susceptible to oxidation and therefore important for meat flavor development.

The importance of phospholipids rather than triglycerides in explaining the contribution of lipids to cooked meat flavor has been shown by Elmore et al. (1999). Removal of the intramuscular triglycerides had relatively little effect on the pattern of volatiles, but subsequent removal of the phospholipids caused a loss of aliphatic aldehydes. There was also a marked decrease in pyrazines, which suggests that, in cooked meat, lipids are normally involved in Maillard reactions and inhibit the production of pyrazines. Using model systems, Campo et al. (2003) assessed the importance of oleic, linoleic, and alpha-linolenic acids, with or without the presence of cysteine and ribose, in relation to the development of odor in cooked meat. "Fishy" notes were experienced only with mixtures including linolenic acid, an effect exacerbated by the presence of ferrous iron.

Many carbonyl compounds have been identified as meaty odorants in oven roast beef (Rochat and Chaintreau, 2005), cooked pork (Elmore et al., 2001), goat meat (Madruga et al., 2009), and sheep meat (Bueno et al., 2011; Watkins et al., 2013). They have not been considered as essential contributors to meat aroma due to their high odor threshold values in comparison to heterocyclic compounds although they contribute to specific aroma notes: rancid, grass, green, citrus, fried, fatty, depending on chemical structure (See Fig. 13.2). Traditionally it has been believed that the full flavor of meat cannot be developed without its associated fat, but the possible health hazards of the ingestion of fat have led producers to rear leaner animals.

13.2.2 Maillard reactions and Strecker degradation

The Maillard reaction between a reducing sugar and amino compound produces a high number of volatile compounds in thermally processed foods. This reaction is favored by high temperatures and low moisture and results at the first stages in the generation of furanones, furfurals, dicarbonyl compounds, and others. Furthermore, reactions of the compounds with reactive compounds such as amines, amino acids, hydrogen sulfide, thiols, ammonia, lead to the generation of heterocyclic compounds (pyrazines, oxazoles, thiophenes, thiazoles) with low-odor threshold and high aroma impact (Mottram, 1998; Toldrá

FIG. 13.2 Volatile components of cooked meats characterized as main meaty odorants in different species (beef (B), pork (P), lamb (L), Goat (G) and duck (D)). References: 1. Farmer and Patterson (1991), 2. Cerny and Grosch (1992), 3. Rochat et al. (2007), 4. Madruga et al. (2009), 5. Watkins et al. (2013), 6. Bueno et al. (2011), 7. Kerscher and Grosch (1998), 8. Rochat and Chaintreau (2005), 9. Du et al. (2020), 10. Thomas et al. (2014), 11. Frank et al. (2016a,b), Kosowska et al. (2018).

and Flores, 2007). The Strecker degradation of amino acids by dicarbonyl compounds originated from Maillard reaction produces a deamination and decarboxylation of the amino acid. This reaction results in an aldehyde with fewer carbons than the original amino acid and an alpha-aminoketone. The compounds produced by the Strecker degradation of several amino acids have been reported as aroma contributors in different cooked meats (Table 13.1). The degradation of sulfur containing amino acids produces important reactive intermediates (hydrogen sulfide, ammonia, acetaldehyde) for sulfur compounds formation. The contribution of Maillard and Strecker reactions to odor notes is related to roasted, toasty, caramel, fried, potato, meaty, etc., odors (See Fig. 13.2). In the case of Strecker degradation of methionine, it produces methional. The

TABLE 13.1 Aldehydes derived from amino acid degradation and detected by GC-O (gas chromatography olfactometry) in different cooked meat species.

Amino acid	Aldehyde	Odor threshold (mg/kg)[a]	Odor description[b]	Pork references	Beef references	Lamb references
Leucine	3-Methylbutanal	0.009	Ethereal, peach, fatty	Thomas et al. (2013) and Ramírez et al. (2004)	Rochat and Chaintreau (2005), Guth and Grosch (1994), Resconi et al. (2012) and Vasta et al. (2011)	Vasta et al. (2007) and Resconi et al. (2010)
Isoleucine	2-Methylbutanal	0.003	Musty, chocolate, nutty	Ramírez et al. (2004)	Rochat and Chaintreau (2005), Guth and Grosch (1994) and Resconi et al. (2012)	Vasta et al. (2007) and Resconi et al. (2010)
Valine	2-Methylpropanal	0.04	Fresh, aldehydic, floral, pungent	Thomas et al. (2013)	Rochat and Chaintreau (2005), Guth and Grosch (1994) and Resconi et al. (2012)	Vasta et al. (2007) and Resconi et al. (2010)
Phenylalanine	Phenylacetaldehyde	0.004	Honey, floral rose, sweet	Ramírez et al. (2004)	Rochat and Chaintreau (2005), Guth and Grosch (1994) and Vasta et al. (2011)	Vasta et al. (2007)
Alanine	Acetaldehyde	0.025	Pungent, ethereal aldehydic fruity	Thomas et al. (2013)	Rochat and Chaintreau (2005) and Guth and Grosch (1994)	Vasta et al. (2007)
Threonine	Propionaldehyde	0.14	Earthy, alcoholic, whiskey, cocoa, nutty	–	–	–
Methionine	3-Methylthiopropanal	0.0018	musty potato tomato earthy vegetable	Thomas et al. (2013) and Ramírez et al. (2004)	Rochat and Chaintreau (2005), Guth and Grosch (1994), and Resconi et al. (2012)	Vasta et al. (2007)

[a] *Odor threshold in water by Van Gemert L and Nettenbreijer A. Compilations of odor threshold values in air and water, Boelens Aroma Chemical Information Services (BACIS), BACIS, Zeist (2004).*
[b] *Odor description by http://www.thegoodscentscompany.com/.*

degradation of this sulfur compound can lead to the formation of hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, widely reported as odor contributors in meat (Fig. 13.2).

13.2.3 Carbohydrate degradation reactions

The carbohydrates of meat are also important in producing flavor on heating. They lose the elements of water in two stages (at 180°C and 220°C), forming furfural from pentoses and hydroxymethylfurfural from hexoses. As indicated, the caramelization of sugars is performed at temperatures higher than those normally found during meat cooking except for surface areas when the meat is grilled or roasted (Mottram, 1998). So, Maillard reactions between sugars and amino acids are the ones involved in aroma development during meat cooking.

13.2.4 Thiamine degradation

Apart from amino acids, carbohydrates, and fats, thiamin appears to be an important precursor of meat aroma as its degradation produces furans, thiophenes, thiazoles, and aliphatic sulfur compounds (Vermeulen et al., 2005). The thermal degradation of thiamin depends on many factors, such as temperature, time, pH, matrix composition, etc., and several studies done on model systems have shown the production of sulfur heterocyclic compounds. The thermal degradation of thiamin produces at least three key odorants in cooked ham including 2-methyl-furanthiol, 2-methyl-3-methyldithiofuran, and bis(2-methyl-3-furyl) disulfide (Thomas et al., 2015). The production of these three compounds has been related to the increase in thiamin concentration and in the intensity in meaty-cooked ham aroma. Furthermore, the meaty impression, especially umami and kokumi perceptions, is related also to thiamine degradation products. However, the relation is due to the formation of nonvolatile taste modulating compounds that possess a thiol group linked to the aminopyrimidine moiety of the thiamine molecule (Brehm et al., 2019).

13.2.5 Degradation of ribonucleotides

The degradation of ribonucleotides by the enzymatic dephosphorylation and deamination of adenosine triphosphate in postslaughter muscle contributes to the generation of flavor compounds by the production of ribose from inosine monophosphate and 5′-ribonucleotides (Toldrá and Flores, 2007). The ribose generated will take part in Maillard reactions while 5′-ribonucleotides are per se umami compounds. Many sulfur compounds have been obtained from the reaction of different forms of ribose and cysteine under different conditions. The relative amounts of the different forms of ribose, free sugar, sugar phosphate, and inosine 5′-monophosphate in meat affect meat flavor development (Mottram and Nobrega, 2002).

13.3 Methodology for meat aroma volatile identification

13.3.1 Extraction of volatile compounds

The isolation and identification of the volatile compounds present in the meat are necessary to elucidate which of them contribute to the aroma (Fig. 13.3). The volatile profile will depend on the technique used although the objective is to obtain an extract with a good aroma representing the original sample and without artifacts (Flores and Olivares, 2014). The most widely used techniques are those applied to extract the volatiles from the meat matrix and from the headspace surrounding the meat. The techniques used to extract volatiles from the matrix are solvent extraction and distillation, thermal desorption, and supercritical fluid extraction. This technique results in the complete flavor of the food, but its main disadvantages are the formation of artifact due to heat, decomposition of compounds, and loss of highly volatile compounds. Therefore, solvent extraction and distillation have been modified in the past years using high vacuum distillations and low temperatures to reduce the formation of artifacts (d'Acampora Zellner et al., 2008).

Nowadays, headspace techniques are frequently used and consist of the sampling of the air surrounding the food while kept in a closed vial. Their main advantages are the simplicity, elimination of solvent use, and heat is not used, avoiding artifact formation. However, the concentration in the headspace will be affected by the different volatility of the compounds and the food matrix composition as it is necessary to reach equilibrium between the matrix and the headspace. In addition, due to the low sensitivity of the static headspace techniques, dynamic techniques were developed where trapping materials are used with different absorbing capacities to concentrate the volatile compounds prior to the chromatography analysis. So, the volatile profile obtained will depend on the absorbing capacity of the materials used. The dynamic techniques are named as purge and trap (P&T) where an inert gas is purged through the sample and trapped by using materials such as Tenax, carbopack, etc. A widely applied technique is solid-phase microextraction (SPME), which consists on the extraction from the headspace using fused silica fibers coated with different phases. Both techniques, P&T and SPME, depend on the material used and extraction conditions (T^a and time), and their volatile profile will not be representative of the meat analyzed.

As indicated in Fig. 13.1 and Table 13.2, the identification of potent odorants in cooked meat has been mainly done by solvent extraction and distillation and P&T although latest studies were done using SPME extraction. However, it is generally recommended to combine solvent extraction with headspace analysis to avoid the loss of highly volatile compounds and, in this way, obtain the whole meat aroma profile (d'Acampora Zellner et al., 2008). After the extraction, the separation of volatile components remains a challenge for the detection of odor components at trace levels in order to minimize background interference. Therefore, classical gas chromatography technique is used for the separation

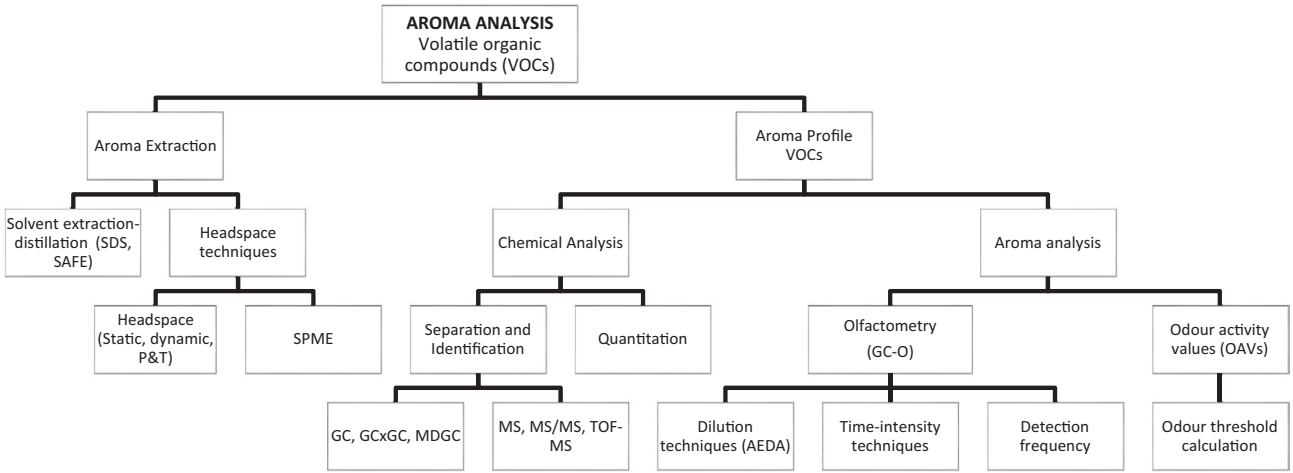

FIG. 13.3 Techniques used for isolation and identification of volatile compounds in aroma analysis. *AEDA*, aroma extraction dilution analysis; *GC*, gas chromatography; *GC-MS*, gas chromatography-mass spectrometry; *GC-O*, gas chromatography olfactometry; *MDGC*: Multidimensional (MDGC); *P&T*, purge and trap; *SAFE*, solvent-assisted flavor evaporation; *SDE*, solvent distillation extraction; *SPME*, solid-phase microextraction; *TOF-MS*: Time-of-flight mass spectrometry; *VOCs*, volatile organic compounds.

TABLE 13.2 Effect of cooking processes on meat volatiles.

Cooking	Compound	Odor	Technique
Beef			
Stewed beef (4h in water at 200°C)	12-Methyltridecanal	Tallow and beef-like, aroma of stewed beef	Solvent extraction and distillation-GC-MS, GCO-AEDA (Guth and Grosch, 1993, 1994, 1995)
Stewed beef (4h in water at 200°C)	Methanethiol, acetic acid, acetaldehyde	Stewed beef juice odor	Solvent extraction and distillation-GC-MS GCO-AEDA (Guth and Grosch, 1994)
Stewed beef (4h in water at 200°C), boiled beef	4-Hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)	Stewed beef juice odor	Solvent extraction and distillation-GC-MS (Guth and Grosch, 1994; Kerscher and Grosch, 1997) GCO-AEDA
Boiled beef	2-Furfurylthiol, 2-methyl-3-furanthiol, 3-mercapto-2-pentanone, 1-octen-3-one, (E)-2-nonenal	Odor of boiled beef	Solvent extraction and distillation-GC-MS GCO-AEDA (Kerscher and Grosch, 1997)
Pork			
Cooked ham with/without nitrite	Hexanal aroma low in nitrite cured ham Hexanal aroma high in nitrite free hams.	Fatty, green, grassy	Dynamic HS, GC-MS-O (Thomas et al., 2013)
Cooked ham with nitrite (69°C for 2h)	2-Methyl-3-furanthiol, 2-Methyl-3-(methyldithio)furan bis(2-methyl-3-furyl) disulfide	Meaty, cooked ham	SPME-GCxGC-MSTof, GC-O (Thomas et al., 2014, 2015)

Cooked ham with nitrite (cured)	Hexanal, 1-octen-3-one, 2,6-dimethylpyrazine, 2-methyl-3-(methyldithio)furan, furfuryl mercaptan 3-Methylthiopropanal, benzaldehyde, (E,E)-decadienal, guaiacol, 2-acetylthiazoline	Fatty, rancid, broth, toasted, meat-like, ham, fatty, potato-like, almond, broth	Simultaneous distillation extraction GC-MS-O (Benet et al., 2016)
Fried pork in different fat (olive oil, butter, and pig lard)	Olive oil: pentanal, hexanal, (Z)-2-heptenal, benzaldehyde, (E)-2-octenal Butter: 2-heptanone, 2-nonanone, 2-undecanone, tridecanone, 2-heptadecanone Pig lard: 2-Methylbutanal, methional, dimethyl disulfide		SPME-GC-MS (Ramírez et al., 2004)
Mini-pig roasted and cured with nitrite and spices	3-Methylbutanal, pentanal, 3-hydroxy-2-butanone, (E)-2-pentenal, hexanal, 2,5-dimethylpyrazine, benzaldehyde, 2-acetylthiazole	Unpleasant, pungent, sour, green, buttery, herbaceous, roasted, floral, meaty	SPME-GC-MS, solvent extraction distillation-GC-MS, GC-O (Xie et al., 2008)

AEDA, aroma extraction dilution analysis; *GC*, gas chromatography; *GC-MS*, gas chromatography-mass spectrometry; *GCO*, gas chromatography olfactometry; *HS*, headspace; *MSTof*, mass spectrometry time of flight.

of the volatile compounds, but other techniques offer excellent separation efficiency such as multidimensional gas chromatography (MDGC), beginning from conventional heart-cut (MDGC) to the most recent comprehensive two-dimensional gas chromatography (GCxGC). This technique has unmasked co-elution of odor-active compounds in cooked meat and revealed the contribution of sulfur- and nitrogen-containing compounds to the aroma (Giri et al., 2015).

13.3.2 Identification of aroma compounds

The analysis of the potent odorants in meat has been a difficult task due to the complexity of the food matrix. The first step in elucidating the odorants is to select the compounds with strong aroma impact from those odorless. For this purpose, the concept of odor activity value (OAV) was introduced as the ratio of compound concentration versus its flavor threshold. However, these OAVs are difficult to obtain since it requires the calculation of the threshold for each compound, and the calculation of the compound threshold should be made in a medium similar to the food (Grosch, 1993). On the other hand, the screening of the compounds eluted from a gas chromatograph (olfactometry GC-O) by using a sniffing port with a human panelist was selected as a sensory tool in flavor analysis (Delahunty et al., 2006). In GC-O, the end of the chromatographic column is split into different detectors: MS, FID, and olfactory port, and the signals from all the detectors are represented together in an aromagram. The results obtained will reveal among the hundreds of volatile compounds identified which of them contribute to the aroma. The olfactory analysis is a sensory evaluation that will be affected by panel performance and sniffing method. Different olfactometry methods are used such as dilution, detection frequency, and direct intensity techniques (Delahunty et al., 2006). These techniques will result in an aromagram with aroma description, intensity, or duration. In dilution techniques [AEDA (aroma extraction dilution analysis)], the food extract is diluted and the odorants are ranked based on the last dilution where the aroma was detected in the sniffing port. In time intensity methods, the panelists indicate the intensity perceived at the sniffing port for each compound. Finally, in the detection frequency technique, the intensity of the aroma compound is based on the number of panelists who detect the aroma. As observed in Table 13.2, the dilution techniques (AEDA) have been used for the identification of potent meat aroma compounds.

To understand the processes that take place during food production or consumption, several techniques have been developed to monitor in real time the release of volatile compounds from food or during the eating process. These techniques have been named as "direct mass spectrometry techniques," and the first one was developed in the 90th APCI-MS to study flavor release also named as "nose space" analysis, in which the air expelled from the food during mastication is collected and analyzed by gas chromatography-mass spectroscopy. Then other direct techniques differing in the ionization process were developed: PTR-MS (proton transfer reaction-mass spectrometry) and SIFT-MS (selected ion flow

tube-mass spectrometry), and use in food flavor studies (Biasioli et al., 2011). These techniques have been applied to study the release of volatile compounds in raw and cooked meat and meat products (Flores et al., 2013).

13.3.3 Aroma compounds in meat from different animal species

Raw meat has very little aroma, and its flavor developed on cooking. However, the identification of compounds with a unique meat odor is a difficult task and has been widely accepted that meat flavor is a balance of multiple components generated through multiple reactions.

Clearly, whether derived from water-soluble or fat-soluble precursors, the pattern of volatiles produced on heating meat must be important for flavor. In the last century, many volatile compounds were identified and many different chemical compounds were reported in beef (Macleod, 1998; Elmore et al., 2004) and pork (Elmore et al., 2001). The use of olfactometry techniques together with the screening procedure developed by Grosch (1993) (AEDA) allowed the selection of the key aroma compounds by diluting the flavor extract and selecting those chemical compounds detected by the human nose. Then, the aroma compounds are rated based on the last dilution where the compounds are detected (flavor dilution factor). Many other olfactometry techniques were developed and contribute to improve the technique as reported above. In addition, these techniques allowed the selection of aroma compounds based on high sensory significance. Since the 90s, they began to be applied to the study of meat flavor and contribute to the identification of potent odorants in different meat species (Fig. 13.2).

The contribution of carbonyl compounds derived from lipid oxidation processes, linear aldehydes, 2-alkenals, and 2,4-dienals is reported in oven roasted beef and lamb meat contributing to green, grass, and citrus notes, but the increase in the carbon chain contributes to fatty odor notes (Fig. 13.2). Also, several ketones contribute to mushroom (1-octen-3-ol) and buttery notes (2,3-butanedione). Though, heterocyclic compounds are identified as meaty character compounds. Many sulfur compounds are described as contributors of meaty notes, toasty notes in beef, pork, and lamb (2-acetyl-thiazoline), cooked vegetable notes in beef, sheep, goat, and duck (methional), meaty in bovine (2-furfuryl2-methyl-3-furyldisulfide, bis(2-methyl-3-furyl)disulfide, 3-(methyl) thiophene, 2-methyl-3-[(2methylbutyl)thio]furan, 2-phenyl-thiophene, 3-phenyl-thiophene, 4-isopropyl-benzenethiol, 4-(methylthio)benzenethiol, furaneol), and nutty roasted notes in sheep, lamb, and beef (2-acetyl-1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine). In addition, specific aroma notes are described such as the stable, animal odor (4-methylphenol) and mutton-like odors produced by 4-ethyl-octanoic acid, 4-methyloctanoic acid, and methylnonanoic acid in sheep meat (Fig. 13.2).

The meaty odor notes reported in cooked meat are related to the incorporation of sulfur into heterocyclic ring systems (e.g., thiazoles, thiophenes, etc.) and generally related to roast processing where high temperature is used

(Rochat et al., 2007). Kerscher and Grosch (1998) found as potentodorants in-heated meat 2-methyl-3-furanthiol and 2-furfurylthiol, 2-mercapto-3-pentanone and 3-mercapto-2-pentanone although they indicate that 2-methyl-3-furanthiol can be easily oxidized. Among the different sulfur compounds present in roasted beef, 2-methyl-3-furanthiol is also a powerful meaty aroma contributor in cooked beef, pork, and lamb, boiled beef, and cured cooked pork ham (Table 13.2). The formation of 2-methyl-3-furanthiol in beef muscle is related to the availability of H_2S and pentose and/or glucose degradation (Parker et al., 2006) although in addition to thiamine degradation (Resconi et al., 2013). Moreover, other thiol and disulfides containing 2-furanylmethyl moieties are found in heated meat models contributing to meaty and roasted aromas (Mottram, 1998).

In addition, furaneol (4-hydroxy-2,5-dimethyl-3(2H)-furanone) is reported as an important key odor compound in beef (Cerny and Grosch, 1992) produced from Maillard reactions. Roasted aromas are related to heterocyclic compounds such as pyrazines, thiazoles, and oxazoles (Mottram, 1998). The formation of pyrazines is probably from the condensation of two alpha-aminoketones producedin the Strecker degradation of amino acids by dicarbonyl compounds. The more severe heating involved in roasting appears to be associated with increased production of both thiazoles and pyrazines. Nevertheless, many factors such as pH affect its generation as pyrazines are formed from amino acid heated models at increasing pH values (pH>5) (Meynier and Mottram, 1995).

The contribution of carbonyl compounds derived from lipid oxidation processes, linear aldehydes, 2-alkenals and 2,4-dienals, and ketones to the aroma (Fig. 13.2) is of a less importance than the previous compounds due to their high threshold values. However, these carbonyl compounds are important as they will take part in the Maillard reaction during the cooking process (Mottram, 1998). Aldehydes, from lipid oxidation, take part in the reaction to yield 2-alkyl thiazoles, alkylthiophenes, alkyl pyridines, several of them reported as important key odorants in cooked meat (2-acetyl-2-thiazoline, 3-methyl-thiophene in Fig. 13.2). Elmore and Mottram (2000) report the formation of 2-alkyl-(2H)-thiapyrans when (E,E)2,4-decadienal and hydrogen sulfide are heated.

Lipid oxidation during heating contributes to desirable aroma of meat. Among the lipid fraction present in meat, phospholipids are essential contributors. Polyunsaturated fatty acids (PUFAs) have a high propensity to oxidize due to their double bonds and are restricted to the phospholipid fraction in ruminant muscle and adipose tissues (Wood et al., 2004). Their oxidation reaction leads to a high number of carbonyl compounds with high aroma impact such as alkenals and 2-alkenals, and 2,4-decadienals. Elmore et al. (1999) reported high levels of these compounds after cooking in aroma extracts of beef steaks with increased PUFA content. In this sense, 12-methyltridecanal and (E,E)2,4-decadienal have been reported as key odorants in goat meat and stewed beef (Fig. 13.2 and Table 13.2). The effect of individual fatty acids: oleic, linoleic, and linolenic acids on odor development was assayed in heated systems together with cysteine and ribose and reported the presence of a pronounced meaty odor when cysteine and ribose were

present. But the presence of the fatty acids contributed to different odor notes such as the fishy odor found in the presence of linolenic acid (Campo et al., 2003). Therefore, the presence of fat induces species-specific flavor, this fact is confirmed by the removal of the phospholipids from lean beef that results in marked chemical and sensory differences and also by the use of model systems where the role of PUFA in aroma development is assayed as indicated above. Based on all these observations, the phospholipids present in beef are essential for aroma, but triacylglycerols are not essential (Macleod, 1998).

13.4 Pre- and postslaughter factors affecting aroma

Many different factors affect meat aroma. Several antemortem factors are: age, breed, sex, nutritional status, stress level, fat level, fat profile, and composition (see Chapter 2) while postmortem are slaughter process, carcass handling, aging, cooking, and storage after cooking (see Chapters 4 and 10).

13.4.1 Breed, sex, aging

The flavor of meat is subject to variability due to both intrinsic and extrinsic factors. Of the former, differences due to species have already been referred to. There is some evidence for differences due to breed (Elmore et al., 2004; Watkins et al., 2013). Elmore et al. (2000) found that levels of pyrazines and sulfur-containing compounds (volatiles derived from Maillard reactions) were much higher in the meat from Soay than in that of Suffolk lambs. In contrast, Elmore et al. (2004) found a high effect of diet on aroma volatiles in beef muscle than the effect of breed.

Increasing animal age is associated with increased intensity of flavor, as the bland flavor of veal and the characteristic flavor of beef testify. In sheep meat flavor, the term mutton is related to the flavor of cooked meat from older animals while pastoral term is related to pasture diet (Watkins et al., 2013).

Strength of flavor in beef tends to increase up to 18months of age and thereafter to reach a plateau. This fact must be presumed to be determined by age-related changes in the precursors. Since the meat of older animals tends to contain more fat and that of a more saturated character, no doubt fat-soluble precursors are among those involved. As the percentage of intramuscular fat increases in porcine longissimus dorsi, there is a concomitant increase in the percentage of monounsaturated fatty acids and a decrease in that of PUFA, and this is associated with improved flavor (Cameron and Enser, 1991). Nevertheless, although fat is clearly essential for flavor, there is an optimum. In grilled beef with a high IMF content (> 10% IMF), marbling affects volatile generation and release of lipid-derived volatiles producing an intense sensory flavor (Frank et al., 2016b; Frank et al., 2017). Moreover, fat participates in the chewing process making the matrix softer and easier to chew, leading to more rapid oral breakdown and to greater volatile release (Frank et al., 2017).

Since there are systematic biochemical differences between muscles, it is not surprising that these should have different flavors when cooked, as the relative blandness of that from *psoas* muscle (tenderloin) and the strength of that from diaphragm (skirt) indicate. Again, bovine longissimus dorsi has a stronger flavor than *semitendinosus*. A ranking of muscles for beef flavor intensity demonstrates the differences between different beef muscles although they are relatively small (Calkins and Hodgen, 2007).

There is also one major biochemical variable that affects muscle flavor, the ultimate pH. As reported previously, Maillard reactions are affected by pH values because pH increase favors pyrazine formation. However, the meat has a narrow pH range (5.5–6.0), and meat with high pH is perceived with low flavor intensity. This can be due to a different production of sulfur compounds from amino acid degradation (Calkins and Hodgen, 2007). It has been proposed that meat at high pH has high water retention ability, and during cooking, less water-soluble proteins are lost.

A second important biochemical variable arises from the changes occurring when meat is held to aging for some time after the ultimate pH has been reached to "age" or "condition" it. During this period, meat becomes more tender, but the flavor also tends to increase or alter. Objective study has shown that there is, concomitantly, a marked increase in amino acids and peptides during pork meat aging that is also affected by ultimate pH. Changes in the free fatty acids during aging no doubt contribute to the observed flavor changes. Moreover, progressive nucleotide breakdown, whereby ADP (adenosine diphosphate) and AMP (adenosine monophosphate) are ultimately split to ribose, hypoxanthine, phosphate, and ammonia, was reported as a contributing factor especially on pork meat taste (Flores et al., 1999). During beef aging, the most significant changes are the increase in ribose, methionine, and cysteine, and the increase observed in free amino acids was higher than those observed for sugars (Koutsidis et al., 2008a). All of these compounds will take part in Maillard reactions producing pyrazines and Strecker aldehydes and therefore affect the flavor. However, the generation of these water-soluble precursors can be affected by diet as those animals fed on grass have shown a higher free amino acid concentration than those fed on concentrate diets (Koutsidis et al., 2008b).

The intrinsic factors considered above tend to cause expectable, and generally desirable, variability in meat flavor although what is desirable is partly determined by culture and traditions. Watkins et al. (2013) reported consumer's perspectives in terms of the world variation in sheep meat liking.

13.4.2 Farming, feeding practices

Of extrinsic factors causing variability in flavor, diet can be significant, although this often refers to relatively undesirable features derived from specific components rather than to the level or intensity of feeding. Flavor differences between the cooked meat from grass-fed animals and those fed intensively on concentrates were reported especially the pasture including plants with specific flavor-inducing compounds (Watkins et al., 2013) (Table 13.3).

TABLE 13.3 Effect of feeding on meat and fat flavor.

Feeding process	Extraction technique	Flavor	Diet effect
Beef Muscle			
Pasture, Pasture plus corn grain, concentrate	DHS-SPE (dynamic headspace-solid phase extraction)	Grilled: 1-octen-3-one, (E)-2-octenal, methional, hexanal	Concentrate: high in methional Pasture: high in (E,E)-2,4-heptadienal (Resconi et al., 2012)
Different types: grazed pasture, grass silage and concentrate	SPME-GC-MS	Meat was put at 70°C for 10min and VOCS extracted for 30min	Concentrate fed discriminated by: Skatole, 3-undecanone, cuminic alcohol, 2-methyl-1-butanol Germacrene D: marker of grass feeding (Vasta et al., 2011)
Lamb Fat			
Pasture vs. grain	Dynamic headspace (P&T-Tenax)	Lamb flavor: 4-methyloctanoic and 4-methylnonanoic acids	Pasture indicators: 2,3-octanedione, 3-methylindole (skatole) (Young et al., 1997, Watkins et al. (2014))
Pasture	Dynamic headspace (P&T-Tenax)		Beta-caryophyllene (Priolo et al., 2004)
Lamb Muscle			
Pasture vs. concentrate	P&T	Grilled flavor: heptan-2-one, oct-1-en-3-one	Pasture: low lipid derived unsaturated aldehydes, ketones and Strecker aldehydes (Resconi et al., 2010)
Pasture vs. concentrate	Dynamic headspace (P&T-Tenax)		Concentrate: high 2-ketones, alkanes No terpenoids and 2,3-octanedione found in muscle from pasture (Vasta et al., 2007)

A number of off-flavors are detected in the meat of sheep when they graze certain pastures for some weeks before slaughter. The effects are more noticeable at particular times of the year, at certain stages of growth of the plants, and within specific soil conditions. A period of about 1–2weeks on neutral feed will generally overcome any such problems in lambs (Watkins et al., 2013). The effect of

different brassicas diet on sheep meat flavor is important although not always affects the flavor of lamb cooked meat (Frank et al., 2016a).

Watkins et al. (2013) realized a meta-analysis of the aroma studies in lamb meat reducing the number of volatile compounds to only 15 volatiles where just one of them is characterized as mutton-like odor (4-ethyloctanoic acid). This compound is absent from beef profiles demonstrating that the odor and flavor of sheep meat are specifically related to branched chain fatty acids (e.g., 4-ethyloctanoic acid). In the past years, other branched chains acids are also related to mutton flavor (e.g., 4-methyloctanoic, 4-ethyloctanoic acid, and 4-methylnonanoic acids) (Watkins and Frank, 2019). Nevertheless, the mutton intensity is increased by the presence of other compounds related to pastoral flavor, such as 3-methylindole and 4-methylphenol derived from tyrosine degradation present in pasture. Mutton flavor is related to a lower consumer acceptance, and therefore, these compounds can be used to predict it. In this sense, Watkins and Frank, 2019, proposed the use of heptadecanoic acid as a marker of mutton flavor based on the association found between it and the branched fatty acids, especially for 4-methyloctanoic and 4-methylnonanoic acids. The choice of heptadecanoic acid is due to its presence in sheep fat in such a proportion that makes it easy to be measured through simpler analytical methods.

Taken into account the different extraction techniques employed, the cooking conditions, and differences in analyses, several studies have shown the effect of different feeding systems in meat aroma. The studies performed on lamb fat indicate that 2,3-octanedione and 3-methylindole (Young et al., 1997) and beta-caryophyllene (Priolo et al., 2004) can be used as pasture indicators. However, when the studies are performed in lamb muscle, these compounds are not found as indicators of the pasture feeding, and those muscles fed with pasture result in low abundance of lipid-derived unsaturated aldehydes, ketones, and Strecker aldehydes.

An international investigation by Sañudo et al. (2000) showed, however, that Spanish consumers prefer the flavor of lamb containing a relatively high content of n-6 PUFA derived from concentrates, whereas British consumers prefer the stronger flavor of lamb associated with n-3 PUFA derived from grass, but consumer preferences are clearly dominated by previous experience and traditions.

In beef meat, the presence of methional in concentrate feeds is seen while pasture is characterized by a high content of (E,E)-2,4-decadienal (Resconi et al., 2012) although other compounds such as skatole, 3-undecanone, cuminic alcohol, and 2-methylbutanol are able to discriminate the concentrate feed while germacrene D is a marker for grass feeding (Vasta et al., 2011).

The origin of these compounds in ruminants can be related to the profile of unsaturated fatty acids (Wood et al., 2004). Although both diets have a similar content of unsaturated fatty acids, the content of PUFA differs as grass feeding is high in n-3 PUFA (alpha-linolenic acid) while grain feeding is high in n-6 PUFA content (linoleic acid is the most common n-6). The lowest lipid oxidation detected in grass feeding has been attributed to the antioxidants present in

grass (vitamin E), which are deposited in the tissue and produce a benefit in terms of low oxidation and color.

Elmore et al. (2000) varied the content of n-3 PUFA in the muscles of lambs by feeding supplements of linseed oil (which increased the level of alpha-linolenic acid) and of fish oil (which increased the levels of eicosapentenoic and docosahexaenoic acids). These also increased the amount of aromatic volatiles derived from autooxidation of the PUFA on cooking. However, latest studies indicate that the increase of n-3 PUFA content in terms of increasing the nutritional value of meat may produce off-flavors. The reasons are that the breakdown products of n-3 PUFA are more reactive than those derived from n-6 PUFA and will interact with the Maillard reaction products reducing the levels of meaty aroma compounds (thiophenes and furans) (Elmore et al., 2002). In addition, the high content of n-3 PUFA can initiate the free radical oxidation increasing the levels of breakdown products and alter the aroma of cooked meat.

13.4.3 Cooking

The duration and temperature of cooking influence the nature and intensity of odor and taste in meat. The formation of meaty aroma compounds is favored by the presence of flavor precursors such as free amino acids and free fatty acids obtained from proteolysis and lipolysis processes, respectively, which will take part in thermal reactions. The reaction of the precursors (free amino acids and fatty acids) in different cooking conditions is seen by the lower content of free fatty acids in cooked meat than in raw meat except for microwave cooking where the precursors content is not decreased because the cooking conditions are not high enough to promote chemical reactions.

The generation of soluble water precursors depends on several factors such as postmortem treatment, aging, gender, and breed although the changes observed during aging as reported above produce high increases of these compounds, although cooking temperature is by far the most important factor. In pork flavor, frying temperature of 150°C produces volatile compounds from lipid reactions while the use of 250°C increases the formation of Maillard products (Meinert et al., 2007). Several strategies have been directed to increase flavor precursors through diet such as the use of fasting in terms of having high glycogen concentrations as a source of monosaccharides although they are not sensory appreciated.

Different cooking processes have been applied to beef meat and the aroma compounds elucidated. As reported before, roasted beef contributes to the generation of sulfur and carbonyl compounds with high odor impact (Fig. 13.2 and Table 13.2), while stewed beef is characterized by the presence of 12-methyltridecanal, methanethiol, acetic acid, acetaldehyde, and furaneol (4-hydroxy-2,5-dimethyl-3(2H)-furanone) (Guth and Grosch, 1993, 1994, 1995). Recent studies done in cooked ham with and without nitrite reveal a high hexanal content in nitrite free hams that affect the flavor by

affecting the perception of sulfur key odor compounds (Thomas et al., 2013, 2014, 2015). On the other hand, the fat used during cooking of pork meat can also affect the generation of aroma compounds as reported when olive oil fat, butter, or pig lard is used. Different volatiles are generated such as linear aldehydes and 2-alkenals when using olive oil, 2-ketones with butter and methional, dimethyl disulfide, and 2-methylbutanal when using pig lard (Table 13.2).

13.5 Off-flavors

13.5.1 Warmed over flavor

The off-flavor referred to as "warmed over flavor" (WOF) in cooked meat was ascribed to the oxidation of lipids and, more specifically, to that of phospholipids catalyzed by both heme and nonheme iron (Table 13.4). The rancidity develops much faster than that in uncooked meat during refrigerated storage. Any process that damages muscle membranes, such as chopping or emulsification, exacerbates the condition, but it can be retarded by the use of antioxidants, such as nitrite, phosphate, and naturally occurring herbs and spices (e.g., rosemary), which contain various factors that inhibit rancidity. The compounds responsible for the WOF odor have been identified and related to an increase of n-hexanal and *trans*-4,5-epoxy-(E)-2-decenal (Kerler and Grosch, 1996) although other aldehydes have been also related to the cardboard flavor (Table 13.4).

The use of natural plant extracts (grape seed and pine bark extracts) applied to cooked ground beef has been proposed to avoid WOF development. They produce a high antioxidant effect during refrigerated storage, but its use may be limited to a level of applications at 0.02% as the sensory quality can be affected (Ahn et al., 2002).

Free fatty acids, produced by microbial action or otherwise, accelerate the development of oxidative rancidity that later can occur even at −10°C during long storage. The conditions predisposing toward oxidative rancidity in intramuscular fat have been thoroughly investigated (Wood et al., 2004). Species differences in the development of off-odors and tastes arise from the different spectra of fatty acids produced by lipolysis and of carbonyls produced during oxidative rancidity. The phospholipids of meat fats are the most unstable constituents, and they may well play a major role in accelerating flavor deterioration (Campo et al., 2003). However, it is essential to remember that phospholipids and not triacylglycerols are the main contributors to the typical meaty aroma through Maillard reactions in the presence of ribose and cysteine (Mottram, 1998).

In addition, in the study of the relationship between fatty acids and off-flavors, the sensory terms associated with off-flavor in beef are beefy and tallow and are related to 2-octenal and 2,4-decadienal products originated from linoleic acid degradation (Stelzleni and Johnson, 2010). However, other off-flavors such as grassy and gamey are not related to the phospholipid fraction.

TABLE 13.4 Volatile compounds related to undesirable odors in meat.

Description of off-flavor and compounds	Precursor	Origin-cause
Warmed Over Flavor (WOF): Cardboard and Metallic Aromas		
Odorants (AEDA): Hexanal, 1-octen-3-one, (E)and (Z)-2 octenal, (Z)-2-nonenal, (E,E)-2,4-Nonadienal, *trans*-4,5-epoxy-2-decenal	Oxidation of unsaturated fatty acids	Stored boiled beef (Konopka and Grosch, 1991; Konopka et al., 1995)
Loss: 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 3-hydroxy-4,5-dimethyl-2(5H)-furanone Increase: n-hexanal, *trans*-4,5-epoxy-(E)-2-decenal	Cardboard and metallic aromas	Oxidation of refrigerated beef patties (Kerler and Grosch, 1996)
Irradiated Odors: Pungent, Rancid, Fatty, Musty Odors, Rotten, Egg, Sweet, Bloody, Liver-Like Odors		
Pentanal, hexanal, E-2-heptenal, octanal, (Z)-2-octenal, (E,Z)-decadienal	Lipid oxidation of unsaturated fatty acids	Irradiation (Brewer, 2009)
Sulfur compounds: dimethyl disulfide, dimethyl trisulfide, methyl mercaptan, hydrogen sulfide	Degradation of sulfur containing amino acids	Irradiation (Brewer, 2009)
Boar Taint: Urine and Fecal Odors		
5a-androst-16-ene-3-one (urine odor); and skatole (3-Methyl indole) (fecal odor)	Hormone and tryptophan	Androstenone is a testicular steroid. Skatole is a tryptophan degradation product (Bonneau, 1998)
Short chain fatty acids Phenolic compounds (4-phenyl-3-buten-2-one)		From lipid oxidation process (Fischer et al., 2014) from intestinal digestion (Rius and García-Regueiro, 2001)
2-Aminoacetophenone	Skatole metabolite	Use of entire male (Fischer et al., 2014)
Off-Flavor: Fermented, Rancid, Sulfurous, Moldy		
Branched alcohols (3-methyl-1-butanol), linear aldehydes (hexanal, nonanal), sulfur (dimethyl disulfide), 1-octen-3-ol	Microbial metabolism and degradation of meat components	Microbial meat spoilage in air and vacuum package (Casaburi et al., 2015)

13.5.2 Irradiation odors

The use of irradiation for pathogen reduction is allowed in United States in uncooked chilled red meat, uncooked frozen meat, and fresh and frozen meat (Brewer, 2009) (Table 13.4) (see Chapter 8). However, the irradiation of fresh meat can result in the development of off-odors described as rotten egg, bloody, fishy, burnt, sulfur. Several factors affect the development of off-flavor during irradiation: type of meat, temperature, oxygen exposure, packaging, and antioxidant substances (Ahn et al., 2013). Depending on the conditions, several volatile compounds have been produced, but aldehydes are affected by packaging, and sulfur compound such as dimethyl trisulfide is responsible for off-flavors. Several techniques are used to prevent the off-flavor formation, such as low temperature to control the free radical generation, vacuum packaging to exclude oxygen, use of inert gas, and addition of antioxidants (Brewer, 2009).

13.5.3 Boar taint

When the flesh of certain pigs is heated, an unpleasant odor arises, which is commonly referred to as boar odor although it has been reported in the flesh of both sexes (Bonneau, 1998). Two compounds are the main contributors to boar odor: androstenone and skatole. Both compounds are accumulated in the adipose tissue evoking urine and fecal, bitter, or manure odors. In addition, other minor compounds contributing to boar taint odor are androstenols, indole, 4-phenyl-3-buten-2-one, phenolic compounds or aldehydes, and short-chain fatty acids (Table 13.4). Androstenone (5α-androst-16-en-3-one) is a steroid synthesized in boar testes, and therefore the taint has been related to sexual maturity, and their levels depend on age, weight, and genotype. On the other hand, skatole (3-methyl indole) is an L-tryptophan metabolite produced through microbial intestinal degradation and degraded by the liver, so its production depends on environmental factors. Different strategies have been used to reduce skatole concentration such as carbohydrates use in diet (inulin, raw potato starch, etc.) and enzymes or sorbent material. However, androstenone concentration cannot be easily reduced unless immunocastration is carried out. A vaccine called Improvac is available although it produces several effects such as lower drip loss and darker meat than entire male meaning a lower propensity to develop pale, soft, and exudative meat (Bonneau and Lebret, 2010).

Consumer perception of boar taint odor depends highly on the boar compounds as 99% of consumers are sensitive to skatole, whereas a high number of consumers are anosmic to androstenone. Around 44% of men are unable to detect "boar odor," but only about 8% of women cannot do so.

An international assessment of the significance of skatole and androstenone in causing boar taint was undertaken by a group of seven collaborating European countries. The study was based on the analysis of data from 4000 entire male pigs and 400 gilts. Overall, most consumers were dissatisfied with

the odor and flavor of the flesh from entire males. High levels of skatole were the predominant causes of boar odor, whereas skatole and androstenone contributed equally to unpleasant flavor. There were national differences. Thus, whereas British consumers were generally satisfied with the odor and flavor of meat from both entire males and gilts, Danish and Dutch consumers strongly objected to such odor from males. French, German, Spanish, and Swedish consumers found both the odor and flavor of the meat from entire males unacceptable. It was concluded that, in the short term, a reduction in the levels of skatole would achieve a limited improvement in consumer satisfaction but that, in the longer term, a reduction in the levels of both skatole and androstenone would be necessary to overcome the problem of boar taint (Bonneau and Lebret, 2010).

The prevalence of boar taint in Spain has been recently studied, and the percentage of carcasses above the high thresholds of androstenone and skatole (androstenone is 0.5–1.0μg/g fat and skatole is 0.20–0.25μg/g) are around 10.2% that represents around 1.6 million carcasses with high boar taint levels per year (Borrisser-Pairó et al., 2016. This fact indicates the necessity of introducing a system to classify carcasses according to boar taint presence to give carcasses an adequate use. In the past years, advancements are made in rapid boar taint detection methods based on the development of techniques such as sensors, e-noses, and laser diode thermal desorption combined with tandem mass spectrometry, which can be used for online detection (Burgeon et al., 2021).

Castration causes a fall in the androstenone level and an increase in the titer of the enzyme cytochrome P4502EI, which enhances the breakdown of skatole in the liver. Since neither animal age nor weight is correlated with taint in the average boar of potential commercial value, a simple test to make an early detection of taint involves the heating of a sample of fat to about 375°C using an electrically operated soldering iron.

13.5.4 Microbial odors

Odors produced by microorganisms growing on meat surfaces are not so objectionable as those due to the metabolic products of anaerobes; the former tend to be sour rather than putrid. The lipases of such microorganisms will attack fat, splitting-off fatty acids with more or less unpleasant consequences according to their nature. The exact nature of the off-odors will, of course, depend on the types of microorganisms growing, and these in turn will be determined by such factors as the temperature of storage and the nature of the product (fresh, cured, comminuted). Relatively high temperatures and the absence of oxygen will produce putrid off-odors through the breakdown of proteins, in bone taint in those deep-seated portions of the carcass, which have not been cooled sufficiently quickly after death, and where there is a reservoir of suitable microorganisms in the lymph nodes.

Microbial spoilage of meat depends on storage and packaging conditions that will allow the development of differential bacterial populations (Casaburi et al., 2015; Mansur et al., 2019) (see Chapters 6 and 10). The development of volatile organic compounds during meat storage may have an aroma impact if their concentration increases. Many different volatile compounds such as alcohols, ketones, esters, acids, and sulfur compounds increase during aerobic chill meat storage. The microorganisms *Pseudomonas fragi*, *Brochothrix thermosphacta*, *Shewanella putrefaciens*, *Moraxella*, and *Carnobacterium* spp. are the major producers of ester compounds while the production of aldehydes and acids is mainly due to *Enterobacteriaceae, Pseudomonas* spp., *Br. thermosphacta*, and *Carnobacterium* spp. On the other hand, the same volatile compounds have been found in meat stored under vacuum, but the microorganisms associated with the volatile production were *Enterobacteriaceae* (mainly *Serratia*), *Carnobacterium* spp., *P. fragi*, and clostridia. However, the production of sulfur compounds in vacuum-stored meat is due to *Serratia proteamaculans*, *Serratia liquefaciens*, *P. fragi*, *Carnobacterium maltaromaticum*, *Hafnia alvei*, *Lystrosaurus curvatus*, *Lactobacillus sakei*, *Clostridium algidicarni*, *Clostridium putrefaciens*, and *Clostridium perfringens*. In summary, the spoilage of meat depends on storage conditions, and the main bacteria involved are *P. fragi*, *Br. thermosphacta*, *Carnobacterium* spp. *Enterobacteriaceae*, other lactic acid bacteria (LAB), and clostridia. An aroma wheel representing the volatiles and aromas produced during storage of meat has been developed by Casaburi et al. (2015) (Fig. 13.4) revealing the effect of the different storage conditions. The aroma wheel shows the development at early stages under air storage of esters and fatty acids contributing to fruity and dairy odor notes while they are not detected under vacuum storage.

13.6 Meat product flavor

Attempts for the deliberate enhancement of meat odor and taste have mainly been confined to cured and comminuted meats and sausages, which frequently contain added spices, condiments (including sodium glutamate), sugars, etc. (Toldrá and Flores, 2007). These products are based on traditional practices where many factors from raw material to processing parameters affect flavor development. Among meat products, two main groups based on technology process can be distinguished: wet curing and dry curing. Since the main component of meat products is meat, the differences in flavor depend on meat composition and precursors and their interactions together with processing conditions (Flores, 2018).

13.6.1 Wet-cured meat products

The acceptability of wet-cured products depends on its flavor developed under mild cooking conditions. It is widely accepted that the flavor of cured cooked

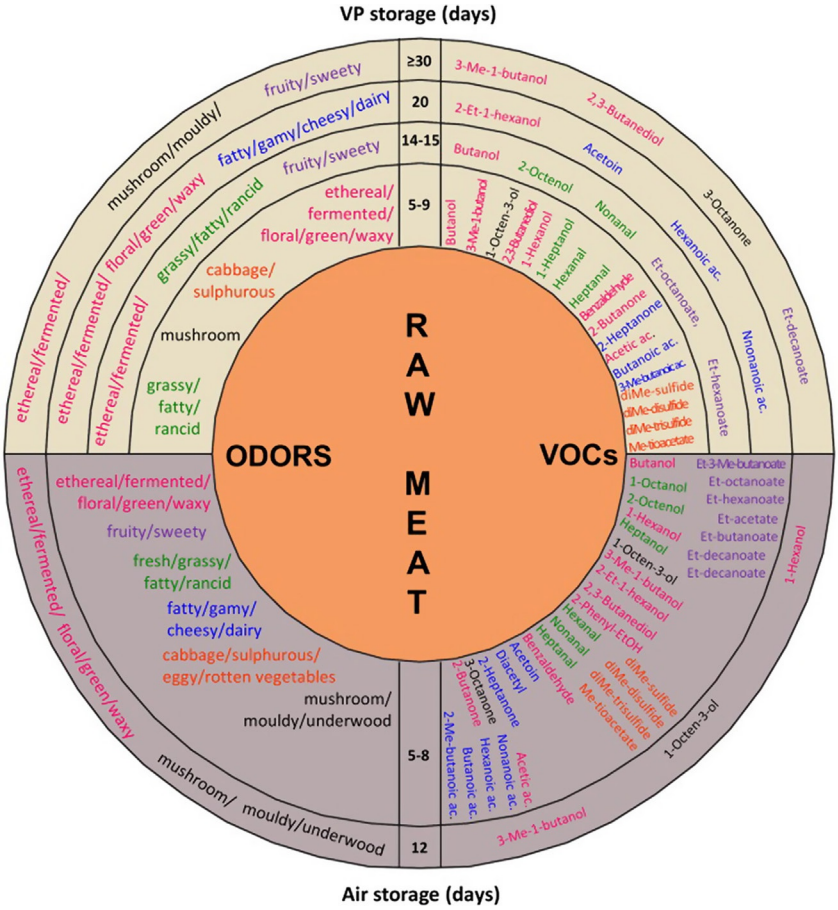

FIG. 13.4 Representation of volatile fraction evolution during chill storage of meat in air and vacuum package (VP). *VOC*, volatile organic compounds. (*Reproduced from Casaburi, A., Piombino, P., Nychas, G.J., Villani, F., Ercolini, D., 2015. Bacterial populations and the volatilome associated to meat spoilage. Food Microbiol. 45(PA), 83–102.*)

meat is mainly caused by nitrite through the inhibition of lipid oxidation as shown by the inhibition of carbonyl compounds (see Chapter 9). Nevertheless, curing also causes an increase in free amino acids, which is further enhanced on cooking, and their conversion to various volatiles no doubt contributes to flavor development.

In fact, hundreds of volatile compounds have been identified in wet-cured meat products, and several studies reported the presence of aldehydes, alkane, ketones, esters, terpenes, sulfur, furans, pyrazines, etc., in products such as cooked ham, frankfurter, and bacon. Regarding cured aroma flavor, a comparison of the aroma from uncured and cured cooked ham shows that curing strongly inhibits hexanal formation in comparison with other carbonyls but also affects

the flavor by affecting the perception of sulfur key odor compounds (Thomas et al., 2013, 2014, 2015) (Table 13.5). The olfactometry evaluation of hexanal odor is very weak in nitrite-cured ham and strong when nitrite is not used; thus, hexanal plays an important role in cooked ham aroma as its formation masks the cooked ham odor. In addition, one sulfur compound is assigned as a key aroma

TABLE 13.5 Odorants identified in meat products.

Compound	Odor description	Extraction technique and GC-O
Cooked Ham		
Ethanethiol Hexanal Isovaleric acid 1-Octen-3-one Octanal 2-Nonanone 2-Decenal 3-Methylthiopropanal 2-Methyl-3-(methyldithio)furan 2-Methyl-3-furanthiol bis(2-methyl-3-furyl) disulfide 2-Methyl-thiophene	Sulfurous Fruity fatty, green rancid Cheese Mushroom Fatty, orange Creamy, fatty oily, grassy Potato, earthy Cooked ham, meaty Cooked ham, meaty Meaty, roasted Sulfurous, onion	Dynamic headspace (Tenax) GC-O-Time intensity (Thomas et al., 2013, 2014)
Hexanal 2,6-Dimethylpyrazine Furfuryl mercaptan 2-Methyl-3-(methyldithio) furan 3-Methylthiopropanal Benzaldehyde E,E – 2.4-decadienal 2-Acetylthiazoline	Fatty, rancid Toasty Coffee, meaty Meaty, ham, beef Potato, metallic Almond, sweet Fatty, broth Meat-like, toasty	SDE (simultaneous distillation extraction) GC-O detection frequency (Benet et al., 2016)
Dry-Cured Ham		
3-Methyl butanal Hexanal 2,3-Butanedione Methyl 2-methylpropanoate Acetic acid Methylpyrazine 2,6-Dimethylpyrazines	Cheesy-green Green-grassy Buttery Fruity Vinegar Nutty Toasted	Purge and trap (Tenax) (Flores et al., 1997)
Methanethiol 3-Methylbutanal 1-Penten-3-one Hexanal 2-Methyl-3-furanthiol	Rotten eggs Fruity, almond-like Rotten, fruity Green, acorn-like Cured ham-like, nutty	Purge and trap (Tenax/silica gel/charcoal) (Carrapiso et al., 2002)

TABLE 13.5 Odorants identified in meat products.—cont'd

Compound	Odor description	Extraction technique and GC-O
Dry Fermented Sausage		
Benzothiazole Pyrrole 2-Acetyl-1-pyrroline 2-Acetylpyrrole 2,3-Dihydrothiophene 2,6-Dimethylpyrazine Methional	Green, strange, damp Coffee, sweet Fried corn Toasted Walnut Toasted Cooked potato	SAFE-GC-MS GC-O-AEDA (Corral et al., 2016)
3-Methylbutanal 1-Pentanol 2-Hexenal Heptanal 2-Heptanol Methional 2,4-Heptadienal (E,E) 2-Octenal Heptanoic acid	Rancid, dry ham roasted, roasted meat Salty meat, dry ham Citrus, rancid, cured ham Plastic, pork scratchings brothy, rancid Cooked meat, nutty dry ham, dry sausage Rancid, dry ham	SPME-GC-MS GC-O-detection frequency (Marco et al., 2007)
2-acetyl-1-pyrroline 2,5-dimethylpyrazine dimethyl trisulfide 2-acetyl-2-thiazoline 2-methyl-3-furanthiol methyl-2-methyl-3-furyl disulfide 3-methylbutanal 2,3-butanedione Acetic acid hexanal ethyl 2-methylbutanoate β-Myrcene 3-Carene α-Terpinene linalool	Fried corn Nutty rotten, vegetable Toasted, fried corn Fatty, medicinal Meaty, wet wood Caramel, chocolate Dairy, buttry Vinegar Green, grass Fruity Green Earthy, green, fresh Pine, woody Fresh, floral	SPME-GC-MS GC-O-detection frequency (Perea-Sanz et al., 2020; Aquilani et al., 2018)

AEDA, aroma extraction dilution analysis; *GC-O*, gas chromatography olfactometry; *MS*, mass spectrometry; *SAFE*, solvent-assisted flavor evaporation; *SPME*, solid-phase microextraction.

compound for cooked ham aroma, 2-methyl-3-(methyldithio)furan, although the olfactory perception of this compound is not affected by the presence of nitrite. Moreover, the formation of a specific cured aroma compound cannot be attributed to the use of nitrite as curing agent. In meat products where nitrite is not used, the generation of aldehydes during oxidation reactions masks the aroma of sulfur compounds as they are the key aroma compounds in nitrite-cured products (Thomas et al., 2013).

The production of sulfur compounds with intense meaty and cooked ham odors such as 2-methyl-3-furanthiol, 2-methyl-3-(methyldithio)furan, and bis(2-methyl-3-furyl) disulfide is linked to the thermal degradation of thiamin (Thomas et al., 2014). Furthermore, the concentration of thiamin has been related to the amount of 2-methyl-3-furanthiol in model cooked hams (Thomas et al., 2015). In contrast, the contribution of cysteine as a precursor of these sulfur compounds is not observed when assayed in cooked ham models. In summary, the main reactions involved in the development of cooked cured aroma are lipid oxidation, Maillard reactions, and degradation of thiamin while the thermal degradation of sulfur amino acids is not contributing to the key aroma compounds. Nevertheless, the contribution of intramuscular fat and PUFA to the cooked ham flavor cannot be forgotten. The overall cooked ham flavor is produced by the presence of 2-methyl-3-(methyldithio) furan (Benet et al., 2016), but also cooked hams with high intramuscular fat content have higher aroma compounds from Maillard reactions related to roasted notes than low-intramuscular fat hams.

13.6.2 Dry-cured meat products

Regarding dry-cured meat products, hundreds of volatile compounds have been identified, and the effects of different processing conditions on the production of volatiles have been studied. In terms of key aroma compounds, any compound has been identified as having dry-cured aroma note (Table 13.5). It is generally assumed that the balance of several volatile compounds contributes to the dry-cured aroma. In 1997, the key aroma compounds present in dry-curedham were elucidated: hexanal, 3-methyl-butanal, 1-penten-3-ol, and dimethyl-disulfide with green-grass, cheesy, toasted, and unpleasant odors (Flores et al., 1997). The major aroma contributors are aldehydes that result from amino acid degradation and lipid oxidation and several ketones that also come from lipid oxidation. However, the contribution of ester compounds is minimal as only one ester compound has been described and its presence depends on the processing conditions (Toldrá and Flores, 1998), for example, higher proportions of ester compounds are present in Parma (Italian) than in Iberian or Serrano (Spanish) dry-cured hams. Nevertheless, several sulfur compounds such as methanethiol and 2-methyl-3-furanthiol are important contributors to dry-cured aroma in Iberian dry-cured ham (Carrapiso et al., 2002).

13.6.3 Fermented dry-cured meat products

In contrast, the aroma of fermented dry sausages is not only due to the mechanism indicated above. In this case, the thermal degradation of thiamin or amino acids is substituted by microbial activity (Flores and Olivares, 2014). The main microbial reactions involved in sausage flavor formation are carbohydrate fermentation, amino acid degradation reactions, lipid β-oxidation, and Staphylococci esterase activity. The fermentation of carbohydrates by LAB generates lactic acid, which is responsible for the acidification that produces the coagulation of meat

proteins, but at the same time many volatile compounds with aroma properties are produced by other bacteria and yeasts such as diacetyl, acetaldehyde, acetoin, ethanol, and organic acids, such as formic, acetic, propionic, and butanoic acids (Table 13.5). However, among the chemical reactions described above, Maillard and Strecker reactions may be limited due to the processing parameters (temperature, pH, etc.) as low temperatures are applied in dry fermented sausages. Anyway, the low water activity of the sausages and the long times applied for drying may favor them and favor the generation of substrates (free amino acids and dicarbonyl compounds). In this sense, microbial degradation of amino acids is a source of aroma compounds, and different microbial groups take part in it. Coagulase-negative staphylococci, LAB, and yeasts (*Debaryomyces hansenii*) degrade amino acids and produce volatile compounds with high aroma impact (Flores et al., 2015). The microbial degradation of amino acids by transamination and decarboxylation can be followed through the production of the respective branched aldehydes, alcohols, and/or acids. Several potent sulfur and nitrogen aroma compounds producing meaty odors are detected also in fermented sausage aroma, such as 2-methyl-3-furanthiol, 2-acetyl-1-pyrroline, methional, dimethyl trisulfide, 2-acetyl-2-thiazoline, and methyl-2-methyl-3-furyl disulfide, which are mainly derived from the degradation of sulfur amino acid and thiamine (Table 13.5). These reactions are favored by the long ripening times, as well as the low water activity and acid pH (Flores et al., 2021)).

In addition to the aroma mechanisms of formation in meat products, the contribution of spices and smoking processing cannot be overlooked. By itself, spices are a source of volatile compounds contributing to particular flavors that depend on traditions (Toldrá and Flores, 2007). The most frequently used spices in meat products are black pepper, paprika, garlic, onion, mustard, nutmeg, and oregano, among others. They contribute to the presence of volatiles in meat products such as terpene hydrocarbons and sulfur compounds (Aquilani et al., 2018). Terpene compounds impart fruity, green, herbal pine, pungent aroma notes to meat products, while the sulfur compounds produced from the use of mainly garlic are related to unpleasant and garlic notes (Flores and Olivares, 2014). On the other hand, smoking process imparts a desirable flavor based generally on phenols and methoxyphenol compounds.

It seems that very considerable advances have been made in the enhancement and control of meat product odor and taste through the addition to the comminuted product of controllable microorganisms or even by flavor-producing chemicals. However, the origin and formation pathways of the aroma active compounds in meat products have not been completely elucidated and are necessary to select the appropriate conditions for obtaining improved meat product flavor.

13.7 Conclusions and future trends

Meat flavor has been studied for many years, but due to the meat matrix complexity its advancement has been a challenge. Nevertheless, a remarkable

progress was made in the past years in the identification and quantification of the characteristic meat aroma compounds. Thousands of volatile compounds were identified as volatile constituents, but the use of olfactometry methodologies and mass spectrometry contributed to increase the knowledge of the main meat odors. Among them, the impact of sulfur-containing compounds in meat aroma was of main importance, but the contribution of other aroma compounds should not be underestimated due to possible synergisms among aroma components. Moreover, many different factors affect the generation of flavor, and all of them should be taken into consideration to improve the quality of cooked and processed meat. This meat flavor knowledge is very helpful for the development of savory flavors in flavor creation. Although other features should be taken into account such as the sensory perception of flavor, the effect of meat matrix in flavor interactions, and the online monitoring of flavor release to link invivo results to consumers attitude. All of these aspects will help in understanding the complexity of flavor perception. Finally, the actual trend to demand natural flavor ingredients in foods makes flavor scientist to look for precursors and biotechnology processes for the production of natural meat flavors.

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Chapter 14

The eating quality of meat: IV—Water holding capacity and juiciness

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14.1 Introduction

Water-holding capacity (WHC) of fresh meat determines the visual acceptability, thus influencing the consumers' willingness to purchase the product. WHC also determines the loss of water during transport, storage, processing, and cooking. Meat juiciness, which is in part determined by WHC, is also an important trait and contributes to eating quality as well as playing a role in texture. Juiciness is a uniquely subjective property of meat.

Muscle comprises approximately 75% water at rigor and the addition of water to meat, and the hydration of the meat after processing or cooking, is closely related to taste, tenderness, color, and juiciness. The consequence of poor WHC is low cook yields and often "dry (lack of juiciness)" meat, so these can also be used to indirectly measure WHC. Poor WHC results in high drip and purge loss from meat and meat products, which can represent significant loss of weight from carcasses and cuts and may affect the yield and quality of processed meats. Water is also important because of its role in molding muscle structure and the consequent effects on quality. As water is lost from the muscle structure during heating and cooking, the proteins become less pliable and more rigid. Although when longer heating times are used, some proteins, such as sarcoplasmic and collagen, will become gelatinized and able to retain water.

14.2 Definition of water-holding capacity and juiciness

WHC is defined as the ability of meat and meat products to bind water (Pearce et al., 2011) during slicing, mincing, and pressing and also during transport, storage, processing, and cooking (Hamm, 1986). The water released can be described as drip, purge, weep, exudate, or cook loss, and these are inversely related to WHC (Warner, 2014). The water released from a processed meat

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product is often described as cook yield, and this is directly related to WHC. Each of these traits is related to each other but there is not always a strong correlation between different methods for measuring WHC. In particular, cook loss can have quite different influencing factors and thus should not be inferred from measurements of the other traits. Using the definition above, WHC refers both to the bound water contained within the meat and also to water added during various operations connected with meat processing. Sometimes the term water-binding capacity (WBC) is used. Where both terms are used, usually WHC points to the potential ability to bind water in raw meat, while WBC refers to the water bound by meat during its processing combined with heating (Pospiech and Montowska, 2011).

The content of water in muscle by chemical composition is about 75%; the other components being protein (~20%), lipids (~5%, but can vary, influencing water content), carbohydrate (~1%), and vitamins and minerals (~1%, often left as ash) (Offer and Knight, 1988). There is a direct relationship between water and fat content by percent such that as fat % increases, the water % decreases. Furthermore, as about 85% of the volume of the muscle cell is myofibrils, it is evident that the majority of the water is associated with myofibrils. About 1% of the water in meat is classed as "bound" water and is tightly bound by proteins (Huff-Lonergan and Lonergan, 2005). This water has reduced mobility and is resistant to freezing and heating (Fennema, 1985). The amount of bound water is thought to show little change in postmortem muscle (Huff-Lonergan and Lonergan, 2005) although the bound water undergoes continual exchange with the surrounding water molecules (Pearce et al., 2011), and Devine et al. (2014) showed that the bound water fraction changes with aging. Another fraction of water in meat is termed "immobilized" or "entrapped" water (Fennema, 1985) and the water is held by steric effects or by attraction to the bound water (Huff-Lonergan and Lonergan, 2005). This water does not easily leave the structure but can be removed by drying, be lost during the rigor process, and by changes in the physical protein structure, e.g., through protein degradation or denaturation. This fraction comprises about 85% of the total water (Pearce et al., 2011). "Free" water is the fraction of water that can flow unimpeded from the structure when conditions allow this to occur, independent of the charged groups (Pearce et al., 2011). It exists in the sarcoplasmic fluid and is also held by capillary forces between and within myofibrils (Table 14.1). It has been found that predominantly, WHC varies with water in this extra-myofibrillar fraction, as well as through the loss of intra-myofibrillar water through shrinkage. The extra-myofibrillar water, and a small fraction of the intra-myobrillar water, is easily mobilized such as by myofibrils and cells shrinking during the rigor process, but this water does not flow freely in pre-rigor or high ultimate pH meat (Pearce et al., 2011). The changes in the water content of these compartments in the conversion of muscle to meat are shown in Table 14.1. In Table 14.1, protein-bound water is the same as described above, and water is lost from the intra-myofibrillar and extra-myobrillar compartments, subsequently appearing in the

TABLE 14.1 Water distribution in muscles of live animals (pH ~ 7) and meat (pH 5.3–5.8). All values are approximate.

	% Water	
	Muscle	Meat
Protein-bound water	1	1
Intra-myofibrillar	80	75
Extra-myofibrillar	15	10
Extracellular water	5	15

Reproduced and adapted from Honikel, K. O. 2009. Moisture and water-holding capacity. In: Nollet, L. M. L. & Toldra, F. (eds.) Handbook of Muscle Foods Analysis. Boca Raton, Florida: CRC Press.

extracellular space where it is free to flow out of the meat as drip loss (Honikel, 2009). A shift in "free" and bound water occurs with aging and is discussed further in Section 14.5.3 below.

Water held within the meat by capillary action refers to a mechanism that is similar to water immobilized in a sponge or other porous material. The magnitude of the force immobilizing the water is inversely related to the pore size (see Table 14.2). For the water to be removed from the meat, an external pressure must be applied, which is greater than the pressure generated by the capillaries (Trout, 1988) (see Table 14.2). The water held by capillary action in the microstructure of meat is predominantly in pores between the thick and thin filaments of the myofibrils, which range from 0.02 μm radius in the A-band to 0.05 μm radius in the I-band. Some water is also held by capillary forces in the pores between myofibrils, which are approximately 0.5 μm in diameter. Thus in order for the water to be removed from meat, the force applied must be in the order of 200 to > 2000 psi (See Table 14.2). In meat products, the microstructure is quite different and the process of comminution (and heating) and addition of salt and/or phosphate destroy the muscle structure and the proteins rearrange and aggregate into a three-dimensional protein lattice, resulting in the predominance of pores being 0.1–1.0 μm in diameter (Trout, 1988), thus requiring less force in order for water to be removed. In this chapter, the focus is on the structural determinants of WHC. Water holding in meat can also be considered from a surface and colloidal chemistry perspective, which is discussed in a recent review by Puolanne and Halonen (2010).

Juiciness is a sensory trait and is determined through consumer or trained sensory panels (see Chapter 15). Unlike other measures of texture (e.g., tenderness), juiciness remains a uniquely subjective property of meat. In consumer grading systems, juiciness is calculated to contribute to 10% of the variation in overall acceptability of meat by a consumer (Watson et al., 2008).

TABLE 14.2 Effect of capillary radious on the hydrostatic pressure generated in small pores: expressed in cm of water and PSI (pounds per square inch). Calculated at 25°C and assuming a contact angle of 0°C.

Capillary Radius (μm)	Hydrostatic pressure (cm water)	Hydrostatic pressure (psi)	Approx. range in diameter of pores in muscle cell structures	Approx. range in diameter of pores in comminuted meat products
100	15	0.2		
10	150	2.0		
1	1500×10^3	20	Pores between myofibrils ~ 0.5 μm	Pores in microstructure of three-dimensional protein lattice
0.1	1.5×10^4	200		
0.01	1.5×10^5	2000	Pores between thick and thin filaments	
0.001	1.5×10^6	20,000		

From Trout, G. 1988. Techniques for measuring water-binding capacity in muscle foods—a review of methodology. Meat Sci. 23, 235–252.

Meat juiciness is defined as the impression of moisture and lubrication when meat is chewed in the mouth. Meat juiciness can be separated into two components. The first being the impression of wetness during the initial chews, produced by the rapid release of meat fluids, and thought to be related to the water content of the meat. The second component is the impression of juiciness during sustained chewing and is thought to be related to the fat content of the meat as it is considered to be a result of the stimulating effect of fat on salivary flow (Winger and Hagyard, 1994).

14.3 Structural influences on the WHC of uncooked, cooked, and processed meat

In the living animal, water is kept in cells by the sarcolemma (cellular membrane) and is maintained by various membrane pumps. Post-slaughter, water is moved to the sarcoplasm by longitudinal and transverse shrinkage of the myofibrils and kept in the cell until the pH falls, adenosine triphosphate becomes unavailable, and water and ions can move and pass through the sarcolemma into the extracellular space between the cells. In muscle, approximately at pH 7, of a live animal, the myofibrils take up most of the space in the muscle cell. Postmortem, the myofibrils shrink both in transverse and longitudinal directions, the intracellular sarcoplasmic space increases, and over time, water moves to the extracellular space (Hughes et al., 2014). In a muscle at approximately pH 7, more than 95% of the water is within cells; some days postmortem and at pH 5.5, approximately 15% is in the extracellular space (see Table 14.2), and the water appears as drip at the surface of the meat.

Weight loss of meat, during storage, freezing, and thawing, is related to how much water is available and how easily it can leave the muscle structure network. Water flow from a cut of meat is a time-dependent physical process that requires a driving pressure. The rate of flow depends on the permeability of the material and the dimensions and length of spaces, with the majority of the water held by capillary forces. From a structural perspective, water loss from meat is mainly influenced by (a) the longitudinal and transverse shrinkage of myofibrils, which changes interfilament spacing, (b) breakdown in the cell membrane structure making it more permeable to water, (c) integrity of the intracellular cytoskeleton, which affects shrinkage of the whole cell, (d) development of spaces between cells allowing fluid to accumulate and flow (Hughes et al., 2014), and (e) development of a network between sarcoplasmic and myofibrillar proteins, which entraps water (Liu et al., 2016).

In the living animal, there is very little extracellular space between muscle cells. Postmortem, during the process of rigor onset and fall in pH down to a normal pH (~5.5), the extracellular space between muscle cells increases (Heffron and Hegarty, 1974), allowing channels for fluid flow to develop. These channels conduct the fluid to the meat surface, predominantly along the muscle fiber direction (Hughes et al., 2014). Muscles exhibiting high drip

loss generally have earlier development of drip channels postmortem, and the extracellular spaces are larger (Hughes et al., 2014). The muscle cell membrane forms a barrier between the intracellular and extracellular spaces. Thus fluid can flow more freely to the extracellular space when the membrane becomes more permeable. Muscle cells with higher permeability in their membranes exhibit greater drip and fluid loss from the meat (Hughes et al., 2014). Furthermore, intracellular antioxidants, such as polyphenols or Vitamin E, can prevent membrane breakdown postmortem and reduce water lost as drip or purge during storage (Shakeri et al., 2020; Asghar et al., 1991). Myofibrils are linked to each other and to the sarcolemma through the cytoskeleton, which is comprised of a network of linked fibrous proteins. These proteins are differentially degraded postmortem, and once degraded, the linkage between myofibrils and the cell membrane is lost and shrinkage in myofibrils can no longer be transferred to shrinkage in the cell as a whole, thus influencing water loss from the muscle cell structure (Straadt et al., 2007). During aging, there is a progressive breakdown in the myofibrillar structure and an increase in concentration of small peptides, resulting in increased WHC in raw muscle, which is discussed further in Section 14.4.3 below.

Sarcoplasmic proteins are known to be degraded during the curing of dry-cured hams and also during aging of fresh pork and beef (Stoeva et al., 2000; Olmo et al., 2013; Lametsch et al., 2003). Drip loss from beef muscles is correlated to the protein concentration of the drip (Den Hertog-Meischke et al., 1997), and a positive role of sarcoplasmic proteins in WHC of raw muscle has been demonstrated (Liu et al., 2016). The mechanism appears to be the formation of a network linkage between sarcoplasmic and myofibrillar proteins, allowing water to be entrapped.

In cooked meat, the structural influences on WHC also dominate and are driven by the shrinkage that occurs in the structure as the temperature in the meat increases and proteins denature at different temperatures. This is discussed further in Section 14.5 below. In processed meat products, the influence of different ingredients on swelling in the myofibrillar lattice is important as well as the development of a structured gel network. As shown in Table 14.2, the pores in this three-dimensional protein network hold the water, and this is discussed further in Section 14.7 below.

Thus water loss from the muscle structure of raw, cooked, and processed meat products is related to the force applied (shrinkage of myofibrils, cells, and gels), membrane permeability, cytoskeletal and myofibrillar protein degradation, development of channels for water flow, network development between proteins, protein denaturation, and effect of ingredients of myofilament spacing. Variations in thick filament spacing can cause significant changes in volume, and the shrinkage force will squeeze water out of the intra-myofibrillar structure. Sarcomere length, myosin and sarcoplasmic protein denaturation, pH, ionic strength, ions, and osmotic pressure can cause changes in thick and thin filament spacing, and these are described in following sections.

14.4 Factors influencing WHC in raw muscle

WHC of raw muscle changes as a result of animal genetics, pre-slaughter stress, antemortem and postmortem factors, which are discussed below. Postmortem glycolytic metabolism and pH fall (see Chapters 4 and 5) are fundamental determinants of WHC. Variation in postmortem muscle metabolism is often the underlying mechanism behind many genetic effects and observed variations in metabolomic and proteomic profiles in muscles of the animal which are linked to meat quality and WHC.

14.4.1 pH fall postmortem, PSE, and DFD

The rate and extent of pH fall, associated with postmortem anaerobic muscle glycolysis (see Chapter 4), are major determinants of the WHC of raw, processed, and cooked meat products. Associated with the fall in pH in "normal" muscle postmortem from 7.0 to 5.5 is the transverse shrinkage in the myofilament lattice and the expulsion of water, with consequent loss of water from the meat as drip, exudate, or purge. This can be explained by changes in the structure as a result of changes in the chemical charge on the proteins. During rigor, as the pH of the muscle approaches 5.4 or lower, the net charge on proteins in the myofibrils diminishes and filaments can approach each other, causing transverse shrinkage in the myofibril. The isoelectric point, defined as the point of minimum charge, of the myofibrillar proteins occurs at pH 5.0–5.2. As the ultimate or final pH approaches the isoelectric point of the muscle proteins, the WHC of the muscle reaches a minimum (Fig. 14.1). Thus, there is always some loss of WHC when the pH of muscle drops from 7 to 5.5 (Fig. 14.1). Meat of high ultimate pH (namely dark-cutting or dark, firm, dry (DFD), see below) does not undergo shrinkage in the myofibrils and muscle cells postmortem due to the pH being well above the pI and repulsion between the myofilaments due to the net negative charge (Fig. 14.1). In contrast, meat of low ultimate pH with denatured proteins (PSE, see below) has excessive shrinkage in the myofibrils and muscle cells. This is shown in Fig. 14.1 where the decline in % bound water from pH 7.0 to pH 5.0 is demonstrated.

Meat that reaches a low pH of 5.2–5.3 (i.e., large extent of muscle pH fall, pH drops excessively postmortem), such as pig meat from animals carrying the RN gene (Rendement Napole gene; common in the Hampshire breed of pig), will lose more fluid and have lower WHC. Conversely, meat that only has a small drop in pH (low extent of pH fall, pH does not drop very far postmortem) will lose less fluid due to the high ultimate pH, described as DFD (dark, firm, dry) or dark-cutting. Dark-cutting (beef, sheep meat), also DFD (pigs) meat, is defined as meat with a high ultimate pH (>5.8), and this meat loses less drip than meat of normal pH. Dark-cutting meat is caused by low muscle glycogen at slaughter as a consequence of pre-slaughter stress and is described in Chapter 5.

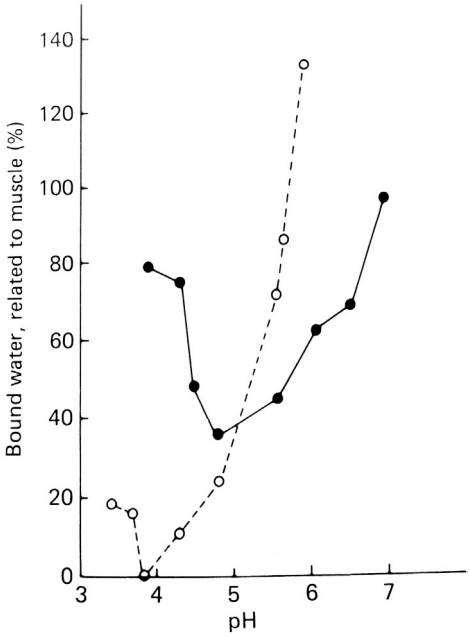

FIG. 14.1 Influence of pH on the water-holding capacity of salted (open circle; 2% NaCl) and unsalted (closed circle) comminuted beef (50% added water; filter paper press method). *From Hamm, R. 1986. Functional properties of the myofibrillar system and their measurements. In: Bechtel, P. J. (Ed.) Muscle as Food. New York: Academic Press.*

In muscle prone to the pale, soft, and exudative (PSE) condition, the high temperatures shortly after slaughter (35–42°C) and the low pH, caused by rapid glycolysis, lead to myosin denaturation along with early membrane destruction. When the myosin head denatures, it shrinks from 19 to 17 nm causing a transverse shrinkage in the myofibrillar lattice in addition to the shrinkage due to the low pH (Offer and Knight, 1988). In PSE meat, these phenomena cause excessive transverse shrinkage in the myofibril, sufficient to explain the excess water loss in PSE meat. PSE in pig carcasses is generally caused by pre-slaughter stress, genetic stress susceptibility (halothane gene), and some forms of stunning (Channon et al., 2000) (also see Chapter 5). In PSE muscles, the three conditions of low pH, denatured myosin, and damaged membranes cause drip loss to occur virtually without any time lag (Hughes et al., 2014). The strongest increase in drip loss occurs in the first 2 days. This seems to be less of an issue in beef and lamb, although increased drip has been observed in beef and sheep muscle going through high temperature at rigor (Warner et al., 2014a,b).

14.4.2 Rigor, cold, and heat shortening

With the onset of rigor, and the formation of the actomyosin rigor bond, there is some shortening in the sarcomere. In addition, pre-rigor (pH > 6–6.2) muscle

temperatures below 12°C cause membrane failure, calcium release from the sarcoplasmic reticulum, and significant sarcomere shortening. Conversely, if the muscle enters rigor at a high temperature (>25–30°C), sarcomere shortening can also occur. These phenomena are discussed in more detail in Chapter 4. Of relevance is the effect on WHC. When the sarcomere shortens, due to rigor, cold, or heat shortening, the myofibril shortens longitudinally, with water being expelled to the intracellular space. This can be prevented by ensuring the muscle is stretched.

Thus, meat from stretched muscles, with long sarcomeres, has higher WHC as measured by drip loss, purge, and cooking loss. This is shown in Fig. 14.2 and Fig. 14.3 where the increased WHC at longer sarcomeres is evident for porcine *psoas* and ovine *gluteus medius,* respectively. Fig. 14.3 also demonstrates the reduction in WHC in lamb muscle when myosin is denatured due to high pre-rigor temperatures and PSE conditions.

14.4.3 Electrical stimulation

Electrical stimulation of beef and sheep is used to accelerate postmortem glycolysis, reduce the time to rigor onset, and prevent cold shortening (see Chapter 5). Electrical stimulation of pig carcasses, using 200–400 mA, results in an increase in both drip loss and purge during storage of pork and thus is not recommended for pig carcasses. In general, there have been very few studies showing a detrimental effect of electrical stimulation on beef or lamb muscle

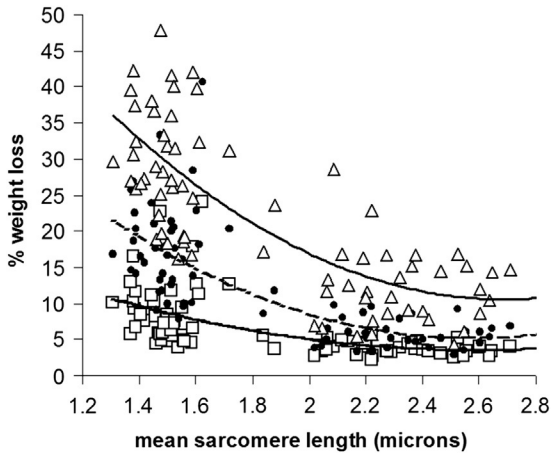

FIG. 14.2 Drip from porcine *psoas* muscle varies with sarcomere length and time postmortem. Percentage weight loss, also showing trend lines, after 48 h (*open squares, dotted line*), 72 h (*filled circles, dashed line*) and 144 h (*open triangles, solid line*) postmortem versus the mean sarcomere length. *From Hughes, J. M., Oiseth, S. K., Purslow, P. P., Warner, R. D. 2014. A structural approach to understanding the interactions between color, water-holding capacity and tenderness. Meat Sci. 98, 520–532.*

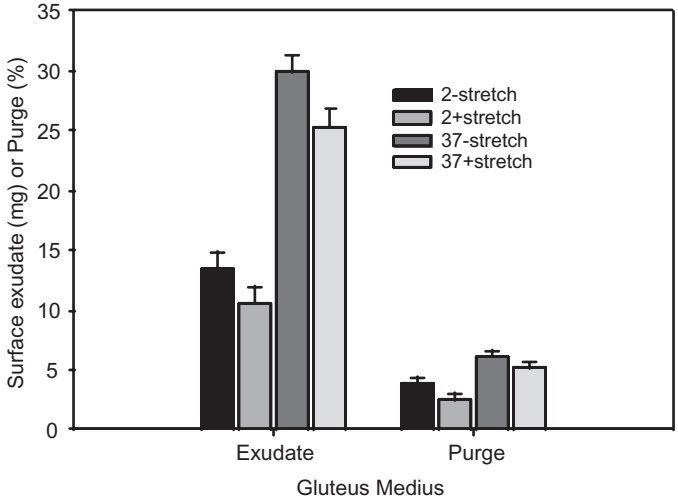

FIG. 14.3 Surface exudate (mg) and purge (%) from ovine hot-boned *gluteus medius* muscle subjected to pre-rigor stretching (− stretch, + stretch; sarcomere lengths 1.95 vs. 2.8 μm respectively) and pre-rigor temperature treatments of cold or hot (2°C, 37°C; myosin denaturation measured by myofibrillar ATPase activity 0.091 vs 0.122 μmol/min/mg protein). The data demonstrate that meat with longer sarcomere length (+ stretch) has greater water-holding capacity and that meat subjected to high pre-rigor temperatures, with associated myosin denaturation, has reduced water-holding capacity. *Derived from Warner, R. D., Kerr, M., Kim, Y. H. B., Geesink, G. 2014b. Pre-rigor carcass stretching counteracts the negative effects of high rigor temperature on tenderness and water-holding capacity using lamb muscles as a model. Anim. Prod. Sci. 54, 494–503.*

WHC. Conversely, most studies on beef and lamb have only examined the *longissimus thoracis et lumborum* muscle. Muscles deep in the hindleg of the beef carcass are likely to cool more slowly and thus be more susceptible to exhibiting pale, weepy beef (Tarrant and Mothersill, 1977). Den Hertog-Meischke et al. (1997) showed that although electrical stimulation of beef carcasses did not influence the WHC of the *longissimus thoracis* muscle, the *semimembranosus* from electrically stimulated carcasses had higher drip loss and filter paper wetness and lower myofibrillar WHC. Reduced myofibrillar WHC and increased drip loss are found at greater depths in the hind limb muscles of the beef carcass such as the *adductor, semimembranosus, biceps femoris, and semitendinosus*. The problem can occur in beef and sheep carcasses if excessive electrical stimulation is used, inducing very rapid pH fall or if electrical stimulation is used on carcasses where the muscle metabolism is already rapid, such as in heavy grain-fed beef carcasses (Warner et al., 2014a). Thus electrical stimulation of beef carcasses is likely to exacerbate the occurrence of pale, weepy beef, particularly in hind limb muscles. For this reason, the total electrical inputs into the beef carcass, including inputs at the immobilizer (post-stunning) and hide puller, as well as any electrical stimulation applied to the carcass, need to be considered. An increase in total slaughter-floor electrical inputs has been shown to increase the

rigor temperature and increase the purge (weep score) of the beef loin muscle at grading (Warner et al., 2014a) as shown in Fig. 14.18.

14.4.4 Changes in WHC during aging

Aging of meat was originally thought to increase its WHC, along with an increase in pH during aging. Kristensen and Purslow (2001) observed destruction of the cytoskeletal proteins (especially talin, desmin, vinculin) due to proteolysis during aging which was hypothesised to remove the linkage between shrinkage of the muscle fibers per se and that of the myofibrils. With the removal of the cytoskeletal linkages, the force expelling water from within the cell is eliminated and re-entry of the water is potentiated, thus muscle cells and myofibrils are observed to "swell" during aging. Many authors have shown an increase in purge from meat during aging/storage in a vacuum bag, and also as a consequence of cooking meat which has previously undergone an aging period (cook loss, discussed further in Section 14.5 below) (Shanks et al., 2002; Straadt et al., 2007; Warner et al., 2007, 2014b). The changes in water loss with aging can be dependent on the method used and the muscle studied. Over a 23-d aging period, bovine *longissimus lumborum* (L) and *semimembranosus* (SM), but not *psoas major* (PM), increase in purge but decrease in drip loss in LL and SM, but not PM (Ma and Kim, 2020) (Table 14.3). Farouk et al. (2012) showed a decrease in gravitational and centrifugal water loss in ovine *longissimus* aged for 9 weeks although there was an initial increase over the first 1–2 weeks (Fig. 14.4) This decrease in water loss with aging reported by Farouk et al. (2012) may be explained by the progressive degradation of myofibrillar proteins with aging and their "sponge" hypothesis, as illustrated in Fig. 14.5. The increase in purge with aging shown in Table 14.3 is not explained by the "sponge" hypothesis. Likely the pressure applied during vacuum packing shifts the water distribution in the muscle, and as proteins progressively degrade during aging in a vacuum pack and the cells "swell," this is not expressed as an increase in WHC but in fact the reverse, a decrease in WHC with the continual increase observed in purge with aging. Devine et al. (2014) showed that as aging of lamb loin progresses, there is an increase in free water and a decrease in bound water (Fig. 14.6), although for muscles entering rigor at the ideal temperature of 15°C, this shift was less pronounced than for muscles entering at lower or higher temperatures. Thus the effect of aging on WHC varies with method of measurement and muscle and is discussed further in Section 14.5.

14.4.5 Cutting, packaging, and temperature during storage

A major problem with any centralized packaging system for retail cuts of fresh meat is drip. When large primals or chunks of meat are cut into smaller pieces, water in the muscle structure is able to flow out as drip, and greater surface area after cutting will result in greater water loss. Increased handling of meat and

TABLE 14.3 Effect of bovine muscle (LL, *longissimus lumborum*; SM, *semimembranosus*; PM, *Psoas major*) and aging period on water-holding capacity traits. SE is the standard error of the mean. $N = 8$ for each mean.

			Muscle (M)			
	Aging (A)	LL	SM	PM	SE	*P*-values
Cook loss (%)	2 d	22.77	30.39	28.99	1.08	M, $P<0.0001$ A, $P=0.02$ M×A, $P=0.007$
	9 d	21.98	30.74	22.24		
	16 d	23.01	27.25	26.15		
	23 d	22.22	28.06	24.59		
Purge loss (%)	2 d	1.61	2.22	2.89	0.54	M, $P=0.006$ A, $P<0.0001$ M×A, $P=0.002$
	9 d	2.40	3.21	6.14		
	16 d	3.29	4.97	3.82		
	23 d	4.31	5.48	4.53		
Drip loss (%)	2 d	3.15	3.79	1.28	0.29	M, $P<0.0001$ A, $P<0.0001$ M×A, $P=0.0005$
	9 d	3.29	3.48	1.91		
	16 d	1.70	2.39	1.96		
	23 d	1.60	2.10	1.49		

Derived from Ma, D., Kim, Y. H. B. 2020. Proteolytic changes of myofibrillar and small heat shock proteins in different bovine muscles during aging: their relevance to tenderness and water-holding capacity. Meat Sci. 163, 108,090.

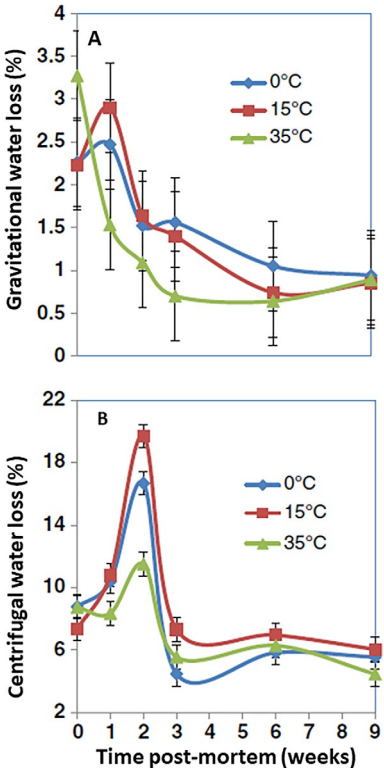

FIG. 14.4 After storing bovine *longissimus* samples pre-rigor at 0°C, 15°C, or 35°C, changes in water loss with aging time at −1.5°C (weeks) of *semimembranosus* measured (A) gravimetrically (drip loss) and (B) through centrifugation. Error bars = standard error of the difference between means. $N = 18$ for each data point. For gravimetric water loss, ~50–100 g samples were suspended in netting held inside a plastic dish and then stored at 4°C for 72 h (Honikel, 1998). For centrifugal water loss, muscle strips 4 mm wide and 15 mm long, along length of muscle fibers, were centrifuged at 40 g at 4°C (Kristensen and Purslow, 2001). *Derived from Farouk, M. M., Mustafa, N. M., Wu, G., Krsinic, G. 2012. The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with aging. Meat Sci. 90, 670–677.*

also pressure changes can increase drip loss, as each can exert a force to the muscle, which compresses the structure.

Different packaging processes can apply various physical compression forces as well as brief heat treatments, which may influence water loss. Meat packs can contain a pool of fluid, which is unsightly and often disliked by the consumer. This is the reason soaker pads are used to absorb any fluid exuding from the meat during storage and display. A major determinant of weep in the pack is the surface-area-to-volume ratio, with less weep occurring with a reduced surface area. In addition, less weep occurs with longitudinal rather than transverse cutting of muscle fibers (Mcmillin, 2008).

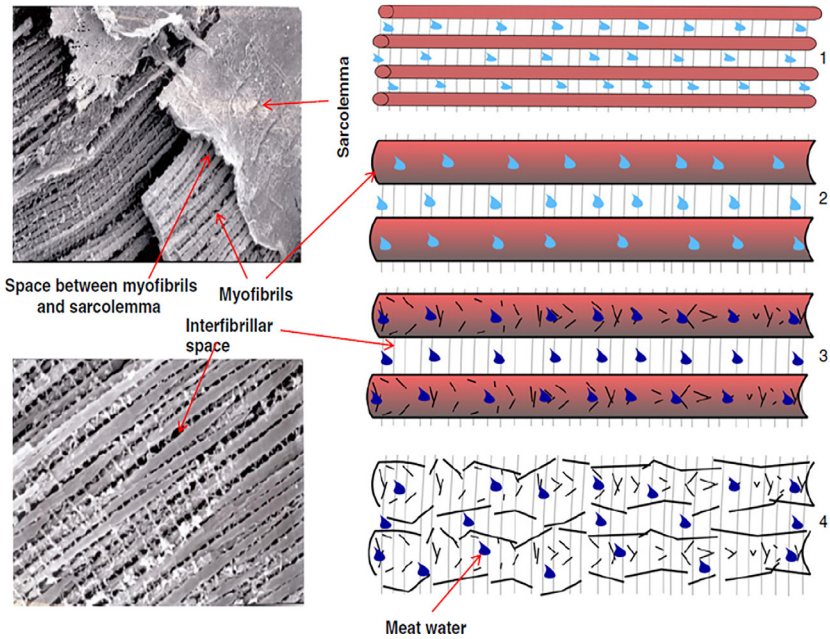

FIG. 14.5 LEFT: The images were from a *longissimus* derived from a carcass infused with a tenderizing solution, which accelerated the breakdown of the collagenous tissue substances within 24 h. The tenderness improved by about 25% within 24 h. Both images were taken on a scanning electron microscope (SEM) at a magnification of 2000. Top image is of the muscle freeze fractured at 0 h postmortem and shows the presence of a layer of endomysial-sarcolemmal sheath covering the surface of the myofibrils. The bottom image is the same sample removed at 24 h, and then freeze fractured, with the sheath absent and the surface of the myofibrils exposed. RIGHT: Pictorial representation of the "sponge" effect hypothesis for the improvement in meat water-holding capacity with aging. The structural proteins within and between myofibrils progressively breakdown with aging (1–4); the viscosity of drip water continue to increase with aging time resulting in greater force being required to expel water from the meat by gravity. *Derived from Farouk, M. M., Mustafa, N. M., Wu, G., Krsinic, G. 2012. The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with aging. Meat Sci. 90, 670–677, with addition of explanatory notes on SEM from first author.*

The negative pressure applied during vacuum packing results in extraction of liquid from the meat, resulting in increased exudation and purge in the bag (Gariepy et al., 1986). Samples of beef, pork, and lamb *longissimus* stored in vacuum lose between 2% and 6% of weight in the form of drip during a storage period of 5–7 days at 0°C. The weight loss will be higher if the samples are from animals stressed pre-slaughter or at stunning, from the halothane genotype (in the case of pigs) or exposed to PSE conditions or high rigor temperature post-slaughter (Channon et al., 2000; Warner et al., 2014b). Increasing the storage period of meat in a vacuum bag to 3 or more weeks can double or even triple the weight loss compared with 1 week

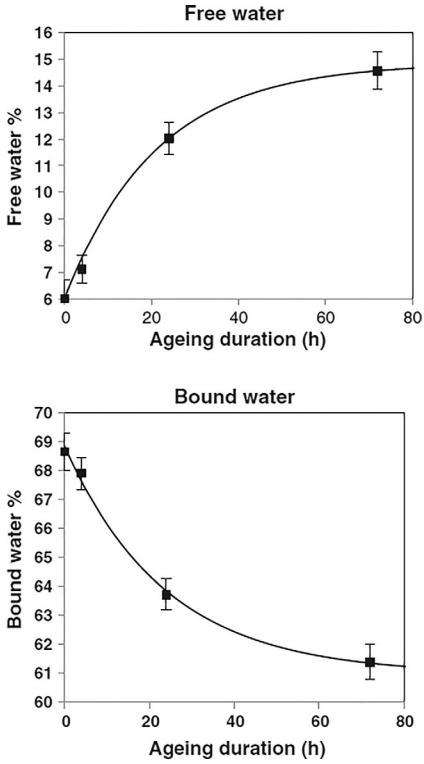

FIG. 14.6 Changes in free water and bound water for ovine longissimus muscles held at 15°C postmortem. Each mean is comprised of $n = 14$ and the vertical bar represents ± standard error. Zero time represents rigor mortis, not time of slaughter. Free water was measured by centrifuging 2 g muscle sample for 15 min at 1800 G, and the water lost was measured by weight difference (Kristensen and Purslow, 2001). Bound water was the water that was "dried off" from the sample after oven-drying the centrifuged sample. *From Devine, C., Wells, R., Lowe, T., Waller, J. 2014. Pre-rigor temperature and the relationship between lamb tenderization, free water production, bound water and dry matter. Meat Sci. 96, 321–326.*

storage (Warner et al., 2007). Elevated or fluctuating temperatures during storage increase the drip loss from meat. Hertog-Meischke et al. (1998) demonstrated that there is an increase in drip loss for meat stored at 3°C, compared with 0°C, with the effect being more pronounced in the *semimembranosus*, then in the *longissimus*. Others have reported an increase in drip loss when the storage temperature was elevated from 0°C to 10°C, with a more rapid increase in drip loss for storage temperatures between 5°C and 10°C (O'Keeffe and Hood, 1981).

In comparison to vacuum packing, modified atmosphere packaging (MAP) generally results in less drip loss or weep in the pack (Mcmillin, 2008), most likely as no pressure is applied during packing. Conversely, meat is only stored

in MAP for a short period, < 8–10 days (Mcmillin, 2008), which may explain a portion of the lower water loss.

14.4.6 Thaw rigor

Thaw rigor or thaw contraction describes the shortening that occurs when meat, which has been frozen pre-rigor, is thawed. It is characterized by massive shortening and drip loss up to 30% (Pearson and Young, 1989). Due to the excessive toughening and drip loss, it is of great practical significance to the meat industry. Thus it is recommended that meat is not frozen while in the pre-rigor state. Thaw rigor appears to result from an extensive release of Ca^{2+} so that the sarcoplasmic reticulum becomes saturated and the Ca^{2+} spills into the extracellular space, causing muscles to contact and shorten (Pearson and Young, 1989). The onset of thaw rigor occurs when the concentration of ATP is still high (~40%) and appears to be similar to cold shortening, except on a larger scale. Dransfield (1996) has suggested that thaw rigor is not caused by reduced sarcomere length and muscle shortening, but by variation in calpain activity. Electrical stimulation of carcasses, which hastens rigor onset, is a proven method to prevent thaw rigor.

14.4.7 Freezing and thawing

Freezing and thawing affect the amount of exudate, thaw loss, and drip loss from the meat. As the time to reach a frozen meat product increases above 12–20 min, the amount of exudate that forms on thawing becomes markedly higher (Ngapo et al., 1999). This phenomenon has been associated with the size and distribution of the ice crystals that form along the freezing gradient. However, prolonged freezer storage has been found to level out effects of freezing rate, which is suggested to be a consequence of recrystallization of small crystals into bigger crystals during long-term freezer storage (Ngapo et al., 1999). Denaturation and oxidation of myofibrillar proteins during freezing and thawing result in a lower pI and in reduced WHC and higher purge loss (Zhang and Ertbjerg, 2018). The condition of the muscle prior to freezing influences the water loss upon thawing. Bovine *semimembranosus* aged for 0–6 weeks prior to freezing showed a progressive decline in thawing water loss and expressible water with aging, and this was shown to be correlated with protein extractability and spreadability, supporting the "sponge" hypothesis (Farouk et al., 2012) (Table 14.4). There is also a correlation between the rate of thawing and the extent of exudate formation. A decrease in thawing time (e.g., to < 50 min) results in a decrease in exudate (Ngapo et al., 1999). This is attributed to the melting of ice in the extracellular spaces causing an increase in water activity, resulting in the net flow of water into the intracellular spaces and its subsequent reabsorption by the dehydrated fibers. Refrigerated thawing for 28 h produces the greatest drip loss, and rapid thawing of meat by submergence in water decreases

TABLE 14.4 Bull *semimembranosus* water-holding and structural changes after aging for 0, 1, 3, or 6 weeks and then freezing for 5 days. $N = 8$ for each mean.

	Storage time (weeks)					
	0	1	3	6	SED	*P*-value
Thaw loss (%)[a]	11.7	11.4	10.8	7.9	1.16	0.01
Expressible water (%)[b]	65.4	62.6	62.89	56.1	2.55	0.01
Spreadability (%)[b]	34.6	37.4	37.2	43.9	2.55	0.01
Protein extractability (%)[c]	7.4	7.1	9.6	10.3	1.30	0.05

[a] *Thaw loss is the weight lost after thawing.*
[b] *The filter paper press method was used for spreadability and expressible water where ~0.5 g sample was subjected to 60 kg pressure. After the pressure was removed, the areas on the filter paper were measured using imaging software, where the area of the outer circle represents the expressible water and the inner circle represents spreadability.*
[c] *Protein extractability was measured by homogenizing 5 g sample of mince in 150 mL of 4% (w/v) NaCl and the protein in the supernatant was measured.*
Derived from Farouk, M. M., Mustafa, N. M., Wu, G., Krsinic, G. 2012. The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with aging. Meat Sci. 90, 670–677.

drip loss (Ambrosiadis et al., 1994). Water lost during thawing of meat is an important influence on industry economics due to potential weight loss and can be minimized with careful attention to pre-freezing muscle condition and freezing and thawing rates.

14.5 Changes in WHC during cooking of raw meat

WHC changes as a result of cooking and heating, and this is shown in Fig. 14.7, and the influence of structure, protein denaturation, aging, and high pressure processing is discussed below. Water is lost during the cooking process of fresh and processed meat products, as a result of muscle proteins denaturing at various temperatures, inducing transverse and longitudinal shrinkage. Greater understanding of the interplay between muscle structure, functionality, protein denaturation, and cooking conditions will enhance development of innovative ways to prepare novel meat products of high water-binding capacity. These are discussed below.

14.5.1 Relationship between cooking temperature and water loss

During cooking, the muscle proteins denature, leading to a decrease in their WHC and to shrinkage of the protein network. The shrinking network exerts a mechanical force on the water between the fibers (Van Der Sman, 2007). In the

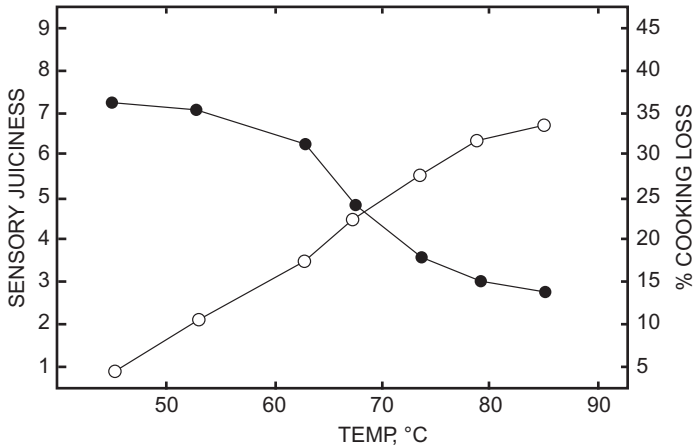

FIG. 14.7 Juiciness and cooking loss of 1 cm thick beef *semimembranosus* slices as a function of heating temperature. Samples were heated to the specified temperature at 1.2°C/min and once the temperature was reached, were held at the temperature for 5 or 20 min. Each point is an average of 6 animals and of the two holding times. Open circles are cooking loss % (average error of the mean, AEM = 1.01) and closed circles are sensory juiciness (AEM = 0.16). *From Martens, H., Stabursvik, E., Martens, M. 1982. Texture and color changes in meat during cooking related to thermal denaturation of muscle proteins. J. Texture Stud. 13, 291–309.*

presence of pressure gradients, the excess interstitial water is expelled to the surface of the meat, and the expelled fluid is commonly known as cooking loss (Van Der Sman, 2007). Thus meat can lose a large quantity of its mass in the form of meat juice, and this is known to occur in a temperature- and time-dependent manner (Martens et al., 1982). Water loss determines the technological yield of the cooking operation, making it a critical factor in the industry. An increasing rigidity of the myofibrillar structure occurs with cooking, due to the denaturation of proteins, and this is associated with increased water loss during cooking. A temperature dependent increase in % cooking loss occurs over the range from 45°C to 80°C, and above 80°C, the % cooking loss usually tapers off (Figs. 14.7–14.9) (Tornberg, 2005; Martens et al., 1982; Vaskoska et al., 2021). Furthermore, the steepest increase in cooking loss occurs over the temperature range of 50–65°C (Van Der Sman, 2007), which corresponds to the temperature range over which the greatest change in volume of the muscle cell occurs (Hughes et al., 2014). The cooking loss in whole muscle and in minced meat is similar at all temperatures from 45°C to 80°C, except at 65°C where whole muscle has greater cook loss than hamburger (Fig. 14.8) (Tornberg, 2005). At 65°C, there may be a cooperative shrinkage between collagen and muscle fibers as this is the temperature of peak denaturation for collagen (see below) (Tornberg, 2005). A temperature of 65°C is very close to the temperature at which there is a major change in the state of water in meat during heating (66°C) (Micklander et al., 2002). Muscles with predominantly red muscle fibers (type I) such as the *masseter* have a peak in myosin denaturation above 60°C, whereas muscles comprised of predominantly white fiber types (type IIb) such as the *cutaneous truncii* show a peak in myosin denaturation at about 55°C (Vaskoska et al., 2020b). This influences the pattern

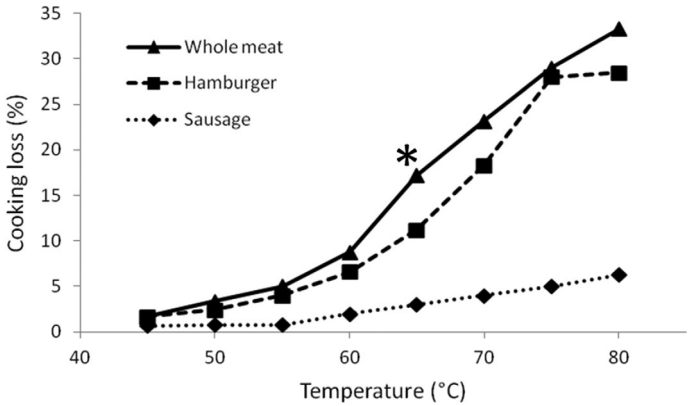

FIG. 14.8 Cooking losses (%) as a function of cooking temperature for whole meat, hamburger, and an emulsion sausage. The meat used in all cases was *biceps femoris*. * shows the only temperature point where there was a difference in cooking loss between whole meat and hamburger. *From Tornberg, E. 2005. Effects of heat on meat proteins—implications on structure and quality of meat products. Meat Sci. 70, 493–508.*

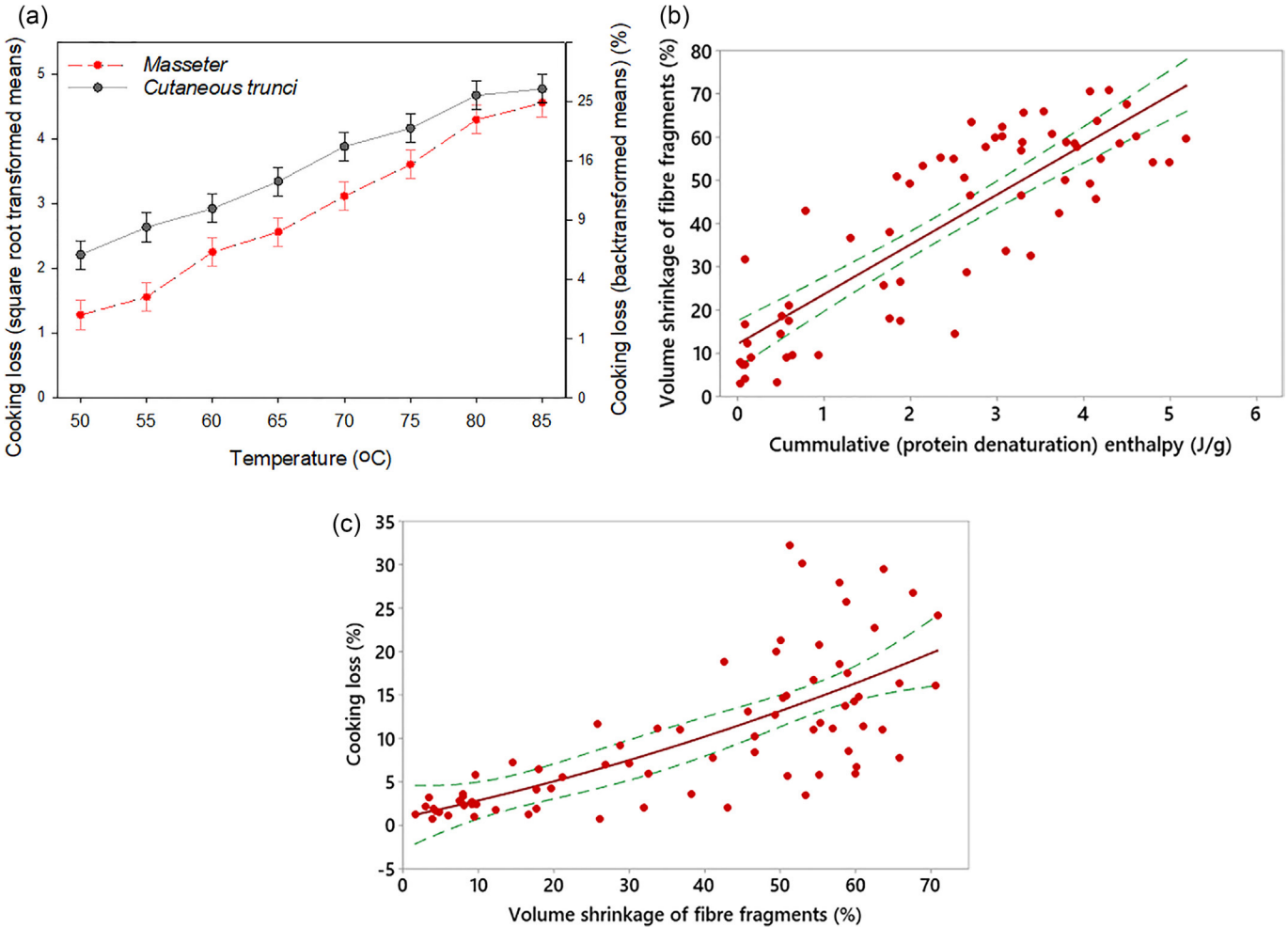

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FIG. 14.9 A consistent heating rate of 5°C/min was applied to muscle cuboids (50 mm L, 10 mm W, 10 mm H) for measuring cooking loss, muscle fibers homogenized in mannitol buffer (380 mM mannitol, 5 mM potassium acetate, pH 7) and viewed under a microscope during heating to measure volume shrinkage and 20 mg of muscle placed in aluminum pan and subjected to differential scanning calorimetry (DSC) to measure cumulative enthalpy (protein denaturation) ΔH. (A) Effect of bovine muscle (*Masseter*, 100% fiber type I; *Cutaneous truncii*, 94% fiber type IIa and IIb) and cooking temperature (55–85°C) on cooking loss from muscle cuboids. Data was transformed due to heteroscedastic variance and back-transformed values are shown on right-hand axis. (B) Relationship between cooking loss in cuboids and cumulative enthalpy in DSC ($R^2 = 70.94\%$; $P < 0.001$). (C) Relationship between cooking loss in cuboids and volume shrinkage in muscle fiber fragments ($R^2 = 52.24\%$; $P < 0.001$). $n = 10$ for each mean and the standard error of the difference is shown ± each mean. *Derived from Vaskoska, R., Ha, M., Ong, L., Chen, G., White, J., Gras, S., Warner, R. 2021. Myosin sensitivity to thermal denaturation explains differences in water loss and shrinkage during cooking in muscles of distinct fiber types. Meat Sci. 179, 108,521.*

of water loss during cooking as water loss follows protein denaturation temperatures and the associated volume shrinkage as shown in Fig. 14.9 (Purslow et al., 2016; Vaskoska et al., 2020a,b, 2021). A final phase of water loss during cooking occurs around a temperature of 70–80°C. FT-IR has been used to identify a sharp transition in chemical conditions in muscle tissue around 70°C (Kirschner et al., 2004), and NMR has been used to identify a transition in the state of water at 76°C (Micklander et al., 2002).

14.5.2 Water loss in relation to protein denaturation

Most of the water loss during cooking is from the juice expelled by protein denaturation and contraction of muscle structures, and this changes with the temperature of cooking (Kondjoyan et al., 2013). Although collagen contraction is thought to lead to meat shrinkage and water transport during cooking (Bouhrara et al., 2012), there is some debate over the role of collagen in muscle shrinkage and fluid expulsion during cooking (Purslow et al., 2016). As discussed above, the majority of the water is held in myofibrils, and both NMR and cooking studies have shown that the water loss during cooking of minced or hamburger muscle is similar to that of intact muscle (see Fig. 14.8). As the connective tissue structure is destroyed by mincing, this illustrates the lack of a role collagen may have in the formation of cook loss (Bertram et al., 2004; Tornberg, 2005).

Muscle tissue is known to shrink laterally and longitudinally by different degrees at different temperatures (Tornberg, 2005), indicating that not one but several proteins are involved in shrinkage and water expulsion observed during cooking. Fig. 14.9 shows the change in volume of a muscle fiber fragment when heated from 25°C to 75°C, and it is evident that the muscle fiber shrinkage is directly related to the cumulative enthalpy due to protein denaturation. Fig. 14.9 also clearly shows that the loss in water during heating is directly related to the loss in volume of the muscle block, which is a consequence of the shrinkage.

In the temperature range from 40 to 60 °C, transverse shrinkage occurs in the myofibrils as well as the muscle cell, if the cytoskeleton is intact. Myosin denatures in this temperature range (Bertazzon and Tsong, 1990), and the shrinkage in myofibrillar structure is generally attributed to this protein, although other proteins such as desmin may play a role (Hughes et al., 2014). Actin denatures in the region of 70–80°C (Bertazzon and Tsong, 1990) and is generally attributed to the longitudinal shrinkage in structure seen at these temperatures. However, as the denaturation of titin occurs at 75–78°C, contributions from titin denaturation should also not be discounted (Hughes et al., 2014). The video taken on a confocal microscope and available in supplementary material in Purslow et al. (2016) clearly shows the peak in transverse shrinkage in a muscle fiber fragment at ~55°C and then a peak in longitudinal shrinkage at ~80–85°C.

14.5.3 Cook loss changes with aging

Although there is an increase in WHC associated with the swelling of muscle fibers during aging and a decrease in drip loss (see Section 14.4.3 above), this is not translated into lower cooking loss which corresponds to the increase in purge (Straadt et al., 2007). Upon cooking, aged meat has pronounced shrinkage of muscle fibers as well as lower myofibrillar water (measured after cooking) (Straadt et al., 2007). Generally, the water lost during cooking is higher in meat that has been aged for at least 3–6 days relative to unaged meat and varies with the aging period. Fig. 14.10 shows an initial decline in cooking loss over 1–5 days of aging of beef *longissimus* followed by an increase in cooking loss over 5–35 days aging (Shanks et al., 2002). Others have shown an increase in cooking loss with aging (Warner et al., 2007; Straadt et al., 2007), and this

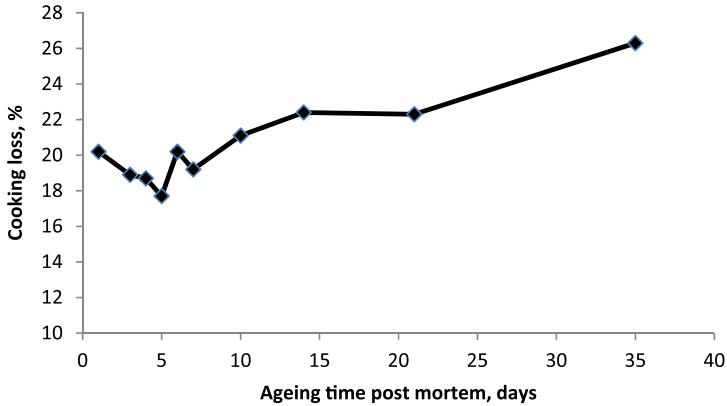

FIG. 14.10 Means for percent cooking loss of beef *longissimus* steaks at several different postmortem aging periods ($n = 20$ at each time point). A measure of the variation is indicated by RMSE in the original article. *Adapted from Shanks, B. C., Wulf, D. M., Maddock, R. J. 2002. Technical note: The effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. J. Anim. Sci. 80, 2122–2125.*

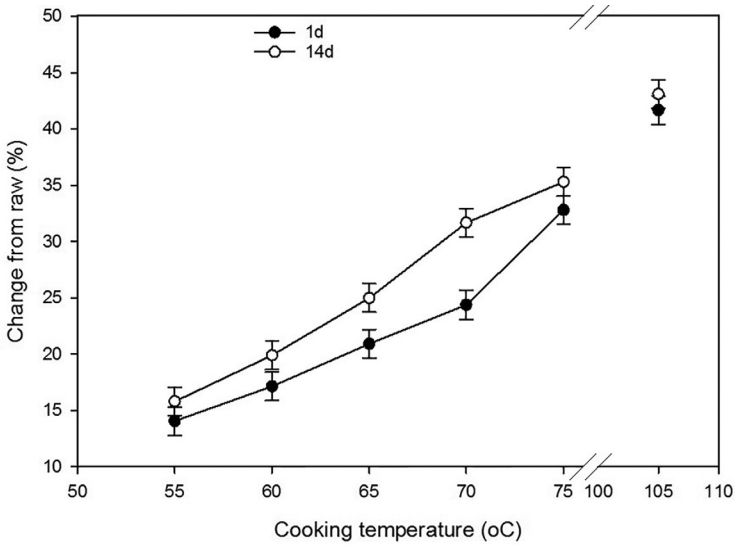

FIG. 14.11 The effect of cooking temperature (55–105°C) and days aged (1 d vs. 14 d) on the cooking loss (change from raw %) of beef *semitendinosus*. *From Purslow, P. P., Oiseth, S. K., Hughes, J. M., Warner, R. D. 2016. The structural basis of cooking loss in beef: variations with temperature and aging. Food Res. Int. 89, 731–748.*

increase in cook loss in aged meat is consistent across cooking temperatures of 60–75°C, as shown in Fig. 14.11 (Purslow et al., 2016). Thus in contrast to the decrease in drip loss seen with aging in raw muscle, cooking loss increases and is most likely caused by the proteolysis during aging producing protein fragments, which are more easily lost from the structure during storage and cooking, along with water (Purslow et al., 2016). If the lamb carcass is conventionally chilled, cook loss of 7-day aged lamb *semimembranosus* and *gluteus medius* muscles has been shown to be 3%–5% higher than cook loss for 1-day aged muscles (Warner et al., 2014b). Conversely, if the muscles were exposed to high pre-rigor temperatures resulting in myosin denaturation, the post-cooking water loss was high, regardless of the aging period (Warner et al., 2014b). The increase in cooking loss in aged meat relative to unaged meat varies with pre-rigor temperature conditions in the muscle as well as with sarcomere length (Warner et al., 2014b). It would appear that the weakened protein structure in aged meat cannot retain or trap water during cooking (Wu et al., 2006).

14.5.4 Influence of sarcoplasmic proteins

During cooking, water is lost from the muscle structure, and if proteins are large, they are more likely to be retained in the structure. The positive role of sarcoplasmic proteins in WHC of raw muscle has been demonstrated (Liu et al., 2016), and there is evidence for a role for sarcoplasmic proteins in determining

cook loss. In order to explain their greater cooking loss from aged meat, Purslow et al. (2016) proposed that as a consequence of the degradation of sarcoplasmic proteins during aging, these smaller proteins are lost more easily from the structure during cooking, along with water. This follows from the previous studies that showed that sarcoplasmic proteins are influential in retaining water in the muscle structure by providing a networked linkage with each other and with myofibrillar proteins, enabling more water to be trapped in the structure (Liu et al., 2016).

At heating temperatures below 50°C, sarcoplasmic (water soluble) proteins exhibit low solubility and high viscosity (Chen et al., 2015), and temperatures just below 50°C are associated with higher cooking loss than just above 50°C (Vilgis, 2015). Although Vilgis (2015) attributes this to changes in myosin structure and unfolding, Chen et al. (2015) attribute this to aggregation of sarcoplasmic proteins. At temperatures above 60°C, sarcoplasmic proteins can unfold, resolubilize, and stabilize to form a network, contributing to WHC (Chen et al., 2015).

14.5.5 Influence of high-pressure processing on WHC

High-pressure processing (HPP) is a form of processing where a pressure is applied statically to a product, at or above 100 MPa by means of a liquid transmitter (see Chapter 8). The application of high-temperature (HT > 25°C) high-pressure (200–600 MPa) processing (HPP) to fresh, post-rigor meat can result in higher cook yields and lower cook losses (Sikes and Tume, 2014), depending on the temperature and pressure applied (Sikes and Warner, 2016). One possible explanation for the increased WHC of fresh post-rigor meat undergoing HT-HPP appears to be that the meat proteins unfold under pressure and water populations undergo a shift and change in their populations. The high temperature causes aggregation and irreversible denaturation of proteins, and when the pressure is released, these proteins do not return to native configuration, and some water remains trapped in the muscle structure. In situations where there is preheating of the meat prior to HT-HPP, water loss occurs as a consequence of preheating and improved cook yield/WHC does not occur (Anita Sikes, unpublished results). Low-temperature HPP exhibits either similar or increased cook loss relative to nontreated meat (Hong et al., 2005).

Pressure treatment of pre-rigor muscle also results in lower water loss from meat after cooking relative to controls, which have not been pressure-treated, and the moisture content of the cooked or raw meat is also higher (Macfarlane, 1973,). The drip loss and purge (water lost during storage) of high-pressure-treated pre-rigor meat are also lower (Souza et al., 2012). The mechanism for the increase in WHC is generally attributed to the arrested glycolysis that occurs during HPP treatment of pre-rigor meat, resulting in a higher ultimate pH in muscle subjected to HPP. Thus

application of high pressure to pre-rigor and high temperature and high pressure applied to post-rigor meat both result in increased water retention in the meat structure.

14.5.6 Influence of sous vide cooking

Low-temperature long-time cooking (LTLT) techniques have recently increased in popularity. Sous vide is an LTLT method where the food/meat is first sealed in a heat-stable vacuum bag and then cooked at temperatures from 50°C to 85°C for prolonged times of up to 48 h (Zielbauer et al., 2016). The influence of sous vide cooking of meat on water loss is dependent on many factors including the age of the animal as well as the cooking temperature and time, as shown in Table 14.5 (Naqvi et al., 2021). Naqvi et al. (2021) showed that total water content (TWC, %) in bovine *semitendinosus* was lower after sous vide cooking at 65°C and 75°C relative to 55°C, and the older cows had lower TWC than the younger animals at 65°C and 75°C (Naqvi et al., 2021). This indicates a role for collagen in the WHC, as the authors also showed that the older cows had lower levels of soluble collagen than the young cows after cooking, as would be expected. In addition, younger cattle generally had lower cook loss at low and intermediate sous vide temperatures (55–65°C) in bovine *biceps femoris* and *semitendinosus* and cook loss increased with both temperature (55–75°C) and time of cooking (1–18 h) (Table 14.5). We postulate that at 55°C for 1 h, transverse shrinkage has occurred due to myosin denaturation, but there has been no collagen denaturation or actin denaturation and associated longitudinal shrinkage of muscles (see above); hence, cook loss is very low. For longer cooking times and higher cooking temperatures, denaturation of all proteins occurs (Zielbauer et al., 2016), resulting in volume shrinkage of fibers and muscles and increased cook loss (Purslow et al., 2016; Vaskoska et al., 2021).

14.6 Juiciness: Influencing factors and interactions with WHC

As cook loss increases with heating, there is a consequent reduction in juiciness (Fig. 14.7). While intuitively, juiciness should be positively correlated with the WHC of raw meat, results of studies comparing sensory assessment of juiciness to measures of WHC often show a lack of relationship and are often contradictory (Winger and Hagyard, 1994). The correlation between cooking loss and juiciness is higher, but does depend on the temperature to which the meat is cooked (Bejerholm and Aaslyng, 2004), as is evident from Fig. 14.7.

Sensory studies show that the juiciness (water release on first one or two chew cycles) and perceived moisture content (sensory evaluation of meat wetness/dryness after several chew cycles) vary between different muscles. Aaslyng et al. (2003) found that, in porcine *longissimus*, juiciness is influenced

TABLE 14.5 Effect of age of the animal, *sous vide* cooking temperature, and cooking time on cooking loss (%) in bovine *biceps femoris* and *semitendinosus*.

Temperature	55°C			65°C			75°C		
Time	1 h	8 h	18 h	1 h	8 h	18 h	1 h	8 h	18 h
Biceps femoris									
Young	16.3[b]	24.4[c]	27.4 d	27.9[d]	33.6[ef]	37.4[g]	36.6 [g]	41.0[h]	41.3[h]
Older	12.7[a]	19.1[a]	22.9[c]	25.2[cd]	31.4[e]	35.3[fg]	34.7[fg]	40.8[h]	41.4[h]
Semitendinosus									
Young	17.9[a]	24.8[bc]	27.8[d]	27.4[d]	34.2[ef]	35.8[ef]	36.3[f]	38.5[gh]	40.2[h]
Older	16.0[a]	22.6[b]	24.4[bcd]	25.2[cd]	32.3[e]	35.5[fg]	35.3[fg]	39.7[h]	40.1[h]

Data are presented as least squares means. Superscripts (letters a–h) refer to significant differences ($P<0.05$) in mean cooking loss within each block of two rows within young and older animals. The separation of the means was done using Fisher's least significant differences (LSDs).
From Naqvi, Z. B., Thomson, P. C., Ha, M., Campbell, M. A., Mcgill, D. M., Friend, M. A. & Warner, R. D. 2021. Effect of sous vide cooking and aging on tenderness and water-holding capacity of low-value beef muscles from young and older animals. Meat Sci., 175, 108,435.

by cooking loss ($R^2 = 0.46–0.52$), but this can vary with cooking procedure and the initial pH of the sample. Sensory appreciation of cooked meat tenderness is not uniquely related to juiciness or moistness, but in general there is a positive correlation between the two, whereas objective shear force measures are less correlated to sensory measures.

The sensory perceptions of both initial and sustained juiciness increase with the intramuscular fat content of muscle, as shown in Fig. 14.12. As meat of high IMF has higher scores for tenderness and flavor, as well as for juiciness, this explains the consumers' preference for high-IMF meat. Higher prices per kg are also paid for highly marbled (high IMF) meat (e.g., meat from cattle of the Hanwoo and Wagyu breed).

Consumer perceptions of juiciness are influenced by the pre-rigor muscle metabolism. Beef carcasses going through rigor at an elevated temperature have been shown to have lower consumer scores for juiciness in the *longissimus lumborum* and *gluteus medius* muscles (Warner et al., 2014c). Also, decreased juiciness has been reported in frozen-thawed beef, relative to chilled beef although differences were too small to be detected by consumers (Lagersted et al., 2008).

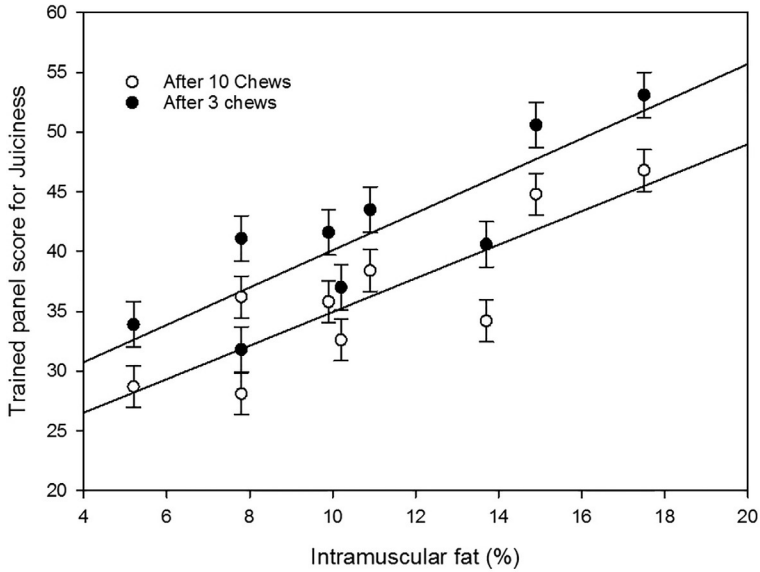

FIG. 14.12 Effect of the intramuscular fat (%) content of beef *longissimus* on the trained panel score for juiciness after three or 10 chews. The standard error of the difference is shown as ± on each mean and the estimated lines of best fit are shown after three chews *(top)* and after 10 chews *(bottom). Derived from Frank, D., Ball, A. J., Hughes, J. M., Krishnamurthy, R., Piyasiri, U., Stark, J. L., Watkins, P. E., Warner, R. 2016. Sensory and objective flavor characteristics of Australian marbled beef: the influence of intramuscular fat, feed and breed. J. Agric. Food Chem. 64, 4299–4311.*

14.7 Factors influencing WBC of meat products

14.7.1 WHC of comminuted meats

Sausage and comminuted meats are, on the one hand, more liable to exude fluid (even if the WHC of the proteins is intrinsically high) because the structure of the meat is destroyed in their preparation, thus removing its contribution to the physical retention of fluid. On the other hand, however, the nature of these products permits direct manipulation of the meat to enhance its WHC artificially. Before considering special effects, the behavior of water itself as an additive should be noted. The ratio of water to meat affects the overall WHC of the mix. The latter, as measured by a centrifugal method (Sherman, 1961), is maximal when the ratio is about 2:1 (see Table 14.6).

14.7.2 Salt

Various salts (particularly sodium chloride) have been added to processed meat formulations for their known effects on improved functionality such as better gelation, higher WHC (see Fig. 14.1) and fat retention, improved flavor, reduced cooking losses and for reducing microbial growth during storage (Desmond, 2006). Meat emulsion and sausage-type products are dependent on the extraction of salt-soluble myofibrillar proteins for physical stability and functionality of the product. The solubility of these proteins is markedly enhanced when sodium chloride and/or other salts are blended into the batters. These solubilized proteins form connections between proteins and lipids, and when heated, the proteins denature and aggregate, resulting in gelation and water retention (Gordon and Barbut, 1992).

NaCl is a neutral salt, and the main effect of addition to muscle is an osmotic effect. As membrane integrity disappears and the membrane becomes permeable, the salts migrate into the muscle fibers. Meat of normal' pH (5.3–5.7) is on the basic side of the isoelectric point of the muscle proteins, hence the proteins carry a net negative charge, as discussed in Section 14.4.1 above. The addition of NaCl results in binding of the Cl^- ions to these positively charged protein side groups and screens the positive charge, breaking salt bridges and allowing the filaments to separate resulting in greater hydration. Binding of these anions shifts the isoelectric point to a lower pH (Fig. 14.1). The more strongly ions are bound within the myofibril, by proteins, the stronger will be the hydrating effect (Hamm, 1986). The effect of anions in shifting the isoelectric point to more acid values and in enhancing the WHC above the original isoelectric point was first shown by Hamm in 1960 (Hamm, 1986) (see Fig. 14.1). It has been presumed that the WHC of connective tissue proteins is similarly enhanced by ions. Offer and Trinick (1983) suggested that the water uptake by myofibrils in strong salt solutions is caused both by expansion of the lattice of thick and thin filaments as the increasingly negatively charged components repelled one another and also by disruption of the forces, which determine the regular arrangement of the

TABLE 14.6 Influence of water-to-meat ratio on water retention of pork muscle.

Water: meat ratio	No water added	1:2	1:1	3:2	2:1	3:1	4:1	5:1	6:1
Per cent water retention at 0°C	−4.0	−1.0	−1.5	0.5	9.5	5.0	−5.0	−5.0	−6.0

Derived from Sherman, P. 1961. The water binding capacity of fresh pork. Food Technol. 1, 79–87.

filaments at the *Z*- and M-lines and between the heads of the myosin molecules and the adjacent actin filaments. Both sodium chloride and pyrophosphate exert these two effects (Greene, 1981).

Adding up to 2% NaCl to meat increases swelling of muscle when water is added. Fig. 14.13 shows the effect of varying NaCl concentration from 0.4% to 3.0% (*w*/v) in beef homogenates. As the NaCl concentration increases, the

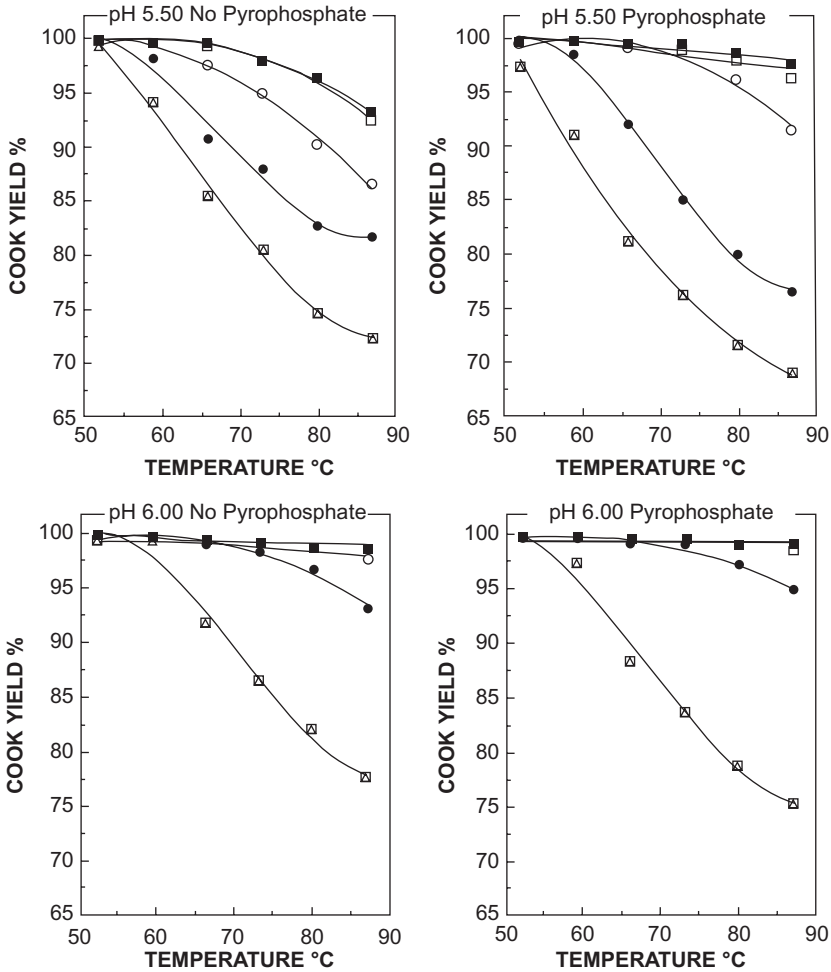

FIG. 14.13 Effect of cooking temperature, pH (5.5, top row; 6.0, bottom row), pyrophosphate (0, no pyrophosphate, left column; 0.31%, pyrophosphate, right column) and ionic strength (triangle in circle 0.12, closed circle 0.22, open circle 0.32, open square 0.42, closed square 0.52; ionic strength adjusted by varying the NaCl concentration from 0.41% to 3.04%, see paper for details) on cook yield of beef homogenates. LSD for comparison between treatments is 1.5%. *From Trout, G., Schmidt, G. R. 1987. The effect of cooking temperature on the functional properties of beef proteins: The role of ionic strength, pH and pyroposhate. Meat Sci. 20, 129–147.*

cook yield increases demonstrating the importance of salt in retaining moisture in meat products. This graph also shows that the effect of NaCl on cook yield is larger at a pH 6, relative to pH 5.5.

Salt alters the electrostatic, hydration, and water structuring effects of muscle myofibrillar proteins, resulting in enhanced solubility (salting-in) or insolubility (salting-out). Since NaCl at concentrations from 0.3 to 1.0 M induces a salting-in effect of myofibrillar proteins, it is thought that Cl^- ions bind to the filaments and increase the electrostatic repulsive force between the filaments, allowing the filament lattice to expand (Offer and Trinick, 1983). With increasing salt concentration greater than 1 M, the solubility begins to decrease, most likely resulting from the "salting-out" phenomenon. This is generally ascribed to the loss of stable hydrophilic surface, causing the exposed hydrophobic areas of protein to interact, inducing aggregation and hence loss in solubility (Chen et al., 2015). The propensity of muscle fibers to swell in hypertonic solutions is initially opposed by the restraint of the endomysium, but with increasing time postmortem, the degree of swelling increases. Wilding et al. (1986) attributed this to a weakening of the endomysium per se during conditioning, but Knight and Elsey (1989) demonstrated that the proportion of fibers without endomysial sheaths increases at this time. Such stripped fibers swell more in hypertonic media than those in which the endomysium is intact. This confirmed earlier evidence of a weakening in the link between muscle fibers and their endomysium during aging, which was given by Stanley (1983), who showed that endomysial tubes empty of myofibrillar material could only be prepared after aging. It would thus appear that myosin extraction by salt would be more readily effected from fibers that have lost their endomysia. Muscles may contain fibers of several types, which vary in their pH and myosin extractability and solubility. Hence it is not surprising that the myofibrils within muscle fibres react differently to hypertonic treatment (Offer and Trinick, 1983), a feature that could be important in explaining and controlling the variability of meat in curing.

14.7.3 Application of salts and bicarbonates pre-rigor

Pre-rigor WHC can be retained for several days by adding NaCl to pre-rigor muscle subjected to comminution, in spite of the fact that NaCl and particle size reduction increase ATP breakdown and glycolysis. Salting of pre-rigor meat maintains the high WHC for several days during refrigerated storage. This "hot" salted meat has enhanced WHC and fat-binding properties in sausages (Pearson and Young, 1989). Pre-rigor salting of meat results in increased solubilization of myofibrillar proteins, and the high WHC can be explained by the inhibition of rigor mortis, thus preventing the actomyosin bond and also preventing the drop in pH. It is important to mix the salt quickly and thoroughly while the meat is still in early pre-rigor state, before ATP becomes depleted and the pH drops. The enhancement of WHC with salt concentration increases up to ~1.8% salt addition, with no further improvement beyond this (Pearson and Young, 1989).

The injection of 0.2–0.4 M sodium bicarbonate into pork muscle pre- and post-rigor has been found to reduce water loss, prevent PSE, and increase the ultimate pH, resulting in more favorable consumer panel juiciness scores (Kauffman et al., 1998). The mechanism for the improved WHC is explained by the elevated ultimate pH. The total expressible fluid from emulsion sausages is lowest (i.e., highest WHC) for pork leg muscles which have been hot-boned pre-rigor, relative to sausages made from pork leg meat which have been cold-boned or frozen for 30 days (Fig. 14.14) (Wang et al., 2009). Increasing the phosphate addition from 0.1% to 0.5% significantly decreased the total expressible fluid for hot-boned pre-rigor sausages, but the effect was very small for sausages made from cold-boned or frozen meat. Hence, hot-boning and treating meat pre-rigor with various brine solutions are an excellent method to retain the inherently high WHC of pre-rigor meat.

14.7.4 Ions and ionic strength

The strength of the impact that various ions have on proteins and their ability to bind water is described by the Hofmeister series. The anions with the greatest stabilization abilities are PO_4^{3-} anions followed by $SO_4^{2-}>CH_3COO^->Cl^->Br^->NO_3^->I^-$. For cations, the series is $(CH_3)_4N^+>NH_4^+>K^+>Na^+>Mg^{2+}>Ca^{2+}$.

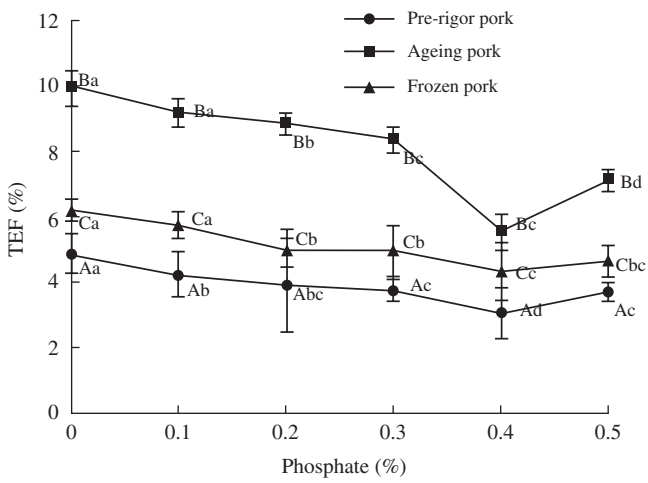

FIG. 14.14 Effect of type of meat and phosphate level in an emulsion-type sausage on the total expressible fluid (TEF). The emulsion sausage was made from pork hindquarter meat and pork tallow in ratio of 8:2. Pre-rigor pork was hot boned within 1 h postmortem. Aging pork was conventionally boned at 24 h postmortem. Frozen pork was stored at −18°C for 30 days and then thawed in running water. For the same phosphate level, means without similar capital superscripts are different ($P<0.05$) and for the same meat type, means without similar lowercase superscript are different ($P<0.05$). *From Wang, P., Xu, X.-L., Zhou, G.-H. 2009. Effects of meat and phosphate level on water-holding capacity and texture of emulsion-type sausage during storage. Agric. Sci. China 8, 1475–1481.*

The Hofmeister series is correlated with the ions' capacity to increase water binding in meat (Pospiech and Montowska, 2011). In addition, the mechanism by which various ions increase WHC can be described by classifying the ions into chaotropic compounds, that destabilize the proteins native structure, and kosmotropic ions, which stabilize the protein structure. Strong chaotropic cations, such as K^+ and NH_4^+, and weak kosmotropic anions, such as carboxylic groups of amino acids, stabilize biological systems and increase WHC of meat (Puolanne and Halonen, 2010).

Ionic strength has long been considered highly important because it influences myofibrillar protein solubility, WHC and other physicochemical and functional properties of foods, such as their emulsifying, foaming and gelling properties, and viscosity. Myofibrillar proteins are more soluble at higher ionic strength over the range of 0.2–0.6 M (Chen et al., 2015; Trout and Schmidt, 1987) with variations dependent on pH, which can be influenced by the phosphate used, as discussed below. Only 50% of myofibrillar proteins are soluble at ionic strengths <0.2 M (Chen et al., 2015). High-ionic-strength extraction solutions will dissociate the actomyosin complex more completely, resulting in higher mole ratios of actin and myosin than lower-ionic-strength solutions. Commercially, it is generally accepted that a high ionic strength of about 0.47–0.68 M (2% to 3% salt) is required to fully develop the functional properties of muscle tissue foods (Chen et al., 2015).

At high ionic strength, salt has a dehydrating effect and is at a maximum when the ionic strength is about 0.8–1.0, corresponding to the initiation of the "salting out" of the myofibrillar proteins, which become insoluble at >1.0 (see above). This ionic strength corresponds to 5% and 8% of sodium chloride for meat without and with 60% added water, respectively (Hamm, 1986). Knight and Parsons (1988), however, in observing the swelling of myofibrils in concentrated salt solutions, attributed the effect to entropic swelling pressure caused by a steric resistance to the rotational movement of the tails of myosin molecules imposed by the actin filaments to which they were attached: the swelling was greatest in ~6% NaCl. They attributed the apparent dehydrating effect of higher salt concentrations to the precipitation of myosin, a feature that would reverse its depolymerization in sodium chloride and cause shrinkage.

14.7.5 Phosphates

The trend to reduce sodium content in meat has resulted in phosphates being used as a method to increase water binding. For this reason, phosphates and polyphosphates are often added to comminuted meats to enhance WHC. Polyphosphates are widely used to improve the WHC of meat products, and tetrasodium polyphosphate (TPP) is generally the most effective phosphate for increasing WHC, both in the presence and absence of NaCl (see Table 14.7). Sodium tripolyphosphate (STP) is the next most effective, and the phosphates vary dramatically in their pH, which affects their functionality. The relative pH

TABLE 14.7 Percentage of fluid (gram retained per 100 g absorbed) by chicken breast marinated, tumbled for 30 min, stored in bags for 24 h at 2-3 °C, then fried at 171 °C for 5 min to reach endpoint temperature of 68 °C.

No salt							8% salt						
Control	PP[a]		TP		HMP		Control	PP		TP		HMP	
	Low[b]	High	Low	High	Low	High		Low	High	Low	High	Low	High
Marinade retained in raw muscle immediately after tumbling (g per 100 g muscle)													
4.6	29.0	35.1	29.8	25.0	16.4	15.5	26.3	37.8	41.5	34.5	29.1	28.4	27.9
Percentage retention in raw muscle after 24 h (g retained per 100 g absorbed)													
24.1	67.4	80.0	79.9	71.6	67.8	59.4	81.7	85.7	78.9	76.8	73.1	76.8	67.7
Yield after cooking (cooking yield, %)													
77.3	106.0	112.0	103.9	100.4	88.7	90.0	93.5	114.9	119.4	111.9	108.3	95.3	97.5

[a] *PP, sodium pyrophosphate; TP, sodium tripolyphosphate; HMP, sodium hexametaphosphate.*
[b] *Low = 1.6% and high = 3.2% phosphate.*
From Brewer, M. S. 2014. Chemical and physical characteristics of meat | Water-holding capacity. In: Dikeman, M., Devine, C. (Eds.) Encyclopedia of Meat Sciences (second ed.). Oxford: Academic Press.

of a 1% (*w*/v) solution of TPP, STP, sodium hexametaphosphate, and sodium acid pyrophosphate is 10.5, 9.8, 7.0, and 4.2, respectively. The efficacy of tripolyphosphate addition appears to depend upon its being enzymically broken down to diphosphate (Hamm, 1986). Bendall (1954) concluded that the effects of most of these phosphates were largely one of ionic strength and pH. The exceptional properties shown by pyrophosphate (in the presence of 1% NaCl) in increasing water binding are specifically due to the splitting of actomyosin into actin and myosin in the presence of ATP and to the formation by the myosin of a gel, thus trapping water.

Improved cook yields of beef homogenates are evident when pyrophosphate is added as well as salt (e.g., 0.31% pyrophosphate in Fig. 14.13). In this context, Offer and Trinick (1983) have shown that pyrophosphate substantially reduces the salt concentration required to produce maximum swelling when myofibrils are placed in solutions of sodium chloride. In the absence of pyrophosphate, the protein in the middle of the A bands is extracted, but, in its presence, all of the A-band protein dissolves. In a subsequent study by Knight and Parsons (1988), when myofibrils were supported between grid bars rather than resting on a coverslip, it was observed that some material in the center of the sarcomeres resisted extraction by sodium chloride: it appears to be titin or nebulin (see Chapter 4). The extraction of both A-band and *Z*-proteins, which occurs in sodium chloride, is progressively inhibited at higher salt concentrations, presumably because myosin is salted out.

14.7.6 Marination

The preservation of foods by organic acids, which is the traditional preparation of marinated meat by vinegar and spices, involves conditions that enhance the WHC of muscle proteins on the acidic side of their isoelectric point (pH < 5.0). The acidification process is most often carried out using lactic acid, which occurs in meat naturally, or ascorbic, citric, or tartaric acid (Pospiech and Montowska, 2011). Rao et al. (1989) made a detailed study of such systems using various beef muscles in acetic acid solutions of 0.01–0.25 M. The WHC increased over the range from pH 5.1 down to pH 4.0 in the six muscles investigated. The *longissimus* had a significantly higher swelling ratio than that of the other muscles in the range between pH 4.3 and 4.0. Between pH 5.1 and 4.4, swelling increased in all muscles, both along and across the fiber axis. As the pH of the marinating solution approached 4.0, however, fiber swelling occurred predominantly in the "white"-type muscles, whereas fiber shrinkage occurred in predominantly "red"-type muscles. Interactions between the swelling of the muscle fibers and that of the connective tissue determined the total swelling of the muscles between pH 4.5 and 4.0.

Marination can also occur using phosphates and salt and often tumbling. Tumbling is used to enhance the distribution of the marinade mixture through the meat. The combination of salt and phosphate concentration, and phosphate

chemical composition, will determine the effectiveness in retaining the fluid applied during marination. In both the absence and presence of 8% salt, Xiong and Kupski (1999) showed that chicken breasts exhibited maximum cooking yield when sodium pyrophosphate was used at 3.2% in the marinade, relative to using a lower concentration and to using other phosphates (Table 14.7). Furthermore, juiciness scores given by a sensory panel, were highest for chicken breasts with tripolyphosphate added, relative to those with hexametaphosphate added.

The relative swelling of muscles on addition of marinades, also reflects their total content of protein, and the proportion of the total protein which is connective tissue. Thus the swelling of muscle fibers dominates over that of collagen in muscles with a relatively high content of total protein relative to collagen, such as *longissimus* at pH levels below 4.3. In this same pH range, however, the swelling of supraspinatus, in which the total protein content is relatively low and that of connective tissue is high, is dominated by collagen (Gault, 1991). Both the perimysial collagen and that of the reticular fibers of the endomysium swell during marination, but the effect is more marked in muscles in which the endomysium is thin (e.g., *longissimus*). Generally, however, the effect of marinating in swelling the myofibrillar proteins appears to be more important than that of the connective tissue proteins.

14.7.7 Amino acids and other additives

Some of the amino acids display unique water-binding abilities, particularly aspartic and glutamic acid. These amino acids can bind 4–7 water molecules, and the high water-binding ability of the myosin molecule is explained by the fact that it is rich in these amino acids (Pospiech and Montowska, 2011). Thus these amino acids are sometimes included in mixtures for processed meat, to increase the water binding.

Compounds that assist in gelling or thickening can sometimes be used to reduce drip from meat products, including soy, agar, pectin, starch, carrageenans, inulin, etc. Other compounds that can assist in water binding and should be considered by food technologists for increasing water binding in meat products include blood plasma, egg white, and milk proteins (Pospiech and Montowska, 2011). Flours made from grains are well known to increase water binding in meat products, and flours which are made from novel sources, such as lupins, or from by-product materials generated during processing of a raw material, have potential to be valuable water-binding agents. Collagen extracted from hides has been recommended for addition to meat products due to high water binding as well as the functional role as an extender/filler (Ranganayaki et al., 1982). In fact, collagen is widely used in the preparation of meat products owing to its functionality and effects on water-binding ability. The addition of commerical collagen mixtures to the brine mixture injected into pork loins prior to tunbling and smoking/cooking resulted in increased cook yield, moisture content, and WHC (Choe and Kim, 2019). Sugarcane fiber is a by-product of sugar refining

and has excellent water-binding properties resulting in increased yield and reduced water loss in chicken sausages (Fang et al., 2019). Lupin is a legume, which has the potential as a neutraceutical for addition to meat products, and lupin flour has been shown to decrease the water released from sausages during cooking (Leonard et al., 2019) Many other natural and engineered compounds can be used to increase water binding in meat products; some are well known to the meat industry, and some are yet to be discovered.

14.7.8 High-pressure processing

Enhanced meat binding through extraction of salt-soluble proteins is an essential step in the formulation of meat products such as sausages and emulsion-type products. HPP has been used as a possible means of improving the functional properties of muscle proteins as it has been shown to increase the solubility of certain myofibrillar proteins (Macfarlane and McKenzie, 1976) and also to increase binding between meat particles in patties following heat denaturation (Macfarlane et al., 1984). In a beef batter, the absence of salt results in 35% cook loss, but when salt is added at 2%, this cook loss is reduced to 10%. If the salt content is reduced to 0.6%–0.8% and HPP is applied at 200 MPa, the cook loss is reduced to less than 10% (see Fig. 14.15) (Sikes and Tume, 2014). The ability to reduce salt and achieve high binding and water retention through use of HPP is important in being able to produce healthier foods.

14.8 Methods to measure WHC and juiciness

The measurement of WHC usually involves the application of force to measure the water released. The force can either be natural, through gravimetric means, or be applied externally as pressure, through centrifugation, compression, or taking advantage of capillary action. Only methods currently in use are discussed as some methods are no longer used, but are described elsewhere (Kauffman et al., 1986a; Trout, 1988).

14.8.1 Methods for measuring WHC applying no external force

Gravimetric (drip loss): This method involves the measurement of weight loss in free drip, bag drip, or cube drip, where the meat is left in storage under different environmental conditions. The most commonly used method involves using a standard size and weight block of meat (approximately 30–100 g) and suspending it in a bag, for 1–2 days at 1–4°C, ensuring that the meat does not touch the sides of the bag (Honikel, 1998) (Fig. 14.16). Variations of this method include chopping the meat and placing it in a tray, so that all the drip can be collected in the bottom of a tray, thus the drip is not in physical contact with the meat, including the EZ-DripLoss (Christensen, 2003) method. Each method

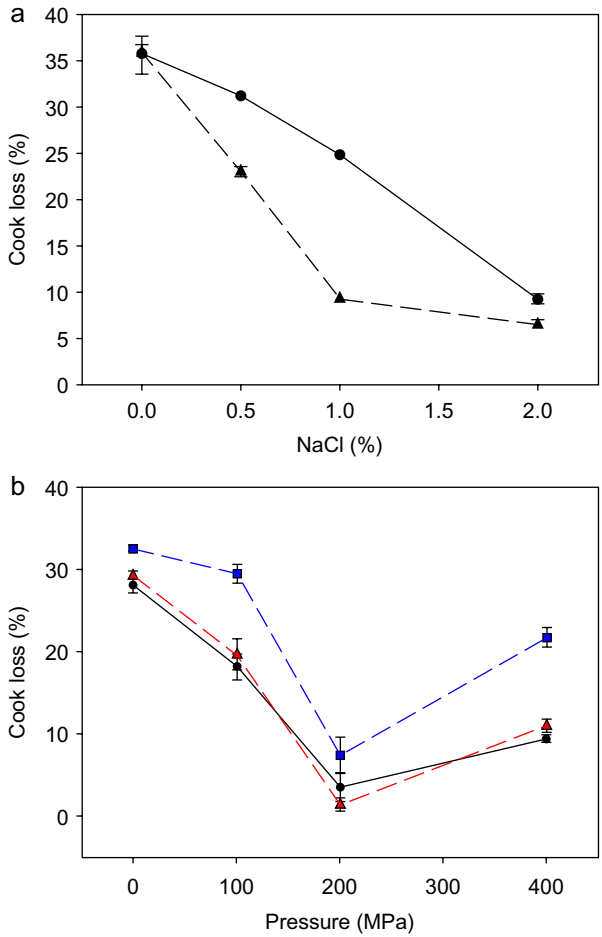

FIG. 14.15 Changes in cook loss of pressure-treated beef batters containing various NaCl concentrations; (A) beef batters subjected to high pressure, 0.1 MPa (*black circle*) or 200 MPa (*black triangle*); and (B) beef batters containing 0.6% (*black solid line*), 0.8% (*red dashed line*) and 1% NaCl (*blue dashed line*). *From Sikes, A. L., Tobin, A. B., Tume, R. K. 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. Innov. Food Sci. Emerg. Technol. 10, 405–412.*

involves weighing the meat at the start and end, and the drip loss is expressed as a loss in weight of the sample over the defined period, expressed as a percentage of the initial weight. The surface area of the meat can influence the results. This method is widely used because of its simplicity.

Weep or purge: Purge, or weep, is defined as water lost from meat or muscle during storage post-rigor, including during storage in trays (overwrap or modified atmosphere packs) on retail shelves (Warner, 2014) and during aging of

FIG. 14.16 Illustration of the gravimetric method for measuring drip loss, involving suspension of a meat sample in an inflated bag, and storage at 2–4°C for 1–2 days. *Credit: Robert Kauffman, University of Wisconsin-Madison.*

meat in a vacuum bag. Measuring purge, or weep, involves measuring the loss in weight of meat over a defined period. Thus, the meat is weighed before being placed in the bag or tray, then weighed again once the bag is opened or the packaging is removed, at the end of the storage or display period. At the completion of the storage period, the storage pack is opened, the fluid is mopped up, then the meat is reweighed. This is a method that can be used when the meat is stored in a vacuum bag or retail-ready tray. The appearance of weep or purge, in the tray or bag, is unsightly and implies an inferior product.

Subjective scoring of exudate: Visual scores for exudate have been used to assess the surface muscle of beef and pork carcasses (Kauffman et al., 1986b; Warner et al., 2014a). The score can be a simple "yes" or "no," or a score from 0% to 100%, similar to that used for the rapid filter paper method discussed in the following section.

14.8.2 Methods for measuring WHC applying external force

The press method or compression: This was the first method developed to measure WHC where the unbound water is quantitatively removed from a sample by pressing using either a weight applied manually, or applied using an Instron Universal Testing Machine (Trout, 1988). The predefined circular piece of meat (usually 0.5–30 g) is placed on filter paper, and the defined pressure is applied. The water squeezed out is absorbed by the filter paper and is related to the amount of "loose" water in the sample. The amount of water released is either measured (1) indirectly as the area of the ring of expressed juice or (2) directly by weighing the filter paper. Although this method became less used over a number of years, as the results were variable and were highly dependent on the

texture of the meat, the quantification system has been refined, and the method has gained in popularity. Barbera (2019) reported low coefficient of variation (CV) when they used a video image analysis method to quantify the marks on the filter paper. Unfortunately a low CV does not ensure a more reliable method as the reason for low CV may be low standard deviation caused by failure of a method to measure the full range in WHC of samples (Huiling Huang and Robyn Warner, unpublished results).

High-speed centrifugation: This involves subjecting samples of 1–20 g to centrifugal forces of 6000–40,000 g (Trout, 1988). Water release is determined by weighing the water released or weighing the sample before and after centrifugation. This method removes more water than most of the other methods, due to the high centrifugal forces involved. But due to the elastic nature of meat, some of the water is reabsorbed once the centrifugal force is removed. Similar to the press method, the results obtained are influenced by the texture of the meat. This method has had very limited use.

Low-speed centrifugation: This method involves subjecting 3–15 g samples at 100–10,000 g for 15–30 min (or even longer) and measuring the weight loss of the sample or weight of the exuded fluid (Kristensen and Purslow, 2001). The advantage of this method over high-speed centrifugation is that it uses specially designed centrifuge tubes with a perforated disc in the middle (e.g., Mobicols from MoBiTec). This disc allows moisture to spin down to the bottom while leaving the tissue on the top, thus preventing reabsorption of the fluid. One of the problems with this method, which has introduced inaccuracies in the results, has been the blocking of the pores in the membrane. This can be overcome by using superglue to glue the piece of meat to the bottom of the lid of the tube (Kristensen and Purslow, 2001). This method has become popular as it correlates well with drip loss measurements, is relatively simple to conduct, and samples can be centrifuged regularly (e.g., every 15 min) to follow changes in WHC.

Rapid filter paper method: This method relies on capillary suction forces of the filter paper applied to the meat surface. It is extremely rapid and involves placing a filter paper on a freshly cut surface at a defined time post-cutting (e.g., 10 min), smoothing it out, then either scoring the filter paper for wetness (0%–100% wet) or weighing the filter paper (Fig. 14.17). Fig. 14.18 shows the relationship between the filter paper score, a weep score, and temperature at rigor.

Application of heat (cooking loss): Cooking loss is defined as the loss in weight as a result of cooking, and is expressed as a percent of the precook weight (Honikel, 1998). A common method is to combine a measure of weight loss during cooking with the objective measurement of tenderness. Thus, samples destined for objective measurement of cooked meat are weighed before cooking, and then after cooking, the samples are cooled before removal from the bag, blotted, and then reweighed in order to determine cooking loss. Cooking loss is strongly related to degree of aging, cooking temperature, and cooking conditions and is quite an ill-defined property of meat. Of all the WHC

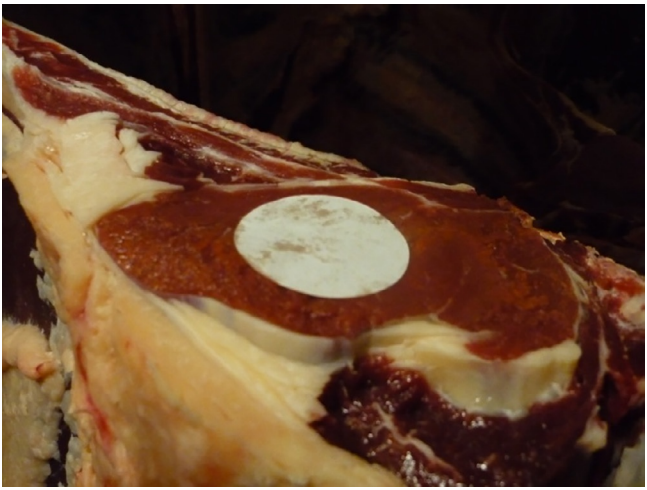

FIG. 14.17 Illustration of the rapid filter method for measuring weep or exudate on a beef loin surface. The filter paper can either be weighed, for amount of fluid absorbed, or scored for % of wetness. *Credit: Athula Naththarampatha, Department of Primary Industries, Victoria, Australia.*

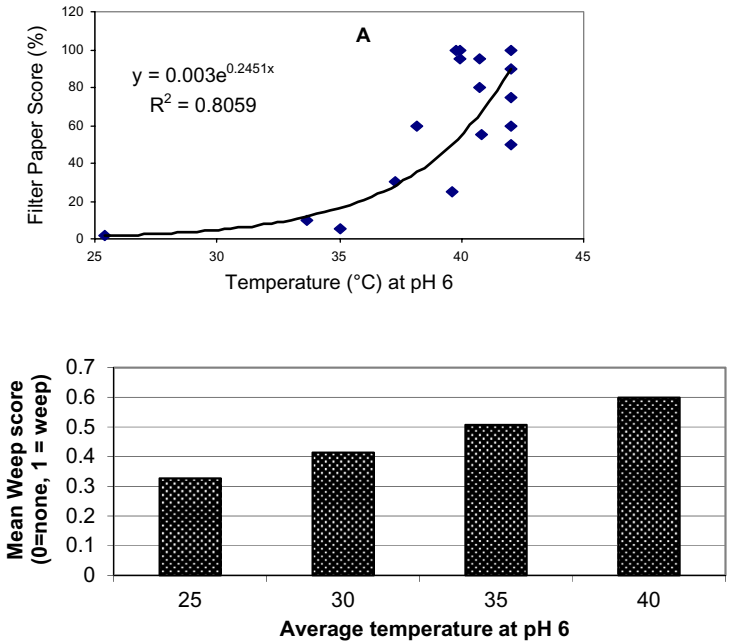

FIG. 14.18 *(Top)* The relationship between filter paper score (rapid filter paper method) and temperature at pH 6 (rigor temperature) in the beef loin muscle. *(Bottom)* The effect of temperature at pH 6 on the mean weep score (exudate; 0 = none, 1 = weep present) of the *longissimus thoracis* at the time of grading. Top *From Warner, R. 2014. Measurement of meat quality | Measurements of water-holding capacity and color: objective and subjective In: Devine, C., Dikeman, M. (eds.). Encyclopedia of Meat Sciences (Second Edition). Oxford: Academic Press and* bottom *from Warner, R. D., Dunshea, F. R., Gutzke, D., Lau, J., Kearney, G. 2014a. Factors influencing the incidence of high rigor temperature in beef carcasses in Australia. Anim. Prod. Sci. 54, 363–374.*

measurements, cook loss has the highest correlation with the sensory trait juiciness, which is a complex and ill-defined trait in itself.

Cook yield: Cook yield tests are carried out on small samples, and in essence, duplicate procedures are used in commercial practice but on a smaller scale. With these procedures, small representative samples of meat (250–800 g) are homogenized in a food processor, filled into containers, cooked to a predetermined internal temperature, cooled, and then weighed for cook yield determination (Trout, 1988). Cook yield is determined by measuring the amount of water lost or the decrease in the weight of the sample during cooking. One of the better containers for cooking are small cans, since they completely prevent evaporative loss. Other containers that have been used for this purpose include: sausage casings, centrifuge tubes, and sealable aluminum trays. In all cases, though, the product must be removed from the container immediately after cooling to prevent reabsorption of water lost during cooking.

14.8.3 Indirect methods for measuring WHC

Protein-based methods: Protein solubility has been used as an indirect indicator of WHC, particularly to quantitate the low WHC of PSE meat muscle (Penny, 1969). Measurement of sarcoplasmic and myofibrillar protein solubility of muscle involves homogenizing 1–2 g of meat in a low-ionic-strength and high-ionic-strength buffer, for sarcoplasmic and total protein solubility, respectively, then measuring the protein concentration in the supernatant after centrifugation at 1500 g and 4°C for 20 min. Measurement of myosin denaturation through measuring myofibrillar or myosin ATPase activity is also an indirect indicator of low WHC as when myosin denatures, WHC is lower due to myofibrillar lattice shrinkage (Offer and Knight, 1983).

Low-field NMR: Because many of the above methods involve pressure and damage to the structure of muscle, low field NMR has been used to measure free water rather than measuring WHC (Bertram et al., 2001). It was shown that low field NMR transverse relaxation (termed T_2) is an effective noninvasive alternative for determining free water in muscle and meat. The technique revealed a component at 30–45 ms (referred to as T_{21}) and one at 100–180 ms (T_{22}), representing changes occurring during rigor mortis and in vivo extracellular water, respectively.

Raman spectroscopy: Raman spectroscopy can be used as a rapid, noninvasive, in-line measurement technology and has potential for measuring WHC as well as other parameters of importance such as proteins and lipids. Handheld and portable Raman spectrometers have been developed as well as a large-volume Raman probe, and these have shown reasonable prediction of drip loss (Andersen et al., 2018). The importance of representative sampling and lack of reproducible reference methods in the field have hampered further development of this technology. Andersen et al. (2021) used a method where the Raman spectra were recorded at the exact same position in porcine *longissimus* as drip

loss and found that Raman spectroscopy could only be used for rough screening for drip loss. Although showing considerable potential and undergoing much active research, the future of this technology for predicting WHC is yet to be determined.

Scattering: There is a well-recognized correlation between the surface lightness (L^*)/visual appearance of meat and the drip loss. Fiber optic probes have been tested for their capacity to measure scattering in muscles when the probe is inserted into the muscles of a carcass, but the correlations with drip loss ($r=0.61$) are not generally high enough for commercial consideration (Brøndum et al., 2000). Hughes et al. (2019) measured scattering by quantifying global brightness from bovine longissimus thoracis muscle fibers viewed under a microscope in reflectance mode. They reported that drip loss (1.7%–5.2%) and scattering were not significantly correlated ($r=0.33$ in transverse sections; $P>0.05$), although drip loss was correlated with glycolytic enzyme activity in supernatant fractions ($P<0.05$) and more highly correlated with myosin-myosin spacing measured using a synchrotron ($r=-0.64$; $P<0.05$) and surface lightness ($r=0.69$; $P<0.05$). Hence, although scattering is an indicator of WHC, it does not appear to be practical for implementation and is not necessarily highly correlated to WHC.

Electrical conductivity or impedance: Electrical conductivity can be measured on intact muscle or carcasses and hence, is not destructive or time-consuming. The principle of using electrical conductivity is that it essentially measures the breakdown in membranes postmortem. Muscles with highly intact membranes have high impedance and low electrical conductivity, and vice versa for muscles with degraded membranes (see Hughes et al., 2014 for discussion and diagrams). Pork and poultry muscles with low WHC (measured as drip loss and cook loss) have high electrical conductivity (Lee et al., 2000). Lee et al. (2000) show that when electrical conductivity is measured at 24 h postmortem, it is a very accurate predictor of WHC and is better than measuring ultimate pH.

14.8.4 Relationships between methods

How well do the various methods for measuring WHC correlate with each other? The data in Table 14.3 and Figs. 14.4, 14.8, 14.9, and 14.13 demonstrate that the WHC measured using various methods varies with a number of parameters including days aged, muscle, rigor temperature of the muscle, cooking temperature, cooking conditions, state of the muscle (raw, mince, processed), added ingredients, etc. Although drip loss often declines with days aged, purge increases with days aged, and the effect of days aged on cook loss varies with muscle, mostly independent of drip loss and purge (Table 14.3). Hence, the lower drip loss from aged meat due to protein degradation and the "sponge" effect implies high WHC. Conversely, the higher cook loss from aged meat due to protein degradation and smaller peptides and molecules flowing more easily out of the structure of aged meat upon cooking implies lower WHC. It is not

surprising that many WHC methods are not highly correlated as they are measuring different aspects of WHC. Illustrating the variation between muscles, the relationship between WHC measured by exudate, and ultimate pH has been found to be highest in the *gluteus medius* and *semimembranosus* ($r = -0.75, 0.76$ respectively; $P < 0.001$) but lowest in the *rectus femoris* ($r = -0.12$; $P < 0.05$) (Warner et al., 1993). If total water loss is calculated, and the methods used are all weight-based, raw samples with low WHC (eg. high drip, or high thaw loss), generally have lower cook loss, as the water is already lost from the muscle structure prior to sample weighing precooking.

The most important feature of any method to measure cook yield is that the procedure accurately predicts the results obtained with large-scale equipment (Trout, 1988). Thus techniques for measuring cook yield need to show the same trends in cook yield behavior as the larger-scale procedures when such processing parameters as salt concentration, phosphate concentration, pH, and homogenization temperature are varied (Whiting, 1984). One potential application for these procedures is to use them to obtain meaningful bind values for predicting the processing potential of meat from different sources.

14.8.5 Methods for measuring juiciness

The only reliable and consistent measure of juiciness is achieved using sensory methods—either consumer or trained panels (Winger and Hagyard, 1994) as described above and in Chapter 15. The correlation between WHC measures and juiciness has been reported to be very variable, likely due to inconsistencies in methodologies applied to measure WHC (Trout, 1988). Fig. 14.7 shows a relationship between juiciness and cooking loss, but this is not always the case. Considering the lack of correlation and relationship between various methods for measuring WHC discussed above, it should not be surprising that WHC methods are often poor predictors of juiciness. NMR can be considered as a gold standard for measuring WHC, as it measures the free, loosely bound, and tightly bound water. In 1986, Fjelkner-Modig and Tornberg (1986) found only a weak correlation between sensory attributes for juiciness and NMR relaxation characteristics for pork *longissimus*. More recently, differences in pork sensory juiciness were explained by differences in pore size measured by NMR in relation to mobility of extra-myofibrillar water and water expulsion (Bertram et al., 2005, 2007). Hence, if a true measure of juiciness is required, conducting a sensory panel is essential. Making NMR measurements alongside sensory panels, to understand underlying mechanisms, should be very helpful.

14.9 Conclusions and future trends

WHC of meat and meat products determines the visual acceptability, weight loss, and cook yield as well as sensory traits upon consumption. Muscle structure and changes in muscle proteins pre-rigor, and during packaging, storage, and

processing influence the WHC of raw, processed, and cooked meat. Postmortem glycolysis and metabolism are highly influential in determining the WHC and are influenced by genetics, pre-slaughter stress, and post-slaughter interventions such as electrical stimulation and HPP. In raw muscle and in processed and cooked meat, the main factors determining water loss from the structure, or the reverse being water retention in the meat structure, are (1) the shrinkage and swelling occurring in the myofibrillar structure and forces assisting the flow or retention of water and (2) the unfolding, network formation and aggregation of proteins, which determine the formation of gels or separated insoluble proteins.

In order to reduce water loss from meat, care must be taken with both the animal and the raw material. Postmortem treatments should ensure that moderate pH fall occurs in the muscles of the carcass and attention to the handling, packaging, and storage conditions needs to ensure optimum WHC. Pre-rigor treatment of muscles with salts, phosphates, or HPP is successful in increasing muscle WHC by causing the cessation of glycolysis, resulting in a higher muscle ultimate pH. As pre-rigor processing also has energy (chilling) and production schedule advantages, it should be considered in the installation of innovative modern meat processing plants.

PSE meat, which has excessive loss in water, has commonly been described in pig carcasses, but the problem can also occur in beef and sheep carcasses if excessive electrical stimulation of carcasses is used, inducing very rapid pH fall. PSE meat can also occur if electrical stimulation is used on beef carcasses where the muscle metabolism is already rapid, such as in heavy grain-fed beef carcasses. Hence, electrical stimulation of beef carcasses is likely to exacerbate the occurrence of pale, weepy beef, particularly in hind limb muscles. Consumer perceptions of juiciness are influenced by the pre-rigor muscle metabolism and occurrence of PSE. Hence, implementation of QA programs for assuring eating quality need to ensure consideration is given to postmortem muscle metabolism including the total electrical inputs into the carcasses.

Variation in postmortem muscle metabolism is often the underlying mechanism behind many genetic effects and observed variations in metabolomic and proteomic profiles in muscles of the animal. Investigations of links between proteomic, metabolomics, and genomic profiles and WHC/meat quality often overlook the importance of postmortem muscle metabolism as a causal mechanism. Developing collaborative program between "omic" and animal/meat/food scientists will enhance the opportunity for this to occur.

Meat stored in a vacuum bag loses about 2%–6% of weight in the form of drip over 1 week, and the weight loss can be much higher when exacerbating pre-slaughter stress occurs, or exposure of the post-slaughter muscle to PSE conditions. Increasing the storage period of meat in a vacuum bag to 3 or more weeks can double or even triple the weight loss. Future endeavors between researchers and commercial industry should focus on reducing these water losses during storage as this would enable a reduction in waste and increased visual and sensory quality.

Water is lost during the cooking process of fresh and processed meat products, as a result of muscle proteins denaturing at various temperatures, inducing transverse and longitudinal shrinkage. Greater understanding of the interplay between muscle structure, functionality of added ingredients, protein denaturation, and cooking conditions will enhance development of innovative ways to prepare novel meat products of high water-binding capacity, e.g., *sous vide*.

Muscles may contain fibers of several types, and it is thus not surprising that the myofibrils they contain react differently to hypertonic treatment, a feature that could be important in explaining and controlling the variability of meat in curing. Many natural and engineered compounds can be used to increase water binding in meat products; some are well known to the meat industry, and some are yet to be discovered. The ability to reduce salt and achieve high binding and water retention through use of HPP, and other possible emerging technologies, is an important consideration for the meat industry in being able to produce healthier foods. In the future, in order to understand the role that heat, salt, phosphates, pH, and other ingredients play in water retention in cooked and processed meat products, advances in biophysical and biochemical knowledge of meat from different species and different muscles will be required. To ensure continued consumption of meat in the future, the desire for novel, healthy, and innovative meat products will be partially fulfilled through ensuring WHC and juiciness in the products.

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Chapter 15

The eating quality of meat: V Sensory evaluation of meat

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15.1 Introduction

Sensory evaluation has been defined as a scientific discipline used to evoke, measure, analyze, and interpret human reactions to meat sensory characteristics as perceived by sight, smell, taste, touch, and hearing by the Institute of Food Technologists (IFT). Sensory science has been recognized as a scientific discipline, and sensory evaluation of meat is used as a component of meat quality, shelf-life, and consumer acceptance studies. It has been long recognized that the sensory attributes of meat are important for consumer acceptability, and by understanding these relationships, the meat industry can improve or decrease variability in specific meat sensory properties and increase consumer satisfaction.

One of the greatest challenges for meat scientists is that there are multiple sensory techniques or tools that can be used where each tool has strength and weaknesses and provides different types of sensory information. Additionally, evaluating meat using sensory techniques requires product knowledge and unique product, environmental, and panel controls that are important to understand prior to conducting meat sensory evaluation. The American Meat Science Association has published cookery and sensory guidelines for whole muscle meat and ground meat products and provides an extensive reference for sensory evaluation (AMSA, 2015). This guideline along with major sensory resources from the American Society of Testing Materials subcommittee E18 on Sensory Evaluation (ASTM, 1968, 1981, 1992, 1996, 2004, 2005, 2006, 2008a,b, 2009, 2010a,b, 2011a,b, 2012), Institute of Food Technologists (IFT, 1995) and major sensory textbooks (Lawless and Heymann, 2010; Meilgaard et al., 2016) can provide base level knowledge on how to conduct sensory evaluation using different sensory methods.

The objectives of this chapter are to discuss pertinent issues related to sensory evaluation of meat and to provide tools and references for meat sensory evaluation.

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15.2 Why sensory evaluation of meat is unique

Sensory evaluation or the understanding of inherent eating properties of meat has been discussed and used in meat science since the early 1900s. Watkins (1936) presented the need for understanding consumer preferences of beef, variation in consumer beef preferences, and factors that were related to those preferences. Meat scientists have long recognized that the eating quality of meat is related to consumer acceptance and subsequent consumer demand. Interestingly, Watkins (1936) did not present sensory data, but did present support for the relationship between carcass fatness, specifically marbling, and meat palatability. Even as early as 1939, understanding consumer knowledge of meat and the importance of meat selection by consumers was discussed by Scott (1939). Scott (1939) presented the concept that tenderness and flavor were important attributes to consumers and that tenderness was the most important factor with flavor second in importance for consumer acceptability. Neither of these authors presented data or research to support their hypotheses, but their work established that meat palatability or meat eating quality was related to consumer acceptance and that meat tenderness and flavor were important components of meat palatability.

Meat science research has established that for whole muscle meat, meat palatability has been defined as tenderness, juiciness, and flavor as these factors are related to consumer acceptance. Sensory work in meat science evolved to the examination of these three palatability traits using trained sensory panelists and was defined as Meat Descriptive Sensory Evaluation by AMSA (1978, 1995). Much of the trained meat descriptive research used these methods where juiciness could be evaluated as initial or sustained or overall juiciness; tenderness was defined as muscle fiber tenderness, connective tissue amount, and overall tenderness; and flavor was evaluated as overall flavor intensity. Some researchers in the 1990s began expanding flavor to evaluate more specific components of flavor such as species-specific flavors (pork brothy, beef brothy, lamb brothy) or defined attributes (fat flavor, serumy/bloody, grainy, grassy, cardboard, painty, fishy, metallic, for example). A formal introduction of evaluating specific flavor attributes of meat was introduced in AMSA (1995), and examples of use of these expanded flavor evaluations can be seen in Johnson and Civille (1986). Flavor evaluation has evolved to the development of measuring specific attributes of flavor using either defined terms or existing lexicons. Lexicons are defined as a group of terms where attributes are defined and references to clarify the attribute are given. Examples of meat lexicons are the Beef Whole Muscle Lexicon (Adhikari et al., 2011) and the Pork Whole Muscle Lexicon (Chu, 2015). AMSA (2015), Meilgaard et al. (2016), and Lawless and Heymann (2010) discuss the use of lexicons, how to train sensory panelist using a lexicon, and how to report data.

As descriptive attribute sensory evaluation has evolved from measuring tenderness and flavor to more extensive attributes and the use of defined references, consumer sensory evaluation has evolved as well. Measuring consumers'

response to meat was not extensively reported in the scientific literature prior to the 1980s. Consumer research was used within the meat industry and by marketing personnel, but meat scientists tended to not use consumer research as a tool due to the inability to always repeat results. Consumer sensory methods have evolved and are extensively used and reported by meat scientists. The evolution of the use of consumer research tools has enabled meat scientists to tie descriptive sensory data with consumer preference.

This evolution of the use of meat sensory research and new tools for sensory evaluation provide meat scientists with the ability to understand pre- and post-harvest factors that impact meat palatability. Meat is unique in that meat requires the knowledge of preparation and inherent physical components across species and muscle, as well as an understanding of sensory science techniques to conduct sound sensory methods.

15.3 Overview of how sensory is perceived and defining sensory attributes

The five human senses of sight, smell, taste, touch, and hearing are used in the perception of meat. Information from all five senses are taken in or perceived by the sensory organs, and information is related to the brain via the nervous system. Once information arrives at the brain, a perception is formed and a response is formulated by the brain. Each sense organ is perceived at different locations in the brain. Depending on experience, training, acuity of each sensory organ, and communication skills, individuals may understand sensory perceptions across the senses or they may not. Interactions are common across the five senses. For example, a consumer who eats well done meat may not understand that when they look at a meat piece that is slightly red, they formulate perceptions on what the meat is going to taste like and their acceptance prior to consuming the meat. The meat may not have all of the sensory characteristics that the consumer describes in the meat, as they may not be able to independently evaluate the flavor perceptions without the preconceived perceptions influencing their flavor evaluation. This makes conducting sensory evaluation a challenge. Knowledge of how the senses are perceived is important prior to conducting meat sensory evaluation so that perception of each sense is understood and potential interactions between senses can either be controlled or understood. A more thorough discussion of how the senses are perceived can be found in Meilgaard et al. (2016), Lawless and Heymann (2010), or an extensive human anatomy and physiology textbook.

Sight includes color and visual appearance of meat and may include packaging interactions, product size or portion, lean and fat levels or ratios, or any aspect of the visual appearance of meat either raw or cooked. Sight is perceived by the eyes and is recognized as the first component of perception. The adage that we eat with our eyes first is important for the sensory scientist as visual aspects of meat provide information to humans that may or may not affect their

perceptions using other senses. It should be noted that many times humans do not conscientiously know that visual appearance impacts their perceptions.

Sound is not traditionally considered important in meat sensory evaluation as cutting and consuming meat usually does not involve creation of sound. When sound is created during consumption of foods, the attribute is usually defined as a texture attribute. An example would be the sound created when biting into an apple that is a component of crispness. In unique products, sound may be a sensory attribute and should be considered. For example, when chewing woody chicken breasts (woody chicken breasts is a quality defect), there can be a crackling sound that is perceived during mastication. The extent of detection of the sound provides a measurement of the extent of the quality defect.

Smell or odors are perceived through the nose by the regio olfactoria on the olfactory bulb at the base of the sinus cavity. Compounds that are volatilized are carried by air through the nose where they are warmed and filtered through the nasal passage. When volatile compounds come in contact with the olfactory bulb, if there are receptors that recognize the compound, they are perceived. Some volatile compounds are not perceived and are defined as nonaromatic volatile compounds. Aromatic volatile compounds are those that are perceived by the olfactory bulb, but the amount or concentration of each compound needed for detection varies across individuals. Threshold values have been published for aromatic volatile compounds that indicate base levels of concentrations that are needed for detection. However, humans vary in the amount or types of receptors that they have on the olfactory bulb. Some humans have a heightened sense of smell. These individuals have more receptors for detection. Factors can affect an individual's ability to sense aromatic volatile compounds. Some diseases and medications interfere with detection and small amounts of some compounds, sulfur-based compounds for example, override the ability to detect or sense other aromatics. Any component that would coat the nasal passages and the subsequent olfactory bulb can also interfere in the ability of humans to detect smells or odors. Examples of this are mucous production or environmental contaminants such as ash. In sensory science, volatile aromatic compounds are commonly called odors.

Flavors are detected by the olfactory bulb as previously described except the aromatic volatile compounds originated in the mouth during consumption of the meat. The aromatic volatile compounds may be the same compounds detected as odors or new compounds that may be released during chewing or swallowing and may be detected. These aromatic volatile compounds come in contact with the olfactory bulb through the back of the throat and may also be derived from gustation after swallowing, many times referred to as aftertastes. The response to aromatic volatile compounds derived from the mouth are defined as flavor aromatics. Aftertaste flavor aromatic are those derived after swallowing by sensory professionals and can be evaluated as a component of sensory evaluation.

Basic tastes of sweet, salty, sour, bitter, and umami are those attributes detected by the taste buds on the tongue and in the mouth cavity. Receptors on the

tongue are stimulated when water-soluble compounds are in contact. Tongue maps were previously used to describe basic tastes, but it is now recognized that all four basic tastes are detected along the tongue, but there are areas of higher concentration of some receptors at different locations. Humans vary in the number of basic taste receptors on the tongue, and so like flavor aromatics, humans vary in their acuity for basic tastes. Environment and health conditions can greatly impact a human's ability to detect basic tastes as previously discussed for flavor aromatics, as well. More recently umami, the taste initiated by monosodium glutamate or glutamic acids has been recognized as a basic taste. Recent discussion in the sensory literature provides evidence that fat in food products may provide sensations that could be basic taste. Currently, fat is not defined as a basic taste but is characterized as a mouth feel and a flavor aromatic. Basic tastes for meat are defined as sweet, salty, sour, bitter, and umami.

The sensory of touch is used in sensory evaluation of meat in two ways. The sense of touch includes the tactile and the kinesthetic senses. The tactile senses have receptors in the skin for pressure, light and heavy touch, pain, and temperature. For meat, information on how heavy a piece of meat is when picking it up, juiciness during mastication, astringency or metallic mouthfeels while chewing, particle orientation, presence or amount, and how warm or cold the sample is when touching it or chewing are examples of use of the tactile sense. Sensory attributes for the tactile sense can include attributes while handling the meat prior to chewing, attributes in the meat during chewing and mouthfeels in the mouth during mastication. The kinesthetic sense gives the perception of resistance and is the deep pressure perceived by the tension and relaxation of muscles. In whole muscle meat, the perception of tenderness or resistance during mastication uses the kinesthetic sense. Another use of the kinesthetic sense for meat tenderness that is sometimes overlooked is the information the human perceives while cutting meat prior to consumption. Many times, panelists are served precut meat where the amount of force required to cut into the meat is not available. If provided a portion of meat to cut and then consume, information from the kinesthetic sense is provided from the cutting process and the mastication process. In processed meats, hardness and springiness are examples of key sensory attributes that use the kinesthetic sense. Attributes associated with the sense of touch are defined as texture and mouthfeel or feeling factors. While classically, tenderness is the most recognized texture attribute of meat, other attributes related to the texture of whole muscle and processed meats are important to consider.

The five senses are extensively involved in meat sensory evaluation. As meat scientists, it is our responsibility to understand what sensory attributes are important within a product and recognizing that sensory attributes are not the same across products. A partial list of trained and consumer sensory attributes and their definitions are listed in Table 15.1. Whole muscle and processed meat may have different sensory attributes depending on raw meat source, nonmeat ingredients added, processing procedures, packaging and storage, cooking, and

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense.

Attributes	Definition	Reference
Flavor (F) and odor (A) aromatics		
Apricot	Fruity aromatics that can be described as specifically apricot	Sun sweet dried apricot = 7.5 (F)
Asparagus	The slightly brown, slightly earthy green aromatics associated with cooked green asparagus	Asparagus water = 6.5 (F); 7.5 (A)
Animal hair	The aromatics perceived when raw wool is saturate with water	Caproic acid = 12.0
Barnyard	Combination of pungent, slightly sour, hay-like aromatics	White pepper in water = 4.0 (F); 4.5 (A)
	Associated with farm animals and the inside of a horn	Tinure of civet = 6.0 (A)
Beef identity	Amount of beef flavor identity in the sample	Swanson's beef broth = 5.0
		80% lean ground beef = 7.0
		Beef brisket = 11.0
Beet	A dark damp-musty-earthy note associated	Food Club sliced beets juice with1 part juice with canned
		Red beets to 2 parts water = 4.0 (F)
Bloody/ serumy	The aromatics associated with blood on cooked meat products	USDA choice strip steak = 5.5
	Closely related to metallic aromatic	Beef brisket = 6.0
Boar taint	Aromatic associated with boar taint; hormone-like; sweat, animal urine	0.1 g 3-methylindole, sniffed = 13.0 (A)
		Androstenone wafted directly from bottle = 15.0 (A)

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Brown/ roasted	A round, full aromatic generally associated with beef suet that has been broiled	Beef suet = 8.0
		80% lean ground beef = 10.0
Buttery	Sweet, dairy-like aromatic associated with natural butter	Land O'Lakes unsalted butter = 7.0 (F)
Burnt	The sharp/acrid flavor note associate with overroasted beef muscle, something over-baked or excessively browned in oil	Alf's red wheat Puffs = 5.0
Cardboardy	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging	Dry cardboard, 1 in square = 5.0 (F), 3.0 (A)
		Wet cardboard, 1 in square steeped in 1 cup water for 30 min = 7.0 (F), 6.0 (A)
Chemical	The aromatics associated with garden hose, hot Teflon pan, plastic packaging and petroleum based product such as charcoal liter fluid	Zip-Loc sandwich bag = 13.0
		Clorox in water = 6.5 charcoal lighter fluid
Chocolate/ cocoa	The aromatics associated with cocoa beans and powdered cocoa and chocolate bars. Brown, sweet, dusty, often bitter aromatics	Hershey's cocoa powder in water = 3.0
		Hershey's chocolate kiss = 8.5 (F)
Cooked milk	A combination of sweet, brown flavor notes and aromatics associated with heated milk	Mini Babybel original Swiss cheese = 2.5
		Dillon's whole milk = 4.5

Continued

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Cumin	The aromatics commonly associated with cumin and characterized as dry, pungent, woody an slightly floral	McCormick or Shilling ground cumin = 7.0 (F); 10.0 (A)
Dairy	The aromatics associated with products made from cow's milk, such as cream, milk, sour cream, or butter milk	Dillon's reduced fat milk (2%) = 8.0
Fat-like	The aromatics associated with cooked animal fat	Hillshire farms Lit'l beef smokies = 7.0
		Beef suet = 12.0
Floral	Sweet light, slightly perfume impression associated with flowers	Welch's white grape juice, diluted 1:1 with water = 5.0 (F)
		Geraniol = 7.5 (A)
Green	Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, and fresh cut grass	Hexanal in propylene glycol (5000 ppm) = 6.5 (A)
		Fresh parsley water = 9.0
Green-hay like	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley, and tea leaves	Dry parsley in medium snifter = 5.0 (A)
		Dry parsley in ~30-mL cup = 6.
Heated oil	The aromatics associated with oil heated to a high temperature	Wesson Oil, microwaved 3 min = 7.0 (F and A)
		Lay's potato chips = 4.0 (A)
Leather	Dusty, old leather (like old book bindings)	2,3,4-Trimethoxybenzaldehyde = 3.0 (A)
Liver-like	The aromatics associated with cooked organ meat/liver	Beef liver = 7.5
		Oscar Mayer Braunschweiger liver sausage = 10.0

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Medicinal	A clean sterile aromatic characteristic of antiseptic like products such as band-aids, alcohol, and iodine	Band-Aid = 6.0 (A)
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons	0.10% potassium chloride solution = 1.5
		USDA choice strip steak = 4.0
		Dole canned pineapple juice = 6.0
Musty/earthy/humus	Musty, sweet, decaying vegetation	Sliced button mushrooms = 3.0 (F); 3.0 (A)
		1000 ppm of 2,6-dimethylcyclohexanol in propylene glycol = 9.0 (A)
Nutty	Nutty characteristics are: sweet, oily, light brown, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc.	Diamond shelled walnut, ground for 1 min = 6.5 (F)
Overall sweet	A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet	Postshredded wheat spoon size = 1.5 (F)
		Hillshire farms Lit'l beef smokies = 3.0
		SAFC ethyl maltol 99% = 4.5 (A)
Painty	Aromatics associated with paint	Wesson oil placed in covered glass container in
		100°C oven for 14 days = 8 (F); 10 (A)
Petroleum-like	A specific chemical aromatic associated with crude oil and its refined products that have heavy oil characteristics	Vaseline petroleum jelly = 3.0 (A)
Pork identity	Amount of pork flavor identity in the sample	Boneless Pork Chop, 175°C = 7.0 (F), 5.0 (A)
		80/20 Ground Pork, 71 °C = 6.0 (F); 5.0 (A)

Continued

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Rancid	The aromatics commonly associated with oxidized fat and oils	Microwaved Wesson vegetable oil (3 min at high) = 7.0
	These aromatics may include cardboard, painty, varnish, and fishy	Microwaved Wesson vegetable oil (5 min at high) = 9.0
Refrigerator stale	Aromatics associated with products left in refrigerator for an extended period of time and absorbing a combination of odors (lack of freshness/flat)	Ground beef cooked over medium-high heat to 165°F, grease drained, store overnight in covered glass container at room temperature = 4.5 (F); 5.5 (A)
Smoky charcoal	An aromatic associated with meat juices and fat drippings on hot coats which can be acrid, sour, burned, etc.	Wright's Natural Hickory seasonings in water = 9.0 (A)
Smoky wood	Dry, dusty aromatic reminiscent of burning wood	Wright's Natural Hickory seasoning in water = 7.5 (A)
Soapy	An aromatic commonly found in unscented hand soap	Ivory bar soap in 100 mL water = 6.5 (A)
Sour aromatics	The aromatics associated with sour substances.	Dillon's buttermilk = 5.0
Sour milk/ sour	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream	Laughing cow light Swiss cheese = dairy 7.0
		Dillon's buttermilk = 9.0
Spoiled-putrid	The presence of inappropriate aromatics and flavors that is commonly associated with the products. It is a foul taste and/or smell that indicates the product is starting to decay and putrefy	Dimethyl disulfide in propylene glycol 10,000 ppm = 12.0 (A)
Vinegary	Aroma notes associated with vinegar	1.1 g Vinegar in 200 g water = 6.0 (F); 4.0 (A)
Warmed-over	Perception of a product that has been previously cooked and reheated	80% lean ground beef (reheated) = 6.0

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Basic tastes		
Bitter	The fundamental taste factor associated with a caffeine solution	0.01% caffeine solution = 2.0
		0.02% caffeine solution = 3.5
Sour	The fundamental taste factor associated with citric acid	0.015% citric acid solution = 1.5
		0.050% citric acid solution = 3.5
Sweet	The fundamental taste factor associated with sucrose	2.0% sucrose solution = 2.0
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides	0.035% accent flavor enhancer solution = 7.5
Salty	The fundamental taste factor of which sodium chloride is typical	0.15% sodium chloride solution = 1.5
		0.25% sodium chloride solution = 3.5
Mouthfeels		
Astringent	The chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as a puckering/dry and associated with tannins or alum	Lipton Tea, 1 bag in 1 cup boiling water and steeped for 3 min = 6.0 (F)
		Lipton Tea, 3 bags in 1 cup boiling water and steeped for 3
		3 min = 12.0 (F)
Meat whole muscle texture attributes		
Juiciness	The amount of perceived juice that is released from the product during mastication	Carrot = 8.5; mushroom = 10.0; cucumber = 12.0; apple = 13.5; watermelon = 15.0
		Choice top loin steak cooked to 58°C = 11.0
		Choice top loin steak cooked to 80°C = 9.0

Continued

TABLE 15.1 Examples of meat basic tastes, flavor aromatics, mouthfeels, and texture descriptive attributes that have been used to evaluate whole muscle beef and pork samples; definitions, reference standards, and their intensities where 1 = none; 16 = extremely intense—cont'd

Attributes	Definition	Reference
Muscle fiber tenderness	The ease in which the muscle fiber fragments during mastication	Select eye of round steak cooked to 70°C = 9.0
		Select tenderloin steak cooked to 70°C = 14.0
Connective tissue amount	The structural component of the muscle surrounding the during mastication muscle fiber that will not break down	Cross cut beef shank cooked to 70°C = 7.0
		Select tenderloin cooked to 70°C = 14.0
Overall tenderness	Average of muscle fiber tenderness and connective tissue amount when connective tissue amount is 6 or less	If connective tissue amount is 12–15, then overall tenderness = the value of muscle fiber tenderness; If connective tissue amount is then overall tenderness is the average of connective tissue amount and muscle fiber tenderness
Ground and processed meat texture attributes (other texture references in Meilgaard et al., 2016)		
Springiness	Degree to which sample returns to original shape after a certain time period	Marshmallow (miniature, Walmart Inc. AR, United States) = 9.5
Juiciness	Amount of wetness/juiciness released from sample	Apples (Red Delicious) = 10.0
Hardness	Force required to bite through sample	Hard candy (Life Savers, WM. Wrigley JR. Co., Chicago, IL, United States) = 14.5
Cohesiveness	The amount the sample deforms rather than shears/cuts	Candy chews (Starburst, Masterfoods USATM, Hackettstown, NJ, United States) = 12.5
Denseness	Compactness of the cross section	Malted milk balls (Whopper, The Hershey Co., PA, United States) = 6.0

Data from Adhikari, K., Chambers IV, E., Miller, R., Vazquez-Araujo, L., Bhumiratana, N., Philips, C., 2011. Development of a lexicon for beef flavor in intact muscle. J. Sens. Stud. 26, 413–420 and Chu, S.K., 2015. Development of an Intact Muscle Pork Flavor Lexicon (MS thesis). Texas A&M University, College Station, TX. Adapted from Laird, H.L., 2015. Millennial's Perception of Beef Flavor (MS thesis). Texas A&M University, College Station, TX are defined.

how the meat is consumed. Additionally, humans vary in sensitivity or acuity of all five senses, and humans may have similar acuity, but do not process information in their brain similarly. Understanding what senses are involved, how meat is prepared and consumed, and what are the important sensory attributes in a product are key to conducting valid sensory tests.

15.4 Sensory controls for meat

As information from all five senses are used when humans consume meat, it is important to understand what information is taken in by humans through the senses. Some information may be unwanted and some information may be key to the hypothesis of the study. For example, cooking meat to a standard end temperature measured by inserting thermocouples into the geometric center of each product during cooking allows for control over cooking and heat transfer. However, other factors affect heat denaturation of myoglobin during cooking and subsequent visual appearance of degree of doneness such as meat pH. Therefore, a decision has to be made, do you allow humans to see the difference in myoglobin denaturation or do you mask it. There is no right or wrong answer. It depends on the hypothesis of the test and the objectives. If the visual appearance of the meat is masked using red lights, the sensory evaluation is based on the inherent properties of the meat during consumption. If the panelists are allowed to see the meat without masking the visual appearance, this will most likely influence perceptions of flavor, juiciness, and tenderness for panelists, and that influence will most likely not be similar across panelists. For panelists who prefer meat cooked to lower degrees of doneness, upon seeing the meat cooked as they prefer, they begin the evaluation with positive thoughts and anticipation of a positive eating experience. For panelists who prefer meat cooked to higher degrees of doneness, upon seeing the meat cooked different than their preferences, they begin the evaluation with negative thoughts and some level of dread of a negative eating experience. These psychological factors may be important in the study or it may be important to remove them. Based on the objective and knowledge of how the meat is consumed, the meat scientist must determine what factors to control and not control and understand potential effects on the sensory results.

Sensory controls are defined as product, environmental, and panelist controls. These controls can influence sensory perceptions of humans. After consideration of product, environmental, and panelist controls, sensory procedures that account for these controls should be defined and closely followed during sensory evaluation. An example of sensory procedures for a whole muscle steak, chop, or breast is provided in Table 15.2. While these procedures may not be an exhaustive list, it provides an example of controls that need to be considered when conducting sensory evaluation, regardless of the testing method. Meat science knowledge of the product and resources listed as sources for further information provides more extensive discussion on controls that may be needed for conducting high-quality sensory testing.

TABLE 15.2 An example of sensory preparation procedures used to control product, environment, and panelist controls for a whole muscle steak or chop.

Control	Procedure
Product	Storage temperature and length of storage
	Packaging materials and atmosphere
	Standardize cooking methods including temperature of cooking instrument, initial product temperature, and turning conditions, if required
	Cook temperature monitoring devises and placement within the product for temperature monitoring
	Cooking, handling, and cutting utensils and containers unique for each product to eliminate cross-contamination
	Consistent serving containers, sample size and temperature, and serving utensils
	Coding of samples to easily ascertain sample and treatment
Environment	Odor free area that may include filtered air with standardized temperature and humidity for sample and panel areas
	Neutral colored surfaces, wall, and ceilings
	Low noise
	Surface material that do not absorb odors or impart odors into the panel or sample preparation areas
	Sample evaluation area separated from the sample preparation area
	Consistent lighting in the evaluation area
	Booths or evaluation room that provides sufficient room and is comfortable
	System to either pass or present samples to panelists that do not impart information about the sample or transfer noise or odors from the sample preparation area
Panelists	Panelist nor allowed in the sample preparation area
	Sufficient training to consistently evaluate products for trained sensory panelists
	Palette cleanser to be used between products
	Sufficient time between samples to reduce sensory fatigue
	Length of testing sessions
	Use of three-digit codes to not impart information about the sample
	Randomize order of sampling to reduce order biases across treatments

15.4.1 Product controls

Product controls include factors that may influence the visual, sound, odor, flavor aromatics, basic tastes, or texture of a meat product. As meat is mainly consumed after cooking, standard cooking protocols are imperative. For some studies, cooking methods may be a treatment and protocols for each treatment must be defined. Temperature of the heating surface and variations associated with the temperature of the heating surface should be known and/or defined. Monitoring of cooking using internal temperature monitoring devices, such as thermocouples, to determine beginning temperatures, midpoint temperatures, and final temperature are imperative to assuring that cooking does not influence sensory properties or that cooking and heat treatments are consistent across meat products. Internal temperature prior to cooking is an important aspect of cooking that may be overlooked. If meat is below freezing, the extra heat required to transition from frozen to thawed (28°F for whole muscle meats) may influence the time of cooking and the subsequent impact of heat on meat sensory characteristics. Efforts should be implemented to assure that meat is a standard temperature at the beginning as well as throughout and at the end of cooking. How much time after cooking prior to serving and serving needs to be standardized regardless of sensory method. Steaks from the same loin may have different sensory properties if one is served within 5 min of removal from the cooking apparatus versus the steak that is held for 30 min at 120°F prior to being served to panelists. As meat and sensory scientists, conditions that will not appreciably affect the sensory properties need to be considered, examined, and defined. For example, it was determined that pork chops cooked to one of four internal temperatures (145°F, 155°F, 165°F, and 175°F) could be held in glass bowls with glass lids in a 120°F oven for 5–20 min prior to cutting and serving to either a trained or consumer sensory panel without affecting the flavor, tenderness, and juiciness. If the holding temperature was higher, the pork chops would have more oxidized flavors and be drier and tougher. Additionally, it was determined that chops should be held, not cut pieces as oxidation, and dehydration occurred more rapidly after cutting. These parameters were determined in preliminary tests prior to conducting the trained and consumer sensory tests. Serving temperature should be monitored to assure consistent serving temperature within a reasonable range. Scientists have used preheated glass containers, yogurt makers that maintain temperature, preheated stones, or heating units to place samples on for serving. Time for cutting and serving should be monitored to be consistent, and the length of time needed for the panelists to evaluate the product should be considered. The goal is to reduce unwanted variation associated with handling and serving so that the scientist is assured that the meat sample was the same temperature and expressed the same sensory attributes for each panelists.

Conditions may not be the same for each study, and product controls should be considered for each project and they should also be practical. When running

a large beef roast and steak consumer study in four locations, cooking procedures were clearly defined and equipment was transported to each location, including a 300 lb. stainless steel electric foodservice grill and 20 crock pots. All steaks and roasts were thermocoupled, and cooking time and temperatures were monitored. Cooked samples were placed on white Corning ware plates, also transported to each location, and covered with aluminum foil. Steaks and roasts were placed in a 120°F oven, temperature monitored to assure that opening the door during evaluation to place samples in or to remove samples did not alter the holding temperature of the oven more than ±2°F during evaluation. Samples were cut and served in soufflé cups within 4 min of removal from the holding oven and within 20 min of being placed in the holding oven. The target meat temperature for consumer sensory panelist was 120°F. Some sensory scientists may not approve of this serving temperature as ASTM (1996) recommends that serving temperature of meat, especially chicken, to be 165°F. First, in this study, some meat samples were cooked to 145 °F internal temperature so it was not practical to serve at 165°F as it would alter the study design hypothesis. The next question was if serving at 145°F was advisable. To ascertain the temperature consumers normally consume meat, a small study was conducted where steaks and chops were provided to consumers along with a meat thermometer. Internal cook temperature was monitored after cooking and at the time of eating in their homes. Additionally, cooking method and time from the end of cooking to eating the steaks and chops were determined. These data were used to determine product controls. As locations changed, assurance that these conditions could be maintained during evaluation were tested; temperature and times were adjusted to assure compliance to the study protocol. Therefore, if location effects were found, these effects were due to consumer differences, not how the study was conducted. A more complete discussion of product controls is provided by AMSA (2015).

The amount of product served to either trained or untrained sensory panelists need to be considered for each study. For trained panelists, 2 or 3—1.27 cm sized samples may be presented for each experimental unit. The sample size should be sufficient to represent at least one bite each and enough sample should be provided for 2–3 evaluations per sample. The major concern is that sufficient sample is provided for evaluation of all attributes and that sufficient training has occurred so that panelists are familiar with use of the samples. For consumers, the amount and how samples are presented may differ depending on the objectives of the study. Samples as previously defined may be presented, but consumers may not be familiar with cubed samples. While cubed samples represent one bite as most consumers cut their meat into pieces and then consume it, information about the sensory properties of a sample may be obtained during the cutting process for consumers. While there is no standardized method of sample presentation, whether consumers are served cubed samples or larger portions of meat that they have to cut and sample should be considered. As more than one consumer evaluates from one steak, chop, breast, roast, ham, etc., how

much sample is available and how the sampling effects the experimental design should be considered. For example, in a large pork consumer test, it was determined that four chops would be obtained from one pork loin where the pork loin represented an experimental unit. Consumers in four cities were recruited where four chops from each pork loin were evaluated in each city as pork chops were cooked to either 145°F, 155°F, 165°F, or 175°F internal cook temperature. It was determined that four consumers evaluated each pork chop within a city as consumers were the greatest source of variation. While it would have been optimal to serve half of a pork chop to each consumer so that the consumers cut their own samples, there was not sufficient meat to have four consumers evaluate meat from each loin across the four internal temperature endpoints. Therefore, it was determined to serve each consumer two—1.25 cm cubes from each chop. The researchers understood that they may have given up some information, but their main objective was to understand the relationship of the loin quality traits and internal temperature endpoint on consumer acceptance. After the amount and size of sample to be presented to panelists within a study is determined, consistency and adherence to protocols are key to successful sensory evaluation.

Meat and sensory scientists also consider standardization of storage conditions including packaging materials, type of packaging, length of storage, temperature, and its fluctuations during storage. Packaging materials may impart flavors and may or may not be a concern. For example, storing top loin steaks in a commonly used packaging material may impart plastic flavors. The decision is whether to have this effect in the study or not. If this is a typical flavor in meat when consumers purchase the steaks, then allowing this effect across all treatments may be warranted. However, it may be determined that the plastic flavor masks or influences the study of sensory objectives and the steaks should be stored in a material that does not impart flavor affects such as aluminum foil or glass. The most important consideration is to understand how packaging and storage conditions may impact sensory attributes in the product and either eliminate the effect or control conditions so that all products are similarly affected.

15.4.2 Environmental controls

The environment that panelists, either trained or consumers, evaluate meat during sensory evaluation can influence sensory verdicts. The objective is to eliminate or control environmental factors that may influence sensory verdicts so that the sensory response is a measure of the sensory properties of the sample, and it is not influenced by information or conditions within the environment. Extensive discussion on environmental controls is provided in AMSA (2015), Meilgaard et al. (2016), ASTM (1996, 2008b, 2010b), and Lawless and Heymann (2010). The major environmental controls to be standardized include lighting, temperature and odor, and standardized facilities where panelists will be seated either in booths or in groups. Lighting should be standardized and the

type of lighting should be representative of normal lighting conditions unless masking of color or appearance of the sample is desirable for the test. A light meter should be used to assure consistent lighting in the area where panelists are seated so that lighting affects each panelist similarly. For meats, red lighting can be used to mask differences in visual appearance so that degree of doneness does not influence the sensory verdict. Trained panelists may or may not evaluate in red lighting conditions. An understanding of how long sessions should be prior to onset of mental and sensory fatigue are needed so that sensory verdicts are not influenced. Red lights are not normal conditions for consumers. Consumers should be allowed time to adjust to red lights, and length of sensory sessions should be controlled. A general rule is that consumers are not fatigued by 20–40 min per session under red lights, but consumers vary in their tolerance. If multiple locations are used, lighting should be standardized across locations.

The location where panelists are seated can influence sensory verdicts. The use of sensory booths is a common practice, and construction of booths is extensively discussed in Meilgaard et al. (2016), Lawless and Heymann (2010), and ASTM (2008b). Sensory booths should be neutral in color, odorless, and free from noise; have standardized temperature, humidity, and negative airflow from the booth into outside areas; have sufficient room for samples, palette cleansers, computers, and sinks if needed; and have consistent lighting. Booths need to be separated from the sample preparation room and a pass-through mechanism that eliminates or minimizes transfer of odors and air from other areas, especially the sample preparation room, are needed. If panelists are seated in a room, the aforementioned parameters are important as well. For trained panelists seated in a room, a round table with centrally located turn table to easily pass or share samples is recommended. Panelists need to be trained to not speak or provide signals that communicate their perceptions to other panelists. For consumer panelists, whether seated in booths or a room, the same issues apply. Consumers may be seated in a centralized room, but usually tables where panelists do not face each other are used. A moderator should be present at all times to assure that panelists do not talk or communicate during evaluation and that neutral conditions are maintained.

15.4.3 Panelist controls

Panel controls for consumer and trained panelists are similar for some aspects, but trained panelists have the added step of panel training. Information directly or indirectly provided to panelists can influence their sensory verdicts. The goal is to have only sensory aspects of the product being evaluated influencing the sensory verdict. Factors that can influence sensory verdicts such as physiological factors of adaptation, enhancement. and suppression; psychological factors such as expectation error, error of habituation, stimulus error, logical error, halo effect, order of presentation of samples, mutual suggestion, lack of motivation, capriciousness, and timidity; and poor physical condition

needs to be understood by sensory analysis. These factors can affect panelists bias and increase variability within a sensory study. AMSA (2015), Meilgaard et al. (2016), Lawless and Heymann (2010), and ASTM (1981) provide discussion of these factors and how to control them. For meat scientists, standardizing serving containers and sample size, randomizing order of presentation of samples, use of palette cleansers between samples, standardizing time between sample servings, use of three-digit numerical codes for samples, assuring the number of samples served within a session do not affect sensory and mental fatigue, length of sessions and number of sessions per day, limit communication between panelists, assure health of panelists, provide a warm-up to help standardize panelists each day, be aware of panelist attitudes and comfort level, and monitor panelist performance for identification of re-training needs are needed. While this is an exhaustive and some of the aforementioned factors are also components of product and environmental controls, it is important to assure that panelists are comfortable, positive, willing to work, have no outside influences or biases, and provide accurate sensory verdicts.

For trained sensory panelists, the extent and amount of training for a sensory test affects the reliability of the data. AMSA (2015), Meilgaard et al. (2016), Lawless and Heymann (2010), and ASTM (1981) provide extensive discussion of panelist training methods. The key to successful panelist training is first identifying the objective of the study including what attributes are to be included and what testing methods will be used, prescreening and screening potential panelists, identifying the number of suggested testing days knowing that the number of training days most likely will change based on response of panelists to training, and a proposed schedule for each training day including the objective for the training day and a schedule of activities. Table 15.3 provides an example of how an existing meat flavor descriptive attribute panel would be trained for a new study. For each sensory study, panel training days are needed. For example, an expert trained flavor and texture descriptive attribute sensory panel with over 25 years of experience of evaluating whole muscle beef flavor and texture would need training days prior to initiation of a new sensory project, even if they had just completed a similar project. While the panel may only need six to 10 days of training, samples that represent the treatments for the study and samples that provide lower and higher levels for attributes within the study should be presented to the panelist during training. A similar panel with less training may need additional and more extensive training for the same study. Panels should be verified prior to initiation of a study. AMSA (2015) and Meilgaard et al. (2016) provide methods for panel verification.

15.5 Sensory techniques

In conducting sensory, different tools or techniques are available. The major sensory techniques and what type of questions that can be answered by each sensory method are described. AMSA (2015) provides a decision tree that can

TABLE 15.3 Examples for training panelists for descriptive attribute sensory evaluation.

Session	Suggested exercises for approximately 2 h sessions
1	Introduce panelists to the sensory facilities; explain the program and the importance of the program to the objectives of the company or institution; explain how sensory information is obtained through the five senses of sight, smell, feel, hearing, and basic tastes; explain basic procedures such as panelist requirements, use of palette cleansers, serving procedures, rules for procedures, how to use booths, lighting options, etc.; answer questions
	Introduce Universal scale (Meilgaard et al., 2016) or basic scale to begin understanding how to scale
2	Work to understand scaling and expand exercises and experiences with different attributes
	Present the Universal scale and one basic scale for sweet, salty, bitter, and sour (Meilgaard et al., 2016). Concentrate on each scale and discuss. Have them re-taste as needed. Re-enforce use of palette cleansers between samples. Give them one of the references as an unknown labeled with a three-digit code. Have panelists each call out numbers and ask to discuss what they found. Have them retaste the references for that attribute. After discussion, tell them the intensity level of the attribute. Have them re-evaluate the unknown and the references to anchor the attribute. Give them a second unknown that is in between the references, for example, if you are using references 0, 2, 5, 10, and 15, give them an 8 that you make using intermediate concentrations. Have panelists each call out numbers and ask to discuss what they found. Have them retaste the references for that attribute. After discussion, tell them the intensity level of the attribute. Have them re-evaluate the unknown and the references to anchor the attribute. Introduce a second basic taste scale
3.	Re-enforce scaling for basic tastes and introduce unknowns from basic taste solutions to begin applying scaling
	Present the solutions for the 2 basic tastes used in session 2. Have panelists taste one set of solutions, using palette cleansers. Give an unknown marked with a three-digit code (one of the solutions that they just tasted) and have them rank it for the attribute. Have panelists each call out numbers and ask to discuss what they found. Have them retaste the references for that attribute. After discussion, tell them the intensity level of the attribute. Have them re-evaluate the unknown and the references to anchor the attribute. Give them a new unknown that is in between the references but different from the unknown given in session 2. Continue with calling out their scores, discussing, re-evaluating the references, and telling them the expected level. Give the references for the second basic taste, give an unknown that is the same as one of the references and then an unknown that is in between. Provide the scale for the third basic taste. Positively re-enforce panelists

TABLE 15.3 Examples for training panelists for descriptive attribute sensory evaluation—cont'd

Session	Suggested exercises for approximately 2 h sessions
4.	Re-enforce scaling for basic tastes and expand use of unknowns from basic taste solutions
	Repeat exercises from session 3 changing the unknowns to be along the scale. Introduce the fourth basic taste and repeat the exercises as previously discussed. Repeat these exercises until panelists are consistently scaling and recognizing differences. Introduce a food product that contains basic tastes (level of basic tastes for food products are presented in Meilgaard et al., 2016) and have panelists score different basic tastes. Work with panelists to recognize the attribute in a more complex system where more than one attribute is present. This trains the panelist to concentrate on one attribute or pull it out in a more complex system
5.	Repeat session 4 exercises changing up products used to identify basic tastes asking panelists to rate only one basic taste. Encourage panelists to use references to rate the basic taste. Always re-enforce use of palette cleansers between samples and have a discussion for each exercise. When panelists are proficient, ask panelists to rate more than one basic taste in a product. Use the same method of calling out values, re-evaluating references and coming to consensus
6.	Repeat session 5 exercises except continue to change products and add evaluation of three basic tastes. When the panelist are proficient, ask them to rate four basic tastes. This may take more than 6 sessions depending on panelists. Continue with sessions until panelists can consistently evaluate the four basic tastes in food products consistently and know how to use the references
7.	Introduce the first meat flavor attribute. Select an attribute that is easy to identify like beef, pork, or chicken identity
	Repeat the process of introducing the attribute, give the definition, and provide references. Give an unknown and ask the panelist to rate the attribute of interest. Have panelists give their score, discuss as a group, have panelists re-taste references, and come to consensus. Give them the level of attribute that was in the product. Give them time to anchor and adjust their understanding of the attribute
8.	Continue to add new attributes following the aforementioned process. Add complexity by asking panelists to rate multiple attributes. Build the ability of the panelist to rate attributes until all attributes are introduced and panelists are rating attributes similarly using unknowns. This will take multiple sessions. Examples of products for training are provided in AMSA (2015)
9.	After panelists are scaling attributes, place them in booths for short periods of time asking them to rate unknowns. Add sessions to increase the amount of time. This increases their familiarity with the booths, the testing environment and continues to expand their ability to identify attributes and scale each attribute
10.	Conduct validation study over 3 days as defined in AMSA (2015)

be used to determine what type of sensory test can be used depending on the sensory objectives. Fig. 15.1 shows a reduced decision tree that can be used by sensory and meat scientists to understand the three general areas of sensory tools available. First, the main objective or outcome of the test needs to be defined so that the test and the objectives can be matched. For example, if a new product that is lower in fat has been developed, the first objective may be to test if the low-fat product differs from the regular fat control. Discriminative tests provide sensory methods to address that question, but discriminative tests most likely will not characterize what differences exist and how much difference exists in each attribute. Descriptive tests provide sensory tools that assist the meat scientist in understanding what attributes differ from visual appearance, odor, flavor, and texture. Additionally, how much difference in each attribute between the low-fat and regular fat product can be determined. This information can be used to either reformulate the low-fat product or the meat scientist may ask the question, "are these differences important to consumers?" If reformulation is the direction after descriptive testing, the sensory process may repeat until differences in specific attributes are either not detected by discriminative or descriptive analysis. Consumer testing is conducted when the question of what consumers prefer or find acceptable is important. Meat and sensory scientists

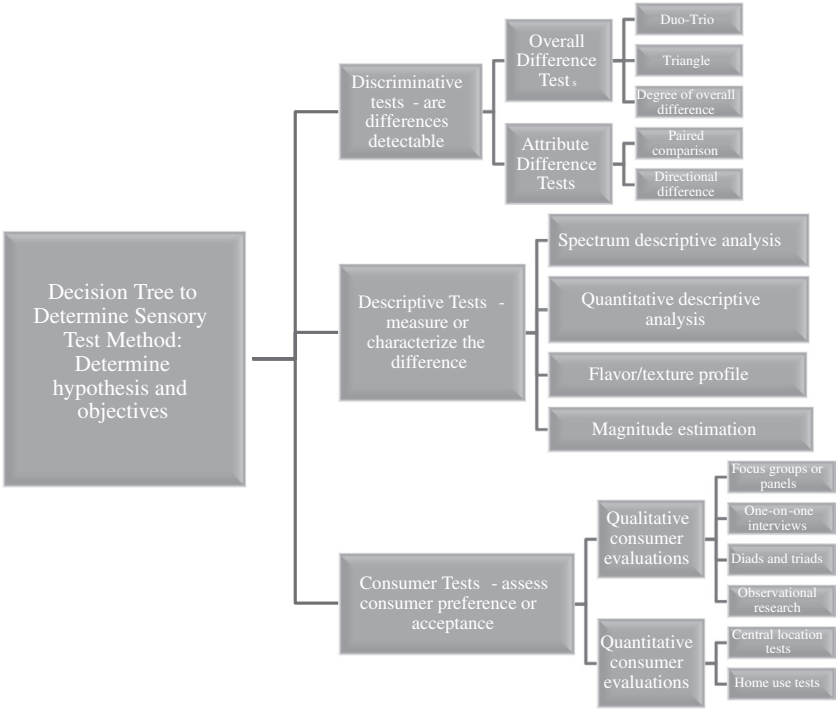

FIG. 15.1 Sensory decision tree illustrating sensory tools for three different sensory procedures.

continue this process until the final product is deemed acceptable or ready for market. It should be noted that differences may exist between products for specific attributes, but the decision to market the product may be positive with acceptance of the difference. By using sensory evaluation, the team of meat and sensory scientists and marketing professionals can make informed decisions.

15.5.1 Discriminative analysis

The purpose of discriminative analysis is to understand if two or in some cases more products differ. Consumer panelists are commonly used to conduct these tests so that results are related to the sensitivity for consumers. However, trained sensory panelists can also be used. When trained sensory panelists are used, the panelists will be more sensitive to differences than consumers. As this may be desirable for the test, it should be clear to professionals involved in using the results. For example, if the two aforementioned products, a low-fat and regular fat meat product, were developed. The first iterations of the low-fat product may be tested against the regular fat product using trained sensory panelists in discrimination testing. The results of these tests can be used by product development scientists to adjust the low-fat product to be more similar to the regular fat product. These tests are easy to conduct and are usually conducted using in-house trained panelists where results can be obtained the same day of testing. After the product has been more closely developed, discriminative tests with consumers that would use a larger number of panelists could be conducted to understand if consumers could detect differences. Discriminative tests are divided into overall difference tests and attribute difference tests. Overall difference tests are used to determine if differences exist and differences may be due to any attribute. Attribute difference tests provide information on if a specific attribute of two products differ and allows the researchers to concentrate on attributes of interest. Discriminative tests are generally easier to conduct, statistical analyses are more straight forward and well defined, and the number of participants are defined in predetermined tables for ease of design. Lawless and Heymann (2010) and Meilgaard et al. (2016) have extensive discussions on discriminative tests and provide statistical tables for determination of results. Prior to conducting discriminative tests, regardless of method, α and β for the test need to be determined so that a full understanding of the strength of the test is understood. α is the probability of concluding that there is a difference in the products evaluated and there is not. β is the probability of missing a difference that exists. As most meat scientists use statistical tools that concentrate on, it is important to note that for discriminative tests, controlling for β may be considered more important than controlling for α depending on the objective of the test. P_d or $P_{\max}$, defined depending on the test selected, are used to account for the proportion of the population that will detect differences and are related to the interpretation and magnitude of the population that inference can be projected. Defining levels for α and β prior to conducting the test, selecting the appropriate number

of participants based on P_d or P_{max}, and conducting the study based on these parameters allow maximum success of conclusions and use of these tests.

The three most common discriminative tests are triangle, duo-trio, and paired comparison tests. Paired comparison tests can be used for overall difference but are most commonly used for attribute difference testing. Full discussions on the use of these tests using the American Society of Testing Materials (ASTM), Subcommittee E18 on sensory analysis, the AMSA sensory guidelines, and major sensory textbooks are provided in the Sources for Further Information section for more details on how to conduct these tests.

Triangle test are used to determine overall difference between two products. Panelists are presented three samples, either at one time or separately and are asked to define the sample that is different. They are told that two samples are the same and one is different. There are six presentation schemes that are randomized by panelists. For two products, A and B, the six serving possibilities are ABB BAB BBA AAB ABA and BAA. These six serving possibilities should be randomized so that each sample has the opportunity to be the difference sample and that order of serving for the different sample is randomized to first, second, and third. Panelists are asked to guess if they do not know and that statistics for Triangle tests accounts for a 33% guessing rate. An example of a triangle ballot is presented in Fig. 15.2. Product, environment, and panelist controls should be carefully monitored so that only the sensory attributes of the samples influence selection of the odd or different sample. Samples should be identified with three-digit random numbers, and palette cleansers should be used between samples. Directions should be clearly communicated to panelists either on the ballot or through a panel moderator present during testing. Panelists can be asked to define why the odd or different sample was identified. While an open-ended response will not provide statistically analyzed differences, it can provide insight into why differences were detected. As panelists are presented with three samples, preliminary tests to assure that adaptation and fatigue are not issues during testing are needed. The total number of triangle tests presented to one panelist should be limited as each test requires evaluation of three samples.

DuoTrio tests use the presentation of three samples, but panelists are asked to determine differences slightly differently that results in a 50% guessing rate. Two samples are used in the test, but one sample is presented to the panelist as the control or reference. In random order, panelists are provided two samples, and they are asked to identify the sample that is the same as the control. There are four randomizations possible for DuoTrio tests for products A and B. A is defined as the reference, and samples are presented as either AB or BA, and B is defined as the reference, and samples are presented as either AB or BA. Adaptation and fatigue can be issues with DuoTrio tests as discussed for Triangle tests. The two samples being evaluated should be identified with random three-digit codes, and palette cleansers should be provided. Directions should be adequately communicated to the panelists on how to conduct the test

TRIANGLE TEST BALLOT
INSTRUCTIONS: Taste the samples from left to right. Two are identical; determine which is the odd sample. Please cleanse your palate with a sip of water and a bite of cracker in between samples. If no difference is apparent, you must guess.

Set of Three Samples	Indicate Odd Sample	Comments – why sample was different
459 731 976	_____	_____

DUO TRIO TEST BALLOT
INSTRUCTIONS: Taste samples from left to right. The left hand sample is a reference. Determine which of the two samples matches the reference and indicate by placing an X. If no difference is apparent between the two unknown samples, you must guess. Provide comments as to why the sample differed in as much detail as you can.

1. Reference	Code 345	Code 834
☐	☐	☐

Comments _____

PAIRED COMPARISON BALLOT: ONE-TAILED
INSTRUCTIONS: Please take a bite of the cracker. Now, please take a sip of water. You are now ready to sample the meat. Taste the two samples from left to right. You have been provided a knife and fork to cut the sample. Eat as much of the sample as you would like. After you have finished tasting a sample, please do not go back and retaste the sample. Determine if the two samples are different and mark your response below.

_____ **YES** _____ **NO**
(NOTE: Some of the sets consist of two identical samples.)

Comments: Please provide any information about the sample that you would like us to know.
_____

PAIRED COMPARISON BALLOT: TWO-TAILED
INSTRUCTIONS: Please take a bite of the cracker. Now, please take a sip of water. You are now ready to sample the meat. Taste the two samples from left to right. You have been provided a knife and fork to cut the sample. Eat as much of the sample as you would like. After you have finished tasting a sample, please do not go back and retaste the sample. Mark the sample that is more tender.
_____ 345 _____ 834
(NOTE: Some of the sets consist of two identical samples.)

Comments: Please provide any information about the sample that you would like us to know.
_____

FIG. 15.2 Examples of triangle, DuoTrio, and one- and two-sided paired comparison ballots for discriminative testing.

either orally or on the ballot, or both. An example of a DuoTrio ballot is presented in Fig. 15.2. A panel moderator should be in the test environment to assure panelist understand and conduct the test as defined. Product, panelist, and environment controls should be considered, defined and applied as previously discussed while conducting DuoTrio tests.

Paired comparison tests are the most commonly used discriminative tests and can be either one- or two-tailed tests. Two-tailed tests also are called directional difference tests. There are variants of this test described in sources for further information that are useful in determining modifications to increase power of the test or to address issues with sensory fatigue or adaptation. For meat

products, the challenge with using paired comparison tests is usually whether to use one- or two-tailed tests. Examples of ballots for both types of tests are presented in Fig. 15.2. For both types of tests, two samples, A and B identified with random three-digit codes, are presented to panelists and they determine if the two samples are the same or different, and these tests have a 50% guessing rate. There are four presentation of samples for paired comparison tests: AA, AB, BB, and BA. These should be served in random order, and the number of tests a panelist completes is based on pretesting for fatigue and adaptation responses.

Degree of difference testing as described by AMSA (2015) is a discriminative test that can not only provide information on if the samples differ but also an assessment of how different the samples are for specific or overall attributes. New discriminative test methods continue to be developed that provide information on if two or more products differ. One of the newest discriminative tests is the Triad. In this method, four samples are presented to the panelist; two of each product A and B. The panelists are asked to identify the two samples that are the same for each pair. This test has a lower guessing rate, and therefore, fewer panelists are needed to have the same power for determining differences. Meilgaard et al. (2016) discuss the use of this test and provide tables for assessing the number of panelists and for determining if differences exist.

In summary, multiple discriminative tests can be used by meat scientists. However, the selection of a discriminative test should be based on knowledge of the strengths and weaknesses of the test, of the product and panelist adaptation and potential sensory fatigue, and determination of the type of information needed from the test.

15.5.2 Descriptive analysis

Descriptive sensory analysis uses trained sensory panelists to describe and quantify sensory attribute differences between products. These methods depend on statistical analyses to determine differences and methods vary in the amount of training required for panelists. However, the common thread is that descriptive methods identify attributes within the products being tested and provide the sensory or meat science professional the ability to conclude if there are differences in these attributes. There are three general categories of descriptive sensory analysis used for meat products, Flavor and Texture Profile, Spectrum Descriptive Attribute Analysis, and Quantitative Descriptive Attribute Analysis.

Panelist training is a component of descriptive analysis and depending on the method, the extent of training may vary. AMSA (2015), ASTM STP758 (1981), and Meilgaard et al. (2016) provide guidance on how to select and train descriptive sensory panelists. Panelists should have normal acuity for the attributes to be evaluated and should be able to follow directions and make judgments. A prescreening questionnaire should be developed that accesses the potential panelist general health, availability, and interest. Panelist who are deemed acceptable during the prescreening interview should be scheduled for

screening tests. If panelists are late, do not call if they need to reschedule or do not show a positive attitude and interest during screening, regardless of outcome of screening tests, these panelists should not be invited back for further training. Screening tests should include logic and judgment tests that ascertain the ability of the panelist to follow directions and tests that evaluate their ability to discriminate attributes of interest. Meilgaard et al. (2016) provide logic tests. Sniff tests help to determine if potential panelist can identify or describe flavor aromatics. Essential oils for a varying array of flavors can be obtained. Filter paper, cut into strips, can be dipped in the essential oil and placed in a small glass container like a glass custard dish with a glass concave cover or a baby food jar. Potential panelists are asked to sniff the samples and write down the flavor or items that they identify. Matching tests, ranking tests, or triangle tests that are analyzed using sequential testing are evaluations that can be used to determine if panelists can ascertain differences in sensory attributes. Examples of how to conduct these tests for meat and poultry are explained in AMSA (2015).

After selection of potential panelists, training begins. The purpose of training is to familiarize panelist with the test procedures, improve each panelists' ability to recognize and identify sensory attributes, and improve panelist's sensitivity and memory of attributes that allow for precise and consistent sensory judgments. The amount of training is panel and product dependent and may take up to 6 or 12 months. The key is that panelists consistently recognize attributes and scale each attribute similarly across multiple days. A trained panel will have up to 200 h of training, and an expert panel will have over 200 h of training. Table 15.3 provides suggestions on panel training exercises.

One of the greatest challenges with using descriptive analysis is what attributes to measure and what scales to use. Each method has similarities but may require differences in length of training and may use panelists to measure attributes differently. These differences are discussed below. Generally, the meat and/or sensory scientists can use either published lexicons or a product-specific lexicon can be developed. The key to developing a product-specific lexicon is that each attribute in the lexicon is defined and references to understanding scaling for each attribute are provided. For whole muscle meat, lexicons have been developed for beef (Johnson and Civille, 1986; Adhikari et al., 2011) and pork (Chu, 2015). AMSA (2015) discussed each of these lexicons and use of lexicons in sensory evaluation are provided in Meilgaard et al. (2016) and ASTM (2011a). Table 15.1 provides components from these lexicons, as examples. For repeatable descriptive sensory evaluation and adequate training of panelists, the attributes and references for scaling provide a base for descriptive sensory evaluation.

15.5.2.1 Flavor and texture profile methods

The flavor profile method was originally developed to provide a quantitative method for assessing differences in food products. In this method, four to six

panelists are selected according to their abilities to determine differences in odor and flavor within the product category. Each panelist evaluates the product and identifies the aroma, flavor, and aftertaste attributes, intensity of each attribute, and order of appearance called character notes using a 7-point scale. Each panelist provides an individual score, and a panel leader leads a discussion on each attribute and a consensus intensity is derived. References can be brought in for identification of attributes. Data can be analyzed if sufficient replication is obtained, but panelist effects are not determined. The flavor profile method can have undue influence by dominant panelists or panel leaders, and small differences may not be determined as a 7-point scale may not offer enough points of discrimination.

The texture profile method evolved from the flavor profile method except the texture or structural components of the sample are evaluated. The geometric, mechanical, moisture, and fat-related attributes can be measured with this method. Geometric attributes related to shape, size, and orientation of a sample before, during and after chewing can be determined. Mechanical attributes are those attributes that change in the meat sample while stress, or chewing, is applied. Moisture and fat attributes are identified by mouth feel during or after chewing. While the procedures are similar, some modifications were implemented for the texture profile method. More clearly defined definitions of attributes, scaling references, and procedures for evaluation were developed (ASTM, 1992). Additionally, individual panelist scores can be used in this method without obtaining consensus that allows for more extensive statistical analysis of the data.

15.5.2.2 Meat descriptive attribute sensory evaluation

Meat scientists through the American Meat Science Association published the first sensory methods document in 1978 (AMSA, 1978) to address how to evaluate meat palatability attributes. These attributes were defined as juiciness, connective tissue amount, muscle fiber tenderness, and overall tenderness and the revisions in 1995 included overall flavor intensity (AMSA, 1995). This attribute, however, cannot be uniformly referenced and scaled and is not repeatable across panels. As flavor has evolved as a major component of palatability, species-specific flavor lexicons have been developed (Johnson and Civille, 1986; Adhikari et al., 2011; Chu, 2015) where each flavor attribute can be referenced and scaled. Lexicons are dynamic and new attributes can be identified and added to any lexicon. As attributes are product and study specific, other attributes or components of attributes identified in a product can be used based on the study objectives. The key is to define attributes and to use references for scaling. It should also be understood that if an attribute is expressed in a study and not included in the lexicon, important aspects of the sensory differences may be missed. Therefore, an attribute labeled other should be included on all sensory ballots so that panelists have the opportunity to describe flavor or texture attributes identified but not listed on the ballot. Table 15.1 includes

meat descriptive attributes evaluated on a 16-point scale, but traditionally these attributes have been evaluated on an 8-point scale (ASMA, 2015). An example of a meat descriptive attribute ballot using the 8-point scale is presented in Figs. 15.3 and 15.4, provides an example of a similar ballot using the 16-point scale. Either scale is acceptable as long as the scale is defined and the panel is sufficiently trained to use the scale. The 16-point scale provides more points of determination, and if the panel is evaluating flavor attributes using a 16-point scale, there is an advantage of using the scale across all attributes evaluated.

For processed meats and ground meats, texture attributes vary from those of whole muscle meats. Some texture attributes used for ground and processed meats are presented in Table 15.1, and an example of a flavor and texture attribute ballot used for ground beef is reported in Fig. 15.4. The texture attributes also are defined and scaled using solid oral texture attributes from Meilgaard et al. (2016). Flavor and texture attributes included on this ground beef ballot are not inclusive of all attributes found in ground beef, but are the attributes that were important to evaluate in the study. For processed meats, flavor aromatics, basic tastes, mouthfeels, and texture attributes that are expressed or important for a project can be included on the ballot.

15.5.2.3 Spectrum descriptive attribute analysis

The Spectrum descriptive analysis procedure was developed by Gail Civille to provide a uniform method of measuring intensity of aroma, flavor, and texture using a universal scale (Meilgaard et al., 2016). Sensory attributes of a product are identified either by developing or using an existing lexicon of terms that defines each term and provides references for assistance in scaling. For meat products, the whole muscle beef flavor lexicon (Adhikari et al., 2011), the

						Flavor Aromatics							
Sample	Juiciness	Muscle Fiber Tenderness	Connective Tissue Amount	Overall Tenderness	Flavor Intensity	Beef Identity	Cooked Beef Fat	Serumy/ Bloody	Livery	Grassy	Soda	Metallic	Off Flavors
341	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
860	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
112	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
938	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____

Description of Attributes

Juiciness	Muscle Fiber & Overall Tenderness	Connective Tissue Amount	Flavor Intensity	Off-Flavor Character
8 Extremely Juicy	8 Extremely Tender	8 None	8 Extremely Intense	A Acid
7 Very Juicy	7 Very Tender	7 Practically None	7 Very Intense	BR Browned
6 Moderately Juicy	6 Moderately Tender	6 Traces	6 Moderately Intense	C Cardboard
5 Slightly Juicy	5 Slightly Tender	5 Slight	5 Slightly Intense	CW Cowy
4 Slightly Dry	4 Slightly Tough	4 Moderate	4 Slightly Bland	F Fish-like
3 Moderately Dry	3 Moderately Tough	3 Slightly Abdt	3 Moderately Bland	SW Sweet
2 Very Dry	2 Very Tough	2 Moderately Abdt	2 Very Bland	SO Sour
1 Extremely Dry	1 Extremely Tough	1 Abundant	1 Extremely Bland	SD Soured
				N Nutty
				P Putrid
				X Other (describe)

FIG. 15.3 Example of a meat descriptive attribute ballot using 8-point scales.

SAMPLE NUMBER	_____	_____
Flavor Aromatics - Major Notes:		
Beef Flavor Identity	_____	_____
Pork Flavor Identity	_____	_____
Brown/Roasted	_____	_____
Bloody/Serumy	_____	_____
Fat-Like	_____	_____
Metallic	_____	_____
Cardboardy	_____	_____
Painty	_____	_____
Fishy	_____	_____
Liver-Like	_____	_____
Putrid, sulphur-like	_____	_____
Umami	_____	_____
Overall Sweet	_____	_____
Sour milk/Sour Dairy	_____	_____
Sweet	_____	_____
Sour	_____	_____
Salty	_____	_____
Bitter	_____	_____
Aftertaste	_____	_____
Other	_____	_____
Meat Descriptive Attributes		
Juiciness	_____	_____
Muscle Fiber Tenderness	_____	_____
Connective Tissue Amount	_____	_____
Overall Tenderness	_____	_____

0 * 1 * 2 * 3 * 4 * 5 * 6 * 7 * 8 * 9 * 10 * 11 * 12 * 13 * 14 * 15

Slight | Moderate | Strong

Other Flavor attributes:

Asparagus	Apricot	Barnyard	Beet	Buttery	Burnt
Chemical	Chocolate/Cocoa	Cooked Milk	Cumin	Dairy	Floral
Green-haylike	Heated Oil	Refrigerator Stale	Rancid	Warmed-over	

Juiciness		**Muscle Fiber & Overall Tenderness**		**Connective Tissue Amount**	
15	Extremely Juicy	15	Extremely Tender	15	None
13	Very Juicy	13	Very Tender	13	Practically None
11	Moderately Juicy	11	Moderately Tender	11	Traces
9	Slightly Juicy	9	Slightly Tender	9	Slight
7	Slightly Dry	7	Slightly Tough	7	Moderate
5	Moderately Dry	5	Moderately Tough	5	Slightly Abundant
3	Very Dry	3	Very Tough	3	Moderately Abdt
1	Extremely Dry	1	Extremely Tough	1	Abundant

FIG. 15.4 Example of a beef and pork whole muscle descriptive flavor and texture attribute ballot using a 16-point scale.

pork flavor lexicon (Chu, 2015), and warmed over flavor in beef (Johnson and Civille, 1986) have been developed. Many of these attributes are presented in Table 15.1. The use of the universal scale and how to develop and use a lexicon is discussed in Muñoz and Civille (1992). A 16-point universal scale or line scale are used to rate the intensity of each attribute. An existing lexicon can be used and product-specific attributes can be added. For example, when conducting a study on pork flavor where the live animals were fed acorns as a component

of their diet, nutty flavor may not adequately describe the flavor found in the subsequent pork chops. A new attribute may need to be added. ASTM (2011a) provides descriptions and suggestions on attributes. AMSA (2015) presents the current whole muscle meat lexicons and provides a more detailed discussion on how to use the lexicons. Lexicons for processed meats are more product specific. The trained panel may use components from an existing lexicon, but develop attributes that are identified in the product. The panel leader is a key component of this method. They provide the lexicon and design exercises to assist panelists in understanding each attribute, how to use references and how to scale. It is critical that the panel be exposed to the range of products within the project and similar products that are outside of the range of the project. In other words, provide panelists with the extensive range of the product. Once the panel is trained and has experience, they can help in identifying attributes that may be product specific and not listed in a lexicon. Ballot development sessions should be conducted where the panelists are exposed to the product within the treatments for the study and other similar products. The panel with the assistance of the panel leader identifies attributes. The panel leader then presents potential references for the attribute, and the panelists and panel leader come to agreement on the attribute, the definition, and references with their intensities for the attribute. Extensive panel training is conducted with the references for each attribute until panelists can identify and scale each attribute consistently across products. An example of a ballot using a flavor and texture attributes for ground beef is presented in Fig. 15.5.

An alternative method using the same principles for the spectrum method can be used, called the Short-Version Spectrum Descriptive Method. In this method, key attributes may be evaluated by the panel and not the full scope of descriptive attributes present in the product. Muñoz et al. (1992) discuss the use of this method. This method uses less panel time and provides the ability of panelists to evaluate a greater number of samples per session. This method applies all the same principles that each attribute is defined, and references are presented for scaling and assistance in identifying the attributes.

15.5.2.4 Quantitative descriptive attribute analysis

This method was developed by the Tragon Corp. and is identified as the Tragon QDA method to provide stronger statistical evaluation of sensory data. Use of this method is discussed in Stone and Sidel (2004). Training as previously described is conducted with methods and terminology defined. Panelists are more free to score and identify terms for a product. Usually this method uses a panelist pool so that as products are changed, such as whole muscle steaks to poultry sausage, panelist may not be the same, but they have been similarly trained. Panel results are usually not discussed, and the panel leaders become more of a facilitator and does not influence the panelists to the same degree as in the Spectrum method. Data are presented in a spider web where a spoke for each

SAMPLE NUMBER	741	206	567
Flavor Aromatics			
Beef identity	_____	_____	_____
Cooked Beef Fat	_____	_____	_____
Serumy/Bloody	_____	_____	_____
Grainy/Cowy	_____	_____	_____
Cardboard	_____	_____	_____
Painty	_____	_____	_____
Fishy	_____	_____	_____
Liver	_____	_____	_____
Soured	_____	_____	_____
Browned/Burnt	_____	_____	_____
Sorghum	_____	_____	_____
Other (describe)	_____	_____	_____
Feeling Factors			
Metallic	_____	_____	_____
Astringent	_____	_____	_____
Basic Tastes			
Salt	_____	_____	_____
Sour	_____	_____	_____
Bitter	_____	_____	_____
Sweet	_____	_____	_____
Aftertastes			
Astringent	_____	_____	_____
Fat Mouthfeel	_____	_____	_____
Bitter	_____	_____	_____
Browned/Burnt	_____	_____	_____
Sour	_____	_____	_____
Sweet	_____	_____	_____
Other (describe)	_____	_____	_____
After Feeling Factors			
Lip burn	_____	_____	_____
Metallic	_____	_____	_____
Texture			
Springiness	_____	_____	_____
Hardness	_____	_____	_____
Sandy/Gritty	_____	_____	_____

FIG. 15.5 Example of a flavor and texture descriptive attribute ballot for ground beef.

attribute is represented. An example of this form of data reporting is shown in Fig. 15.6. As a visual, differences in flavor are easy to explain.

Regardless of method used, descriptive analyses provide tools for describing differences and the magnitude of those differences in products. These methods use trained panelists, and depending on method may involve differing levels of training and panel leader interaction.

15.5.3 Consumer analysis

Consumer sensory evaluations are methods that determine consumers' perceptions of preference or acceptance, also defined as liking. Consumer techniques include qualitative and quantitative methods. While qualitative methods are

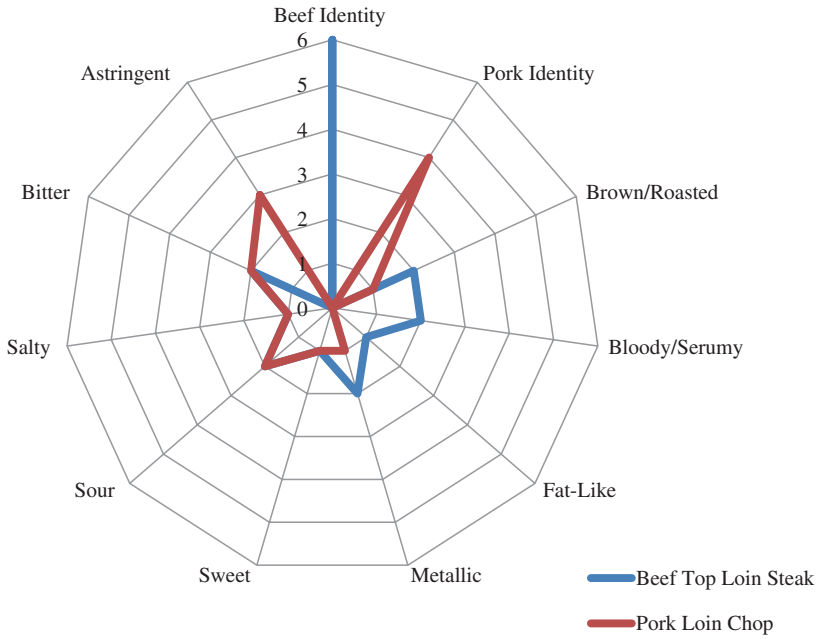

FIG. 15.6 Spider graph showing flavor attributes of a beef top loin steak and a pork loin chop.

valuable tools for meat scientists, they are not quantitative and will not be discussed. For an understanding of qualitative consumer methods see Meilgaard et al. (2016). The most common quantitative consumer sensory methods used by meat scientists are central location tests (CLT) and home use tests (HUT). Both methods provide strong consumer results, but differences in the testing methods result in differences in interpretation of the results. More complete discussion of conducting consumer sensory evaluation can be found in AMSA (2015) and Meilgaard et al. (2016).

Selection of consumers, defined as respondents, is a key to conducting strong consumer evaluation, regardless of methods. It is recognized that a minimum of 50 consumers, but ideally 80–100 consumers, are needed in one location for valid consumer sensory evaluation. Consumer sensory data are more variable than discriminative and descriptive sensory evaluation data so larger numbers of respondents are needed. Consumer recruitment parameters should be defined. Examples of parameters to potentially consider are usage level of the product, age, geographical location, sex, ethnicity, income, product purchase decision maker, and product-specific issues. Product usage is commonly the most important factor in selecting consumers. For example, Glascock (2014) used consumers that ate beef 3 or more times per week to understand factors that affected heavy beef eaters' perceptions of beef flavor. However, Luckemeyer (2015) selected consumers who ate beef 1 to 2 times per week, or light beef eaters, to

understand factors that affected perceptions of beef flavor for consumers who were light users of the product. Laird (2015) selected consumer on age and beef usage level to understand how millennials and nonmillennials that were either light (eat beef 1–2 times per month) or heavy (eat beef 3 or more times per week), and beef eaters were affected by beef that differed in flavor. While the three studies found similar trends, the results were slightly different due to the consumer population defined for use in the study and based on the study objectives. Demographic information on sex, age, income, usage level, and other criteria should be obtained for consumer used in studies to ascertain the effect of the consumer population demographics on results of the study.

Consumer recruitment can occur through many different methods. The method of recruitment should provide a random sampling of the population of interest and not provide bias toward the selection of specific populations unless desired. For example, to test light beef eaters, Luckemeyer (2015) selected consumers in Olathe KS, College Park PA, and Portland OR. These locations were selected to represent the east, west, and Midwest regions of the United States where beef is consumed. Each location randomly selected the consumers across their city either using an existing consumer data bank, random calling, or email solicitation of major groups. To obtain 80 consumers, over 20,000 consumers in one location were contacted. Luckemeyer (2015) could have conducted the study with students at Texas A&M University where light beef eaters would most likely be easier to find as many students have financial restraints. However, if consumers were only selected from a student population, the data would only infer back to students at Texas A&M University and not light beef eaters from across the United States. Once decisions on where to recruit consumers and the requirements of the study, a consumer screening questionnaire is needed. Meilgaard et al. (2016) provide examples of consumer screening questionnaires and what to included. The key in developing these questionnaires is to select for the important components of the study such as age, sex, and usage level of the product.

15.5.3.1 Central location tests (CLT)

Consumer CLT are conducted in a central location where consumers travel to participate in the study. Sessions are usually up to 1 h long, longer sessions can be conducted as long as consumers are aware of the time requirements and are provided sufficient time between samples and are given rest periods. The location of the test should be easily accessible, have adequate parking, provide environmental controls as previously discussed, and be comfortable for consumers. Sensory booths can be used or a room where tables or desks are set up for consumers. It is important to have a moderator in the room where the testing is being conducted to assist panelists in their evaluation and to answer any questions. Panelists are asked to not communicate during the evaluation and to proceed through the testing protocol as defined. Samples are prepared and presented to consumers assuring that product and environment controls are in

place. Consumers should not have information about the study samples. In CLT, consumers are in an artificial environment, and results may differ from HUT tests as the effect of handling, cooking, and any family member opinions are not influencing the sensory verdict of the consumer.

15.5.3.2 Home use tests (HUT)

Home use tests are where consumers are selected, and they test the product in their home. Samples are provided in standard packaging materials and identified with unique codes. Ballots and instructions are provided with the product, and consumers usually mail their completed ballot. All samples or partial or single samples may be provided for evaluation. In HUT tests, consumers prepare the product, so information on how consumers handle, cook, and what recipes are used can be ascertained. Additionally, the influence of handling the product, cooking it, smelling it cook, serving it, and eating it are components of the evaluation. The family opinion most likely influences the sensory perception for those with more than one individual living in the home. Data from HUT studies are the most variable and are considered the most real life evaluations. However, it is assumed that consumers followed directions and completed the study as requested.

15.5.3.3 Ballot development and use of scales

Regardless of consumer testing method, construction of a ballot that will adequately provide sensory input from the consumer is critical to successfully conducting consumer sensory evaluation. A standard consumer sensory ballot is presented in Figs. 15.7 and 15.8. A well-constructed ballot is important in consumer testing as the ballot is the sensory instrument that consumers will be using to record their sensory response. The ballot should include demographic questions and directions. Examples of demographic questions are given in Fig. 15.7, and the use of demographics to recruit consumers was previously discussed. The ballot should be easy to read, actionable, be consistent in structure and scales, and provide enough points of determination to measure responses but not too many points so that consistent use of the scale by consumers across products is compromised. Meilgaard et al. (2016) provides extensive discussion of these issues and ballot development. The ballot in Fig. 15.8 is recognized as containing the most commonly used questions and scales for consumer testing, regardless of method. Questions that are product specific can be added and consumers can be asked to select the product that they preferred after evaluation of more than one product. If consumers are asked to select products for preference when they have been served over 4 products, there will be strong order biases toward the last product evaluated.

The ballot presented in Fig. 15.8 uses 9-point hedonic liking questions and scales (questions 1–4), a 5-point just about right (JAR) question and scale (question 5), and a 9-point intensity scale (question 6). The scales are end and/or middle anchored and are presented as category scales. Line scales similarly

Please circle each appropriate response.

1. Please indicate your gender.
   Male Female
2. Which of the following best describes your age?
   20 years or younger 46 - 55 years
   21 - 25 years 56 - 65 years
   26 - 35 years 66 years and older
   36 - 45 years
3. Please specify your ethnicity.
   African-American Latino or Hispanic
   Asian/Pacific Islanders Native American
   Caucasian (non-Hispanic) Other
4. Which of the following best describes your household income?
   Below $25,000 $75,000 - $99,999
   $25,001 - $49,999 $100,000 or more
   $50,000 - $74,999
5. How many people live in your household including yourself?
   1 2 3 4 5 6 or more
6. Please indicate your employment level.
   Not employed Part-time Full-time
7. How many times a week total do you consume the following protein sources?

Beef	0	1-2	3-4	5-6	7 or more
Pork	0	1-2	3-4	5-6	7 or more
Lamb	0	1-2	3-4	5-6	7 or more
Chicken	0	1-2	3-4	5-6	7 or more
Fish	0	1-2	3-4	5-6	7 or more
Soy Based Products	0	1-2	3-4	5-6	7 or more

FIG. 15.7 Example of demographic and consumer attitude questions for meat products to be included on a consumer sensory ballot.

1. How much do you like or dislike this meat **OVERALL**?
   ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
   Dislike Extremely Neither Like or Dislike Like Extremely
2. How much do you like or dislike of the **OVERALL FLAVOR** of this meat?
   ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
   Dislike Extremely Like or Dislike Neither Extremely Like
3. How much do you like or dislike of the **JUICINESS** of this meat?
   ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
   Dislike Extremely Neither Like or Dislike Like Extremely
4. How much do you like or dislike of the **TENDERNESS** of this meat?
   ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
   Dislike Extremely Neither Like or Dislike Like Extremely
5. Please indicate your opinion on the **TENDERNESS** of this meat?
   ☐ ☐ ☐ ☐ ☐
   Too Tough Just Right Too Tender
6. Please indicate the intensity of the **TENDERNESS** of this meat?
   ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐
   Extremely Tender Extremely Tough

FIG. 15.8 Example of a consumer ballot for meat products.

marked can be used. Line scales are considered to have more uniform spacing across increasing or decreasing levels within the scale as consumers tend to not use the ends of category scales. Also, consumers may not perceive uniform and consistent changes in intensity from box to box for the category scales presented in Fig. 15.8. Numbers can be replaced by the boxes presented in Fig. 15.8 to reduce this effect. It is important to select a scale and uniformly use it across the ballot. By including the JAR question, penalty analysis can be conducted as discussed in AMSA (2015).

Consumer sensory evaluation provides opportunity to understand consumers' perceptions of meat products. Careful consideration for selection of consumers, ballot development, and sensory method are needed for reliable consumer results. Meat scientists began using more consumer evaluation in the scientific literature in the 1990s, and understanding the relationships between pre- and post-harvest factors that affect consumer perceptions has strengthened. These results have provided valuable direction for industry, government, and academic meat scientists as they address how to improve consumer satisfaction.

15.6 Emerging or underutilized sensory techniques

Some of the newest trends in sensory research are understanding the relationship between emotions and sensory responses. As the brain receives signals from the olfactory bulb that is close to the emotional center of the brain, many aromas and smells are associated with emotional responses. Additionally, other sensory characteristics, such as sight, touch, taste and sound, are also associated with emotion responses. For example, if you have a negative experience or illness associated with peppermint, an individual may not be able to tolerate peppermint flavored products without a negative response. The putrid odor from the production of sulfur-based compounds by microorganisms during meat spoilage is associated with negative flavor of meat and many times other flavor attributes, even if present will not be perceived. There are also positive emotions associated with meat. The smell of grilled meat or positive smoke aromas often evolve salivation and hunger when smelled. Methods to monitor brain signals when exposed to either positive or negative aspects of meat in conjunction with perception of sensory attributes is an emerging area of meat science research.

Sensory scientists commonly use multivariate statistical techniques to help understand sensory relationships. In many of the aforementioned sensory techniques, especially the descriptive analysis tools, panelists are trained to take an initial sensory response that is multivariate or has multiple factors, and to dissect the response into individual, univariate responses. Meat scientists analyze the univariate response and determine if differences are reported. While this information is invaluable, the use of multivariate techniques provides a second step to understand relationships between variables and in some cases what factors are major drivers of positive attributes of a meat product. Meilgaard et al. (2016) provide discussion of major techniques as well as AMSA (2015).

However, principal components analysis and partial least squares regression are easily understood tools that can be used to show these relationships. Data from both of these methods are presented in biplots that show interrelationships on two axes. Fig. 15.9 shows a partial least squares regression biplot adapted from Laird (2015), where millennial and nonmillennial consumers were asked to evaluate beef bottom round roasts, beef top loin steaks, pork loin chops, and chicken breasts in a HUT test in four cities. Trained descriptive attributes flavor and texture analysis was conducted with an expert trained panel. Attributes that cluster or segment into one of the four quadrants in close proximity are related. Using the biplot in Fig. 15.9, consumer attributes that are closely related to overall consumer liking can be understood. Cooked appearance was most closely associated with overall liking or if the consumers liked the cooked appearance, they liked the product. Relationships between cuts and trained and consumer attributes can also be ascertained. For example, beef bottom round roasts were closely associated with liver-like, cardboard, sour, spoiled putrid, and warmed-over flavor attributes. Whereas pork chops were closely associated with pork identity and nutty flavors, and chicken was closely associated with chicken identity, burnt, and tenderness characteristics. Positive and negative descriptive flavor attributes associated with consumer liking or disliking

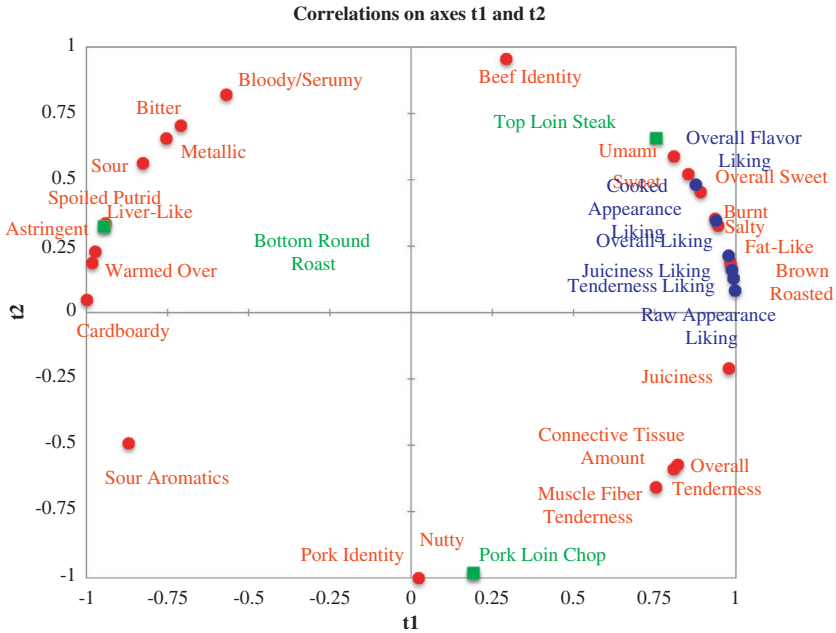

FIG. 15.9 Example of a partial least squares regression biplot ($R^2 = 0.97$) where relationships between consumer sensory attributes *(in blue)*, meat source *(in green)*, and trained descriptive sensory attributes *(in red)*. *(Modified from Laird, H.L., 2015. Millennial's Perception of Beef Flavor (MS thesis). Texas A&M University, College Station, TX.)*

can also be interpreted. Sour aromatics, metallic, bloody/serumy, warmed-over, liver-like, cardboard, sour, and spoiled putrid were negative consumer attributes and brown/roasted, salt, fat-like, umami, sweet, juiciness, and tenderness were positive consumer attributes. These techniques can provide further insight on relationships between sensory methods and ultimately consumer perceptions and preferences.

In understanding segmentation of consumers and their preferences, multivariate statistical tools are available. Tools that categorize consumers by similar characteristics can be useful in understanding how and why consumers respond differently to the same product. Use of agglomerative hierarchical cluster and k-means analysis can be useful tools to segment consumers into groups based on similar responses. The group can then be used as a treatment to understand differences between consumers.

Consumer responses are complex and when up to 95% of consumer attitudes are subconscious, the use of evolving emotional response tools, traditional univariate statistical techniques, and multivariate techniques provide opportunities to understand complex consumer relationships.

15.7 Conclusions

Sensory evaluation of meat can be complex. There is a wide variety of tools available and variation within each tool on how sensory responses are evoked and measured that effect the interpretation and validity of the results. Identifying the objective of the study and selecting the appropriate sensory tool are imperative to successful sensory evaluation. New sensory tools are emerging or have been underutilized that can provide additional insight into sensory properties of meat across the wide range of products meat scientist work with.

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Chapter 16

Meat safety—I Foodborne pathogens and other biological issues

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16.1 Introduction

The safety of meat and meat products, which is delineated by a series of challenges associated with either microbial pathogens or other (biological or not) issues, has been regarded as an ongoing public health concern. Various events have been identified as potential explanations for the rising meat safety concerns of recent years including changes in animal production, product processing and distribution, globalization and increased international trade, increase of human population and urbanization, the per capita income, increased worldwide consumption of animal products including meat, changing consumer needs and consumption trends and dietetic preferences (e.g., preference for minimally processed foods, high-protein diet, etc.), higher numbers of consumers at risk for infection, and increased interest, awareness, and scrutiny by consumers (Dhama et al., 2013; Sofos, 2008).

Although various nonbiological concerns have been, and are expected to continue to be, related to meat safety, such as food additives, chemical residues, and genetically modified organisms, microbial pathogens are traditionally associated with the most serious meat safety issues in terms of both foodborne illness and product recalls (Heredia and García, 2018; Sofos, 2008). Indeed, well-identified causes of concern with regard to the safety of fresh meat and poultry are enteropathogenic bacteria, such as Shiga toxin-producing *Escherichia coli* (STEC) and nontyphoidal serotypes of *Salmonella enterica*, whose primary reservoirs are food-producing animals (Heredia and García, 2018; Omer et al., 2018; Rhoades et al., 2009; Sodagari et al., 2020). On the other hand, the pathogenic bacterium *Listeria monocytogenes* has been regarded as the pathogen of concern in ready-to-eat (RTE) meat and poultry products exposed to postprocessing contamination

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and supporting the organism's growth during storage (USFDA/USDA-FSIS, 2003). Nevertheless, additional bacterial species, such as *Campylobacter* spp., *Clostridium* spp., and *Yersinia enterocolitica*, may also constitute meat safety concerns, while viral pathogens, parasites, and other biological issues, such as prions and biogenic amines, should also be taken into consideration (Alban et al., 2020; Guo et al., 2016; Heredia and García, 2018; Jairath et al., 2015; Markantonis et al., 2018; Nastasijevic et al., 2020; Omer et al., 2018; Sher et al., 2021).

This chapter describes the main characteristics, epidemiology, and transmission routes to humans of foodborne pathogens pertinent to meat safety, with a particular emphasis being placed on bacterial pathogens. Other biological issues as well as current and emerging challenges to meat safety also are discussed.

16.2 Biological meatborne hazards: Prevalence, transmission, and foodborne disease surveillance

Although the zoonotic potential of foodborne pathogens has been well established, the prevalence of biological hazards in food-producing animals and their transmission in the meat production and supply chain have been reported to be highly variable in different countries around the world. Beyond the anticipated effect of distinct livestock farming practices, parameters that have been identified as also contributing to the heterogeneity observed among herds and/or individual animals include geographical location, season of the year, as well as the applied detection methodologies (Brashears and Chaves, 2017; Guo et al., 2016). A similarly extensive prevalence variability has also been recorded in subsequent (to farming) segments of the meat supply chain (i.e., processing, distribution, and retail display), as demonstrated by systematic literature review and metadata analysis approaches (Golden and Mishra, 2020; Liu et al., 2020).

Despite its undoubtful value in the identification and prioritization of appropriate food safety interventions, attribution of foodborne illness to specific food-producing animals and related food commodities is not an easy task, being frequently hindered by methodologic limitations and gaps in the available data (Martínez-Avilés et al., 2019; Omer et al., 2018). The situation is even more difficult when the global burden of foodborne disease outbreaks is to be estimated since comparing global outbreaks maybe rather challenging (Omer et al., 2018). Various approaches and data are used for the purpose of food attribution including, among others, analysis of outbreak data, case-control studies, microbial subtyping, and source tracking methods, as well as expert judgment when outbreak data are lacking, sparse, or highly uncertain (Batz et al., 2005). Although reports of outbreak investigations are considered as providing the most comprehensive data for determining the foods responsible for illnesses, pathogens that rarely cause outbreaks may be considerably underrepresented. Such limitation can be overcome by the information provided by case-control studies, which are of particular value for assessing foodborne attribution of sporadic illness. It has been generally agreed that, since none of the current data sources is likely to be sufficient on its own, the accurate and dependable attribution of foodborne

illnesses to specific foods can only be attained through cooperation among food safety institutions and the development of a comprehensive program that combines all the aforementioned approaches (Batz et al., 2005).

In 2019, a total of 5175 foodborne outbreaks were reported in the European Union (EU) by 27 Member States, with only 716 of them, however, being regarded as "strong-evidence" outbreaks. In these strong-evidence outbreaks, a total of 13,686 cases were involved, with the latter being associated with 1567 hospitalizations (11.5%) and 38 fatalities (0.3%). With the causative agent being identified in approximately 60% of the total outbreaks, bacteria were reported to have caused most outbreaks (26.4%) followed by bacterial toxins (19.3%), viruses (10.7%), other causative agents (3.0%), and parasites (0.6%). The most commonly detected causative agents in strong-evidence outbreaks were *Salmonella* and norovirus (and other calicivirus). The highest proportion of hospitalizations and deaths were observed for outbreaks caused by bacteria, with *Salmonella* being responsible for the highest number of hospitalizations and *L. monocytogenes*, alone, causing more than half of the fatal illnesses. The majority of the strong-evidence foodborne outbreaks and illnesses reported in 2019 in the EU were associated with the consumption of foods of animal origin. Specifically, meat and meat products accounted for an important proportion of strong-evidence outbreaks, namely 21.1%, with the categories of "meat and meat products, unspecified," "pig meat," "poultry meat," "bovine meat," "sheep meat," and "other or mixed red meat and products thereof" being associated with 5.7%, 5.9%, 5.3%, 2.0%, 0.3%, and 2.0% of the total strong-evidence outbreaks, respectively (EFSA-ECDC, 2021a).

It has been estimated that more than 9 million cases of foodborne illness, caused by major pathogens, are acquired annually in the United States (Scallan et al., 2011). Using data from outbreak-associated illnesses for 1998–2008 (i.e., 4589 outbreaks with an implicated food vehicle and a single etiologic agent), it was estimated that a total of 120,321 outbreak-associated illnesses were caused by 36 agents in this time period (Painter et al., 2013). Norovirus was identified as the etiologic agent of most outbreaks and outbreak-associated illnesses, with the majority of viral illnesses, in general, being attributed to the consumption of leafy vegetables, fruits-nuts, and dairy products. On the other hand, most bacterial illnesses were attributed to dairy (18%), poultry (18%), and beef (13%) commodities. Overall, meat-poultry commodities (beef, game, pork, and poultry) were estimated to account for 22% of illnesses and 29% of deaths. More specifically, poultry was linked to more deaths (19%) than any other food commodity, with most of these fatalities being associated with the bacterial pathogens *L. monocytogenes* and *Salmonella* (Painter et al., 2013).

As demonstrated by the information provided above, foodborne illness attribution to specific food categories may be considerably different in different countries reflecting, most likely, corresponding societal and cultural differences. Indicative outbreaks of foodborne disease, occurring during the last decade and being associated with meat and meat products, are presented in Table 16.1. Specifically for European countries, significant outbreaks that occurred in the last decade have been recently reviewed by Sarno et al. (2021).

TABLE 16.1 Selected foodborne disease outbreaks associated with meat/poultry and meat/poultry products.

Year	Country	Pathogen	Food[a]	Reference/source
2010	France	Monophasic *Salmonella typhimurium* 4,5,12:i:-	Beef	Raguenaud et al. (2012)
2010	Denmark	*Salmonella typhimurium*	Salami	Kuhn et al. (2011)
2010	United States	*Escherichia coli* O157:H7	Beef	CDC[b]
2010–12	England	*Listeria monocytogenes*	Pork pies	Awofisayo-Okuyelu et al. (2016)
2011	United States	*Salmonella* Heidelberg	Ground turkey	CDC[b]
2011	Switzerland	*L. monocytogenes*	Cooked ham	Hächler et al. (2013)
2011–13	EU (multicountry)	*Salmonella* Stanley	Turkey meat	Kinross et al. (2014)
2012	United States	*Salmonella enteritidis*	Ground beef	CDC[b]
2012	Norway	*Clostridium perfringens*	Beef stew	Wahl et al. (2013)
2013	United States	*Salmonella typhimurium*	Ground beef	CDC[b]
2014	United States	*Salmonella* Heidelberg	Chicken	CDC[b]
2014	Belgium	*Trichinella spiralis*	Wild boar meat	Messiaen et al. (2016)
2015	United States	Multidrug-resistant *Salmonella* I 4,[5],12:i:- and *Salmonella infantis*	Pork	CDC[b]
2015	United States	Drug-resistant *Salmonella enteritidis*	Raw, frozen, stuffed chicken entrees	CDC[b]
2015	United States	*E. coli* O157:H7	Rotisserie chicken salad	CDC[b]
2016	United States	*E. coli* O157:H7	Beef products	CDC[b]
2016	Italy	*L. monocytogenes*	Sliced beef ham	Maurella et al. (2018)
2016–17	Germany	Sorbitol-fermenting *E. coli* O157:H-	Packaged minced meat	Vygen-Bonnet et al. (2017)

2016–17	United Kingdom	*L. monocytogenes*	Cooked chicken	McLauchlin et al. (2020)
2016–17	The Netherlands	*Salmonella bovismorbificans*	Uncooked ham products	Brandwagt et al. (2018)
2018	United States	*L. monocytogenes*	Pork products	CDC[b]
2018	United States	Multidrug-resistant *Salmonella infantis*	Raw chicken products	CDC[b]
2018	United States	*Salmonella* Newport	Ground beef	CDC[b]
2018	United States	*Salmonella* I 4,[5],12:i:-	Kosher chicken products	CDC[b]
2018	United States	*Salmonella* Reading	Turkey products	CDC[b]
2018	United States	*Salmonella typhimurium*	Chicken salad	CDC[b]
2018	Austria	*L. monocytogenes*	Liver pâté (likely source)	Cabal et al. (2019)
2018–20	EU/EEA[c], United Kingdom (multicountry)	*Salmonella enteritidis*	Poultry products	EFSA-ECDC (2021b)
2019	United States	*Salmonella* Dublin	Ground beef	CDC[b]
2019	United States	*E. coli* O103 and O121	Ground bison	CDC[b]
2019	United States	*L. monocytogenes*	Deli-sliced meats	CDC[b]
2019	United States	*E. coli* O103	Ground beef	CDC[b]
2019	Denmark	*Campylobacter* spp.	Chicken meat	Joensen et al. (2021)
2020	United States	*L. monocytogenes*	Deli meats	CDC[b]
2021	United States	*L. monocytogenes*	Frozen, fully cooked chicken products	CDC[b]
2021	United States	*Salmonella enteritidis*	Raw frozen breaded stuffed chicken products	CDC[b]
2021	United States	*Salmonella* Hadar	Ground turkey	CDC[b]

[a] *Food product implicated as the major outbreak vehicle.*
[b] *Source: United States Department of Health and Human Services, Centers for Disease Control and Prevention (www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html). Accessed July 11, 2021.*
[c] *European Union/European Economic Area.*

16.3 Meatborne bacterial pathogens and toxins

16.3.1 *Campylobacter* spp.

Campylobacter species, previously classified as *Vibrio* spp., are zoonotic organisms initially associated only with animal pathologies (i.e., spontaneous abortions in cattle and sheep), with their recognition as a causative agent of human illness being placed in the late 1970s. The genus *Campylobacter* is a member of the Campylobacteriaceae family and consists of 14 species, several of which are considered pathogenic to humans including *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* (de Blackburn and McClure, 2009). Although *Campylobacter jejuni* and *Campylobacter coli* are clinically indistinguishable, the former species has been identified as the one of primary importance with regard to human foodborne disease, being responsible for 80%–90% of campylobacteriosis cases (Jay, 2000).

Campylobacters are Gram-negative, nonspore forming organisms with curved or spiral-shaped cells that exhibit a characteristic rapid, darting, reciprocating motility (known as "corkscrew-like" motion) provided by a single polar flagellum being present at one or both ends of the cell. Although in some cases, growth may occur under aerobic or anaerobic conditions, *Campylobacter* spp. are, in general, microaerophilic (require small amounts of oxygen, i.e., 3%–6%) with their growth being inhibited in the presence of 21% oxygen (Jay, 2000). With the exception of the thermotolerant *Campylobacter* species (i.e., *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, *Campylobacter upsaliensis*) that grow optimally at 42°C, most of campylobacters have an optimum growth temperature ranging from 30°C to 37°C (Cox et al., 2010). Furthermore, these organisms are generally regarded as susceptible to acidic environments, drying and freezing, while their decimal reduction time (*D*-value) has been estimated to range from 1 to 6.6 min at 55°C, depending on the heating medium (de Blackburn and McClure, 2009). Under sublethal adverse environmental conditions, it has been assumed that certain species, including *Campylobacter jejuni* and *Campylobacter coli*, are capable of entering into a viable but nonculturable (VBNC) state, in which bacterial cells, although metabolically and respiratorily active, cannot be resuscitated using conventional culturing techniques (Rollins and Colwell, 1986). The VBNC state, whose significance in human infection and illness remains to be clarified, is considered to be recovered upon entrance of the pathogenic species to a susceptible host (Richardson et al., 2007). Hence, the existence of this state should be certainly taken into consideration when studying the pathogenicity of these organisms and investigating the epidemiology of animal and human campylobacteriosis.

The four abovementioned *Campylobacter* species are naturally present in the gastrointestinal tract of both domesticated and wild warm-blooded animals, with animal feces being regarded as the primary source of contamination of the environment and foods with these organisms (de Blackburn and McClure, 2009). Indeed, high incidence rates of *Campylobacter jejuni* and *Campylobacter coli*

have been reported in fecal samples from all important food-producing animals including cattle, swine, and poultry, with the latter, however, being prominent (Haruna et al., 2013). *Campylobacter jejuni* appears to predominate in cattle, broiler chickens, and turkeys, while *Campylobacter coli* is more commonly associated with pigs (de Blackburn and McClure, 2009). Although contamination with *Campylobacter* spp. can occur throughout the food production chain, carcass contamination at slaughter is regarded as one of the most important routes of meat contamination with these organisms (Ivanova et al., 2014).

Despite the fact that its epidemiology is not well understood, campylobacteriosis has been recognized as a leading cause of bacterial foodborne illness worldwide, with its incidence and prevalence appearing to increase in both developed and developing countries over the last decade (Kaakoush et al., 2015). According to the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), campylobacteriosis has been the most commonly reported gastrointestinal infection in humans since 2005. At EU level, a significant increasing trend for campylobacteriosis in humans was observed over the 7-year period 2008–14 (EFSA-ECDC, 2015), while a stable (flat) trend was recorded during 2015–19. Specifically in 2019, the number of confirmed cases of human campylobacteriosis was 220,682 corresponding to an EU notification rate of 59.7 per 100,000 population (EFSA-ECDC, 2021a). According to the same epidemiological data, *Campylobacter* was the third most frequently reported causative agent of foodborne outbreaks at EU level (by 18 MS), with 319 outbreaks reported to the European Food Safety Authority (EFSA), involving 1254 cases, 125 hospitalizations, and no deaths (EFSA-ECDC, 2021a). Similar trends with regard to foodborne campylobacteriosis have also been reported in the United States (CDC, 2020; Sher et al., 2021). Based on preliminary surveillance data, the 2019 incidence of campylobacteriosis in the United States was 19.5 cases per 100,000 population, significantly increased (by 13%) compared with the years 2016–18 (CDC, 2020). In addition to the alarming increase of the infection's incidence in Europe, North America, and Australia, epidemiological data from parts of Africa, Asia, and the Middle East indicate that campylobacteriosis is endemic in these areas, particularly in children (Kaakoush et al., 2015). Given their relatively sensitive nature, the high association of campylobacters with human illness has been attributed, in addition to their widespread prevalence in meat animals, to their virulence as well as to the great genetic diversity characterizing *Campylobacter* isolates (de Blackburn and McClure, 2009; Zhong et al., 2016). Important virulence factors that have been identified and refer almost exclusively to *Campylobacter jejuni*, include motility, translocation ability, chemotaxis, and toxin production, with the latter referring to the synthesis of either enterotoxins or cytotoxins (Cox et al., 2010).

Campylobacteriosis in humans is usually manifested as an acute, self-limiting enterocolitis that is often preceded by fever, headache, myalgia, and malaise, while other common symptoms include abdominal pain, cramps, and diarrhea (inflammatory or noninflammatory). The infectious dose is considered

to be relatively low (i.e., a few hundred cells), the incubation period is usually 24–72 h after ingestion of the organism (although it may extend up to 7 days), and despite the severity of the infection, the mortality rate is low (de Blackburn and McClure, 2009). Rare complications include reactive arthritis and bacteraemia (Hannu et al., 2002), while infection with *Campylobacter jejuni* is also associated with Guillain-Barré syndrome, an autoimmune peripheral neuropathy that can be fatal (Winer, 2001). Most of the reported campylobacteriosis cases are sporadic, and foodborne infections have been associated either with the consumption of foods of animal origin that are raw or undercooked or with the consumption of foods that are re-contaminated after cooking (de Blackburn and McClure, 2009). Broiler meat has been identified as the predominant source of infection, particularly with regard to the strong-evidence campylobacteriosis foodborne outbreaks (EFSA-ECDC, 2021a; Kaakoush et al., 2015; Nastasijevic et al., 2020). Other risk factors include contact with animals and international travel (Kaakoush et al., 2015), while cross-contamination in the domestic environment has long been identified as a major risk factor resulting in numerous sporadic cases of campylobacteriosis (Bloomfield et al., 2012).

16.3.2 *Clostridium* spp.

The genus *Clostridium* includes Gram-positive, rod-shaped, endospore-forming anaerobic bacteria. Although most clostridia are saprophytes, four species have been identified as human pathogens, namely *Clostridium perfringens*, *Clostridium botulinum*, *Clostridium Difficile*, and *Clostridium tetani*. Among these species, *Clostridium perfringens* and *Clostridium botulinum* are well-established foodborne pathogens, whereas *Clostridioides difficile* (formerly *Clostridium difficile*) has been identified as an emerging human pathogen with an important foodborne transmission potential (Barbosa et al., 2020). As reported by 13 member states, a total of 160 foodborne outbreaks caused by *Clostridium perfringens* (124 outbreaks), *Clostridium botulinum* (9 outbreaks), or unspecified clostridia (27 outbreaks) occurred in the EU in 2014 (EFSA-ECDC, 2015). In 2019, outbreaks caused by bacterial toxins represented an important proportion of all foodborne disease outbreaks reported in the EU (i.e., 19.3% of all outbreaks), albeit most of them were mostly classified as weak-evidence outbreaks. Specifically, six deaths were caused by both *Clostridium perfringens* and other undefined bacterial toxins, while *Clostridium botulinum* was involved in seven outbreaks, 17 cases and 15 hospitalizations and was responsible for one death. Moreover, the pathogen-food vehicle combination of *Clostridium perfringens*-meat/meat products was among the top 10 pairs causing the highest number of strong-evidence outbreaks (as well as the highest number of cases in strong-evidence outbreaks) in reporting EU member states in 2019: a total of 19 strong-evidence outbreaks (and 589 cases) were reported by eight member states, presenting a fairly stable trend as compared to the period 2010–18 for which a mean number of outbreaks/year of 18.4 was estimated (EFSA-ECDC, 2021a).

The first demonstration of the association of *Clostridium perfringens* with food poisoning was made in the 1940s during the investigation of outbreaks linked to the consumption of chicken (McClung, 1945). Based on their ability to form certain exotoxins, five types of *Clostridium perfringens* have been recognized (types A to E), with types A, C, and D being pathogenic to humans and types B, C, D, E, and maybe type A affecting animals. Nonetheless, the strains causing foodborne illness belong mainly to type A and to a lesser extent to type C and are capable of producing an enterotoxin, which is distinct from the exotoxins and constitutes the causative factor of food poisoning by this organism (Jay, 2000; Juneja et al., 2010). *Clostridium perfringens* type A strains are predominantly involved in foodborne toxicoinfection (or toxin-mediated infection) which is caused by a heat-labile enterotoxin produced by ingested cells during sporulation in the intestine. Food poisoning usually results from the ingestion of a high concentration ($>10^6$) of viable vegetative cells of *Clostridium perfringens*, which are usually encountered in temperature abused foods. Indeed, foodborne outbreaks associated with this organism have been most commonly associated with improper handling and preparation of foods at domestic, retail, and food service settings (Juneja et al., 2010). *Clostridium perfringens* is widely distributed in the environment, being found in soil, dust, as well as in the gastrointestinal tract of animals and humans. Although a mesophile, with its optimum growth temperature being between 37°C and 45°C, the organism can grow in the temperature range of 15–50°C, and despite its anaerobic character, it is quite aerotolerant (Jay, 2000; Juneja et al., 2010). With regard to its incidence in foods, *Clostridium perfringens* is frequently found in meats and meat products (beef, veal, lamb, pork, and chicken products) through (i) fecal contamination of carcasses, (ii) contamination from other ingredients such as spices, or (iii) postprocessing contamination (EFSA-ECDC, 2015, 2021a; Juneja et al., 2010). In this context, *Clostridium perfringens* outbreaks have often been associated with the consumption of meat dishes. An important risk factor with regard to food poisoning from this organism is cooling and rewarming foods; when the applied heat treatment is inadequate to destroy the heat-resistant endospores of this organism, cooling and rewarming are expected to favor their germination and growth (Jay, 2000). Symptoms of foodborne illness caused by *Clostridium perfringens* include acute abdominal pain and diarrhea, while the pathogens' enterotoxin has been assumed to also play a role in the etiology of the sudden infant death syndrome (Lindsay et al., 1993).

The foodborne illness caused by *Clostridium botulinum*, known as botulism, is a rare but severe neuroparalytic intoxication, resulting from the ingestion of highly toxic, soluble exotoxins produced by the organism during its growth in foods. *Clostridium botulinum* is a rather heterogeneous species, consisting of four physiologically and genetically distinct bacterial groups (I to IV), while seven different serological types of synthesized neurotoxins (types A to G) have been recognized: group I (proteolytic, producing neurotoxins A, B, and F), group II (nonproteolytic, producing neurotoxins B, E, and F), group III (producing

neurotoxins C and D), and group IV (producing neurotoxin G). The botulinum neurotoxins, which are the most potent substances known, are heat-labile proteins that can, however, survive freezing. Neurotoxin types A, B, E, and occasionally F are the ones associated with botulism in humans (Peck, 2010). Botulism was first described in the 18th and 19th century in Central Europe as a disease associated with the consumption of blood sausage, characterized by muscle paralysis, breathing difficulties, and a high mortality rate. Outbreaks occurring in the 20th century were commonly associated with commercial and home canning processes. Since then, numerous outbreaks of botulism have been reported, with the majority of them being linked to the consumption of inadequately processed foods both at the domestic and commercial levels. Examples of meat and meat products that have been associated with foodborne illness caused by *Clostridium botulinum* (both proteolytic and nonproteolytic) include meat roll, commercial pork sausage, home-cured ham, and reheated chicken. More specifically, nonproteolytic *Clostridium botulinum* has been regarded as the major microbiological safety hazard in minimally heated refrigerated foods (e.g., cook-chill foods, sous-vide foods, and ready meals (Peck, 2010). Symptoms of botulism, whose mortality rate varies between 30% and 65%, may be developed after 12–72 h after ingestion of foods containing neurotoxins and include nausea, vomiting, fatigue, dizziness, headache, skin and mouth dryness, constipation, muscle paralysis, double vision, and finally respiratory failure and death (Jay, 2000). Infection and colonization of the gastrointestinal tract of infants by proteolytic *Clostridium botulinum* strains can lead to infant botulism. In contrast to what is the case in adult botulism (which involves the ingestion of already synthesized neurotoxins), in infant botulism viable spores of the organism are ingested and toxins are synthesized upon their germination in the gastrointestinal tract of susceptible infants. Infants less than 12 months of age are lacking a mature intestinal microflora capable of preventing colonization by *Clostridium botulinum* and as such are rather susceptible to infant botulism (Jay, 2000; Peck, 2010). Infant botulism, which has been proposed as a potential contributing factor to the sudden infant death syndrome (Fox et al., 2005), has been mainly associated with the consumption of honey (Aureli et al., 2002).

Clostridium difficile has been relatively recently identified as a human pathogen (Dawson et al., 2009). The organism may be present in the gastrointestinal tract of healthy adults and infants and is usually kept under control by the normal intestinal microflora (Warren and Guerrant, 2011). However, when the protective gut microflora is disrupted by certain antibiotics, indigenous, or ingested spores of *Clostridium difficile* germinate, multiply rapidly, colonize the gastrointestinal tract, and produce toxins (Dawson et al., 2009; Warren and Guerrant, 2011). Pathogenic strains of this organism produce two distinct toxins: (i) toxin A, an enterotoxin and (ii) toxin B, a cytotoxin. Colonization of the gut by *Clostridium difficile* and toxin production results in an acute inflammatory response and severe damage to the intestinal epithelium (Dawson et al., 2009). Since its initial recognition, the epidemiology of *Clostridium difficile*

has undergone some very important changes, which have resulted in its characterization as a "continually evolving pathogen"; such changes include the emergence of highly virulent strains causing outbreaks of disease of high severity and significant mortality, as well as the onset of community-acquired cases involving low-risk population groups (Gould and Limbago, 2010). Symptoms of *Clostridium difficile* infection may vary from mild diarrhea to life-threatening pseudomembranous colitis, with the population at high risk not being limited to patients on antimicrobial treatment, but also including patients on other therapies that may also alter the balance of the gut microbiota (e.g., antacid/proton pump inhibitors and nonsteroidal antiinflammatory), as well as the immunocompromised and the elderly (Dawson et al., 2009). The organism can be recovered from a wide variety of environmental sources including soil, seawater and fresh water, and food animals, with the latter being suggested as likely to play an important role in the transmission of this pathogen to humans through food (Gould and Limbago, 2010; Marcos et al., 2021; Redding et al., 2021). Indeed, there are several reports suggesting that food animals can be reservoirs for *Clostridium difficile* (Dawson et al., 2009; Thitaram et al., 2011), while a marked overlap between isolates from animals and humans has also been documented (Zudaric et al., 2008). With particular reference to meat, the organism could be either initially present in the muscle tissue or introduced via fecal contamination in carcasses at slaughter or in meat products during subsequent processing (Thitaram et al., 2011). The meatborne transmission potential of *Clostridium difficile* is strongly demonstrated by the fact that ribotypes identified as causative agents of human disease have been also isolated from retail meat products (Candel-Pérez et al., 2019). Given its increasing interest as a potentially foodborne pathogen, *Clostridium difficile* is expected to constitute a rather important objective of food safety research in the future. In this context, the development of standard methodologies for its detection, isolation, and further characterization and typing is vital for the accurate assessment of its prevalence in foods and its foodborne transmission dynamics (Barbosa et al., 2020; Candel-Pérez et al., 2019).

The main characteristics of the aforementioned *Clostridium* species and the foodborne disease caused by them are summarized in Table 16.2.

16.3.3 Enterohemorrhagic *Escherichia coli*

E. coli is a bacterial species of the Enterobacteriaceae family, consisting of Gram-negative, nonsporeforming rods, and including both pathogenic and nonpathogenic strains, with the latter constituting the majority of facultative microflora located in the gastrointestinal tract of most vertebrates (Ramos et al., 2020). With reference to pathogenic *E. coli*, there are six pathotypes associated with foodborne illness (Table 16.3): (i) verocytotoxigenic *E. coli* (VTEC), also known as Shiga toxin-producing *E. coli* (STEC) and including enterohemorrhagic *E. coli* (EHEC); (ii) enteropathogenic *E. coli* (EPEC); (iii) enterotoxigenic *E. coli*

TABLE 16.2 Characteristics of *Clostridium* species associated with foodborne disease.

Clostridium species	Toxins	Type of disease	Symptoms of disease
Clostridium perfringens Type A	Heat-labile enterotoxin	Toxiconfection	Acute abdominal pain and diarrhea, potential involvement of enterotoxin in the etiology of the sudden infant death syndrome
Clostridium botulinum	Neurotoxins	(i) Intoxication (ii) Toxicoinfection (infant botulism)	Nausea, vomiting, fatigue, dizziness, headache, skin and mouth dryness, constipation, muscle paralysis, double vision, respiratory failure, death
Group I (proteolytic *Clostridium botulinum*)	A, B, F		
Group II (nonproteolytic *Clostridium botulinum*)	B, E, F		
Group III	C, D		
Group IV	G		
Clostridium difficile	Enterotoxin (toxin A), cytotoxin (toxin B)	Toxicoinfection	Vary from mild diarrhea to life-threatening pseudomembranous colitis

Based on Dawson, L.F., Valiente, E., Wren, B.W., 2009. Clostridium difficile-a continually evolving and problematic pathogen. Infect. Genet. Evol. 9, 1410–1417; Juneja, V.K., Novak, J.S., Labre, R.J., 2010. Clostridium perfringens. In: Juneja, V.K., Sofos, J.N. (Eds.), Pathogens and Toxins in Foods: Challenges and Interventions. ASM Press, Washington, DC, pp. 53–70.; Peck, M.W., 2010. Clostridium botulinum. In: Juneja, V.K., Sofos, J.N. (Eds.), Pathogens and Toxins in Foods: Challenges and Interventions. ASM Press, Washington, DC, pp. 31–52.

TABLE 16.3 Characteristics of the main *Escherichia coli* pathotypes associated with foodborne disease.

Escherichia coli pathotype	Adhesion site (mediator)	Symptoms of disease	Acute clinical manifestations
VTEC (or STEC)	Large intestine (intimin)	Diarrhea, abdominal pain, vomiting, headache, fever	Hemorrhagic colitis (bloody diarrhea), hemolytic uremic syndrome (renal failure, thrombocytopenia, seizures, comma, death), thrombotic thrombocytopenic purpura (central nervous system disorders, gastrointestinal hemorrhage, blood clots in the brain, death)
EPEC	Small intestine (intimin)	Diarrhea (watery or bloody), fever, nausea, vomiting, abdominal pain	Severe diarrhea, chronic diarrhea, malnutrition
ETEC	Small intestine (fimbrial colonization factors)	Watery diarrhea, low-grade fever, abdominal cramps, malaise, nausea	Cholera-like extreme diarrhea
EAEC	Small and large intestine (fimbrial adhesins)	Diarrhea (watery or bloody), vomiting	Persistent childhood diarrhea, severe dehydration
EIEC	Large intestine (unclear)	Watery diarrhea, chills, fever, headache, muscular pains, abdominal cramps	Profuse diarrhea (dysentery)
DAEC	Gastrointestinal and urinary tract regions (fimbrial and nonfimbrial adhesins)	Diarrhea	Acute childhood diarrhea

Based on Beauchamp, C.S., Sofos, J.N., 2010. Diarrheagenic Escherichia coli. In: Juneja, V.K., Sofos, J.N. (Eds.), Pathogens and Toxins in Foods: Challenges and Interventions. ASM Press, Washington, pp. 71–94; Bell, C., Kyriakides, A., 2009. Pathogenic Escherichia coli. In: de Blackburn, C.W., McClure, P.J. (Eds.), Foodborne Pathogens: Hazards, Risk Analysis and Control, second ed. Woodhead Publishing Ltd., Cambridge, pp. 581–626.

(ETEC); (iv) enteroaggregative *E. coli* (EAEC); (v) enteroinvasive *E. coli* (EIEC); and (vi) diffuse adherent *E. coli* (DAEC). Among the different *E. coli* groups being responsible for gastrointestinal infections, EHEC, and particularly serotype O157, has been recognized as an etiological agent of serious illness and mortality in outbreaks of foodborne illness worldwide (Viazis and Diez-Gonzalez, 2011). In 1982, *E. coli* O157:H7 was first associated with epidemic foodborne disease linked to the consumption of improperly cooked hamburgers in the United States, and a new foodborne zoonosis was defined (Riley et al., 1983). Since then, more than 200 different O serogroups of *E. coli* have been shown to produce Shiga toxins, and more than 100 of these STEC have been associated with human disease (Johnson et al., 2006).

All diarrheagenic *E. coli* bacteria are mesophilic organisms, capable of growing at temperatures ranging from 7°C to 45°C with an optimum growth temperature in the range of 35–42°C. Under optimum temperature conditions, the pathogen is capable of initiating and supporting growth at pH values of 4–10 and in the presence of up to 8% NaCl. Furthermore, *E. coli* O157:H7 can withstand nutrient starvation as well as acid, thermal, and osmotic stresses, properties that allow for its prolonged persistence in various food-related environments (Beauchamp and Sofos, 2010).

The Shiga toxins (also known as Shiga-like toxins or verotoxins), which are similar to those produced by the bacterium *Shigella dysenteriae*, constitute the primary virulence factor of STEC, including *E. coli* O157:H7, and the main cause of hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (HUS) in humans (Viazis and Diez-Gonzalez, 2011), a serious and potentially fatal clinical condition. Patients with HUS, in their majority young children (< 5 years of age), exhibit acute renal failure, anemia, and thrombocytopenia, while seizure, stroke, herniated bowels, and/or chronic renal malfunction may also be associated with severe infections (Beauchamp and Sofos, 2010). In addition to *E. coli* O157:H7, which is detected and diagnosed based on its inability to ferment the carbohydrate sorbitol, sorbitol-fermenting STEC O157:H^- (with H^- indicating nonmotility) strains have also been identified as potential agents of severe human disease. It has been observed that patients infected with these strains tend to develop life-threatening HUS more frequently than patients infected with other EHEC strains, an observation that potentially implies a hypervirulence of these organisms (Nielsen et al., 2011).

Animal hosts shedding *E. coli* in the environment constitute that primary source of these bacteria, with the latter having been recovered from numerous environmental sites, such as ranches, livestock harvesting facilities, water sources, compost, sewage treatment effluent, and urban and rural soils (Beauchamp and Sofos, 2010). Given their widespread distribution in the environment, *E. coli* organisms can easily enter the food supply chain, with animal feces being the principal source of contamination of animal hides, water, and processing equipment in harvesting facilities. Carcasses of meat animals may become contaminated with organisms originating from animal feces or the gastrointestinal tract

during hide removal or evisceration, respectively, and then act as a secondary contamination (or cross-contamination) source for raw meat and meat products, inanimate objects, and workers (Rhoades et al., 2009). Nonetheless, contamination events may also take place at subsequent stages of the food supply chain, including the retail (e.g., during meat slicing, grounding, or repackaging) and domestic (inappropriate food handling practices) levels.

Outbreaks of foodborne EHEC infections have been traditionally associated with raw, undercooked, or RTE beef products (Riley et al., 1983; Tilden et al., 1996). Indeed, the presence of STEC has been confirmed in numerous raw and processed beef products, as well as in various fermented meat products (de Assis et al., 2021; Fayemi et al., 2021; Rhoades et al., 2009). The EU notification rate for STEC infections in 2014 was 2.2 cases per 100,000 population, with the EU/European Economic area trend increasing from 2015 to 2019 and with O157 being the most commonly reported serogroup (26.6% of cases with known serogroup) (EFSA-ECDC, 2021a). Nevertheless, an increasing number of foodborne outbreaks in several parts of the world has been attributed to non-O157 STEC strains (CDC, 2020; EFSA-ECDC, 2021a; Rhoades et al., 2009; Scallan et al., 2011). As demonstrated by preliminary data on the incidence and trends of foodborne infections in the United States, the incidence STEC infection in 2018 was 6.3 cases per 100, 000 population (CDC, 2020). Although non-O157 STEC are a rather heterogeneous group of organisms, consisting of more than 100 serogroups, 6 serogroups have emerged as significant etiological agents of human disease (including HUS): O26, O45, O103, O111, O121, and O145 (Koutsoumanis et al., 2014). Cattle is considered as the major reservoir of clinically significant non-O157 STEC, while meat products that have been linked to illness caused by these organisms are ground beef and fermented sausages (Mathusa et al., 2010). In response to their increasing public health significance, the aforementioned six serogroups of non-O157 STEC have been declared by the United States Department of Agriculture's Food Safety and Inspection Service as adulterants if present in raw beef products (USDA-FSIS, 2011). Finally, the emergence of new strains of multidrug-resistant foodborne bacteria, such as extended spectrum β-lactamase (ESBL)-producing *E. coli*, has been also acknowledged as a rather worrying issue with regard to the organism's evolution and the infection's clinical outcome (Ramos et al., 2020).

16.3.4 *Listeria monocytogenes*

In the last decade, there has been an increasing number of new species from diverse sources being identified as belonging to the genus *Listeria*. Actually, as of 2020, there were 20 recognized species representing the genus *Listeria* (Nwaiwu, 2020), whereas five novel species have been recently described (Carlin et al., 2021). Nonetheless, *L. monocytogenes* remains the main pathogenic species of the genus for both animals and humans. The organism was

initially recognized as a zoonotic pathogen, affecting different species of wild and domesticated animals including cattle, sheep, and goats. Beyond its veterinary importance, *L. monocytogenes* was also identified as an important human pathogen in the late 1920s (Gray and Killinger, 1966), while its foodborne transmission was first demonstrated during the 1980s (Schlech III et al., 1983). *Listeria* spp. are Gram-positive, nonsporeforming rods, demonstrating a characteristic tumbling motility at 20–25°C provided by few peritrichous flagella, and being widely distributed in the natural environment (Farber and Peterkin, 1991; Gray and Killinger, 1966). Decaying plant material, soil, animal feces, sewage, water, and animal feeds and particularly silage have been extensively documented as natural habitats of the pathogen (Gray and Killinger, 1966).

In addition to its ubiquitous nature, the ability of *L. monocytogenes* to proliferate under a wide range of environmental conditions contributes significantly to its transmission via multiple routes to animal food products and, hence, to its importance as a foodborne pathogen. *L. monocytogenes* can grow at temperatures ranging from 1°C to 45°C, with optimal growth taking place between 30°C and 37°C. Apart from being psychrotrophic, the organism also has the ability to grow at a wide pH range (i.e., from 4.4 to 9.6), and although optimum growth is observed at pH 7.0, it can survive at pH values as low as 3.5. Furthermore, unlike the majority of foodborne pathogens, *L. monocytogenes* can grow at water activity values as low as 0.900–0.920, depending on the humectant used (Jay, 2000).

As supported by both survey and epidemiological data, *L. monocytogenes* has been recognized as a serious concern for the meat and poultry industry, mainly as a postprocessing contaminant of products during additional handling such as peeling, slicing, and re-packaging (Lianou and Sofos, 2007). Indeed, the pathogen has been routinely isolated from numerous RTE meat products, including frankfurters, delicatessen meats, as well as fermented meat products (Lianou and Sofos, 2007; Szymczak et al., 2020), with several of these products being traditionally associated with sporadic and/or epidemic listeriosis (Schuchat et al., 1992). Nevertheless, raw meat products should also be taken into account when evaluating the pathogen's dissemination in the meat production and supply chain, since various prevalence levels have been reported among different raw meats, depending on various factors such as animal species, fresh/frozen state, and type of fabrication/processing (Liu et al., 2020; Sanlibaba et al., 2020). Furthermore, given its ability to adhere to a wide range of materials commonly used in food-processing facilities, *L. monocytogenes* can establish persistent contamination on food-processing equipment and/or other sites within these facilities (Lundén et al., 2002), which, in turn, may serve as reservoirs of the pathogen and potential sources of cross-contamination of finished RTE products. The ubiquitous and hardy nature of *L. monocytogenes* render its control a challenge for the food industry and food safety authorities, with the latter having established different approaches in different countries. However,

it has been generally regarded that the highest risk of listeriosis is imposed by RTE products that are exposed to the environment after a lethality treatment and that support the growth of the pathogen within their shelf life (Regulation (EC) No. 2073/2005; USFDA/USDA-FSIS, 2003).

Although a significant increasing trend of listeriosis was observed in the EU over the period 2008–14 (EFSA-ECDC, 2015), the EU trend of confirmed listeriosis cases remained stable in 2015–19. A total of 2621 confirmed invasive human cases of listeriosis were reported by 28 member states in 2019, with an EU notification rate of 0.46 cases per 100,000 population, which was at the same level as in 2018 (EFSA-ECDC, 2021a). The corresponding incidence of infections in 2019 in the United States was 0.3 (per 100,000 population), being practically unchanged as compared to the incidence of listeriosis recorded in the period 2016–18 (CDC, 2020). Although a rare disease, listeriosis can have serious clinical presentations, and between 20% and 30% of the cases are fatal (Rocourt, 1996). The incubation period between consumption of food contaminated with the pathogen and onset of listeriosis symptoms varies from 1 to 90 days (McLauchlin, 1997). Although there is considerable uncertainty relative to its infectious dose, the high levels of the organism detected in foods associated with epidemic and sporadic cases of listeriosis suggest that the minimum dose required to cause clinical infection is high (Vázquez-Boland et al., 2001). More specifically, there is limited scientific evidence supporting that consumption of levels of the organism lower than 100 CFU/g represents a health risk in healthy individuals (Nørrung, 2000). Nonetheless, given the extensive intraspecies variation, as well as potential interactions between parameters such as food matrix, virulence of *L. monocytogenes* strains and host susceptibility, low doses cannot be excluded from causing infection, at least in sensitive individuals (Chen et al., 2006).

Listeriosis is usually manifested as an illness of the central nervous system, as sepsis or flu-like disease, with its exact clinical characteristics being variable. Pregnant women are considered to be one of the high-risk populations for *L. monocytogenes* infection, due to its severe implications for the fetus, although it is usually a self-limited infection for the pregnant female. Nonpregnant adults may experience a life-threatening invasive disease characterized by sepsis, meningitis, or meningoencephalitis, and high-risk populations include cancer patients, transplant recipients, people receiving immunosuppressive therapy, and the elderly (Rocourt, 1996). In addition to invasive disease, which is the most common clinical manifestation of human listeriosis, outbreaks of febrile gastroenteritis in otherwise healthy people indicate that *L. monocytogenes* can also cause typical foodborne gastroenteritis. The incubation period in this type of *L. monocytogenes* infection has been reported to range from 6 h to 10 days, and the infectious dose is also likely to be high. Although the exact relationship between invasive and noninvasive infection remains to be ascertained, it has been assumed that febrile gastroenteritis may be responsible for a significant proportion of foodborne listeriosis in the general population. Hence, it has been

recommended that *L. monocytogenes* should not be overlooked as a potential causative agent of gastroenteritis when routine stool cultures fail to identify another pathogen (Ooi and Lorber, 2005).

16.3.5 *Salmonella enterica*

Salmonellae are Gram-negative organisms, typical members of the family Enterobacteriaceae that can be indistinguishable from *E. coli* under the microscope or when cultured on ordinary nonselective nutrient media (Adams and Moss, 2008). Despite the significant changes that have been made over time with regard to the taxonomy and nomenclature of the genus *Salmonella*, it has been nowadays well established that the genus is composed of only two genomic species, i.e., *Salmonella enterica* and *Salmonella bongori*, with each one of them containing multiple serotypes and with *Salmonella enterica* constituting the type species (Tindall et al., 2005). The species *Salmonella enterica* is divided into 6 subspecies and includes more than 2500 serotypes; the majority of these serotypes (more than 1500 serotypes) and almost all the medically important strains of the organism belong to subspecies I (i.e., *Salmonella enterica* subsp. *enterica*) (Lamas et al., 2018; Velge et al., 2005). Strains belonging to this subspecies (referred to as "*Salmonella enterica*" for simplification purposes) are responsible for 99% of *Salmonella* infections in humans and warm-blooded animals and are usually transmitted through the ingestion of contaminated food or water; on the other hand, strains in the other five subspecies as well as in the species *Salmonella bongori* are usually isolated from cold-blooded animals and the environment and rarely from humans (Lamas et al., 2018; Uzzau et al., 2000). Certainly, as supported by phenotype and genotype information derived from different studies of non-*enterica* subspecies, the latter exhibit poor ability to invade host cells, while important virulence factors are either absent or modified. With this in mind, and also given that the great majority of human infections due to *non-enterica* subspecies are related to immunosuppressed individuals, it has been proposed that these subspecies should be treated only as opportunistic pathogens (Lamas et al., 2018).

Salmonella spp. are facultative anaerobic organisms and are generally motile with peritrichous flagella (Adams and Moss, 2008). Growth of *Salmonella enterica* can take place in a temperature range of 5–45°C, depending on the strain and the food matrix, while its optimum growth temperature is usually between 35°C and 40°C. Although its optimum pH for sustained growth is between 6.5 and 7.5, *Salmonella enterica* growth has been observed at pH values as low as 4.05 when HCl and citric acid were used as acidulants. Furthermore, the organism cannot grow at water activity values below 0.940, while salt concentrations exceeding 9% have been reported to be bactericidal (Jay, 2000).

The gastrointestinal tract of animals (e.g., birds, reptiles, farm animals) comprises the natural habitat of salmonellae, and the presence of these organisms in various environmental niches (including water, waste, animal feeds, farm and

aquaculture environments, and food products) can be the result of either direct fecal contamination or transmission of the organisms excreted in feces through insects, animals, and humans (Jay, 2000). Although asymptomatic carriage by animals delineates their most frequent incidence in the environment, salmonellae are well-established zoonotic agents of major public health and economic significance for both animals and humans. Depending on the host species with which they are associated, *Salmonella enterica* serotypes are usually referred to as (i) "host-restricted," when their habitat is limited almost exclusively to a host species, such as humans (serotypes Typhi, Paratyphi A and Paratyphi B), ovine (Abortusovis), or fowl (Gallinarum); (ii) "host-adapted," when they are associated with more than one related host species (e.g., serotypes Dublin and Choleraesuis); and (iii) "un-restricted," when they are associated with a broad range of unrelated host species (e.g., serotypes Enteritidis and Typhimurium) (Uzzau et al., 2000). With reference to the foodborne sources of *Salmonella enterica*, due to its high association with food animals such as poultry, cattle, and swine, the pathogen has been commonly linked to raw meat from these and other farm animals (EFSA-ECDC, 2015, 2021a; Golden and Mishra, 2020; Rhoades et al., 2009; Sodagari et al., 2020).

Salmonellosis, an illness known for more than 100 years, continues to be a leading cause of foodborne infections in many countries (CDC, 2020; EFSA-ECDC, 2021a; Scallan et al., 2011). In 2019, the EU notification rate for salmonellosis was 20.0 cases per 100,000 population, while a total of 926 salmonellosis foodborne outbreaks were reported by member states, causing 9169 illnesses, 1915 hospitalizations (50.5% of all outbreak-related hospitalizations), and seven deaths (EFSA-ECDC, 2021a). The 2019 incidence of salmonellosis in the United States was 17.1 per 100,000 population, with an associated number of infections, hospitalizations, and deaths of 8556, 2430, and 46, respectively (CDC, 2020). The clinical manifestations of human salmonellosis depend largely on the *Salmonella enterica* serotype being responsible for the infection. Among the "host-restricted" serotypes of the pathogen, the ones that are associated with animal hosts usually elicit mild symptomatology in humans, whereas serotypes Typhi, Paratyphi A, and Paratyphi B frequently cause severe systemic disease in humans known as typhoid or enteric fever (Velge et al., 2005). Ubiquitous *Salmonella enterica* serotypes, such as Enteritidis and Typhimurium, have been generally associated with self-limiting gastrointestinal infection, known as nontyphoidal salmonellosis, which, nonetheless, can result in serious clinical outcomes in susceptible individuals (Velge et al., 2005). The association of *Salmonella enterica* ser. Enteritidis and of multiple-antibiotic-resistant strains of *Salmonella enterica* ser. Typhimurium with human foodborne infections signified major changes in the epidemiology of nontyphoidal salmonellosis in the second half of the 20th century (Velge et al., 2005). Although these two serotypes continue to be rather commonly associated with foodborne infections in humans, surveillance and epidemiological data demonstrate some considerable changes in the trends of salmonellosis infections during the last decade

(Al-Rifai et al., 2019; Castro-Vargas et al., 2020; CDC, 2020; EFSA-ECDC, 2021a). For instance, according to data for the United States, serotype Infantis moved from the ninth most common *Salmonella* serotype among infected persons during 1996–98 to the sixth most common in 2019, with its incidence being significantly higher (i.e., 69% increase) compared with the period 2016–18 (CDC, 2020). Moreover, the emergence and potential association with human disease of rare serotypes of the organism has certainly attracted the attention of the scientific community. Examples of such serotypes include serotype 4,5,12:i:-, which has been associated with a number of human salmonellosis outbreaks and isolated from various animals and foods, as well as serotypes Cerro, Othmarschen, London, Napoli, and Weltevreden (Koutsoumanis et al., 2014). Given the association of these emerging serotypes with animal pathologies and/or foods of animal origin, and taking into account the antigenic and genetic relatedness of some of them with serotypes of well-established public health significance, it becomes evident that close monitoring of their incidence in meat and meat products is warranted. Last but not least, the emergence and dissemination in the meat supply chain of antimicrobial resistance among *Salmonella enterica* clones, stemming from the misuse of antibiotics in human medicine and animal production, is undeniably an ongoing and significant public health issue (Castro-Vargas et al., 2020; Sodagari et al., 2020).

16.3.6 *Yersinia enterocolitica*

The genus *Yersinia*, member of the family Enterobacteriaceae, is composed of at least 12 species, among which three species are regarded as human pathogens: *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* (Sprague and Neubauer, 2005). Despite the fact that foodborne illness caused by *Yersinia* spp., referred to as yersiniosis, is caused by *Y. enterocolitica* or *Y. pseudotuberculosis*, the former species have been associated with the majority of cases of human illness. *Yersinia* spp. are Gram-negative, nonsporeforming rods or coccobacilli, which are facultative anaerobes and are capable of growing at refrigeration temperatures. Although its optimum growth temperature is approximately 30°C, *Y. enterocolitica* can sustain growth at temperatures as low as 0°C. *Yersinia* spp. are generally regarded as being sensitive to acidic pH conditions and exhibit moderate salt tolerance (Fredriksson-Ahomaa et al., 2010).

Animals have been traditionally regarded as the principal reservoir of pathogenic *Yersinia* spp., with slaughtered pigs being one of the most important sources of *Y. enterocolitica*. Additional meat animals (domestic livestock) that have been shown to harbor the organism are cattle, sheep, and goats (EFSA-ECDC, 2021a; Fredriksson-Ahomaa et al., 2010). Due to the high association of *Y. enterocolitica* with swine, raw pork meat, and its products have been extensively investigated as potential vehicles of transmission of yersiniosis to humans. Indeed, as supported by epidemiological data, consumption of raw/undercooked pork has been rather commonly linked to *Y. enterocolitica*, whereas other meat products, including

among others RTE products, have also been associated with foodborne yersiniosis (EFSA-ECDC, 2021a). Yersiniosis was the fourth most commonly reported zoonosis in humans in 2019 in the EU, and its overall notification rate (as reported by 29 EU/EEA countries) was 1.7 cases per 100,000 population, while "pig meat and products thereof" was among the two food categories most reported to cause strong-evidence yersiniosis foodborne outbreaks in the period 2010–19 (EFSA-ECDC, 2021a,b). The 2019 incidence of yersiniosis in the United States was reported to be 1.4 cases per 100,000 population, a significantly increased incidence (i.e., 153%) as compared to the period 2016–18 (CDC, 2020).

Although yersiniosis can cause various symptoms in humans depending mainly on the age of the infected individual, the most common symptoms include fever, abdominal pain, and diarrhea. Moreover, while most of the infections in healthy individuals are localized and self-limiting, complications such as reactive arthritis and skin rash may also occasionally occur. In some cases, and particularly among children, the main symptoms of *Y. enterocolitica* infections are fever and right-sided abdominal pain and may be confused with appendicitis (Fredriksson-Ahomaa et al., 2010).

16.3.7 Other bacterial pathogens

Beyond the aforementioned meatborne bacterial pathogens, there is also research and epidemiologic evidence to support potential contribution of additional bacterial species to human disease caused by the consumption of meat and meat products. An example of such species with meatborne transmission potential is the toxicogenic Gram-positive bacterium *Staphylococcus aureus* with its incidence in various meat products being frequently reported in the scientific literature (Omer et al., 2018). The public health concern with regard to this pathogen is even more pronounced in the case of methicillin-resistant, vancomycin-resistant, or multidrug-resistant strains that, although initially present almost exclusively in clinical environments, have disseminated in the community in recent decades including food and food-producing animals (da Silva et al., 2020; Saadati et al., 2021). Although the findings regarding the presence of resistant to antibiotic(s) *Staphylococcus aureus* strains in meat and meat products have not been conclusive, and the anticipated prevalence of such strains is relatively low compared to that of other bacterial pathogens (e.g., *E. coli* and *Salmonella enterica*), consumer exposure to such strains through meat and poultry consumption cannot be excluded, particularly in the case of complex and multiingredient products (e.g., meat sandwiches) (Mahros et al., 2021). Another bacterial species linked to meat-associated outbreaks, albeit less frequently reported, is the Gram-positive, spore-forming bacterium *Bacillus cereus* (Omer et al., 2018). Lastly, future research should elucidate the meatborne transmission potential of emerging bacterial pathogens whose involvement in the food chain has not been firmly established yet, such as *Arcobacter butzleri* (Ferreira et al., 2019) and *Acinetobacter* spp. (Malta et al., 2020).

16.4 Meatborne viruses

According to the "European Union One Health 2019 Zoonoses Report," viruses were reported to have caused 10.7% of the total outbreaks, being the third most common causative agent after bacteria and bacterial toxins (EFSA-ECDC, 2021a). Among the various groups of viruses that cause disease in humans, hepatitis viruses, the human rotavirus, and the gastroenteritis-causing human noroviruses have been identified as the most important viral foodborne pathogens (EFSA, 2011). Indeed, these viral groups are highly infectious and have been associated with large numbers of outbreaks and numerous affected people (EFSA-ECDC, 2015, 2021a). Although theoretically, viral contamination of food can occur anywhere in the farm-to-fork continuum, it has been principally observed toward the end of the food supply chain, with most of the documented foodborne viral outbreaks being associated with foods that have been manually handled by infected food handlers, either symptomatic or not (Koopmans and Duizer, 2004). In this context, and as also supported by epidemiological data, the meat products in that viral pathogens are most likely to be present are RTE products such as deli meats (Markantonis et al., 2018). Foodborne viruses can be present in meat processing environments, and meat products may serve, either directly (i.e., raw/undercooked products) or indirectly (i.e., cross-contamination vehicle), as important means of human exposure to viral pathogens (Markantonis et al., 2018).

16.4.1 Hepatitis viruses

The hepatitis viruses are transmitted enterically via the fecal-oral route, either directly from person to person or indirectly through the ingestion of fecally contaminated water or food, and replicate and cause disease in the liver. Viral hepatitis is generally an acute infection, with its main symptoms including fever, jaundice, nausea, vomiting, light-colored stools, dark-colored urine, abdominal pain, enlarged tender liver, elevated liver enzymes levels, and occasional diarrhea. Nonetheless, upon resolution of the infection, the affected individuals develop lifelong immunity to future infections (Mattison et al., 2009). The hepatitis A virus (HAV) and the hepatitis E virus (HEV) are both regarded as important viral pathogens with a significant foodborne transmission potential. However, the available data about HAV and HEV prevalence and frequency of food contamination, prevalent viral strains and sources of contamination, are limited, particularly in developing countries (Di Cola et al., 2021).

In contrast to what is the case in developing countries, where HAV infection is endemic, this viral pathogen constitutes a serious and increasing public health concern in many developed countries (Koopmans and Duizer, 2004; Mattison et al., 2009). Given the long incubation period of HAV infection (virus shedding may start 10–14 days before the onset of symptoms), the virus' spreading can be extensive and, at the same time, the identification of its foodborne sources can

be burdensome (Koopmans and Duizer, 2004). However, to the extent that such association is feasible, foodborne outbreaks of HAV have been mainly linked to the consumption of shellfish and produce items (Mattison et al., 2009) rather than meat and meat products.

On the other hand, consumption of raw or undercooked meat of naturally infected animals, both wild and domesticated, has been identified as one of the most important routes of transmission of HEV to humans (Pavio et al., 2015; Di Cola et al., 2021). HEV is a small, nonenveloped, RNA virus, belonging to the Hepeviridae family and including four distinct (i.e., 1–4) human pathogenic genotypes (Mattison et al., 2009). Although HEV is well known for its ability to cause acute clinical hepatitis in the context of waterborne outbreaks and sporadic infections throughout the developing world, recent reports on zoonotic foodborne autochthonous HEV infections resulted in its identification as an emerging clinical problem in industrialized countries as well (Khuroo and Khuroo, 2016; Koutsoumanis et al., 2014). Animals that are primarily regarded as reservoirs of HEV (most commonly serotypes 3 and 4) are domestic swine and wild boars and deer (Di Cola et al., 2021; Khuroo and Khuroo, 2016), while strains circulating in these animals have been also shown to be genetically related to strains identified in cases of human infections (Pavio et al., 2015). With reference to the incidence of HEV in foods of animal origin, this has been confirmed in pig liver, pork meat, and their products such as sausages and pâté (Berto et al., 2012; Di Cola et al., 2021; Szabo et al., 2015), while the same product types have been also implicated as vehicles of foodborne HEV infection (Szabo et al., 2015). In a study investigating the distribution of HEV in different types of sausages sold at retail establishments in Germany, the virus was detected in 20% and 22% of raw and liver sausages, respectively; the detected HEV was characterized by high genetic diversity and belonged to different subtypes of genotype 3 (Szabo et al., 2015). Despite the fact that hepatitis E is regarded as a self-limiting acute infection, the disease may vary in severity and serious clinical manifestations (e.g., protracted coagulopathy and cholestasis) may be developed in sensitive populations groups. Chronic HEV infection resulting in liver cirrhosis and end-stage liver disease has been frequently reported in organ transplant recipients. Moreover, HEV infections in pregnant women have been associated with high rates of fulminant hepatitis and fatality, particularly in the third trimester of pregnancy (Khuroo and Khuroo, 2016; Mattison et al., 2009).

16.4.2 Noroviruses

Viruses causing gastroenteritis have been well recognized as among the most common causes of foodborne illness worldwide, with norovirus (NoV) (or Norwalk virus) ranking number one in many industrialized countries. Indeed, based on active and passive surveillance data, NoV was responsible for 58% of foodborne illness in the United States in the period 2000–08 (Scallan et al., 2011). This group of viruses belongs to the genetically and antigenically diverse

genus Norovirus of the Caliciviridae family, which forms a phylogenetic clade of five genogroups (I to V) (Jaykus and Escudero-Abarca, 2010). Despite the fact that both NoV genogroups I and II have been associated with human disease, genogroup II tends to be more prevalent, with certain emerging strains (NoV genotype II.4 variant) being associated with global outbreaks of gastroenteritis (Bull et al., 2006). In addition to its presence in human fecal material, NoV can also be shed in vomitus of infected individuals, while the transmission routes of these viral pathogens can be either direct (between individuals) or indirect (through the consumption of contaminated food or water or contact with fomites (Jaykus and Escudero-Abarca, 2010). Although attribution of NoV infections specifically to foodborne transmission is generally considered as a difficult task, the food commodities most commonly linked to NoV disease outbreaks are crustaceans, shellfish, and mollusks (EFSA-ECDC, 2015, 2021a). Nevertheless, meat products subjected to postprocessing handling, such as deli ham, should not be overlooked as potential means of NoV foodborne transmission and causative agents of epidemic disease (Markantonis et al., 2018). The acute viral gastroenteritis caused by NoV is characterized by nausea, vomiting, diarrhea, and abdominal pain, while headache and low-grade fever are also occasionally reported. Incubation periods range between 12 and 48 h, while the maximum duration of the infection is 48–72 h. Although severe illness or hospitalization is uncommon, rehydration therapy may be required in some cases, particularly in sensitive individuals such as children, the elderly, or the immunocompromised (Jaykus and Escudero-Abarca, 2010).

16.4.3 Other viruses

In addition to the aforementioned viral pathogens, Avian Influenza viruses and coronaviruses also have the potential to emerge as meat safety concerns. Birds are regarded as the principal reservoir of Influenza A viruses, with viruses containing combinations of the H1, H2, H3, N1, and N2 subtypes being established in the human population, while viruses of the H5, H7, and H9 subtypes being associated with sporadic human infections (Mattison et al., 2009). Humans may acquire Avian Influenza viruses either through direct contact with infected birds or via contaminated poultry products. An Avian Influenza virus subtype that has recently attracted the attention of public health authorities worldwide is the highly pathogenic H5N1 virus. By being able to replicate in the upper respiratory tract, this virus may cause an intense inflammatory response, associated with a mortality rate often exceeding 60% (De Jong et al., 2006). Since H5N1 virus has been recovered from various parts of infected poultry (e.g., blood, bones, and meat), the consumption of raw or undercooked poultry products as a potential source of infection cannot be excluded (Mattison et al., 2009).

Similarly, a particularly virulent coronavirus strain, known as the "Severe Acute Respiratory Syndrome Coronavirus" (SARS-CoV), may also have the potential to be transmitted to humans through the fecal-oral route and food

products. Indeed, this viral strain, which emerged in 2003 and has been associated with more than 8000 cases of systemic infections and respiratory illness, has been isolated from the gastrointestinal tract and feces as well as from sewage (Mattison et al., 2009). With reference to the life-threatening COVID-19 pandemic of infectious disease, caused by the novel coronavirus SARS-CoV-2 that humanity is currently confronting, there has also been skepticism regarding the risk of food products as potential carriers for the virus' transmission. Although the main clinical manifestation of COVID-19 is the development of respiratory symptoms, the fecal-oral transmission of SARS-CoV-2 has also been questioned based on the fact that viral RNA has been detected in feces of several patients contracting the infection and experiencing gastrointestinal symptoms (Hindson, 2020). Furthermore, SARS-CoV-2 exhibits a remarkable stability in various environments including food processing surfaces, and it has been detected on the packaging materials, storage environment, and surface of frozen raw foods (Han et al., 2021). In this sense, SARS-CoV-2 can be infective on contaminated food products, and the latter may act as potential vehicle of the virus due to either carry-through or carry-over contaminations (Yekta et al., 2021). Specifically for meat and meat products, viral transmission indicating carry-through contamination is also conceivable given the fact that there is some evidence, suggesting that SARS-CoV-2 infection can be transpired in pigs and rabbits (Yekta et al., 2021). At present, and as stated by public health agencies such as the United States Food and Drug Administration, no scientific evidence exists to show a relationship between SARS-CoV-2 and food products. Indeed, persistence of the SARS-CoV-2 infectivity after consumption of foods, and subsequent viral invasion to body tissues has not been demonstrated (Thippareddi et al., 2020). Thus, although application of precautious acts in food processing units is strongly recommended, it has been overall concluded that more studies should be conducted in order to elucidate and ascertain the role of food, especially food products of animal origin, in the transmission of SARS-CoV-2 (Yekta et al., 2021).

16.5 Meatborne parasites

During the last decades, foodborne parasitic infections have been regarded as emerging or re-emerging at a worldwide level, due to either true higher incidence or higher detection (Dorny et al., 2009; Koutsoumanis et al., 2014). A potentially (or seemingly) increased human exposure to foodborne parasites can be associated with factors such as climate changes, improved diagnostic tools, increased international travel, changing eating habits, food supply globalization and changes in food production systems, population growth, and particularly increase of sensitive population groups (Dorny et al., 2009). Among the well-known foodborne parasites, *Taenia* spp., *Toxoplasma gondii*, and *Trichinella* spp. are the ones exhibiting the higher relevance and importance for meat and meat products, taking into account the number of cases and/or the severity of

infection (WHO, 2015). Although it has been acknowledged that underestimation of the true burden of foodborne parasitic infections is very likely, such burden is certainly significant in low- and middle-income countries, where a high specificity of parasitic infections to food sources is anticipated (WHO, 2015). In any case, the need for risk-based surveillance for meatborne parasites has been identified; the application of the principles of risk analysis should be of great value, not only for hazard identification and exposure assessment purposes but also for actions' prioritization and effective and efficient allocation of resources (Alban et al., 2020).

16.5.1 *Taenia* spp.

The taeniid tapeworms of the genus *Taenia* are important zoonotic pathogens likely to be transmitted by food, and particularly by meat, to humans. More specifically, the species *Taenia saginata* (main reservoir: cattle), *Taenia saginata asiatica* (main reservoir: swine), and *Taenia solium* (main reservoir: swine) have been associated with human infections, with the latter being, in turn, linked to the consumption of raw or undercooked meat, liver, or viscera (Dorny et al., 2009). Specifically, the parasite *Taenia solium* has been appraised as contributing considerably to the global burden of foodborne disease, with the associated disability-adjusted life year (DALY) metric being estimated at 2.8 million and being placed second in terms of global burden after *Salmonella typhi* (WHO, 2015).

16.5.2 *Toxoplasma gondii*

Toxoplasma gondii is a coccidian protozoan parasite of man and animals, the only species in the *Toxoplasma* genus, and one of the most significant parasites in both Europe and the United States (Almeria and Dubey, 2021). Although the disease caused by *Toxoplasma gondii*, referred to as toxoplasmosis, is a zoonotic disease of global distribution and importance, having a burden similar to that of salmonellosis and campylobacteriosis, its true burden may be actually considerably higher that estimated given that it has been regarded as a significantly underreported disease (Dorny et al., 2009). Among the three genotypes described within *Toxoplasma gondii* (genotypes I, II, and III), the majority of human toxoplasmosis cases have been associated with genotype II (Smith and Evans, 2009).

The parasite *Toxoplasma gondii* is a pathogen of major importance in animal health, causing reproductive failure and clinical toxoplasmosis in many species, particularly small ruminants (Almeria and Dubey, 2021). Human toxoplasmosis can be contracted by the ingestion of sporulated oocysts present in cat feces and the environment (Dorny et al., 2009). Moreover, *Toxoplasma gondii* can be transmitted to humans through the consumption of raw or undercooked meat since viable parasites have been isolated from many food animals including

swine, chicken, sheep, goat, and horse (Dorny et al., 2009; Guo et al., 2016; Smith and Evans, 2009). Indeed, foodborne outbreaks of toxoplasmosis have been frequently linked to raw or rare meat and raw liver, with an important limitation, however, being the lack of sufficient documentation in the available outbreak reports (Almeria and Dubey, 2021; Smith and Evans, 2009). The importance of swine and, in turn, pork meat as possible sources of human toxoplasmosis has been unequivocally established, as supported by both prevalence and epidemiological data (Almeria and Dubey, 2021; Guo et al., 2016). Furthermore, and despite the relatively low prevalence of infection in cattle, quantitative microbial risk assessment has demonstrated that consumption of beef also constitutes an important source of toxoplasmosis in humans (Opsteegh et al., 2011).

It has been estimated that approximately one-third of the global human population is chronically infected by this parasitic pathogen (Almeria and Dubey, 2021). Although toxoplasmosis may be asymptomatic or associated with nonspecific clinical symptoms in healthy individuals, the disease can be severe in pregnant women, who can pass the infection to the fetus, as well as in immunocompromised hosts (e.g., AIDS patients, organ and bone marrow transplant recipients, those with malignancies and on anticancer chemotherapy) (Almeria and Dubey, 2021; Dorny et al., 2009; Smith and Evans, 2009).

16.5.3 *Trichinella* spp.

Trichinella spp. are widely distributed zoonotic pathogens, well known for their association with meatborne infections in humans. Trichinellosis in humans is contracted by the ingestion of larvae of nematodes of the genus *Trichinella* that are encysted in muscle tissue of domestic or wild animal meat, with swine being identified as the most important source of the infection worldwide (Alban et al., 2020; WHO, 2015). Although the majority of infections in both animals and humans were traditionally attributed to the species *Trichinella spiralis*, eight species and four genotypes have been currently identified within the genus *Trichinella* (Dorny et al., 2009). Clinical disease in humans is characterized by (i) an intestinal phase, whose main symptoms are diarrhea, nausea, vomiting, fever, and abdominal pain and (ii) a subsequent parenteral (tissue) phase, which is usually accompanied by heavy muscle pains, fever, and eosinophilia (Dorny et al., 2009).

16.5.4 Other parasites

The coccidian parasites of the genus *Cryptosporidium*, which exhibit a wide range of vertebrate hosts (e.g., mammals, rodents, birds, and reptiles), can be considered as significant contributors of environmental contamination with oocysts, and hence, as potential vehicles of foodborne parasitic disease (Smith and Evans, 2009). The same applies for the protozoan parasites of the genus *Giardia*,

which are capable of infecting multiple animal species, and whose foodborne transmission potential, although not well understood and documented due to limitations of current detection and surveillance methods, is thought to be considerable (Ryan et al., 2019; WHO, 2015). Lastly, driven by parameters such as particular culinary habits, improved transportation and distribution systems as well as tourism, the emergence of various parasites including trematodes, cestodes, nematodes, and pentastomides as foodborne pathogens is also likely. Such parasites can originate from reptiles, amphibians, and snails and transmitted to humans via the consumption of raw or undercooked meat of these animals (Dorny et al., 2009; Koutsoumanis et al., 2014).

16.6 Other biological issues

16.6.1 Prions

Prions, which were defined as proteinaceous, infectious particles lacking genetic material (Prusiner, 1997), constitute well-established infectious agents (along with bacteria, viruses, fungi, and parasites) and an important source of concern with regard to animal and human health. Although first nominated to express the revolutionary concept that a protein could be infectious, the term "prion" has been broadened over the years to describe aggregates irrespective of their infectivity (Scheckel and Aguzzi, 2018). Prion diseases, also referred to as "transmissible spongiform encephalopathies" (TSE), are progressive and invariably fatal neurodegenerative conditions associated with misfolding and aggregation of a host-encoded cellular prion protein (PrP^C), a cell surface glycoprotein occurring in various mammalian species, including humans (Imran and Mahmood, 2011). More specifically, conformational change of the PrP^C (normal prion protein) to a pathological form (PrP^{TSE}) is considered central to pathogenesis and formation of the infectious agent. The current understanding of TSE diseases is depicted in a concept known as "protein-only theory," according to which the infectious agent involved lacks genetic material, and the abnormal prions are both the result and the cause of these diseases (Momcilovic, 2010). Nonetheless, such concept remains controversial, and despite the significant progress that has been made with regard to our understanding of the pathogenesis of prion diseases, many fundamental questions related to the nature of the infectious agent remain unanswered. Recent research findings indicate a much broader role for PrP^C in physiological and disease processes than originally assumed (Manni et al., 2020; Scheckel and Aguzzi, 2018). Specifically, the PrP^C has been implicated in the pathogenesis of other neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), while it has also been reported to play unexpected functions outside the nervous system (Manni et al., 2020).

The TSE in animals include scrapie of sheep and goats, chronic wasting disease of deer and elk, feline spongiform encephalopathy of cats, transmissible spongiform encephalopathy of mink, and bovine spongiform encephalopathy

(BSE) of cattle. Human TSE include kuru, Creutzfeldt-Jacob Disease (CJD), variant Creutzfeldt-Jacob Disease (vCJD), Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia (Imran and Mahmood, 2011). However, among the abovementioned TSE in animals, BSE is the only one known to be transmitted to humans through consumption of food. Indeed, as concluded in the joined scientific opinion of the EFSA and the ECDC on any possible epidemiological or molecular association between TSE in animal and humans, BSE was the only disease demonstrated to be zoonotic (EFSA-ECDC, 2011). BSE, which was first reported in the United Kingdom in 1986, has killed more than 280,000 cattle worldwide, and has been associated with the feeding of recycled prion-infected foodstuff to the animals. In turn, the human TSE of vCJD, which was first detected in 1996 and linked to the BSE epidemic in cattle, has been associated with the consumption of BSE-contaminated meat and other products derived from affected cattle (Lee et al., 2013).

In addition to acquired infections, human prion diseases can also arise spontaneously or genetically (i.e., hereditary diseases) (Imran and Mahmood, 2011). Although not fully understood, neuronal damage is considered central to the clinical manifestation of prion diseases. Furthermore, despite the fact that brain is regarded as the principal target of prion pathology, prion replication in most TSE has also been displayed at extracerebral locations such as secondary lymphoid organs and sites of chronic inflammation (Imran and Mahmood, 2011). A significant amount of research during the last decade has been aimed at (i) defining the phenotypic heterogeneity of the recognized human prion diseases, (ii) correlating this heterogeneity with molecular/genetic features, and (iii) the comparative assessment of the derived classification along with the corresponding biological properties of the agent as determined in animal transmission studies (Head and Ironside, 2012). Studies on human prion diseases and prion molecular mechanisms have been reviewed by Head and Ironside (2012), Lee et al. (2013), and more recently by Wang et al. (2019).

16.6.2 Biogenic amines

Biogenic amines are antinutritional organic nitrogenous compounds of low molecular weight, which are formed mainly through the decarboxylation of amino acids as a result of microbial activity, whereas they can also arise due to amination or transamination of aldehydes and ketones (Durak-Dados et al., 2020; Stadnik and Dolatowski, 2010). Biogenic amines are found at varying concentrations in different food products including fish, cheese, meat, wine, beer, and vegetables, with their formation and concentration in final products depending on the microbiological quality and chemical composition of raw materials, the applied processing, and the anticipated storage/preservation conditions (Flores et al., 2019; Ruiz-Capillas and Herrero, 2019). In particular, prerequisites for the formation and accumulation of biogenic amines in foods are the availability of appropriate precursors, namely free amino acids, the presence of

microorganisms with amino acid decarboxylase(s) activity, and the occurrence of favorable environmental conditions (and mainly temperature) for their proliferation (Jairath et al., 2015).

Biogenic amines are generated from the removal of the α-carboxyl group from the corresponding precursor amino acid, with the latter being commonly depicted in the name of the derived biogenic amines. For instance, histidine is decarboxylated to produce histamine, tryptophane to tryptamine, tyrosine to tyramine, lysine to cadaverine, while the biogenic amine putrescine can be produced from three amino acids, i.e., glutamine, arginine, and agmatine (Stadnik and Dolatowski, 2010). Although biogenic amines are needed for maintenance of cell viability (e.g., energy supply and acid stress resistance) and the proper course of metabolic purposes (e.g., protein synthesis, hormone synthesis, and DNA replication), they can also have toxic or carcinogenic effects when present at excessive levels (Durak-Dados et al., 2020; Jairath et al., 2015; Wójcik et al., 2021). There are two major groups of biogenic amines: (i) aromatic amines including tyramine, phenylethylamine, histamine, and tryptamine and (ii) aliphatic amines including putrescine, cadaverine, and agmatine. The main concern with regard to the production and accumulation of biogenic amines in foods is the practical absence of any effect on their sensory characteristics so that consumers would be "warned" and not consume such products, while the thermal stability of these compounds renders the application of any heat treatment incompetent to alleviate their presence and concentration once allowed to be produced (Durak-Dados et al., 2020; Flores et al., 2019). The potential negative implications of biogenic amines for consumers' health are mainly related to tyramine, histamine, and phenylethylamine that are associated to vasoactive and psychoactive reactions (Flores et al., 2019).

The most prevalent biogenic amines in meat and meat products are tyramine, cadaverine, putrescine, and histamine (Jairath et al., 2015; Stadnik and Dolatowski, 2010), with their exact type and concentration depending on the type of product. With reference to meat products, biogenic amines are mainly formed in fermented meat products (e.g., fermented sausages), with major representatives the compounds tyramine, histamine, and phenylethylamine (Durak-Dados et al., 2020; Flores et al., 2019; Jairath et al., 2015; Sivamaruthi et al., 2021; Stadnik and Dolatowski, 2010). This is the case due to the distinctive biochemical processes delineating the technology of these products as well as their intrinsic characteristics. The main process underlying the formation of biogenic amines in fermented meat products is proteolysis, which is performed either by endogenous meat proteases or via the proteolytic activity of microorganisms being present during the fermentation process. Proteolysis is, in turn, favored by protein denaturation resulting from acidity increase, dehydration, and action of sodium chloride, and the resulting nonprotein nitrogen fraction (including free amino acids that act as biogenic amines' precursors) increases during meat fermentation and drying (Durak-Dados et al., 2020; Jairath et al., 2015; Stadnik and Dolatowski, 2010). In addition, the microorganisms

responsible for the fermentation process may also contribute to the formation and accumulation of biogenic amines. Yet, since the concentration of biogenic amines may vary in fermented products with comparable microbial flora, it has been assumed that complex interactions among different factors are most likely involved in their production (Jairath et al., 2015).With regard to the ability of microorganisms to produce amino acid decarboxylases, it appears to be highly strain specific (Durak-Dados et al., 2020; Jairath et al., 2015). Although species of multiple bacterial genera are capable of decarboxylating one or more amino acids, decarboxylase activity in meat products is principally attributed to Enterobacteriaceae, Pseudomonadaceae, Micrococcaceae, and lactic acid bacteria (Jairath et al., 2015). Given their coexistence and the coevolution of their concentrations with microbial populations, including among others spoilage microorganisms, biogenic amines are commonly utilized as indicators of fresh meat quality and/or acceptability (Ruiz-Capillas and Herrero, 2019).

Taking into account the food quality and human health implications of biogenic amines, significant efforts are made globally to control their prevalence in food commodities. Notwithstanding, the existing pertinent legislation only covers histamine in fish and fishery products. Specifically, the European Commission Regulations (2073/2005, 144/2007, 365/2010) set food safety criteria for histamine in specific fish species throughout their shelf life with a sampling plan comprising nine units, two of which may be between 100 and 200 mg/kg of histamine and none above the limit of 200 mg/kg. This legislation also refers to histamine levels in fish products subject to enzymatic maturation, with a sampling plan comprising nine units, two of which may be between 200 and 400 mg/kg of histamine and none above the limit of 400 mg/kg. In the United States, the FDA has set a general histamine limit of 50 mg/kg for all food products (Ruiz-Capillas and Herrero, 2019; Wójcik et al., 2021). Although, in general, the aforementioned legislations that are applicable to fish and fishery products are also applied in other food products, histamine has been, indeed, identified as the most toxic amine found in food. Various symptoms comprise the clinical manifestation of histamine poisoning, including reddening of the skin, edemas, and rashes, and dilatation of peripheral blood vessels resulting in hypotension, headache, diarrhea, and vomiting. Additional clinical manifestations include nasal congestion, asthmatic breathing, arrhythmia, and urticaria, while excessive histamine accumulation can also lead to respiratory failure, sweating, palpitations, and rash. Finally, the presence of secondary amines, such as putrescine and cadaverine, may elicit a synergistic effect and make the histamine toxicity more potent (Durak-Dados et al., 2020).

16.7 Current and future challenges to biological meat safety

Although microbial, and particularly bacterial, pathogens appear to constitute the most important meat safety issues in terms of both public health and

economy (i.e., recalls of contaminated products), all the biological issues discussed above need to be appropriately controlled by the food industry and public health authorities. In the framework of such a meat safety control scheme, various challenges need to be effectively managed, with numerous recent societal changes interfering with the persistence of well identified and/or the emergence of new challenges.

With reference to bacterial pathogens, an important current challenge to their control is the worldwide increase in the prevalence of multidrug-resistant (MDR) phenotypes among bacterial phenotypes. The overuse of antimicrobials in animal husbandry has, indeed, been associated with a long-lasting, strong selective pressure on bacteria prevalent in intensive production units, leading to the emergence of antimicrobial-resistant strains in food-producing animals that are then transmitted to humans either directly or through the food supply. Hence, the emergence and raised incidence of MDR strains has been a growing concern over the past 30 years, and particularly among *Campylobacter* and *Salmonella enterica* strains exhibiting resistance to several clinically important antimicrobial agents traditionally used to treat bacterial infections in human and veterinary medicine (Castro-Vargas et al., 2020; Zhong et al., 2016). For instance, resistance to multiple antibiotics appears to be significant among *Campylobacter jejuni* isolates from retail foods (Zhong et al., 2016), an observation that supports the concept that campylobacteriosis is likely to challenge global health in the years to come. In addition to effective surveillance, appropriate antibiotic management and improved diagnostics, which have already been proven to be valuable tools in the assessment and monitoring of antimicrobial resistance, additional measures are needed for this important food safety issue to be addressed. Future research elucidating the genetic determinants of antimicrobial resistance as well as the ecology, epidemiology, and evolution of MDR strains is expected to provide a better understanding of the emergence of antimicrobial resistance and, thus, to allow for the development of improved control measures.

As already mentioned for various foodborne pathogens discussed in this chapter, the identification and effective control of "new," "emerging," or "evolving" pathogenic microorganisms is certainly a very important challenge to meat safety. In addition to antibiotic resistance as discussed earlier, such microorganisms may also exhibit increased virulence, low infectious doses, and/or enhanced resistance to food-related stresses, rendering their control burdensome and dubious. Indeed, although considerable efforts have been made from public health authorities to address the rising public health impact of emerging foodborne pathogens, the generally limited available information with regard to their virulence and their responses to stress (e.g., encountered in the environment, foods and during food processing) still constitutes a major limitation for their effective control and prevention. In general, the challenge of pathogen emergence is expected to be successfully handled through the implementation of robust and effective surveillance programs and the development and utilization of

novel molecular techniques for studying foodborne pathogens (Koutsoumanis et al., 2014; Sofos, 2008).

As expected, the implementation of hazard analysis and critical control point (HACCP) systems, based on a series of sound prerequisite programs, can provide effective control of any foodborne pathogen in any environment (manufacturing, retail, or food service). Nonetheless, the complete and routine implementation of HACCP on the basis of proper food handler training and consumer education has been identified as an important challenge to meat safety (Lianou and Sofos, 2007; Sofos, 2008). Although more challenging, the extension of such a comprehensive food safety system beyond the manufacturing level (i.e., at retail and food service operations) is also of major significance for specific foodborne pathogens, namely those that are mainly introduced to foods as postprocessing contaminants (i.e., during preparation and additional handling by food workers). Examples of such foodborne pathogens are the bacteria *L. monocytogenes* and *Clostridium perfringens*, as well as viruses. Certainly, given that viruses are intracellular pathogens and, as such, are not expected to increase in population during processing distribution or storage of foods, it has been acknowledged that the emphasis with regard to their control should be placed in prevention of contamination by proper implementation of good hygiene practices, good manufacturing practices, and HACCP programs. In addition, the development of simple, efficient, and reproducible methods for foodborne viruses' detection and assessment of their survival in different foods have been identified as areas that future research should focus on (Koopmans and Duizer, 2004).

With reference to parasites, it has been suggested that the main challenge to their control is the complexity of foodborne parasitic infections; the various interconnected biological, economic, social, and cultural variables being involved; dictate the development and application of a holistic approach, based on a large amount of high-quality data as well as on systematic collaboration among sectors and disciplines (Broglia and Kapel, 2011). Finally, despite its continuing decline, owing to the intensive surveillance and screening programs applied the last two decades in the western world, BSE is expected to continue to be an area of concern and a target of eradication (Lee et al., 2013; Sofos, 2008).

Other major current or future meat safety challenges include animal identification and traceability issues, the safety and quality of organic and natural products, the development of improved and rapid testing and pathogen detection methodologies for laboratory and field use, regulatory and inspection harmonization issues at national and international levels, determination of responsibilities for zoonotic diseases between animal health and regulatory public health agencies, and establishment of risk-based food safety objectives and integrated meat safety assurance approaches (Blagojevic et al., 2021; Nastasijevic et al., 2020). Lastly, it has been acknowledged that climate change considerations can have a significant impact on risk assessment in the food safety area; thus, such

considerations should be accounted for in hazard characterization, prevalence/incidence evaluation, as well as in the assessment of the fate and distribution of biological hazards in the environment (EFSA, 2020).

16.8 Concluding remarks and outlook

Meat safety comprises one of the most important current and future public health concerns, and various challenges need to be successfully managed by the food industry and public health authorities if the burden of meatborne illness is to be reduced. Nonetheless, in order for such a goal to be attainable, it is crucial that integrated farm-to-fork approaches, extending throughout the food supply chain, are developed and embraced. With bacterial pathogens (including emerging or evolving pathogenic organisms) constituting the most serious meat safety concerns, the development of novel molecular biology approaches for their detection and characterization (based on culture-independent diagnostic tools and "big data" technologies) and the implementation of robust risk-based surveillance programs are the main areas that future research and interventions strategies, respectively, should engage in.

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Chapter 17

Meat safety: II Residues and contaminants

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17.1 Introduction

With the term food safety, we refer to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer. Numerous outbreaks of foodborne diseases have attracted media attention in the last few years and raised consumer concern, indicating the great importance of food safety and food quality. Food contaminants are any harmful substances unintentionally added to food. Food contaminants may be chemicals from natural sources (e.g., biological toxins), environmental contaminants (e.g., PCBs, dioxins, heavy metals), or they can be formed during food processing (e.g., acrylamide, PAHs, etc.). Veterinary drug and pesticide residues in food products are also a major concern in the food safety framework (Lawley et al., 2008).

Meat is defined as the flesh of animals used as food. In practice, this definition is restricted to a few dozen of mammalian species; however, it is often widened to include, except for muscle tissues, offal such as liver, kidney, and brains and other edible tissues. The big majority of the meat consumed in the EU is derived from sheep, cattle, and pigs: rabbit and hare are, generally, considered separately along with poultry. Meat and meat products have an important nutritional value being a great source of protein of high biological value, of essential amino acids, B vitamins, as well as of several essential minerals and trace elements, with particular relevance for iron. Worldwide meat production and consumption has tripled over the last 4 decades and increased 20% in just the last 10 years. The International Assessment of Agricultural Knowledge, Science, and Technology for Development (IAASTD) predicts that this trend will continue, especially because the growing urban middle classes in China and other emerging economies will adapt to the so-called western diet of people in North America and Europe. Therefore, meat safety is a top priority and challenge for

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scientists, regulators, risk managers, local and national authorities, and consumers worldwide.

The possible presence of the above-mentioned contaminants and residues in meat and meat products requires the employment of rapid, high-throughput, effective, and reliable analytical methods to ensure consumer's safety and product quality. A thorough overview of the chemical contaminants and residues possibly present in meat, risk assessment, and analytical strategies for their determination is presented in this chapter.

17.2 Chemical contaminants and residues

17.2.1 Veterinary drug residues

During the last decades, a large number of veterinary drugs have been used in food-producing animals for different purposes such as to prevent or treat bacterial diseases, to prevent stress-induced animal death and also as growth promoters for intensive animal production. Their improper use, nonrespect of withdrawal periods, and cross-contamination can lead to the presence of residues of veterinary drugs in animal tissues. The possible adverse effects on public health include among others allergic reactions in hypersensitive or sensitized individuals and the development of resistant strains of bacteria following the ingestion of subtherapeutic doses of antimicrobials (Rana et al., 2019; Schwarz and Chaslus-Dancla, 2001).

Different legal frameworks ensure that residues are kept in check through the establishment of maximum residue limits (MRLs) and the control of veterinary drug residues in samples taken from food-producing animals. Most recently, EU Regulation No 37/2010 was released on pharmacologically active substances, and MRLs were established in foodstuffs of animal origin (European Commission, 2010). Maximum levels (MLs) for the presence of coccidiostats or histomonostats in food are established in Commission Regulation (EC) No 124/2009 (European Commission, 2009). For the United States, MRLs are published on www.fsis.usda.gov and for Canada on www.hc-sc.gc.ca.

17.2.1.1 Antibacterials

Antibacterials are the most important drugs administered in veterinary medicine. They are classified based on their mechanism of action, chemical structure, and spectrum of activity or source. The main classes are aminoglycosides, amphenicols, β-lactams (cephalosporins and penicillins), macrolides, lincosamides, nitrofurans, quinolones, sulfonamides, tetracyclines, and miscellaneous.

Aminoglycosides

Aminoglycosides are broad-spectrum antibacterials isolated from *Streptomyces* and *Micromonospora* bacteria, and they are characterized by two or more amino sugars linked by glycosidic bonds to an aminocyclitol component (McGlinchey

et al., 2008). Aminoglycosides are administered both therapeutically and prophylactically to treat cattle, swine, and poultry. Improper use of aminoglycosides may generate residues in animal tissues that are potentially harmful due to their oto-, neuro-, and nephrotoxicity (Mastovska, 2011). EU has set maximum residue limits (MRLs) for eight aminoglycosides in muscle ranging from 50 μg/kg for gentamycin to 1000 μg/kg for apramycin. The highest MRLs are set for kidney (up to 20 mg/kg for apramycin). However, EU does not have established MRLs for three aminoglycosides.(Arsand et al., 2016).

Amphenicols

Amphenicols (chloramphenicol, florfenicol, and thiamphenicol) are broad-spectrum antibiotics with a phenylpropanoid structure. They are effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms (Mastovska, 2011). Chloramphenicol was widely used for many years in veterinary practice both therapeutically and prophylactically since it is highly effective and relatively cheap to produce (Amelin et al., 2017). However, it is hematotoxic for humans and may cause serious adverse effects such as bone marrow aplasia and aplastic anemia (Samsonova et al., 2012). Thus, the use of this antibiotic for the treatment of food-producing animals in the EU, the United States, and Canada has been prohibited and MRL cannot be established. Currently, thiamphenicol and florfenicol are used as alternatives to CAP for animal treatment. The MRL set for thiamphenicol is 50 μg/kg in muscle, fat, liver, and kidney, while for florfenicol it ranges from 100 μg/kg (in poultry muscle) to 3000 μg/kg (in bovine, ovine, and caprine liver).

β-Lactams

β-Lactams have been the most widely used antibacterial drugs for more than eight decades and still constitute the most important group of antibiotics. They are divided into two subcategories: penicillins and cephalosporins. They have as their basic structure a β-lactam ring and variable side chains that account for the major differences in their chemical and pharmacological properties. They are used as growth promoters, and chemotherapeutic, and/or prophylactic agents. Their extensive use in veterinary medicine practices causes numerous residues in foodstuffs that present a serious health hazard, mainly regarding the development of resistance in target organisms (Lara et al., 2012).

MRLs for penicillins in meat vary from 25 μg/kg for phenoxymethylpenicillin (penicillin V) in porcine and poultry (muscle, liver and kidney) to 400 μg/kg for clavulanic acid in bovine and porcine kidney. For cephalosporins, MRLs are set for cefalexin (200 μg/kg in muscle, fat, liver—1000 μg/kg in kidney), ceftiofur (1000 μg/kg in muscle—6000 μg/kg in kidney), cefapirin (50 μg/kg in muscle and fat—100 μg/kg in kidney), and cefquinome (50 μg/kg in muscle and fat—200 μg/kg in kidney).

Macrolides and lincosamides

Macrolides are characterized by a macrocyclic lactone ring containing 14, 15, or 16 atoms with sugars linked via glycosidic bonds. Lincosamides (lincomycin, clindamycin, and pirlimycin) are monoglycosides with an amino acid side chain (Jank et al., 2015). According to the EU Regulation No 37/2010, the MRLs for lincosamides range from 50 to 1500 μg/kg (lincomycin in fat and kidney, respectively) and for macrolides and in meat from 20 μg/kg (for gamithromycin in bovine fat) to 8000 μg/kg (for tulathromycin in porcine kidney).

Nitrofurans

Nitrofurans are synthetic antibacterial compounds, which contain a characteristic 5-membered nitrofuran ring in their structure. Nitrofurans belong to the wide category of antibiotics and were mainly used as feed additives for growth promotion, prophylactic, and therapeutic treatment of bacterial and protozoan infections (de la Torre et al., 2015) In 1995, the use of nitrofurans for livestock production was completely prohibited in the EU due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Vass et al., 2008).

Quinolones

Quinolones are synthetic antimicrobials based on the 4-oxo-1,4-dihydroquinolone skeleton that show very high activity against a wide range of diseases in livestock (Mashak et al., 2017). The first-generation of quinolones, such as nalidixic and oxolinic acids, acts against Gram-negative bacteria and is used to treat urinary tract infections (Belotindos et al., 2021). The second-generation quinolones are fluoroquinolones (enrofloxacin, danofloxacin, and ciprofloxacin) that contain a fluorine atom at position 6 and a bulky piperidine at position 7, broadening the antimicrobial spectrum to Pseudomonas species and some Gram-positive organisms, e.g., *Staphylococcus aureus* (Cheng et al., 2013; Er et al., 2013).

Quinolones are also important human drugs, and their extensive use in food-producing animals has contributed to the rapid emergence of resistance worldwide. In the EU, the use of seven quinolones (danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, oxolinic acid, and sarafloxacin) is approved in food-producing animals with the MRLs in meat matrices ranging from 50 μg/kg (for danofloxacin, marbofloxacin, and oxolinic acid in fat) to 1500 μg/kg (for flumequine in bovine, ovine, caprine, and porcine kidney). In poultry, the lowest MRL is established for sarafloxacin (10 μg/kg) in skin and fat and the highest for difloxacinin (1900 μg/kg) in liver.

Sulfonamides

Sulfonamides are one of the oldest classes of antimicrobial drugs, and they have been used for the treatment of humans and animals from the middle of the

twentieth century. They are derivatives of sulfanilic acid (*p*-aminobenzenesulfonic acid), and they are used for prophylactic and therapeutic treatment of infections caused by Gram-positive and Gram-negative bacteria and some protozoa (causative agents of malaria, toxoplasmosis, etc.) (Dai et al., 2017).

The long-term use of sulfonamides has resulted in a large number of sulfonamide-resistant bacterial strains (Dai et al., 2017; Xia et al., 2017). Additionally, the systematic human intake of sulfonamides through foods can cause allergic reactions, disbacteriosis, suppression of enzyme activity, alteration of the intestinal microflora, and promotion of sustainable forms of pathogens. There has also been evidence of hemotoxicity and carcinogenic effects of some sulfonamides, particularly sulfamethazine (Dmitrienko et al., 2014).

The MRL established in the EU Regulation No 37/2010 for sulfonamides in all meat matrices (tissues, fat and offal) is that the combined total residues of all substances within the sulfonamide group should not exceed 100 μg/kg.

Tetracyclines

Tetracyclines are broad-spectrum antibiotics that consist of a substituted 2-napthacenecarboxamide molecule (Araby et al., 2020; Önal, 2011). They are widely used in veterinary medicine for cost-effective prophylactic and therapeutic treatment and also as growth promoters in cattle and poultry (Karami, 2015). However, their usefulness has been reduced with the onset of bacterial resistance (Baghani et al., 2019). Their MRLs in meat matrices vary from 100 μg/kg in animal muscle tissue to 600 μg/kg in kidney.

Other antibacterials

Except for the antibacterials belonging in the precedent groups, there are also several subgroups of antibacterials, such as diaminopyrimidines, pleuromutilins, peptides, quinoxalines, and dapsone, that are widely used in meat-producing animals. Diaminopyrimidines are a class of organic chemical compounds that include two amine groups on a pyrimidine ring. Trimethoprim blocks folic acid synthesis in bacteria at a step later than the sulfonamides (Nelson and Rosowsky, 2001). The MRLs for diaminopyrimidines lie between 10 and 300 μg/kg for baquiloprim in bovine fat and liver, respectively.

Pleuromutilin and its derivatives are antibacterial drugs that inhibit protein synthesis in bacteria. This class of antibiotics includes retapamulin, valnemulin, and tiamulin. Avoparcin, colistin, bacitracin, efrotomycin, polymyxin, and virginiamycin are antibacterials that belong in the peptide group. Most are complex multicomponent compounds that possess large peptide molecules that often contain D-amino acids in contrast to naturally occurring proteins, which are composed of L-amino acids. These peptides disrupt both Gram-positive and Gram-negative bacteria by interfering with cell wall and peptidoglycan synthesis (Dasenaki et al., 2015). Only bacitracin and colistin are regulated in the EU with established MRLs at 150 μg/kg in all meat matrices (200 μg/kg for colistin in kidney).

Carbadox and olaquindox are both quinoxaline-1,4-dioxide antibacterials that are synthetically produced. They are light-sensitive compounds and require special handling precautions during analysis to prevent their decomposition. Metabolism studies have shown that carbadox is rapidly converted into its mono-oxy and desoxy metabolites, whereas quinoxaline-2-carbonic acid is considered to be the last remaining major metabolite and may serve as a marker residue. Both carbadox and its desoxy metabolite are carcinogenic compounds (Kesiunaite et al., 2008), and their use in animal feedings in forbidden in the EU since 1998 (European Commission, 1998).

Finally, dapsone (diamino-diphenyl sulfone), according to its chemical structure, is not comprehended in any antibacterial class, but according to its mechanism of action, it falls onto the sulfonamide group. It is used for the treatment of *Mycobacterium leprae* infections (leprosy) and for a second-line treatment against *Pneumocystis jirovecii* (Dasenaki et al., 2015). Dapsone is prohibited for use in the EU.

17.2.1.2 Anthelmintics

Anthelmintics (also called parasiticides, endectocides, and nematocides) are drugs used to treat parasitic warm infections, including flatworms (tapeworms and flukes) and roundworms (nematodes), which usually infect human, livestock, and crops, affecting food production.

Three main families can be distinguished on the basis of similar chemical structure and mode of action: benzimidazoles, nicotinic receptor agonists, and macrocyclic lactones (avermectines and milbemycins). The benzimidazoles consist of a ring system composed of a benzene ring fused with an imidazole ring. They exert their effect by binding selectively and with high affinity to the beta-subunit of helminth microtubule protein. The target site of the nicotinic agonists (e.g., levamisole, tetrahydropyrimidines) is a pharmacologically distinct nicotinic acetylcholine receptor channel in nematodes. The macrocyclic lactones (e.g., ivermectin, moxidectin) are a group of complex compounds isolated from *Streptomyces avermitilis*. They act as agonists of a family of invertebrate-specific inhibitory chloride channels that are activated by glutamic acid. The most frequently used anthelmintic compounds are levamisole, several compounds from the benzimidazole group (albendazole, cambendazole, fenbendazole, oxfendazole, and thiabendazole), and ivermectin (Romero-González et al., 2014).

Anthelmintic resistance is widespread and a serious threat to effective control of helminth infections, and, therefore, new classes of anthelmintics with new modes of action are being proposed. Thus, a new anthelmintic class named aminoacetonitrile derivative (AAD) has been developed, which is well tolerated and has low toxicity to mammals (Romero-González et al., 2014). Twenty-five benzimidazoles and macrocyclic lactones have been regulated in the EU for use in food-producing animals with their MRLs in meat matrices varying from 10 μg/kg (levamisole in bovine, ovine, porcine and poultry muscle, fat and kidney, and rafoxanide in bovine liver) to 5000 μg/kg (closantel in ovine kidney).

17.2.1.3 β-Agonists

β-Agonists are synthetic phenethanolamine compounds and were originally used as therapeutic treatments for asthma and preterm labor in humans (Lin et al., 2017). However, these compounds have also been misused as nutrient repartitioning agents in livestock, where they served to divert nutrients from fat deposition in animals to the production of muscle tissues (Shishani et al., 2003). β-agonists have been banned as growth promoters in many countries including the European Union and China because of their well-documented adverse effects on human health (European Commission, 1996).

17.2.1.4 Coccidiostats

Coccidiostats are antiprotozoal agents that act upon Coccidia parasites by inhibiting reproduction and retarding the development of the parasite in a host cell. They are most commonly used in poultry populations by addition in the feed at the authorized levels and observing the prescribed hygiene requirements (Dasenaki and Thomaidis, 2019). The disease can also occur in other food-producing animals including pigs, calves, and lambs. Even minor lesions of the intestinal wall due to coccidiosis can lead to poorer growth of the animal and lower feed conversion, reducing economic viability. Coccidiostats can be grouped in two major classes: the polyether ionophore antibiotics (monensin, lasalocid, maduramycin, narasin, salinomycin, and semduramycin) and the nonpolyether ionophores (often referred as synthetic compounds or chemicals). Polyether ionophore antibiotics are produced by fermentation with several strains of *Streptomyces* spp. and *Actinomadura* spp. They have both anticoccidial and antibacterial activity, and they are also used as growth-promoting agents and as an active compound against clostridiosis (Anadón and Martínez-Larrañaga, 2014).

Coccidiostats in food have been regulated in the Commission Regulation (EC) No 124/2009 and the MLs set in this regulation for animal muscle tissue ranges from 2 μg/kg (monensin, salinomycin, semduramycin, and manduramycin) to 300 μg/kg (nicarbazin in liver). In the EU Regulation No 37/2010, MRLs were also established for some coccidiostats ranging from 2 μg/kg for monensin (bovine muscle) to 7000 μg/kg for monepantel (ovine and caprine fat). Most recently, in Commission Implementing Regulation (EU) No 86/2012, an MRL was set for lasalosid in bovine and poultry species. In bovine meat, MRL was set at 10 μg/kg in muscle, 20 μg/kg in fat and kidney, and 100 μg/kg in liver (European Commission, 2012), while in poultry, MRL was set at 20 μg/kg in muscle, 100 μg/kg in skin, fat, and liver, and 50 μg/kg in kidney.

17.2.1.5 Nonsteroidal antiinflammatory drugs (NSAIDs)

Since the 1970s, the use of NSAIDs in breeding practices has progressively increased because of their efficacy as antipyretics, analgesics, and antiinflammatory drugs (e.g. in the treatment of mastitis in lactating cows). However,

NSAIDs are potentially toxic and can also cause other adverse side effects like allergic reactions, hepatotoxicity, hematopoietic, and renal troubles (Thompson, 2005). For these reasons, their use has been strictly regulated by the EU, which has set MRLs in meat from 1 μg/kg (diclofenac in bovine and porcine fat) to 1000 μg/kg (carprofen in bovine and *equidae* muscle, liver, and kidney).

17.2.1.6 Hormones

Anabolic steroids

Anabolic steroids are synthetic derivatives of testosterone with enhanced anabolic activity and reduced androgenic activity. They have been extensively used in husbandry practice to treat nonregenerative anemias, to enhance growth and performance, and to stimulate appetite. However, toxicological/epidemiological studies show that there are harmful effects to consumers; as a result, the public health is placed in risk. As a consequence, the use of anabolic steroids for fattening purposes has been banned in the European Union since 1986 (Kaklamanos et al., 2009).

Corticosteroids

Endogenous corticosteroids are produced by the adrenal cortex (e.g., cortisol) and have important effects on a variety of metabolic events, including glucose and protein metabolism. Nowadays, several exogenous corticosteroids are authorized for therapy in both human and veterinary practices. They are widely used to combat inflammatory diseases in food-producing animals, but they are also frequently employed as growth promoters. The European Union banned their administration for fattening purposes in 1996 (Draiscia et al., 2001). At present, MRLs have been established for betamethasone, dexamethasone, methylprednisolone, and prednisolone in bovine, caprine, porcine, and *equidae* matrices at low ppb levels (European Commission, 2010).

Thyreostats

Thyreostatic drugs, illegally administrated to livestock for fattening purposes, are banned in the European Union since 1981 (Council Directive 81/602/EC). They are orally active drugs, which disturb the normal metabolism of the thyroid gland. The result of thyreostats' use for animal fattening purposes is a weight gain caused by the increased filling of the gastrointestinal tract as well as the retention of water in edible tissues, by inhibiting the thyroid hormone production (Gentili et al., 2016; Wozniak et al., 2018). Lower quality meat is produced and also economical fraud takes place as water is sold for the price of meat (Vanden Bussche et al., 2009). Additionally, xenobiotic thyreostats are listed as compounds with teratogenic and carcinogenic properties and thus pose a possible human health risk.

17.2.1.7 Tranquilizers

Tranquilizers are administered to animals for sedation prior to anesthesia before transport to the market. Stress in animals is known to produce a deterioration of meat quality and pigs easily become stressed during transport. Some tranquilizers have analgesic effects (α2-agonists), but these are the exception since analgesia is not a hall mark of tranquilizers. They are classified into two categories: major and minor tranquilizers. Major tranquilizers include phenothiazides (acepromazine, promazine, and chlorpromazine), butyrophenones (azaperone, droperidol), and α2-agonists (xylazine, detomidine, medetomidine, dexmedetomidine, etc.), while minor consists of benzodiazepines (diazepam, midazolam, and zolazepam). Most tranquilizers are rapidly metabolized in the animal's body, and any residues are concentrated in the liver and/or kidney (Rankin, 2002). Chlorpromazine's use in food-producing animals has been prohibited by the EU Regulation No 37/2010.

17.2.2 Persistent organic pollutants

POPs are all synthetic chemicals, both intentionally or nonintentionally produced and released in the environment. They include pesticides and industrial products or unintended by-products resulting from industrial processes or combustions (Guo et al., 2019; Hernandez et al., 2017). Their persistence in the environment is remarkable as it may take them decades or centuries to be degraded and they bioaccumulate and biomagnified as they move through the food chain (World Health Organization, 2010).

POPs have low water solubility and high fat solubility and thus accumulate in fatty tissues of living organisms. In humans and animals, there are known adverse health effects of exposure to high levels of POPs; the effects may include cancer, damage to the nervous system, reproductive disorders, or disruption of the immune system. There is also increasing concern that chronic exposure to low levels of POPs may contribute to the burden of disease including increased incidence of breast and other cancers, learning and behavior disabilities and other neurodevelopmental problems, and reproductive problems such as decreased sperm quality and counts (World Health Organization, 2010).

17.2.2.1 Pesticides

Pest control in intensive agriculture involves pre- and postharvest treatment of crops with a variety of synthetic chemicals generically known as pesticides. Pesticides include herbicides and insecticides that are mainly used in the preharvest stages, rodenticides that are employed while storage of the crops and fungicides which can be applied at any stage of the process (Tongo and Ezemonye, 2015). Livestock can be contaminated through the consumption of contaminated feed and water, from pesticide application in animal production areas or from treatment of the animals themselves to prevent pest infestations

(Dimitrova et al., 2018; Oliveira et al., 2018). This can result in the presence of pesticide residues in animal-derived food products, and therefore, humans are mainly exposed to these chemicals through ingestion. The chronic effects of human exposure to pesticides from food intake are not completely defined, but there has been increasing evidence of carcinogenicity and genotoxicity, as well as endocrine disruption capacity (LeDoux, 2011).

To avoid any adverse impact on public health, the levels of pesticide residues in any food are controlled in terms of MRLs or tolerances (both are more or less synonyms). These limits are based on good agricultural practices and are designed to ensure that the amount of pesticide residues in food as small as practically possible. A database order by name of pesticide, active substance, or food product can be found in the website of the European Union, providing a search tool for all the EU MRLs set in the Regulation (EC) No 396/2005 (EU Pesticide Database, 2016). The meat products included in the database are animal tissues, offal, liver, kidney, and animal fat. Action may be taken if pesticides are detected above permitted limits, but this is relatively scarce for meat products as animals tend to metabolize modern pesticides very quickly and they do not bioaccumulate in meat (Kim, 2012).

The exception to this is the organochlorine pesticides (OCPs). OCPs are relatively stable, they are not quickly metabolized, and they bioaccumulate in adipose tissue in animals due to their high lipophilicity. Several OCPs (aldrin, chlordane, dieldrin, endrin, heptachlor, DDT, hexachlorobenzene, mirex, toxaphene, alpha-, beta- and gamma- hexachlorocyclohexane, pentachlorophenol, chlordecone, and endosulfan) are targeted for global elimination under the Stockholm Convention (Stockholm Convention, 2001). However, several studies have reported that meat products do not represent a relevant source for organochlorine pesticide intake (Brambilla et al., 2009).

After OCPs were used widely in soil and plants for some years, organophosphorus pesticides (OPPs), which are less persistent in the environment, became alternatives to OCPs. Some OPPs may also concentrate in the food chain and remain in meat, but OPPs are more hydrophilic compounds and are relatively easily degraded in the environment and metabolized by animals (Kim, 2012).

17.2.2.2 Dioxins and polychlorinated biphenyls (PCBs)

The term "dioxins" refers to two groups of halogenated aromatic hydrocarbons (polychlorinated dibenzo-*p*-dioxins = PCDDs; polychlorinated dibenzofurans = PCDFs) different in the number and/or position of chlorine atoms. This results in 210 substitution isomers (congeners), 75 PCDDs and 135 PCDFs, from which 17 congeners of PCDD/Fs, with chlorines at positions 2, 3, 7, and 8, are of toxicological interest. PCBs are chlorinated aromatic hydrocarbons produced by the direct chlorination of biphenyls (Zennegg, 2018). The family of PCBs comprises 209 congeners of which 20 reportedly have toxicological effects. Some of the PCBs have toxicological properties similar to those of dioxins and are, therefore, often referred to as "dioxin-like PCBs" (Lawley et al., 2008).

There are six, nondioxin like, indicator congeners (PCB 28, 52, 101, 138, 153, and 180), the sum of which is used as a parameter of PCB contents in food and environmental samples (Hoogenboom et al., 2021).

PCDD/Fs have never been intentionally produced industrially, and they have no commercial application. Their formation takes place primarily in thermal or combustion processes, resulting in different levels depending on the physical and chemical conditions in which the combustion process occurs. Other sources for the formation of dioxins are certain industrial processes (e.g., metallurgical industry, production of chemicals) or natural processes (e.g., volcanic eruptions, forest fires) or even from accidents at chemical factories (Andree et al., 2010). On the contrary, PCBs have been industrially produced and used in the manufacturing industry since the early 1930s, mainly as cooling and insulating fluids in electrical equipment. Although their manufacture and use have been banned since the 1970s, some PCBs still remain in use in older closed electrical equipment or sealing materials (Lawley et al., 2008).

PCDD/Fs and PCBs are persistent environmental contaminants that can be found ubiquitously in the environment, soil, sediments, and air. They enter the food chain through a variety of routes, with animal exposure occurring through contaminated feedstuffs as well as water and air. The main source of feed plant contamination is either through the soil or through airborne transport. PCDD/Fs and PCBs are practically insoluble in `water, and they tend to accumulate in fatty tissues of animals and fish. Several studies have reported that human exposure to high levels of these contaminants may lead to reproductive and developmental problems, increased heart disease, diabetes, and increased risk of cancer (Kim, 2012).

PCDD/Fs and dl-PCBs from food sources make up more than 90% of total human exposure. The contribution of animal-derived foods is significant, with dairy products contributing almost 40% and meat and meat products 30% to total human exposure. Fish and fish products are generally more contaminated but are consumed in smaller quantities (Kim, 2012). EU has established maximum levels (MLs) concerning the sum of dioxins in PCDD/F-TEQ, ranging from 1.0 pg/g fat for pork to 2.5 pg/g fat for beef and lamb and 4.5 pg/g fat for liver products (terrestrial animals). TEQ stands for toxic equivalents and is a sum parameter, taking into account the relative toxicities of the individual congeners, using the toxic equivalent factor (TEF) concept. The MLs for the sum of dioxins and dioxin-like PCBS range from 1.25 pg PCDD/F-PCB-TEQ/g fat for pigs to 10 pg PCDD/F-PCB-TEQ/g fat for liver and the ML for the sum of the six marker PCBs is 40 ng/g fat for all meat products (European Commission, 2011a).

Several studies have been presented concerning the concentrations of PCDD/Fs and PCBs in meat and meat products throughout the years. During the years 1999–2008, a study was conducted in the EU, and more than 7000 food samples from 19 member states were analyzed. According to this study, the mean concentration of PCDD/Fs in meat and meat products was 2.61 pg

TEQs/g fat for ruminants, 0.47 pg TEQs/g fat for pigs, and 3.34 pg TEQs/g fat for liver products. (Schrenk and Chopra, 2012).

More than 300 representative German samples of meat and meat products, including pork, poultry meat, beef, sheep, raw ham, and different types of sausage, were analyzed on their levels of PCDD/Fs, dl-PCBs, and marker PCBs in a 2009 study in Germany. This study revealed low concentrations of dl-PCBs in poultry and pork samples (more than six times below the action level concentration) in contrast with beef in which higher concentrations were determined (around 1 pg TEQs/g fat). However, lower concentrations were determined in veal, indicating that the uptake and deposition of these compounds are age-related. The median contents of PCDD/Fs-TEQ ranged from 0.09 pg/g fat (pork), 0.11 pg/g fat (poultry), 0.19 pg/g fat (lamb), up to 0.24 pg/g fat (beef) and were significantly below their maximum levels. The higher PCDD/Fs levels in beef and lamb may be again attributed to the different ages of slaughtering in Germany for pork (about 6 months), poultry (about 3 months), lamb (about 6 months), and beef (about 20 months). Finally, sum contents of the 6 marker PCBs in meat increased from pork (1.41 μg/kg fat), poultry meat (1.73 μg/kg fat), to beef (5.33 μg/kg fat). These levels are in every case below the MLs established (Andree et al., 2010).

More recently, a case study on the trends in the concentrations of a number of environmental pollutants, including PCDD/Fs and PCBs in food and the human dietary intake of those pollutants in Catalonia, Spain, is presented (Domingo and Nadal, 2016).

17.2.2.3 Polycyclic aromatic hydrocarbons (PAHs) and other environmental contaminants

PAHs are a large group of stable, lipophilic organic chemical contaminants containing two or more fused aromatic rings, which are considered of priority concern by health and environment administrations all over the world on the basis of their mutagenic and carcinogenic effects. They are produced during the partial combustion or pyrolysis of organic material and are common by-products of a number of industrial processes, including the processing and preparation of food and especially smoking (Lawley et al., 2008).

Smoking is one of the oldest technologies for conservation of meat and meat products. The temperature of smoke generation plays a decisive role because the amounts of PAH contained in smoke increase linearly with the temperature of smoke generation in the interval of 400–1000°C. Apart from the formation itself, the temperature also affects the structure and the number of PAHs (Šimko, 2009), which are mainly adsorbed in the surface of the meat and only slightly permeate the inside of smoked meat products. Different types of meat products are shown to have different adsorption capacities of PAHs on their surface (Andree et al., 2010).

About 660 different compounds belong to the PAH group. Benzo[*a*]pyrene (BaP), being the best-known carcinogenic PAH, has been accepted as the indicator

of total PAH presence in smoked foods. Since the September 1, 2014, the maximum level for BaP in smoked meat and smoked meat products is set at 2.0 μg/kg (European Commission, 2011b).

Other environmental contaminants that may be present in meat and meat products are polybrominated diphenyl ethers (PBDEs), polyfluorinated alkylated substances (PFAS), and polychlorinated naphthalenes (PCNs). PBDEs were first introduced into the market in the 1960s and are still used in some regions of the world as flame retardants. PFAS are widely found in the environment as a consequence of industrial applications, such as industrial polymers, waterproofing agents, stain-resistant coatings for fabrics, and fire-fighting foams, and PCNs are also industrial chemicals used as dielectics, lubricants, and plasticizers. The main pathway to human exposure to these persistent and accumulating chemicals is likely to be through dietary intake. However, the group of meat and meat products does not have the most significant contribution to the dietary exposure to these environmental pollutants, with fish and shellfish occupying the first place among the different food groups (Domingo and Nadal, 2016).

17.2.3 Heavy metals

The term "heavy metal" refers to any relatively high-density metallic element that is toxic or poisonous even at low concentrations. Although there are many elements that are classified as heavy metals, the ones of most concern, taking in mind their biotoxic effects and presence in food, are arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). No essential functions have been described so far for these metals and their consumption, even at low concentration levels, can cause toxic and even carcinogenic effects as they tend to accumulate to the human body (Lawley et al., 2008).

Heavy metals are continually released into the environment from either natural or anthropogenic sources and enter into the food chain, making foodstuffs a major source of human exposure to toxic metals. Mineral mining, energy extraction, irrigation, fertilization, pesticide applications, industrial emissions, and car exhausts are the main anthropogenic sources of toxic metals. Contamination of food can also occur during manufacturing, processing, and storage (Lawley et al., 2008). Cardiovascular, kidney, nervous, and bone diseases as well as carcinogenesis, mutagenesis, and teratogenesis have been reported to be related to chronic exposure to food contaminated with toxic metals (Kim, 2012). Toxic elements, particularly As and Hg, are mainly found in seafood. Human exposure to these metals through the consumption of meat and meat products is relatively low, except for animals raised in severely contaminated areas. Pb and Cd accumulate mostly in offal (liver and kidneys), while the toxic metal content of muscle is generally low (Kurnaz and Filazi, 2011).

For Cd and Pb, maximum levels (MLs) in meat and offal have been established by Commission Regulation (EC) No 1881/2006 (European

Commission, 2006). The MLs set for Pb are from 0.1 mg/kg in meat of bovine animals, sheep, pig, and poultry to 0.5 mg/kg for edible offal of these animals, while for Cd the MLs are 0.05 mg/kg in meat of bovine animals, sheep, pig, and poultry, 0.2 mg/kg in horsemeat, 0.5 mg/kg in liver, and 1 mg/kg in the kidney of these animals. In 2010, the 73rd Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated cadmium and lead intake levels based on findings from a number of recent epidemiological studies. For Cd, the Committee established a provisional tolerable monthly intake (PTMI) of 25 μg/kg bodyweight, based on its long half-life, while for lead it was concluded that the previous PTWI standard (25 μg/kg bodyweight) was no longer appropriate as it was confirmed that lead reduces children's IQ and increases adults' systolic blood pressure. Accordingly, the relevant PTWI standard could no longer be considered health protective and was withdrawn (JECFA, 2010).

Several studies have reported the occurrence of Pb and Cd in meat and meat products. Pb is present at low concentrations in animal tissue (typically between 10 and 200 μg/kg), while higher levels (even above 1 mg/kg) are found in offal. The concentration of cadmium in food-derived animal products depends on the cadmium level in the feed; mean levels are usually <0.01 mg/kg in cattle, pigs, sheep, rabbits, and poultry. However, cadmium accumulation leads to high mean levels in liver at kidney (0.2 mg/kg), where the maximum concentrations can even reach 3 mg/kg (Hartwig and Jahnke, 2012). Concentration levels of heavy metals found in meat and meat products depend on the species, the breeding regions, and the age of the animals (Andree et al., 2010).

17.2.4 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by fungal species with diverse potent pharmacological and toxic effects in humans and animals. They are produced during mold development on plants, either in the field or during storage period, and they can be found as natural contaminants in many vegetal foods or feeds, mainly cereals, but also fruits, nuts, and grains. Mycotoxins have carcinogenic (aflatoxins B1 and M1), hepatotoxic (aflatoxins), immunotoxic (trichothecenes, fumonisins), nephrotoxic (Ochratoxin A), or estrogenic effects (ZON) on humans and animals (Algammal et al., 2021; Bailly and Guerre, 2009; Dada et al., 2020).

For human consumers, the main source of exposure to mycotoxins is cereals and cereal-based products. Mycotoxins in meat products may originate from residue in animal feed, direct growth of toxigenic molds, usually on the outer layer of meat or from the addition of flavoring materials, such as spices (Abd-Elghany and Sallam, 2015). Additionally, the great stability of these compounds allows them to resist to classical process of cooking and/or sterilization, making the consumption of mycotoxin-contaminated animal-derived food a potential health hazard (Bailly and Guerre, 2009).

Among mycotoxins of greatest public health and agroeconomic significance are aflatoxins and ochratoxin A (OTA). Among the four natural aflatoxins (B1, B2, G1, and G2), AFB1, and its phase I metabolite AFM1 are highly carcinogenic. The intense metabolism of AFB1 in the liver is the reason why a very small part of the native molecule can be detected in animal tissues. In muscles, only low levels of AFB1 are found, even after exposure of the animals to aflatoxin B1, while liver and kidney always contain more toxin and metabolites than muscles (Abd-Elghany and Sallam, 2015; Zhao et al., 2015). Several studies have also indicated that meat processing conditions, especially in countries with hot climate, may result in aflatoxin synthesis even though the aflatoxin levels are low (below 10 μg/kg) (Bailly and Guerre, 2009).

The occurrence of OTA in animal products is not generally considered to be a major public health concern since the overall contribution of products of animal origin to human exposure has been estimated to be not more than 3% of the total ingested OTA (Duarte et al., 2012). The same applies for ZON, trichothecenes and fumonisins which, due to their metabolic pathways and kinetic properties, do not represent a significant hazard as residual contaminant of muscle foods (Bailly and Guerre, 2009).

17.3 Risk assessment

Chemical risk assessment is a scientific process, targeted on public health protection, aiming to estimate how much of a contaminant consumer (either in general and/or population-sensitive groups such as children or elderly people) may be exposed to without any considerable risk. It provides the scientific basis for regulatory decisions and legal frameworks aiming at ensuring, preserving, and improving the safety of human exposure to chemicals. Risk assessments that underpin the development of regulatory measures are conducted by authoritative independent committees of scientific experts such as the European Food Safety Authority (EFSA), the Food and Agriculture Organization (FAO), and the World Health Organization (WHO) (Benford, 2012).

Health risk assessment is commonly divided into four steps providing answers to the following questions: (1) hazard identification—what can go wrong? (2) hazard characterization—what are the consequences? (3) exposure assessment—how can it happen? (4) risk estimation—what is the likelihood the adverse effect would happen? (Brambilla et al., 2009).

Hazard identification involves toxicological tests (single and repeat dose exposure to chemical contaminants) to define the potential harm in different stages of the life cycle and to identify adverse effects. Hazard characterization is closely linked to hazard identification and describes the human adverse health effects that may result from the exposure to chemical contaminants. Ideally, it includes quantitative information in terms of a dose-response relation and the probability of adverse outcomes. Dose-response information is essential for quantifying an adverse health effect. This may be graphically

presented as the relationship between the increase of a dose and the increase of a pertinent biological response. Such dose-response curve is essential for identifying a nonactive dose taken as being the no observed adverse effect level (NOAEL), the highest dose of a substance that causes no detectable adverse alteration in line with defined treatment conditions (Lozano and Trujillo, 2012).

For chemicals that are neither genotoxic nor carcinogenic, health authorities recommend maximum acceptable or tolerable levels such as acceptable daily intake (ADI), reference dose (RfD), especially for pesticides, tolerable daily intake (TDI), and provisional tolerable weekly intake (PTWI) for contaminants that may accumulate in the body. In calculating an MRL, the ADI, the residue depletion patterns of a compound in the edible tissues of a particular food-producing animal, and the theoretical food intakes are taken into account (Dasenaki et al., 2015).

The exposure assessment to chemicals in food requires information on the occurrence of the chemical in food products and on the amounts of those foods consumed by different population groups. Occurrence data for contaminants and residues can be obtained by monitoring programs, targeted surveys, or total diet study approaches. Diet exposure to pesticide and veterinary drug residues can be evaluated either before the drug has been approved to use (preregulation) or after it has potentially been in the food supply for years (postregulation). In the preregulation evaluation, the chemical concentration data are obtained by the manufacturer, while in the post regulation evaluation the concentration data are obtained from foods in the marketplace. Consumption data used in exposure assessments must include not only the general population but also vulnerable population groups and groups expected to have different exposures than those of the general population (Beyene, 2015). Finally, risk characterization comprises the comparison of the exposure results with health-based guidance values where one exists (Benford, 2012).

17.4 Analytical methods

The possible presence of the above-mentioned contaminants in food requires the development of robust, fast, efficient, sensitive, and cost-effective analytical methods in order to ensure food safety. The large number of contaminants that have to be monitored has caused a steady increase of the number of multianalyte methods developed in recent years. This appears as a considerable challenge since the great differences in the physicochemical properties of the contaminants belonging to different chemical groups pose difficulties for their simultaneous extraction, clean up, and analytical separation. The scope of the current section is limited to the trend observed toward multicompound/multiclass methods for the analysis of contaminants and residues in meat and meat products, using generic sample-preparation procedures and liquid and gas chromatography (LC and GC) coupled to mass spectrometry (MS) as detection techniques.

This includes mainly the use of solvent extraction (SE), solid-phase extraction (SPE), and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)-based approaches (Dasenaki and Thomaidis, 2019).

17.4.1 Sample preparation techniques

17.4.1.1 Extraction techniques

A number of techniques have been developed for the extraction of analytes from meat samples that are usually prepared initially by grinding before extraction of the analytes from the matrix. The extraction is performed using a solvent or fluid and one of a number of possible approaches (SE, pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), QuEChERS, etc.), and it is influenced by solubility, penetration of the sample by the solvent (mass transfer), and matrix effects (Ridgway et al., 2012).

In the high-throughput determination of contaminants and residues in meat and meat products, SE is frequently reported as the method of choice. The selection of the solvent is not only based on the target analyte but also on the matrix, aiming to maximize the target compound's extraction and to minimize the extraction of matrix constituents in order to prevent excessive matrix effects (Dasenaki et al., 2015; Dasenaki and Thomaidis, 2019). The most commonly used solvents are acetonitrile, methanol, and ethyl acetate, in conjunction with aqueous solvents containing various additives (ammonium or sodium salts, organic acids, etc.) that are added to the aqueous phase to aid extraction and to help to avoid emulsions (Wang et al., 2021). However, SE provides limited selectivity, and this frequently results in the need for further cleanup or analyte enrichment/concentration before instrumental analysis. For multiresidue/multiclass methods, the choice of solvent generally represents a compromise in order to obtain optimum extraction results for the majority of the compounds (Ridgway et al., 2012). Several applications of SE in the analysis of veterinary drug and pesticide residues in meat and meat products are presented in Table 17.1.

PLE and MAE are the most widely used instrumental extraction techniques in food analysis. PLE uses high pressure and temperature to achieve fast and efficient extraction of the analytes from the solid matrix. Depending on the analytes' polarity, the solvents used in PLE can be nonpolar as dichloromethane, hexane, and ethyl acetate or polar as water. The use of water as an extraction solvent offers an environmentally friendly alternative to the use of large volumes of organic solvents. The unique properties of water, due to the level of hydrogen bonding, mean that as the temperature is increased (under pressure) the polarity decreases and therefore extraction becomes more selective (Moga et al., 2021). PLE has been used for the determination of veterinary drugs, pesticides, and other POPs in meat matrices (Bjorklund et al., 2006; Blasco and Masia, 2011; Garrido Frenich et al., 2006).

TABLE 17.1 Applications of multiresidue analysis of veterinary drugs and pesticides in meat and meat products (in the years 2011–21).

Compounds	Matrix	Sample preparation technique
Antibacterials (16) (4 subclasses)	Bovine muscle tissue	ACN extraction, d-SPE with PSA (QuEChERS) compared to PLE
Antibacterials (34) (6 subclasses)	Porcine muscle	ACN extraction with fast partition at very low temperature
Antibacterials (53) (8 subclasses)	Cattle, pig muscle	EDTA-McIlvaine buffer extraction
Antibacterials (38) (3 subclasses), hormones (7), anthelmintics (23), β-agonists (14), pharmaceuticals (19) and dyes (4)	Meat and other food products	Acidified ACN extraction, SPE cleanup
Antibacterials (60) (6 subclasses)	Muscle tissues	Two protocols: ACN extraction and acidified ACN extraction, SPE cleanup
Antibacterials (16) (6 subclasses), anthelmintics (5), coccidiostats (4), pesticides (35), mycotoxins (2) and other contaminants (58)	Meat, liver and other food products	Acidified ACN extraction—QuEChERS
Antibacterials (76) (6 subclasses), anthelmintics (18), coccidiostats (2), tranquilizers (7) and dyes (2)	Muscle, kidney, liver, and other food products	Extraction with ACN and EDTA-succinate buffer, SPE cleanup
Antibacterials (71) (10 subclasses), anthelmintics (12), β-agonists (9), NSAIDs (6), tranquilizers (10) and hormones (12)	Bovine kidney	ACN-H_2O (4:1, v/v) extraction, hexane partitioning
Antibacterials (44) (9 subclasses), anthelmintics (41), β-agonists (5), NSAIDs (7), tranquilizers (9), hormones (9) and mycotoxins (1)	Bovine muscle	ACN/water (4/1, v/v) extraction, d-SPE and hexane cleanup
Pesticides (110)	Meat and other food products	Simplified QuEChERS

Stationary phase	Mobile phase	Detection identification	Reference
XTerra MS C_{18} (100×2.1 mm, 3.5 µm)	A: aqueous 10 mM ammonium formate B: 10 mM ammonium formate in MeOH	LC-ESI-MS/MS (+)	Blasco and Masia (2011)
Zorbax Eclipse XDB C_{18} (150×4.6 mm, 5 µm)	A: H_2O/ACN (95:5 v/v), formic acid 0.1% and B: H_2O/ACN (5:95 v/v), formic acid 0.1%	LC-ESI-MS/MS (+)	Lopes et al. (2011)
AQUA C_{18} (150×2.1 mm, 3 µm)	A: 0.2% formic acid in H_2O B: 0.2% formic acid in ACN	LC-ESI-MS/MS (+)	Bohm et al. (2011)
Zorbax Eclipse XDB C_{18} (100×3.0 mm, 1.8 µm)	A: aqueous ammonium formate 5 mmol/L with 0.1% formic acid B: 0.1% (v/v) formic acid in ACN	UHPLC-QTOFMS (+)	Deng et al. (2011)
RP_{18} Purospher column (125×3 mm, 5 µm)	A: aqueous HFBA 1 mM with 0.5% formic acid B: 0.5% formic acid in MeOH/ACN (50:50; v/v)	LC-ESI Orbitrap MS (+) and (−)	Hurtaud-Pessel et al. (2011)
Hypersil Gold AQ (50×2.1 mm, 1.9 µm)	A: aqueous formic acid 0.1% (v/v) B: 0.1% (v/v) formic acid in ACN	LC-ESI Orbitrap MS (+)	Filigenzi et al. (2011)
Kinetex Core-Shell C_{18} (150×2.1 mm, 2.6 µm)	A: 0.3% (v/v) formic acid and 5% ACN in H_2O B: 0.3% (v/v) formic acid and 5% H_2O in ACN	LC-ESI Orbitrap MS (+) and (−)	Kaufmann et al. (2011)
Prodigy ODS-3 (150×3 mm, 5 µm)	A: aqueous formic acid 0.1% (v/v) B: 0.1% (v/v) formic acid in ACN	LC-ESI-MS/MS (+)	Schneider et al. (2012)
Waters Acquity RP HSS T3 (2.1×100 mm, 1.8 µm)	A: ACN: H_2O 5:95 (v/v) B: ACN both with 0.1% formic acid	LC-ESI-MS/MS (+) and (−)	Geis-Asteggiante et al. (2012)
Zorbax Eclipse XDB C_{18} (2.1×150 mm, 3.5 µm)	A: aqueous ammonium formate 5 mM, 0.1% formic acid, and 0.02% CAN B: MeOH with 5 mM ammonium formate, 0.1% formic acid	LC-ESI-MS/MS (+)	Anagnostopoulos et al. (2013)

Continued

TABLE 17.1 Applications of multiresidue analysis of veterinary drugs and pesticides in meat and meat products (in the years 2011–21)—cont'd

Compounds	Matrix	Sample preparation technique
Phenyl acetanilide pesticides (25)	Beef, cattle liver, chicken, and other food products	Acetone-*n*-hexane (1:2, v/v) extraction, SPE cleanup
Pesticides (47)	Meat and other food products	Simplified QuEChERS
Pesticides and veterinary drugs (>350)	Meat (chicken, pork, beef)	ACN—H_2O (3:1, v/v) extraction
Antibacterials (7) (5 subclasses), anthelmintics (29), β-agonists (1), coccidiostats (13), NSAIDs (1), tranquilizers (3), hormones (5) and other veterinary drugs (6)	Meat and other food products	Extraction with ACN, freezing, d-SPE cleanup
Organochlorine pesticides (33)	Fatty and high water content foods	MSPD—gel permeation Chromatography—SPE cleanup
Pesticides (111)	Pork muscle tissue, cattle fat tissue, and other food products	QuEChERS extraction with ACN: H20 (1:1), SPE cleanup
Pesticides and veterinary drugs (128)	Swine, chicken, bovine muscle, and liver	Modified QuEChERS
Antibacterials (43) (4 subclasses)	Pork, beef, chicken	ACN/water (4/1, v/v) extraction, d-SPE cleanup with C18
128 Antiparasitic drugs and metabolites (46 pesticides, 19 coccidiostats, 9 sulfonamides and 54 anthelmintics)	Swine, chicken, and bovine meat	QuEChERS

	Stationary phase	Mobile phase	Detection identification	Reference
	DB-1701 MS (30 m × 0.25 mm I.D., 0.25 µm film thickness)		GC-MS	Li et al. (2013)
	VF-5 ms (30 m × 0.25 mm I.D., 0.25 µm film thickness)		GC-(EI)-MS-MS	Anagnostopoulos et al. (2014)
	Hypersil GOLD aQ C_{18} (2.1 × 100 mm, 1.7 µm)	A:aqueous ammonium formate 4 mM, 0.1% (v/v) formic acid B: ammonium formate 4 mM, 0.1% (v/v) formic acid in MeOH	UHPLC-Orbitrap-MS (+) and (–)	Gomez-Perez et al. (2014)
	Acquity CSH C_{18} (2.1 × 150 mm, 1.7 µm) Acquity BEH HILIC (2.1 × 100 mm, 1.7 µm)	**RP**: A: MeOH/ACN 3:1(*v*/v) B: H_2O, both with 0.5 mM ammonium formate, 0.5 mM formic acid **HILIC**: A: ACN B: H_2O with 50 mM ammonium formate, both with 50 mM formic acid	LC-ESI-MS/MS (+) and (–)	Chung and Lam (2015)
	Zebron ZB-MR-2 (30 m × 0.25 mm I.D., 0.20 µm film thickness)		GC-MS	Chung and Chen (2015)
	HP-5MS (15 m and 30 m × 0.25 mm I.D., 0.25 µm film thickness) and Acquity UPLC BEH C_{18} (100 × 2.1 mm, 1.7 µm)	A: aqueous MeOH (0.49 mol/L) with formic acid (26.5 mmol/L) B: formic acid (26.5 mmol/L) in MeOH	GC-MS/MS and GC-MS(NCI) and LC-MS/MS	Lichtmannegger et al. (2015)
	Thermo Hypersil C_{18} (2.1 × 150 mm, 5 µm)	A: 12.5 mM aqueous ammonium formate at pH 4 B: 2.5 mM ammonium formate in ACN/MeOH (50/50, v/v)	LC-ESI-MS/MS (+) and (–)	Wei et al. (2015)
	Waters Acquity UPLC (2.1 × 100 mm, 1.6 µm)	A: aqueous formic acid 0.1% (v/v), B: 0.1% (v/v) formic acid in ACN	LC-ESI-MS/MS (+)	Yamaguchi et al. (2015)
	Thermo Hypersil C_{18} (150 × 2.1 mm, 5 µm)	A: 12.5 mM aqueous ammonium formate at pH 4 B: 12.5 mM ammonium formate in ACN/MeOH (50/50, v/v)	LC-ESI-MS/MS (+) and (–)	Wei et al. (2015)

Continued

TABLE 17.1 Applications of multiresidue analysis of veterinary drugs and pesticides in meat and meat products (in the years 2011–21)—cont'd

Compounds	Matrix	Sample preparation technique
Macrolides (5) and lincosamides (2)	Cattle, swine and chicken muscle, and other food products	LLE with ACN
Pesticides (192) and environmental contaminants (51)	Cattle, swine, and poultry muscle	Modified QuEChERS
Thyreostats (6)	Meat-based baby foods	MSPD
Aminoglycosides (10)	Muscle and other food products	Extraction with trichloroacetic acid, clean up with low temperature precipitation and C18 bulk
Antibiotics (28)	Pork meat	Prediffusion of antibiotic residues into agar
b-Agonists (7)	Muscle and viscera	LLE, SPE
Amphenicols (3) and their derivatives	Meat (beef, pork, poultry), meat by-products, and other food products	Extraction with ACN and dilution of the extract twice with H_2O
Pesticides (188)	Beef meat	QuEChERS
17 Common classes of veterinary drug compounds	Bovine muscle, porcine muscle, bovine liver, bovine kidney, and chicken liver	EMR-lipid cartridge cleanup
Veterinary drug residues (10) (fluoroquinolones, sulfonamides, macrolides, and tiamulin)	Chicken muscle	1% acetic acid in ACN, defatting with hexane

Stationary phase	Mobile phase	Detection identification	Reference
Agella Durashell RP (100 mm_2.1 mm, 5 µm)	A: 0.1% formic acid in H_2O B: 0.1% formic acid in ACN	LC-ESI-MS/MS (+)	Jank et al. (2015)
DB-5 ms (15 m × 0.53 mm I.D., 1 µm film thickness) and Waters BEH C_{18} (2.1 × 100 mm, 1.7 µm)	A: 95/2.5/2.5 (v/v/v) H_2O/MeOH/ACN B: 1/1 (v/v) MeOH/ACN, both containing 20 mM ammonium formate and 0.1% formic acid	GC-MS/MS and LC-MS/MS (+)	Han et al. (2016)
Core-shell phase column Kinetex PFP (100 × 2.1 mm; 2.6 µm; 92 Å)	A: H_2O B: H_2O: ACN (50:50, v/v), 5 mM in formic acid	LC-ESI-MS/MS (+) and (−)	Gentili et al. (2016)
WatersX-Terras C18 column (100_2.1 mm, 3.5 mm)	A: 10 mM NFPA in H_2O B: 10 mM NFPA in ACN	LC-MS/MS (+) and LC-QTOF-MS	Arsand et al. (2016)
Phenomenex Luna C18 column (100 × 3 mm, 2.5 mm)	A: ACN B:10 mM HFBA aqueous solution	QTRAP LC-MS/MS (+) and (−)	Ngoc Do et al. (2016)
Agilent Zorbox SB-C18 column (150 mm × 4.6 mm, 5 mm)	A: 5 mM ammonium acetate in H_2O B: MeOH	LC-ESI-MS/MS (+)	Lin et al. (2017)
Acclaim 120 C18 (2.2 µm) column (150 × 2.1 mm)	A: 0.1% solution of formic acid in H_2O with the addition of 5 mM of ammonium formate B: 0.1% solution of formic acid in ACN	LC ESI-QTOF-MS (+) and (−)	Amelin et al. (2017)
Shimadzu Shim-pack XR-ODSII column (2.0 × 100 mm, 2.2 µm particle size) and a Phenomenex Synergi Fusion-RP column (2.0 × 50 mm, 2.5 µm)	A: 10 mM ammonium acetate in 10% formic acid B: MeOH	LC-ESI-MS/MS (+) and (−)	Oliveira et al. (2018)
Poroshell 120 EC-C18 column of 150 × 2.1 mm, 2.7 m	A: 0.1% FA in water. Solvent B: 0.1% FA in ACN	LC-ESI-MS/MS (+) and (−)	Zhao et al. (2018)
Phenomenex Luna C18 RP column (150 mm × 2.1 mm, 5 µm)	A: 0.05% formic acid in ACN B: 0.05% formic acid in water	LC-ESI-MS/MS (+)	Zhang et al. (2018)

Continued

TABLE 17.1 Applications of multiresidue analysis of veterinary drugs and pesticides in meat and meat products (in the years 2011–21)—cont'd

Compounds	Matrix	Sample preparation technique
Coccidiostats (16)	Animal tissues and other food products	SLE with ACN, d-SPE cleanup with C18
Residues and metabolites of pharmacologically active substances (164)	Chicken, porcine and bovine meat	Extraction with 0.1% solution of formic acid in ACN, SPE, d-SPE
Mycotoxins (17)	Dried beef	SLE extraction with ACN/H_2O/acetic acid (79:20:1, v/v/v)
Polychlorinated dibenzo-*p*-dioxins and dibenzofurans, and dioxin-like and nondioxin-like polychlorinated biphenyls	Adipose tissue, meat, and livers from pigs, cows, sheep, and goats	Extraction using microwave heating

Abbreviations: *ACN*, acetonitrile; *d-SPE*, dispersive-solid-phase extraction; *LLE*, liquid liquid extraction; *MeOH*, methanol; *MSPD*, matrix solid-phase dispersion; *MSPE*, magnetic solid-phase extraction; *PLE*, pressurized liquid extraction; *QuEChERS*, quick easy cheap effective rugged safe; *RP*, reversed phase; *SLE*, solid liquid extraction; *SPE*, solid-phase extraction.

	Stationary phase	Mobile phase	Detection identification	Reference
	Acquity BEH HILIC silica column (100 mm × 2.1 mm, 1.7 µm)	A: ACN B: aqueous ammonium formate 1 mM with 0.1% formic (80/20, v/v)	LC-ESI-MS/MS (+) and (–)	Dasenaki and Thomaidis (2019)
	Phenomenex Luna Omega column (100 × 2.1 mm, 1.6 µm)	A: 0.1% formic acid in water B: 0.1% formic acid in H_2O, and C: 0.1% formic acid in MeOH	UPLC Q-Orbitrap HRMS (+) and (–)	Pugajeva et al. (2019)
	Raptor ARC-18 column from Restek (2.7 µm, 2.1 mm, 100 mm)	A: 0.1% formic acid in H_2O B: 0.1% formic acid in ACN:MeOH (50:50 v/v)	LC-ESI-MS/MS (+)	Dada et al. (2020)
	GC 6890 N (GC column DB5 MS 60 m, 0.25 mm, 0.25 mm)		GC-HRMS	Hoogenboom et al. (2021)

MAE is also an attractive extraction method, due to the drastic reduction of extraction time and solvent consumption compared with conventional methods. It uses microwave energy to heat a solvent in contact with a sample, in order to partition analytes from the sample matrix into the solvent. Although MAE is established as a routine, well-developed method for sample preparation in environmental analysis (soils, sediments, etc.), its applications in the extraction of chemical contaminants from meat products are scarce (Hermo et al., 2005; LeDoux, 2011; Purcaro et al., 2009). PLE and MAE had emerged as very promising techniques few years ago, but their use is still limited because they involve additional cost due to the instrumentation needed while they give similar recoveries to SE (Campo and Picó, 2015). UAE is used for the extraction of analytes from solid samples, applying ultrasound radiation in a water bath or with other devices, such as probes, sonoreactors, or microplate horns. The technique is relatively inexpensive, as no specialized equipment is necessary and multiple samples can be extracted simultaneously. It has found several applications in food analysis (Tadeo et al., 2010).

Special mention has to be done to the QuEChERS extraction and its modifications. QuEChERS is a variation of SE that has become very popular for multiclass residue extraction in various food matrices, including meat and meat products. It comprises extraction with an organic solvent (acetonitrile, buffered or not) and phase separation using a high salt content, in some cases followed by dispersive SPE (d-SPE). It was initially developed for pesticide residue determination, but its application fields are continuously extended to other types of contaminants, as shown in Table 17.1. QuEChERS advantages are its simplicity, rapidness, flexibility, and low solvent consumption, while its wide applicability to different contaminants in different matrices makes it an ideal candidate as an universal sample preparation technique (Campo and Picó, 2015).

Alternatively to SE, a matrix solid-phase dispersion (MSPD) approach can be used for the extraction of chemical contaminants from meat matrices. In MSPD, the sample is mixed with a matrix, such as C18 bonded silica, followed by washing and elution with a small volume of solvent. The solid dispersion phase provides a porous structure to enable the solvent to penetrate into the matrix and extract the analytes, and it can also retain unwanted matrix components, such as fats/lipids. MSPD's biggest advantage is that it can combine the procedures of homogenization, disruption, extraction, and clean-up into one simple process and is particularly useful for animal tissue samples, where drying before extraction can be problematic due to the presence of a high proportion of fats (Ridgway et al., 2012).

17.4.1.2 Sample clean-up techniques

When generic extraction approaches are used sample extracts contain a large number of matrix coextractants. An effective clean-up minimizes matrix effects improving sensitivity, achieving more consistent and repeatable results and extending the lifetime of the chromatographic column.

One of the most common techniques utilized for the determination of trace contaminants in food is SPE, which is mostly used as an additional cleanup/preconcentration step after SE. It involves the partitioning of the analytes between the solid sorbent extraction phase and the sample matrix (liquid phase). Matrices can be extracts in nonpolar or aqueous solvents, and the separation is achieved based on the different relative affinities for the two phases based on adsorption, size, or charge (polarity) (Ridgway et al., 2012). With the packing materials available for SPE, it is possible to find reversed-phase octadecyl silica (C18), hydrophilic-lipophilic balance (HLB) phases, ionic exchangers (anionic and cationic, strong and weak), graphitized black carbon (GBC), Florisil, polystyrene-divinylbenzene materials, and carbon nanotubes, as well as selective SPE columns (molecularly imprinted polymers (MIPs) or immunoaffinity columns), which provide unique specificity to isolate the compounds of interest (Campo and Picó, 2015). SPE has found huge applications in residue and contaminant analysis in meat matrices. Most recent applications of SPE in multiresidue analysis of pesticides and veterinary drugs in meat and meat products are presented in Table 17.1.

Another type of SPE is d-SPE, which is typically part of the QuEChERS method, and it involves adding sorbent material (PSA, carbon, or C18) to a raw extract, followed by shaking and centrifugation and subsequent isolation of the supernatant. The sorbent used for d-SPE is critical; PSA is effective at retaining fatty acids and other organic acids present in food, while C18 removes lipophilic compounds. Thus, for meat and meat products, which have higher lipid content, C18 or a combination of PSA/C18 is more effective (Dasenaki et al., 2015).

17.4.2 Instrumental analysis

Methods coupling separation techniques (LC and GC) and MS are characterized by outstanding specificity, sensitivity, high-throughput capability, and ease of automation. Thus, with the detection of residues and contaminants in food at ultratrace concentrations being imperative, in respect to food safety and quality, LC/GC-MS is by far the most suitable analytical techniques.

GC techniques coupled to MS systems are the most used analytical techniques for the detection of PCBs, PBDEs, PAHs, and contaminants related to food processing, while LC-MS methodologies are widely used for the determination of veterinary drugs and pharmaceutical compounds, mycotoxins, and PFAS. Both GC and LC-MS methods are used for the determination of pesticides in food matrices (Campo and Picó, 2015).

GC is more than 60 years old and is particularly suitable for volatile, semivolatile and thermally stable compounds. It has also been applied to a wide range of nonvolatile compounds after a derivatization step, converting them into volatile derivatives. The combination of GC to MS is, most commonly through electron impact ionization (EI), and more rarely through chemical ionization

(CI). Also, comprehensive GC (two dimensional GC×GC) has gained special attention as it can significantly enhance the resolving power of a separation (the co-eluting peaks from the first column could be separated in the second column) (Hoogenboom et al., 2021). Fast-GC-MS and low-pressure gas chromatography (LP-GC) use relatively small columns and are both more rapid than conventional GC (Zhao, 2014). LC-MS has developed into an important analytical instrumentation due to its high analytical sensitivity and specificity and the wide field of applications, ranging from the determination of small molecules, such as food contaminants, up to large molecules, such as proteins, and to the analysis of thermally unstable and polar compounds. The combination of atmospheric pressure ionization tandem mass spectrometry (API-MS/MS), with liquid chromatography (LC), and ultra-performance LC (UPLC) is currently the most frequently used technique in residue analysis in foodstuffs. The most used atmospheric pressure interfaces are atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI). The breakthrough of LC-MS and its broad range of applications in different fields are clearly indicated by the exponential increase of the number of publications since the 1990s in the field of chemical contaminants and residues in food products (Oliveira et al., 2018).

Different types of mass analyzers, such as quadrupole, ion trap (IT), and high resolution mass analyzers (HRMS), such as time-of-flight (TOF) and Orbitrap, have been implemented for the coupling of separation techniques and MS. Hybrid mass combine analyzers of different types, and they include, but are not limited to, triple quadrupole (QqQ), quadruple linear ion trap (LTQ), quadrupole-TOF (QTOF), quadrupole-Orbitrap (Q Orbitrap), and linear quadrupole Orbitrap (LTQ-Orbitrap) hybrid instruments(Amelin et al., 2017). So far, QqQ, coupled mainly with LC but also with GC, has been the most widely used mass spectrometric technique for detecting and quantifying residues and contaminants in meat and meat product samples, as shown in Table 17.1. QqQ's greater advantages are its high sensitivity, selectivity and specificity, wide linear dynamic range, and excellent precision (Campo and Picó, 2015). However, they are restricted in target analysis, and there are limitations regarding the number of analytes that can be detected simultaneously.

In the last years, there is a growing trend toward the use of HRMS for screening analysis of contaminants. HRMS techniques have the major advantage of structure identification and confirmation and the potential to identify unknown molecules, without the use of standards (Arsand et al., 2016). They also provide the possibility of retrospective analysis, which enables the research for "new" contaminants even years after data recording. Table 17.1 presents multiresidue analytical methods developed for the analysis of veterinary drugs and pesticides in meat and meat products using HRMS techniques.

17.5 Future trends and perspectives

In October 2015, the International Agency for Research on Cancer (IARC), the cancer agency of the World Health Organization, classified consumption of

processed meat as "carcinogenic to humans" (Group 1) on the basis of sufficient evidence for colorectal cancer. The IARC also classified the consumption of red meat as being "probably carcinogenic to humans" (Group 2A). The IARC Working Group considered more than 800 studies that investigated associations of more than a dozen types of cancer with the consumption of red meat or processed meat in many countries and populations with diverse diets. The most influential evidence came from large prospective cohort studies conducted over the past 20 years. Except for colorectal cancer, there is also growing evidence for a possible link to both stomach and pancreatic cancers, but this seems to be less clear cut than the link to colorectal cancer.

IARC's evaluation has triggered the imperative need for more future studies on the association between meat and cancer risk. Future research is a requisite in order to collect and report separate data for unprocessed and processed meat, to collect and report data on the cooking method used, and to adequately control for potential confounding factors such as fruit, vegetable, fiber and fat intake, body weight, and physical activity levels.

In order to keep pace with the increased need for expanded analytical capability, extraction, separation, and detection techniques still need to grow. Miniaturized sample extraction methods, which use much less solvent and smaller sample sizes, and automated extraction techniques that allow online extraction have gained ground during the last years. As far as chromatographic separation is concerned, future prospects include the use of hydrophilic interaction liquid chromatography (HILIC) in orthogonal separations, the use of innovative stationary phases, such as monolithic columns and fused-core or core-shell columns and of multidimensional chromatography. Finally, the introduction of high-resolution MS and the enhancement of the selectivity of detection methods enable the development of wide-scope, multiclass, multiresidue methods, as well as nontargeted and profiling approaches used to identify unknown contaminants in meat and meat products.

Finally, in food science, metabolomics has recently become a tool for quality, processing, and safety of raw materials and final products. Advanced chemometric tools can aid to convert detailed data sets obtained from complex samples into useful information.

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Chapter 18

Meat authenticity and traceability

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18.1 General overview

Over the last decades, the supply and the demand for meat and meat-derived products have evolved creating a big gap between the production and the consumption phases. The consumers cannot directly control the production systems, which are driven by the globalization of the food market. Industrialized processes can modify the original raw food materials and increase the number of intermediate steps to obtain a final product. Therefore, suppliers and production chains had to adapt their production systems to meet consumers' requests, and new regulations have been issued at the national and international levels to rule markets and to protect consumers' rights. In addition, production chains and producers have developed new marketing strategies focused on product differentiation based on quality assurance, breed and geographical origin, or specific processing practices usually linked to traditional and unique products. Specific labels and brands have been created to support the added economic value derived by these specifications. Three quality logos developed by the European Union (Protected Designation of Origin or PDO; Protected Geographical Indication or PGI; and Traditional Speciality Guarantee or TSG) are just a few examples of labeling systems aimed to assure authenticity in terms of regional origin or traditional production. In this scenario, the meat market has experienced an increased risk of frauds derived by the substitution or addition of ingredients or components of meat and meat products which are cheaper than those that are directly indicated in the labels and that describe the products. The economic advantage obtained by the fraudsters is the main driver of most frauds that, in turn, cause detrimental economic losses for many production chains and the cheat of consumers. Frauds have also negative impacts on the health of the consumers that are not assured on the origin and safety of the products, in addition to problems that might be derived by religious laws or personal behaviors.

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Therefore, the development and the application of analytical approaches and methodologies that can assure the authenticity and/or the traceability of meat and meat products are fundamental. Their objective is to monitor production chains and meat markets against voluntary or accidental substitutions or mislabeling practices and other problematic situations.

The terms authenticity and traceability have similar general meanings but follow different routes to guarantee food quality and safety (Fontanesi, 2009). Authentication can be defined as the acts of establishing or confirming the meat or meat products as *authentic*, that is, that claims made by or about the subject are true or that their attributes are true. This might involve confirming the identity of a product, its origins, its composition, or assuring that a product is a trusted one (Fontanesi, 2009). Traceability can be defined according to the European Regulation no. 178/2002 as *the ability to trace and follow a food, feed, food producing animal or ingredients, through all stages of production, and distribution* (European Union, 2002). For meat and meat products, authenticity is usually referred to assure general quality attributes independently by the spatial and temporal contexts which define traceability, whose aim is to guarantee the possibility to identify the origin of a product through all stages, mainly for safety and monitoring issues.

This chapter provides an overview of the main concepts that then lead the application of different analytical approaches and methodologies used for meat and meat product authentication and traceability and identifies new avenues for future developments in this area. Analytical methods are considered according to the detection of product components and characteristics (biological characteristics or molecules and chemical elements; physical-chemical characteristics) based on intrinsic or extrinsic factors or properties of the meat. Intrinsic properties are here defined as those directly derived by the biology of the animal from which the meat is originated. Intrinsic properties are determined by biological features that cannot be modified by external factors or factors that are not directly related to the biology of the animals. We can also consider unmodifiable properties of plant products that could be used to substitute animal products in some frauds. For example, intrinsic properties make it possible to identify the species of origin and the breed of origin of the animals (and based on these features, indirectly and in part, the geographic origin of the animals) from which the meat or meat products are obtained. Extrinsic properties are those that derive by external factors and that confer new attributes to the meat not directly linked to biological and unmodifiable features of the animals. Extrinsic properties can be divided into two subcategories: characteristics or elements of the meat and meat products derived or produced by the interaction between environmental (external) factors and the biology of the animals (e.g., feed, microbiota of the animals modified by feeding or husbandry practices, exposure to stable isotopes); compounds and properties generated by processing and preservation technologies (chemical or physical characteristics, microbiota of the products). For example, extrinsic properties can make it possible to identify or infer the

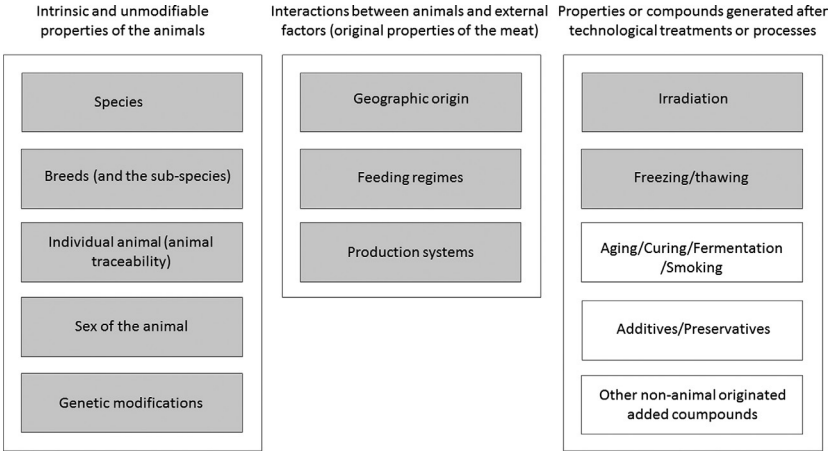

FIG. 18.1 Overview of intrinsic unmodifiable properties of the animals, properties of the meat derived by the interaction between the biology of the animals and external factors, and acquired properties or compounds generated or added by treatments or processing methods applied to the meat. In *gray*, features that are discussed in detail in this chapter.

geographic origin (independently from the species or the breed), feeding and farming practices (e.g., organic productions), treatments and processing practices, and the addition of some adulterants, like additives and water (these latter frauds are not covered in this chapter). Fig. 18.1 reports an overview of the intrinsic and extrinsic properties and attributes of the meat and meat products with related authentication issues.

Most of the analytical methods used in authentication are based on the analysis and detection of biochemical or inorganic components that are present in the meat and meat products (derived by intrinsic and extrinsic factors) and that can answer authentication questions. Table 18.1 summarizes the usefulness and informativity of meat components or properties used for different levels of meat authentication and traceability.

18.2 Intrinsic characteristics of the meat

These properties are derived directly from their constituents (the animals or by substitutes that could be plant-derived). We can identify different levels for which authentication is important, following the biological scale, from the species to the individual animal, including several characteristics of the animals, i.e., the breed, the sex, and eventually genetic modifications of the animals.

18.2.1 Identification of the species of origin

Species substitution and mislabeling of meat products are probably the most common frauds observed in all continents and countries. The percentage of

TABLE 18.1 Definition of usefulness and informativity of meat components and properties for different levels of authenticity and traceability of meat and meat products.

Levels/compounds[a]	DNA	RNA	Proteins[b]	Lipids[c]	Metabolites[d]	Stable isotopes	Trace elements	Other[e]
Intrinsic properties								
Species	+ + +	–	+ + +	+ +	+	–	–	+ +
Breed	+ + +	–	–	–	–	–	–	–
Individual animal	+ + +	–	–	–	–	–	–	–
Sex of the animal	+ + +	–	–	–	+	–	–	–
Genetically modified animal	+ + +	–	–	–	–	–	–	–
Extrinsic properties								
Geographic origin	+	–	–	+ +	–	+ +	+ +	–
Feeding regime	–	–	–	+ +	+ +	+ +	+ +	–
Organic production	–	–	–	+ +	+ +	+ +	+ +	+
Irradiation	–	–	–	–	+ + +	–	–	+ + +
Frozen/thawed meat	+	(+)	–	–	–	–	–	+ + +

An approximate scale is used for this purpose: + + +, highly informative components and established methods; + +, components or characteristics that could provide useful information, methods are proposed but informativity is not absolute; +, low informativity, proposed methods but their use is limited or can have problems; (+), potentially useful but not evaluated into details yet; –, not useful or methods not available.

[a] *A few processing technologies (aging, curing, fermentation, and smoking) and adulteration with nonanimal products are not considered in this table.*

[b] *Including peptides.*

[c] *Including fatty acids.*

[d] *Including xenobiotics.*

[e] *Simple or complex physical-chemical characteristics or undefined components (not characterized by the used analytical approaches and methods).*

mislabeled products varies according to the type of products (with a high rate of mislabeling in game meat, ground meat, and highly processed meat products), countries (based on the presence or absence of specific monitoring programs and agencies deputed to the control and the related legislation), and economic phases (with peaks in depression and recession periods).

The correct identification of the species of origin of meat products has several other implications in addition to the economic aspects. In particular, consumption of some meat species could be against religious rules, personal behaviors, or specific national and international legislations that have been issued for different reasons. For example, in some countries, legislation prohibits the production and use of horsemeat for human consumption. The Convention on International Trade in Endangered Species of Wild Fauna and Flora, integrated in specific national legislations, protects endangered species and restricts the trade of meat derived by these animals (Cites, 1973). Health risk for some people who have meat allergies, even if they are not very frequent, can be a matter of concern (Wilson and Platts-Mills, 2019). The risk of allergies could be higher if meat substitutes have plant origin (Belloque et al., 2002). The 2013 horse meat scandal (Food Safety Authority of Ireland, 2013) and the needs to guarantee the absence of pork in meat preparation for specific markets (Halal and Kosher) have contributed to the development of many methods for the detection of horse and pig in meat products.

The identification of the species of origin of meat and meat products can be obtained by analyzing differences among species that are present in biological molecules, produced by the animals, and that can be isolated from the meat. Species-specific information can be obtained from proteins or peptides, from DNA, and from fat and metabolite components (in particular, fatty acid profiles). Other species-specific information can be derived by the general chemical composition and structure of the meat. Methods can obtain qualitative information (providing a simple positive or negative result on the presence of a species in a sample) or quantitative information (with the percentage or amount of material derived by one or more species in a sample). These two aspects have peculiar definitions and technical aspects according to the method used. The limit of detection (LOD) is also relevant when it is needed to assure the absence of a particular meat species or the presence of plant originated adulteration.

18.2.1.1 Analysis of proteins and peptides

Detection of species-specific proteins is usually more problematic in cooked and processed products where proteins are denatured or degraded. This problem can be in part solved by analyzing peptides. Protein-based analytical approaches useful for meat species identification mainly include electrophoretic techniques, immunological methods, like enzyme-linked immunosorbent assay (ELISA) and immunochromatographic procedures, chromatographic and spectroscopic approaches. Combination of several analytical methods that can obtain a large-scale analysis of proteins in a biological-derived system

(i.e., meat, in our case) defines the discipline of proteomics, which is emerging as a potential useful field for the development of new analytical approaches for meat species identification.

Electrophoretic techniques can obtain information useful for species identification by separation of extracted proteins on different media in the electric field. Electrophoretic methods can be classified according (i) to the type of media and support used in the separation and (ii) to the dimension of the separation. The first approaches used starch gels and then polyacrylamide and agarose gels in one-dimensional separation. Polyacrylamide gels can be used without or with a denaturant agent, like sodium dodecyl sulfate (PAGE or SDS-PAGE, respectively), or with carrier ampholytes in isoelectric focusing (IEF) that are based on gel (gIEF) or on capillary electrophoresis (cIEF). These methods rely on different electrophoretic mobility of myofibrillar and sarcoplasmic proteins among species. Protein bands separated by gel electrophoresis can be visualized by staining with Coomassie Brilliant Blue (less sensitive) or by silver staining. Comparison of electrophoretic patterns can be done using all extracted proteins or only a single protein component present in muscle tissues. For example, electrophoretic pattern of myoglobin, which is a dye-independent species-specific protein, has been one of the first gel-based method to identify the species of origin of meat (Hoyem and Thorson, 1970). Electrophoretic separation in SDS-PAGE of complex muscle protein patterns has shown that troponin I, enolase 3, L-lactate dehydrogenase, triose-phosphate isomerase, tropomyosin 1, and carbonic anhydrase 3 are useful markers for discrimination of mammals from poultry due to their different mobility but not among mammalian species or among avian species (Kim et al., 2017). However, in general, one-dimensional electrophoretic approaches are limited by the poor discriminatory resolution among closely related species, and for this reason they have not been implemented for routine testing.

Analysis of proteins for species identification has been also reported using other techniques and approaches. The first commercialized methods for species identification in meat products have been based on ELISA that, for its simplicity, is still the most common technique used for species identification that targets a protein or several proteins present in a meat sample (Whittaker et al., 1983; Asensio et al., 2008). ELISA is based on antigen-antibody interaction that includes an enzyme that catalyzes a biochemical reaction that, in turn, reveals the presence of the antigen. ELISA methods can be divided in direct assays, indirect assays, and capture or sandwich assays. Direct assays are less sensitive than the other ones. For meat species identification, indirect and sandwich ELISAs have been mainly tested and then commercialized (Asensio et al., 2008). As species determination is usually requested for cooked or highly processed products and considering the structural changes that these treatments can produce on proteins, antibodies (monoclonal or polyclonal) have been raised on denatured meat proteins or on heat resistant proteins (Berger et al., 1988). Several ELISA kits are commercially available and some of them are also

used as prescreening by national regulatory and controlling agencies (e.g., the United States Department of Agriculture Food Safety and Inspection Service) for their simple detection protocols. These kits are usually based on antibodies raised on heat-resistant species-specific, muscle-related glycoproteins with a declared LOD of 1% or lower for the detection of the targeted species (cow, pig, poultry, horse, deer, kangaroo, sheep, goat, rabbit, buffalo, chicken, and turkey) in uncooked or canned, cooked, and processed food products. ELISA methods have been also described for the detection of material of plant origin, usually soybean, in meat products (Koppelman et al., 2004). Commercial immunological-based kits for the detection of soybean in food are available from several producers. Immunochromatographic detection systems based on a lateral flow device (LFD) assay, also known as lateral flow immunochromatographic assays, lateral flow immunoassays (LFIA), lateral flow tests (LFT), or rapid immune strip tests, have been mainly developed to detect porcine immunoglobulins (IgG) as molecular biomarkers that can be simply extracted from meat and meat preparations (e.g., Hendrickson et al., 2021). These systems, in the general concepts, work similarly to the woman pregnancy tests. In these assays, gold nanoparticles as a nano-dispersed label are conjugated with specific antiporcine IgG polyclonal antibodies. Their LOD is usually good (<0.1% w/w) and the time of answer is close to 30 min, including sample preparation, which make them useful for point-of-care screening of meat products to evaluate they authenticity.

Immunological methods should be evaluated for their sensitivity and specificity against the target species, with absence of cross-reactivity with any other species, which is usually a limit if based on polyclonal antibodies. Highly processed products might be also more difficult to be analyzed due to denatured or degraded proteins.

Chromatographic-based approaches have usually high separation potential but, as a drawback, require long extraction and analytical procedures, which limit their applications. The use of chromatographic techniques for meat species identification was first reported by Ashoor et al. (1988) who discriminated beef, pork, veal, lamb, chicken, turkey, and duck meat with a liquid chromatographic (LC) method that produced qualitative and quantitative chromatographic information. Chou et al. (2007) published a method based on high-performance liquid chromatography (HPLC) with electrochemical detection to differentiate products from 15 animal food species based on specific electrochemical profiles. The usefulness of this approach in complex mixed and heat-treated meat samples seems limited by the potential overlapping of the profiles and change of peaks intensity. Myoglobin, as species specific marker separated with anionic ultra-performance liquid chromatography (UPLC), has been used to evaluate the presence of undeclared pork or horse meat in raw beef burgers (with a sensitivity of 5% or 0.1% w/w, respectively; Giaretta et al., 2013).

Further improvements on the detection of species-specific protein markers have been obtained using proteomics techniques and combination of methods.

Proteomics approaches can include a depletion step for the most abundant interfering proteins. Alternatively, they can be designed to take advantage from abundant proteins using a selective enrichment and partial purification of muscle target proteins before the separation steps are performed at the protein and/or peptide level based on two-dimensional gel electrophoresis (2-DE) and/or liquid chromatography (gel-free approach), followed by the final mass spectrometry (MS) analysis (Ortea et al., 2016).

Most of the proteomics approaches used for meat species identification can be considered targeted approaches. Targeted approaches are based on specific MS methods that are set up to detected known marker peptides expected to be present in the sample under investigation. That means also that prior knowledge of the species of origin of the suspected products should be available. When this information is not available, a nontargeted strategy based on shotgun proteomic approaches could be applied to analyze whole protein extracts. In these cases, peptide identification is limited by the technical resolution of the applied MS methods and by the species and protein information available in the database. A shotgun proteomic approach using nanoflow-liquid chromatography/high-resolution mass spectrometry (nLC-HRMS) has been proposed by Claydon et al. (2015) for subsequent identification of heat stable marker peptides for the detection of low levels of horse meat (0.5% w/w) in mixtures with beef.

Alternative untargeted MS methods are based on spectral matching using spectral libraries in a bottom up proteomics approach (Stachniuk et al., 2021). This approach has several advantages over targeted approaches since the comparison and identification are based on the matching or nonmatching of hundreds or thousands of peptides. It is robust against within species protein polymorphisms, and it is an open analysis with no need of genomic information (from which the proteome is deduced) of any species. Spectral libraries cannot only be used for the identification but also for classification of species based on phylogenetic similarity.

Despite the potential of proteomics approaches for meat species identification, most of the published methods are limited by the low number of species that could be determined at the same time, by the low sensitivity of some of these approaches and by the needs of specialized and quite expensive instruments and labs. Thus far, none of these approaches have been implemented as routine protocols for species identification, even if their potential for marker discovery is high.

18.2.1.2 Analysis of DNA: Techniques and approaches

DNA of different species is, by definition, different and analysis of the DNA can easily obtain information useful to attribute the species of origin of meat products. DNA has a higher stability to physical treatments (i.e., cooking) and long-term storage or processing than proteins or other metabolites. DNA degradation should be, however, considered as a possible problem in the analysis of highly processed products. DNA is present in almost all animal cells and thus in

the tissues that constitute the meat. Therefore, the same information can be retrieved independently by the tissue or the part of the animals that constitute the meat and from which specimens for subsequent analyses are sampled. For these reasons, DNA analysis is considered the gold standard for species identification not only in food authentication but also in forensic analysis and systematics. Information useful for meat species identification can be obtained from DNA of the nuclear genome and from DNA of the mitochondrial genome (mtDNA). The size of the nuclear genome is about 3 billion of nucleotides in mammals and 1 billion of nucleotides in birds. It is usually contained in duplicate copies in nucleated diploid cells that usually constitute the meat tissues. The mtDNA is much smaller (about 16.5 kb in all meat species) but is usually present in many more copies per cell. A single muscle cell can contain thousands of mitochondria and thus thousands of mtDNAs. The detection of plant originated DNA can be derived by nuclear DNA, mtDNA, or chloroplast DNA.

Many methods based on DNA analysis for species identification have been published. Methods to identify DNA information useful for meat speciation can be classified according (i) to the technology or approaches used, (ii) to the possibility to provide qualitative or quantitative information, and (iii) to the targeted DNA used in the assay, which in turn may affect the specificity and sensitivity or LOD, the possibility to obtain information from processed products, the possibility to detect more than one species in mixed samples, the cost, and the analytical time.

The preliminary analytical step for all DNA-based assays is the extraction of the DNA that can be done using many different protocols and commercial kits specifically optimized also for food or tissue DNA extraction. DNA extraction is followed by subsequent procedures that are designed to directly or indirectly determine DNA sequence differences among species.

A summary that reports a few characteristics and the pros and cons of DNA-based approaches for meat speciation is reported in Table 18.2.

The first DNA assays for meat species identification were based on DNA hybridization of labeled species-specific nuclear DNA probes (constituted by genomic DNA fragments, total genomic DNA, satellite DNA, amplified DNA fragments, or synthetized oligonucleotides) on nylon membranes on which extracted DNA from the meat samples under investigation is transferred in a slot or dot blot experimental design (e.g., Chikuni et al., 1990). These methods are quite laborious, rely on defined hybridization conditions (difficult to standardize in different laboratories), and on the use of species-specific probes. For these reasons, they have been almost completely abandoned in their original designs, whereas the concept of DNA hybridization has been further refined in applications on macroarrays and microarrays.

Since the advent of polymerase chain reaction (PCR) for DNA analysis, most of the assays designed for species identification use this technique. PCR is an in vitro enzymatic technique that amplifies one or more DNA fragments (the targeted DNA) embedded between two regions (the borders of the fragments)

TABLE 18.2 Summary of characteristics of DNA-based approaches for meat species identification.

Approaches[a]	Targeted DNA[b]	Single species[c]	Multiple species[d]	Unexpected species[e]
DNA hybridization	nDNA	+ + +	+	–
PCR-RAPD	nDNA	+ + +	–	–
AP-PCR	nDNA	+ + +	–	–
PCR species-specific	nDNA/mtDNA	+ + +	–	–
CP-M-PCR	mtDNA	+ + +	+ +	–
PCR-RFLP	mtDNA	+ + +	+	–
PCR-SSCP	mtDNA	+ + +	+	–
PCR melting analysis	mtDNA	+ + +	+	–
PCR + Sanger sequencing	mtDNA	+ + +	–	–
PCR + NGS	mtDNA	+ + +	+ + +	+ + +
All-DNA-seq (NGS)	nDNA + mtDNA	+ +	+ + +	+ + +
PCR + microarray/ microarray	mtDNA	+ + +	+ + +	+ +
qPCR (DNA intercalating dyes)	mtDNA	+ + +	+	–
qPCR (fluorophore-labeled oligos)	mtDNA	+ + +	+ +	–
Digital droplet PCR	nDNA (mtDNA)	+ + +	+	–
LAMP	mtDNA	+ + +	+	–

+ + +, indicates that the method provides good results or it is effective or that it can be easily implemented; + +, indicates that the method has some limits but it can be effective; +, indicates major limits or that it is difficult to be implemented; –, indicates that it cannot be implemented or that it is not useful.

[a] *Approaches and methods are defined in the text. PCR-single strand conformation polymorphism (SSCP) and PCR-melting analysis are not discussed as they have been described only by few studies for meat species identification.*

[b] *Targeted DNA used in the assays. nDNA, nuclear DNA; mtDNA, mitochondrial DNA. The most used target DNA is included. For most methods, even if mtDNA has been more frequently targeted, nDNA regions have been used in the assays or can be used (even if LOD would be worse).*

[c] *It considers the possibility to identify the targeted species that could be the only species constituting the meat product or the possibility to identify one target species in a mixed species meat product.*

[d] *It considers the possibility to detect at the same time more than one species.*

[e] *It considers the possibility to detect at the same time more than one species including also unexpected species that means that the assay can also be not species-specific.*

[f] *It considers the possibility to detect the targeted species without any interference from the presence of other close species.*

[g] *It considers the possibility to obtain reliable results from degraded DNA. It is mainly related to the targeted DNA and, in PCR-based assays, to the length of the amplified product.*

[h] *Limit of detection.*

[i] *It can provide a qualitative evaluation (presence or absence) of a species.*

[j] *It can provide a quantitative measure of the presence of a species.*

[k] *It evaluates the practical possibility, simplicity, and need of specific expertise and instruments for the implementation of the designed assays. (+), the method has been abandoned.*

Specificity[f]	Degraded DNA[g]	LOD[h]	Qualitative[i]	Quantitative[j]	Implementation[k]
++	++	+	++	+	(+)
+	+	+	++	−	+
++	+	+	++	−	++
+++	++	++	+++	−	+++
+++	++	++	+++	−	+++
++	++	++	+++	−	+++
++	++	+	++	−	+
++	++	++	++	−	+
+++	++	++	+++	−	++
+++	+++	++	+++	+	++
++	+++	++	+++	+	+
+++	++	++	+++	−	++
+++	+++	+++	+++	+++	++
++	+++	+++	+++	+++	++
+++	+++	+++	+++	+++	++
+++	+++	++	+++	−	+

defined by complementary oligonucleotides (forward and reverse) that constitute the primers of the reaction. A classical PCR analysis requires the template DNA (from which the DNA is amplified), forward and reverse primers, the four nucleotides (dNTPs) for the construction of the DNA filaments, a thermostable polymerase (*Taq* DNA polymerase), salts, and buffer for the optimization of the reaction environment. About 30–40 temperature cycles (that include alternative temperature steps needed for the template DNA denaturation, for the primer annealing and for the polymerase activity of chain production) are needed to exponentially amplify the target DNA resulting in millions of copies for visualization or subsequent analyses. Detection of the amplified fragments can be obtained (i) by separation of the amplified fragments according to their size or sequence (by gel electrophoresis using agarose or polyacrylamide gels stained with DNA intercalating dyes, by capillary electrophoresis and fluorescent DNA labels, or by other separation methods), (ii) by direct evaluation of the PCR products in gel free systems (by accounting light emission derived by incorporation of dyes in double strands or in other systems), or (iii) by in silico analysis in next-generation sequencing approach or digital PCR. PCR methods can be qualitative (end point PCR), semiquantitative, or quantitative according to when and to the method by which the information on the amplified fragments is collected during the cycles.

Both types of DNAs (nuclear and mtDNA) have been targeted by PCR assays designed for meat speciation. Targeted nuclear DNA can be a single copy DNA region, a family of single copy DNA regions (e.g., a family of closely related genes), repetitive regions of the nuclear genome (short interspersed nuclear elements or SINE; long interspersed nuclear elements or LINE), or undefined multiple regions. The LOD of single copy DNA regions is higher (higher content of material from the targeted species is needed) compared to multiple copy nuclear regions or to mtDNA that is usually present in many copies per cell. Thus, multiple copies nuclear targeted regions and mtDNA are more suitable for detection in degraded or highly processed products. In practice, most of the PCR methods, which have been designed since this technique was introduced amplify mtDNA regions for several other reasons: (i) the sequence of the targeted mtDNA region (or gene) or of the whole mitochondrial genome is publicly available for many species; (ii) short DNA regions in many parts of the mtDNA contain enough sequence differences to discriminate the amplified fragment between species; and (iii) some regions are highly conserved across species and can be used to design universal primers useful to amplify the target region in many species without the need to change primer pairs. This latter possibility is an advantage when it is not known at priori the possible species from which the investigated meat products are obtained. From the other hand, it could create a problem if meat products are obtained from mixed species. In this case, the amplified fragments, in turn, may be actually constituted by a population of different fragments that are more difficult to be analyzed using classical post PCR sequence discriminating analyses, which are highly effective only when the originated amplicons derive from only one species.

Post PCR techniques that have been applied to identify the amplified fragments originated from different meat species can be classified according to the approaches used to detect differences. PCR and post PCR analytical steps are two integrated procedures as the design of the first might be effective only with appropriate subsequent analysis of the second. In particular, separation and then visualization of the amplicons using electrophoretic techniques is one of the simplest approaches based on which many assays have been constructed.

PCR-based analyses coupled with simple post-PCR methods

These PCR-based assays are considered qualitative methods, i.e., they can detect the presence or the absence of a species. They cannot give a precise quantitative estimation of the amount of its presence when meat composite products (potentially originated from more than one species) are analyzed.

One of the first PCR-based approach tested for species identification is random amplified polymorphic DNA (RAPD). Short arbitrary primers are used in a PCR with a low annealing temperature to generate many amplified products separated by gel electrophoresis that provides a sort of species-specific fingerprinting (Koh et al., 1998). This method could be useful to differentiate species for which sequence information is limited and only for internal laboratory analysis (without any across laboratory validation), as interlaboratory reproducibility of RAPD pattern is poor. Meat reference samples might be needed in these cases. A modified version that uses longer degenerate primers in the arbitrarily primed PCR (AP-PCR) has been proposed to increase reproducibility and specificity (Saez et al., 2004). However, both RAPD and AP-PCR cannot be applied in mixed samples where more than one species could be present, as all extracted DNA (independently by its origin) could be targeted by the random primers and might produce bands, altering the species-specific fingerprints. For the described limits, these methods have not been implemented in routine assays for meat species identification.

Other methods are based on PCR designed to amplify only the target species DNA (species-specific PCR). In these protocols, amplification could occur only when the DNA template of the species that may be targeted by specific primers is actually present in the investigated meat. The result would be positive if there is a specific band in a gel or negative in the case of absence of any amplified bands. In methods based only on presence vs absence of a species-specific amplification, the negative results cannot be distinguished from a failure of the PCR. Therefore, multiplex amplification of a control fragment (with another added primer pair to the PCR) that might be always present in any species could provide an internal quality evaluation in the tested samples. The control amplicon should have a different size than that of the targeted species-specific fragment to facilitate its separation through gel or capillary electrophoresis. Multiplex PCR including in the same reaction more than one primer pair, each targeting a different meat species, have been proposed as a possible solution to overcome the problem of mixed samples, making it possible to detect more

than one species in a single reaction (Xue et al., 2016). Amplified fragments of different species are distinguished by their different size in a single electrophoretic analysis. Similar multiplex PCR methods have been proposed with one universal primer (common for more than one species) designed in a conserved mtDNA region and a reverse species-specific primer for each species, obtaining amplified fragments of different size according to the species (Matsunaga et al., 1999). This method is also known as Common-Primer-Multiplex PCR (CP-M-PCR; Hanapi et al., 2015). In these cases, longer fragments amplify less efficiently than shorter fragments in highly processed meat for two reasons: (i) a general lower efficiency of longer fragments due to competitive PCR and (ii) DNA degradation, making more reliable the detection of species for which short fragments are obtained.

Several post-PCR analyses of the obtained PCR fragments have been applied to detect species-specific differences in the amplified regions. For example, species can be distinguished by digestion of the obtained fragments (usually produced with universal primers) with restriction enzymes that cut the DNA in palindromic regions, followed by separation of the digested products through electrophoresis. This approach called PCR-restriction fragment length polymorphism (RFLP) analysis has been applied for meat speciation (Meyer et al., 1994). Combination of several restriction enzymes might be needed to differentiate closely related species or if it would be needed to identify simultaneously more than one species. However, the complexity of the electrophoretic patterns in mixed samples might create problems in the species identification.

Direct sequence information can be obtained by Sanger sequencing of the PCR-generated amplicons produced using species-specific primers or universal primer pairs (e.g., Bartlett and Davidson, 1992). Obtained sequences are then compared to other sequences of the same targeted mtDNA region already available in public databases using sequence alignment tools (BLASTN, https://blast.ncbi.nlm.nih.gov/). The programs compare the query sequence to sequence databases and calculates the statistical significance of matches (% of identical nucleotides and *E*-value) that are used to attribute the species of origin of the analyzed sequence.

Sanger sequencing of barcodes, that derive by the amplification of standardized mtDNA sequences, is at the basis of the DNA barcoding approach. DNA barcoding is a rapid taxonomic assignment of species that in animals relies on sequence differences that are present in the ~650 bp long mitochondrial cytochrome *c* oxidase subunit I gene (COI, COX1 or CO1) (Staats et al., 2016). Identification of the species is based on sequence comparison against a reference database that contains sequences of this gene from a large number of species generated by the International Barcode Of Life project (iBOL; www.ibol.org) and organized in the Barcode Of Life Data (BOLD) Systems (http://boldsystems.org). The DNA barcoding principle for the identification of meat species provides a standardized method for species identification, and for this reason it could be mainly useful if unusual species are suspected to be present.

The amplification of the whole COI gene is problematic when the DNA is degraded, i.e., in highly processed products. In addition, similarly to all other Sanger sequencing-based approaches, this system cannot be applied if more than one species is present in a product, as the heterogeneous generated fragments would produce overlapping and thus unreadable sequences.

PCR and next-generation sequencing

The problems derived by the mixed meat products (i.e., the presence of more than one species) can be overcome by using next-generating sequencing (NGS). The high-throughput of NGS has revolutionized the way to analyze DNA, combining DNA sequencing, and quantification in a single step. Sequence analysis in NGS experiments is not based on any limiting supports as in the case of all previously described PCR methods. Data analysis obtained with appropriate bioinformatics tools gives an enormous flexibility to NGS. NGS platforms, which can be applied to generate sequence data useful for species identification, are Illumina, Ion Torrent, and Oxford Nanopore technologies. These platforms can be applied (i) to analyze target PCR amplified fragments or (ii) in an untargeted way, without previous target amplification. The most common application is based on target analyses, where amplicons obtained from universal PCR primers that targets, for example 12S, 16S rRNA mtDNA genes, are analyzed. Some pros and cons of NGS applied in this context should be mentioned, as deduced by some applications in specific case studies (e.g., Bertolini et al., 2015; Ribani et al., 2018). Barcoding based on specific tags attached to the amplicons derived by different samples gives the possibility to analyze, in the same run, several samples. The sequencing error rate of the different platforms should be evaluated: this should be lower than the sequence difference of the target mtDNA region among species to avoid potential misidentification from the bioinformatic analysis of the sequencing data. A semiquantitative result could be obtained based on the count of reads assigned to different species, providing that equal amplification efficiency of the universal primers can be obtained from all species that contribute to the analyzed mixed meat product. The analytical cost, including the bioinformatic analysis of the data, should be carefully considered and evaluated in the specific context in which information is needed.

Next-generation sequencing can be applied using an untargeted deep sequencing approach. In this method, all DNA present in a food sample is sequenced (All-Food-Seq), following the concept of metagenomics (Ripp et al., 2014). PCR is not included in the preamplification or selection of the targeted DNA (it could be eventually included in the NGS library preparation steps). Bioinformatic analysis of sequence data is the only way to identify the organisms that constituted the investigated food. Deep sequencing, based on Illumina technology, is needed in this approach. Powerful computing facilities and bioinformatic skills are needed for the application of this untargeted NGS approach that can provide not only information on the species but also metagenomic data from microorganisms present or contaminating meat products.

PCR and chip analysis

Other post-PCR methods can discriminate the species of origin of the amplicons by macroarray or microarray analysis (Iwobi et al., 2011). These platforms can make it possible to identify at the same time the presence of more species. However, they could identify only the species that have been previously included in the design of the chip. A commercially available system, produced by Chipron GmbH (Berlin, Germany), is based on a macroarray platform for the simultaneous identification of 32 meat species in food samples. The PCR product, obtained by universal primers designed on the 16S rRNA mtDNA gene, is hybridized on a low-density oligonucleotide probes array (Meat Low Cost and Density (LCD) Array). This system has a LOD of less than 1% (w/w) and can be applied to detect more than one species in mixed samples from raw or highly processed meat products (Cottenet et al., 2016).

Another commercially available system for meat species identification produced by Greiner Bio-One International (https://www.gbo.com/) is based on a lateral flow DNA chip for simultaneous identification of 8 animal species in food products (CarnoCheck) with a LOD from 0.1% to 1% (w/w) depending on the species and sample composition (Iwobi et al., 2011). The detection step (based on hybridization) is preceded by a PCR amplification of a species-specific mtDNA cytochrome B gene region.

Real-time PCR

Almost all methods described above can be considered qualitative, or at best semiquantitative methods, as they are based on end-point PCR. Not all described real-time PCR methods for meat species identification are quantitative methods if the detection is still based on end-point PCR evaluation. In quantitative real-time PCR, also known as quantitative PCR (qPCR), the amplification process is monitored in "real time" using specialized thermal cyclers with an integrated excitation light source that can capture and elaborate (with a dedicated software) the fluorescence generated during the progress of the reaction, derived by the multiplication of the amplified product. Generated fluorescent data can be used to obtain a quantitative analysis of the targeted meat species. Two different approaches can be used for the production of fluorescence (Navarro et al., 2015). One approach uses double-stranded DNA intercalating dyes, such as SYBR Green I or EvaGreen. The second approach includes fluorophore-labeled oligonucleotides that can be divided into three other groups, according to the type of fluorescent molecules used in the PCR: (i) probes acting as primers, called primer-probes, like Scorpions or LUX; (ii) hydrolysis probes emitting fluorescent light upon degradation during the extension phase (TaqMan and TaqMan-MGB or minor groove binder) and hybridization probes producing fluorescent signals when binding to the DNA target during the amplification reaction (Molecular Beacons); and (iii) analogous of nucleic acids (peptide nucleic acids or PNA; not used so far for meat species identification). In general, the LOD of all quantitative real-time PCR methods is lower than many other

methods based on end-point PCR. On the other hand, the risk is to detect minor or accidental contaminations that in most cases are not relevant to assure authenticity of meat samples. Real-time PCR assays with the TaqMan hydrolysis probes have been largely applied for meat species quantification. This system has an inherent increased specificity compared to the use of intercalating dyes. No post PCR melting analysis is requested to confirm the specificity of the correct target amplification. Different fluorophores can be used in the same reaction (each attached to a different probe), making it possible to produce signals for more than one species at the same time (each quantified capturing a different color).

Quantitative real-time PCR assays should solve several problems to obtain an absolute quantification of the meat content of different species in meat mixtures (Ballin et al., 2009). One question derives from the use of mtDNA targets for the quantification, as the number of mitochondrial genomes can largely vary among different tissues. From one hand, the use of mtDNA may give a lower LOD than single copy nuclear DNA that could, however, offer the possibility to obtain more reliable quantification, due to a quite linear relation between the number of nuclei and the muscle mass. Multicopies nuclear DNA components (repetitive sequences) could be useful to increase the sensitivity of methods based on a more stable number of copies of targeted DNA, but sequence heterogeneity might prevent the design of assays with high specificity. These issues are in part also linked to what the quantification should be related to: genome/genome quantification (considering only mammalian or also avian genomes for the different size of the genome of the animals of the two groups of vertebrates); copy/copy quantification if mtDNA is targeted; weight/weight (w/w) quantification, assuming, no bias derived by the characteristic of the targeted DNA in the investigated, and control meat preparations. Different DNA extraction protocols or different efficiencies of DNA extraction among samples and the unknown level of DNA degradation in sampled and control meat might be also other problems that could create biases in the quantification assays.

Digital PCR

Some of the limits described for real-time PCR quantification of meat species could be solved by digital PCR (dPCR). This approach has some advantages over real-time PCR, including (i) the possibility to obtain an absolute quantification of a targeted amplified DNA by end-point PCR, without any external references or standard curves and (ii) the robustness to variations in PCR efficiency (Hindson et al., 2013). Droplet digital PCR (ddPCR) is a method based on a water-oil emulsion droplet system to form the massive sample partition that separates the template DNA into individual nanoliter-sized reaction chambers in which PCR take places. PCR amplification in the droplets can be combined with TaqMan probe-based assays to generate the signal of amplification that is then counted by analyzing each droplet in a flow cytometer. Poisson statistics based on the ratio of positive to total number of droplets can determine the

target DNA concentration in the analyzed sample. Using ddPCR, template DNA presents at very low concentration in a background of nontarget DNA can be quantified with precision. The poor LOD obtained by analyzing single copy nuclear DNA in real-time PCR is no longer a main problem with ddPCR. With this system, relationships between the raw meat weight and DNA weight and between the DNA weight and DNA copy number can be obtained, and formulae and conversion rates are defined to calculate the raw meat weight based on the DNA copy number based on different matrices and analytical procedures (Köppel et al., 2019).

Non-PCR systems

PCR has dominated the field of DNA analysis since its development due to its flexibility, reproducibility, sensitivity, and efficiency. A classical PCR experiment is divided in three phases: DNA extraction, PCR amplification, and then post-PCR analysis of the amplified DNA. These two later phases can be combined in real-time PCR. However, the time that is needed for the amplification step and the needs to use laboratory instruments have reduced the use of PCR for applications where it could be important to obtain a quick answer from the analytical process (i.e., for field application or point-of-care). An alternative DNA amplification method, termed loop-mediated isothermal amplification (LAMP), that rapidly amplifies DNA with high specificity and efficiency under isothermal conditions can be adapted for meat speciation. This method uses a specific DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA assuring high specificity. A typical LAMP reaction lasts for 30–60 min with a temperature of 60–65°C under isothermal conditions. The isothermal characteristic means that the amplification can be conducted with a simple heating block, with controlled temperature, instead of a thermal cycler that change the temperature according to the cycle steps. A few examples of LAMP assays have been reported for meat species identification, coupled in several cases with new systems or devises for the quick analysis of the amplified products (Giris et al., 2020).

18.2.1.3 Analysis of fat, metabolite, and other chemical or physicochemical components or characteristics

Other approaches are based on the analysis of several components (or family of components), and chemical/physical structures of the meat that could be sufficiently different to establish meaningful methods to distinguish the species of origin. It should be, however, highlighted that in most cases a certain amount of variation exists between samples of the same species that can derive from other factors that are relevant for other authenticity questions (e.g., different breeds, different age of the animals, sex, muscle type, feeding, processing methods, etc.). These factors, together with the technical approximation of some instrumentations, might create some problems in the classification of the results

and reference data sets and appropriate statistical and data analysis approaches (e.g., chemometrics) should be used for a comparative evaluation and species identification. Analytical methods are based on nuclear magnetic resonance (NMR), spectroscopy methodologies (i.e., visible-infrared (VIR), UV-visible, near-infrared (NIR) and mid-infrared (MIR) spectroscopy, Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy), mass spectrometry and related metabolomics analytical techniques, electronic nose, and histochemical analyses.

Some other meat components might provide useful information for species identification. For example, triglyceride signature or metabolite profiles could be targeted to identify the species of the meat products that are under investigation. A few methods for species identification of raw meat samples based on these components have been proposed using nuclear magnetic resonance (NMR) and mass spectrometry. Jakes et al. (2015) have described a screening protocol to distinguish beef from horse meat based upon comparison of triglyceride signature obtained by 60 MHz ^{1}H NMR spectroscopy after a simple chloroform-based extraction and principal component analysis (PCA) of peak integration results. Obtained results of the meat species attribution seem not adversely affected by freezing and then thawing the samples. This method is proposed by Oxford Instruments that commercializes a benchtop NMR instrument with applications in the field of meat speciation.

Metabolomic analyses, based on different metabolomic profiles obtained using GC-MS (for the analysis of metabolites involved in primary metabolism), UHPLC-MS (for the analysis of lipophilic metabolite species), and laser-ablation electrospray ionization mass spectrometry (LAESI-MS), have been proposed to identify different grades of raw beef mince and pork mince or to identify different meat species (Trivedi et al., 2016; Zhou et al., 2016). Metabolomic profiles, in several cases partially overlapping, could make it possible to distinguish different meat species, using appropriate chemometric analyses.

All these methods have in common the same drawbacks: (i) they can be used to analyze only raw or fresh samples, due to the limited stability of the analyzed biomolecules and (ii) they cannot be used to detected mixed samples, due to the lack of species-specific biomarkers that could also make it possible to establish quantitative evaluation. Therefore, their applications can be envisaged only when these elements are not relevant.

Other studies have evaluated the use of spectroscopy techniques for species identification. VIR, UV-visible, NIR and MIR, Raman and FTIR spectroscopy were tested for the determination of the species of many different samples, mainly obtained from raw individual meat or raw meat samples derived by mixed species (Mamani-Linares et al., 2012; Alamprese et al., 2016). Minimum preparation of the samples, the nondestructive nature of the methodologies, their speed, and the possibility to implement monitoring systems for industrial plants and the portability of some instruments make these approaches of particular interest. On the other hand, their effective efficiency derives by the construction

of extended spectral databases that include all possible parameters of variation that could be present in the samples under investigation. That means that limited information and a limited number of samples that are analyzed for model construction may reduce the efficiency of the models for correct classification of the samples. A general high misidentification rate has been also reported by most studies that evaluated mixture. In any case, sensitivity of the methods could not go below the 5%–10% w/w, as lowest LOD. Therefore, other methods should be used after a preliminary spectroscopy evaluation that might be useful only to rapidly discriminate problematic samples.

18.2.2 Identification of the subspecies or breed of origin

The identification of the origin of the meat from a lower level (systematically speaking) than that of the species wants to assure the quality of the product that is linked to the consumers' perception derived by the origin of that product to a particular animal subspecies, breed, or line (Fontanesi, 2009). These products are constituted by mono-breed brands (registered or not registered and derived by only one or few defined breeds) or collective or proprietary brands, defined in different ways. Some of these products have also obtained the protected denomination of origin (PDO) or the protected geographical indication (PGI). Other products are derived from the wild relatives of the domesticated animals (considered as subspecies, e.g., wild boar vs domestic pig meat). Most of these productions are fundamental parts of rural identities and agricultural economies. For the declared origin, these meat products are usually sold at a higher price compared to undifferentiated products. The added value of these products contributes to improve the economic incomes of the farmers derived from autochthonous and usually endangered breeds, that are less productive than cosmopolitan and highly selected breeds. Several conservation programs of autochthonous animal genetic resources are based on branded breed-specific products (Fontanesi, 2009). Frauds in this context are mainly driven by economic reasons, stimulated by the added values of these products. Substitutions of the meat are usually from the same species but from different breeds or from undifferentiated animals of lower value. The identification of the breed of origin of the meat could in part answer the question of its geographic origin as it is known that some breeds are raised only in geographic distinct regions or countries.

Methods developed for this level of authentication are mainly constructed on DNA analysis with PCR technologies that can directly detect differences (i.e., DNA markers) that are at the basis of the differentiation between subspecies, breeds, or lines. The level of confidence in the assignment to a particular breed (or in the exclusion of a particular breed) is mainly due to the genetic distance among the groups of animals considered, which is correlated with the history of their constitution.

The constitution of livestock breeds derives from a long selection process of the animals that started from the prehistorical domestication of the

corresponding wild relatives. Then breeding choices of the farmers favored the constitution of close populations with similar morphological traits. These human-driven breeding events led to the fixation of few phenotypes (e.g., coat color, stature, horns, etc.) that differentiated domesticated animals from wild ancestors and, within domesticated species, produced different breeds. These processes left selection signatures in the animal genomes at loci affecting these traits, usually determined by one or few major genes and modified allele frequencies of many other loci in the population that could have minor effects on selected traits or because bottleneck or genetic drift acted in these populations. More recently, divergent selection or specific breeding processes and objectives have differentiated populations within breeds or developed different breeding lines whose level of genetic differentiation might be lower than that is present among breeds. In addition, as subspecies, breeds, or lines are not separated by biological reproductive barriers, separation of genetic pools is not absolute. This aspect further complicates the identification of breed-specific DNA markers useful for this level of meat authentication (Fontanesi, 2009).

Beef and dairy cattle breeds can be easily distinguished by the different muscle mass of the two types of animals. Several mutations in the bovine myostatin (*MSTN*) gene are associated with muscle hypertrophy (Mcpherron and Lee, 1997). Several beef breeds carry different mutations at the *MSTN* gene, determining, however, a similar phenotype known as double muscle. Piedmontese is an Italian originated beef cattle breed that has a breed-specific mutation in the *MSTN* gene. Almost all animals of this breed are homozygous for this mutated allele making it possible to establish a simple PCR-based genotyping test for the identification of meat originated by animals of this breed (Pozzi et al., 2009).

Mutations in a few genes (e.g., melanocortin 1 receptor, *MC1R*; premelanosome protein, *PMEL* or *SILV*; v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, *KIT*) affect coat colors in different species and can be useful to identify or exclude the breed of the meat. For example, in cattle and pigs, the relative abundance of the two main forms of melanin (eumelanin that produces black coat color, and pheomelanin that produces red coat color) are regulated by different alleles of the *MC1R* gene (Klungland et al., 1995; Kijas et al., 1998). Therefore, breeds with red (or brownish) coat color are usually fixed for a recessive *MC1R* allele, whereas animals of breeds with black coat color usually carry at least one copy of the dominant *MC1R* allele. *MC1R* polymorphisms have been used to distinguish meat from different cattle breeds, indirectly by deducing the coat color of the animals from their DNA. The recessive red *MC1R* allele (*e*) is almost fixed in the Hanwoo beef cattle breed, a Korean native cattle breed with red/brown coat color (Sasazaki et al., 2005). Analyzing *MC1R* polymorphisms by PCR-RFLP, it is possible to distinguish its meat from that of other breeds carrying other alleles at this gene, in particular from beef produced by cattle carrying the dominant black E^D *MC1R* allele (e.g., black and white Holstein-Friesian) with poorer meat and carcass quality traits (Chung et al., 2000). A DNA marker in the *PMEL* gene has been proposed to authenticate

meat from the Charolais breed, a French beef cattle breed (Oulmouden et al., 2005). This polymorphism is associated with the dilute coat color that is a characteristic trait of this breed.

Meat from different pig breeds can be differentiated by analyzing polymorphisms in the *MC1R* and *KIT* genes (Kijas et al., 1998; Carrión et al., 2003). The Duroc breed (with reddish/brown coat color) is fixed for the recessive *MC1R* allele (allele *e*). Many local black breeds have high frequency of the dominant black alleles at this gene (E^{D1} or E^{D2}; D'alessandro et al., 2007). The white coat color of many other commercial pig breeds and hybrid lines is determined by a copy number variation at the *KIT* gene that can be easily determined by amplifying the duplication breakpoint of this complex mutation (Fontanesi et al., 2010). The belted coat color phenotype that is present in a few pig breeds is derived by another allele at the *KIT* gene. Fontanesi et al. (2016) identified a polymorphism in this gene that marks the belted allele, making it possible to use a simple PCR-RFLP analysis to identify pork from Cinta Senese breed (an autochthonous Italian belted breed-producing PDO meat) and distinguish it from meat of other commercial hybrids or breeds.

Wild boars are animals of the *Sus scrofa* species (the same species of the domestic pigs, but considered as a subspecies) are usually homozygous for the wild-type allele E^{+} of the *MC1R* gene (Kijas et al., 1998). However, introgression of domestic alleles in wild boar populations in Europe has reduced the discriminatory power of this DNA marker. For this reason, the combined use of *MC1R* markers and of a single nucleotide polymorphism in the *NR6A1* gene can be useful to distinguish meat from wild boars from meat of other domestic pigs (Fontanesi et al., 2014). A mutation in the *NR6A1* gene is associated with an increased number of vertebrae. Domestic pigs of highly selected breeds have more vertebrae than wild boars. Domestic pigs are usually homozygous for the domestic (or mutated) allele of this gene (associated with an increased number of vertebrae), whereas wild boars are usually homozygous for the wild-type allele associated with a lower number of vertebrae (Mikawa et al., 2007). Therefore, the combined use of few DNA markers in genes that were under selection over the domestication process can improve the power for the authentication of wild boar meat (Fontanesi et al., 2014).

The Herd Book of another autochthonous Italian pig breed, Mora Romagnola, has been modified to account the possibility to link DNA markers of the animals with the need to authenticate pork products derived from this breed. Pigs of this breed can be registered to its Herd Book only if they have the allowed genotypes at the *MC1R* and *NR6A1* genes, which can make it possible to distinguish its meat products from those of wild boars and other domestic breeds (Tinarelli et al., 2021).

The use of a larger number of DNA markers (multimarker approach) for breed assignment of individual samples have been proposed when breed-specific markers cannot be identified. Probabilistic approaches are used in combination with the genotyping of customized or commercially available panels of single nucleotide polymorphisms (SNPs), i.e., SNP chips, mainly used in

cattle and pigs (Wilkinson et al., 2011; Bertolini et al. 2018; Schiavo et al., 2020). The most used bovine and pig commercial SNP panels (with 50–70,000 SNPs) can generate redundant information that might complicate the statistical processes needed for the assignment of the individuals to their breed. The statistical analyses are developed to identify the most informative SNPs, starting from SNP data sets already available from all potential breeds to which one individual meat sample could be assigned. The assignment of a meat individual sample to a breed starts from the genotyping of the customized or commercial SNP chips using the extracted DNA from the investigated specimen. Then these genotyping data are compared to the marker preconstituted reference database for allocation of the sample, with a certain level of probability, to one of the breeds for which data are already available in the database.

An application of a multilocus-marker approach is also available in pigs, in which a complication of the process would require the identification of cross-bred animals from two breeds and the estimation of the level of the contribution of the two breeds to the final product (Muñoz et al., 2020). The production of dry-cured Iberian hams, according to Spanish regulations, can be obtained from pigs of the Iberian breed with the possibility to also use cross-bred animals derived by crossing Duroc with Iberian pigs and a maximum of 50% of Duroc genome is admitted for these hams.

One of the drawbacks of the multimarker approaches is that it cannot be used to assign the breed of origin for products constituted by mixtures of several/many animals. This is the case of some meat preparations or processed products where the genotyping results would not be reliable. This problem is less important if only one (or very few) breed-specific markers are analyzed, as all animals of the expected breed might have the same genotype.

18.2.3 Individual identification of the animals: Meat traceability

Individual identification of the animals from which the meat is produced is useful to address the concept of meat traceability, as originally defined. A definition of meat traceability considers this question as the ability to maintain a credible custody of identification of animals or animal products through various steps within the food chain, from the farm to the retailer (McKean, 2001). Meat traceability emerged as a need to safeguard consumers and animal health after the bovine spongiform encephalopathy (BSE) crisis that caused drastic reduction of beef consumption in all Europe, and many other crises that mined the consumers' confidence mainly toward the food of animal origin.

Most traceability systems that are applied in the meat industry are based on documental evidence and electronic transfer of information that rely on classical barcodes, archives of documents, and storage of digital data in proprietary or, in some cases, public databases. Transfer of information follow the different steps of the production, slaughtering, processing, and final distribution of the

meat and thus involves all actors of the production chain that have to keep records of all these events. Despite standard meat traceability processes for different species have been implemented based on several national or international regulations (that consider only parts or all steps of the production chains), it is clear that the unique and unmodifiable identifier that the meat has in all points of the production chain and that links it from the farm to the consumer plate is its DNA. As the genome of each animal is different from that of all other animals (excluding true twins and clones, for which this assumption cannot be directly applied), in theory, individual traceability based on DNA analysis would provide all information for a complete traceability of the animals and of their products. If an animal can be traced, indirectly all other information related to this animal can be traced together, i.e., breed, sex, geographic origin, farming practices, and feeding (for sex see also the following paragraph). Therefore, panels of DNA markers (microsatellites and SNPs) have been proposed to this purpose. These markers can detect the diversity existing within species and whose level of combined interindividual variability within and across breeds would be able to discriminate one animal from another. The informativity of the panels for individual discrimination is based on the match probability that is the probability to find, by chance, two animals with the same genotype at all DNA markers of the analyzed panel. Lower is the match probability, lower is the probability to identify by chance two animals with the same genotypic profile (and thus indistinguishable), and more informative is considered the panel. The match probability is calculated as a cumulative probability of each DNA marker and is derived from the number of markers included in the panel. With a high number of markers with a balanced allele frequency in a considered reference population (and eventually, each with more than two alleles segregating in the population), it is possible to reach a very low match probability, far from the actual number of the heads present in the population (Weir, 1996). The first panels for individual traceability have been based on microsatellites markers that could provide a low match probability by just analyzing a few of them (usually 5–12), considering the fact that they are usually highly polymorphic (i.e., many alleles for each microsatellite). Several studies proposed informative microsatellite panels for beef individual traceability, adapting in some cases panels already developed for parentage testing. These tools are available also for other meat species, like pig, sheep, goat, horse, and poultry and in a few particular cases have been proposed for individual animal identification for meat traceability. Then, again, the introduction of high-throughput SNP genotyping platforms made it possible to design biallelic SNP panels with sufficient markers to assure a comparable low match probability with that of the multiallelic microsatellite panels, but at the same time assuring a higher throughput in the analysis with increased comparability of the results across laboratory (Heaton et al., 2002). At present, commercial SNP chips that use Illumina or Affymetrix (now Thermo Fisher Scientific) genotyping platforms, already available or that could be customized for many meat species, can be used for this purpose.

Individual traceability is effective if the meat product is derived by one animal. In case more than one animal is expected to contribute to a product (e.g., salami, hamburgers, etc.), a few solutions have been tentatively proposed. For example, individuals that contributed to ground beef patties could be traced back by physically separating the individual components of those products, transforming again the problem of a DNA analysis for individual identification with an appropriate statistical approach to reduce the number of analyses for tracing a batch of animals (Vetharaniam et al., 2009).

The implementation of complete individual traceability systems based on DNA analysis at the different steps of a production chain would be too expensive and complicated for their logistic implementation (considering not only the genotyping costs but also the expenses needed for the creation and management of specific databases, the sampling organization and the constitution of biorepositories). The added cost of the production process should be adsorbed by an added value of the meat traced in this way, and this should be also related to the value of the animals and to the willingness of consumers to pay more. In practice, this system can be used to verify other traceability systems or could be implemented in quality assurance programs in which not all animals in the system are analyzed but only a certain number of them and their products to randomly verify the correct procedures already established in other ways.

An approximation of the individual traceability makes use of the way in which DNA of an animal is inherited from their parents. If sires and dams (or only sires) in a farm are genotyped (with microsatellites or SNP chips), it is possible to make inference of the sire or the dam or both parents using genotyping data obtained on the offspring (Hill et al., 2008). This method would reduce the cost as only sires (and dams) might be genotyped. The offspring would be genotyped only in few cases when it would be needed to trace back individual terminal meat animals to their original farm. This simplified approach could be considered as a dynamic batch traceability that requires only to be updated by genotyping new sires (and eventually dams) that are introduced in the farms. The reduction of cost of genotyping and the implementation of genomic selection will probably result in a more frequent application of individual traceability systems than those currently observed in commercial production chains.

18.2.4 Identification of the sex of the animals

The sex of the animals from which meat is produced is important for a few practical reasons. In beef production, for example, carcass and meat quality traits are usually better in steers than in heifers and meat from steers is better valued. This aspect can be relevant when measures such as intervention buying and export refunds are introduced to strengthen the market position of meat producers for which a higher subsidy for male beef is foreseen (Zeleny et al., 2002). In several states of India, slaughtering of cows is usually not allowed

for religious reasons. In pigs, raising entire males that are slaughtered close to the puberty increases the risk of boar taint perception during meat consumption and, in this case, pork is considered as unfit for human consumption. For some pork productions, females are preferred for their higher meat and carcass quality than castrated males. Therefore, in several circumstances, robust methods for sex identification of the meat are needed to avoid frauds. Another application of sexing meat can be for a rapid and simple control of sample identification and matching in a production chain in which both females and males are processed (Fontanesi, 2009).

In mammals, females and males can be distinguished by their sex chromosomes (XX in females and XY in males). Therefore, sex determination methods have been developed to identify these differences in the DNA using PCR analyses. A few methods have been developed by PCR amplification of Y-chromosome specific sequences in male genomic DNA that are present only in animals of this sex. Other methods have been based on the amplification of fragments that could directly obtain information from the presence of only X chromosomes (females) or both X and Y chromosomes (males). For example, the amplification of both *ZFX* and *ZFY* genes (located on the X and Y chromosomes, respectively) with one unique primer pair, followed by RFLP or other methods to identify sequence differences existing between these two forms, has been largely used in many species (Aasen and Medrano, 1990). The most used and convenient methods for sex determination in many mammals take advantage from fragment length differences existing between other two genes present on both X and Y chromosomes, *AMELX* and *AMEL*Y. PCR methods based on the analyses of these genes have been also reported in cattle and pigs (Ennis and Gallagher, 1994; Fontanesi et al., 2008b). Some of the microsatellite panels, not only useful for parentage testing but also for individual identification of the animals, contain also primers for *AMELX*/*AMELY* amplification and thus for sex identification of the animals by capillary electrophoresis of the multiplex generated fragment products. In addition, SNP chips already mentioned in the previous paragraphs, having thousands of SNPs placed on the X chromosome, can directly identify the sex of the genotyped animals. Males might have homozygous (hemizygous) genotype called for almost all markers located on this chromosome.

18.2.5 Identification of genetically modified animals

Despite their potential controversies and the current debate about the use of genetically modified organisms for human consumption, genetically modified animals have not raised a lot of concerns due to their limited availability and their restricted regulation for their use. In this context, genome editing is emerging as an important technology to modify the genome of all organisms, including animals (Tait-Burkard et al., 2018). One of the characteristics of genome edited animals is that they would not be distinguishable from naturally mutated

animals, raising many questions on the debate on the possibility or need to distinguish these animals from conventional animals.

Three categories of genetically modified animals can be defined: (i) genetically modified animals originally designed for human consumption that have been generated for answering farming needs acting directly on genes improving production traits, quality characteristics of the products, disease resistance, or reduced environmental impact; (ii) genetically modified animals used as bioreactors for the production of pharmaceutical active molecules or used as animal models; and (iii) genetically modified animals used as companion animals (Lievens et al., 2015).

DNA-based tests specifically designed for each case, targeting the modified or modifying sequences or their constructs inserted in the genome of transgenic animals, could, in theory, easily identify meat obtained from these animals. However, several problems, which complicate the situation, derive from the fact that (i) the exact modified sequences or the needed sequence information are not available for all genetically modified animals, (ii) multiple integration of a transgene in an animal genome could make it difficult to design simple methods, and (iii) chimerism could not alter all somatic cells and thus not all meat cells could contain the signature of the genetic modification (Lievens et al., 2015). These elements, mainly true for genetically modified animals obtained using traditional transgenic methods, cannot be applied for the new genome editing technologies, like CRISPR/Cas9, which can introduce modification in an animal, replicating variants already present in nature in other animals of the same species.

18.3 Extrinsic characteristics of the meat

Other characteristics or components of the meat derived by the interaction between the animal metabolism and environmental (external) factors or by post mortem treatments of the meat can be used to infer different origins of the animals and, in turn, of the meat. Some modified characteristics of the meat can be useful to detect other adulterations derived from technological processes that usually have as main objective the extension of the preservation time of the meat.

18.3.1 Geographic origin, feeding regimes, and production systems

Compulsory and, in many cases, voluntary indications of geographic origin and feeding practices (used to raise animals) for meat and meat products are in force in many countries or have been adopted as marketing strategies to follow consumers' general demands and perceptions. Moreover, traditional regional products, like Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Speciality Guaranteed (TSG) products, are

defined by specific productions rules and geographic limitations. Measures of stable isotope ratios (SIR) and trace elements in animal products, including meat, have been used to determine their geographic origin and the way in which animals are fed (Franke et al., 2005; Vinci et al., 2013; Camin et al., 2016). Data are elaborated using principal component analysis, discriminatory analyses, or other statistical approaches needed to handle multiple information. The isotopic ratios of the main elemental constitution of bio-organic materials (i.e., $^{2}H/^{1}H$; $^{13}C/^{12}C$; $^{15}N/^{14}N$; $^{18}O/^{16}O$; $^{34}S/^{32}S$) provide signatures of geographic origin, climatic conditions, soil pedology, and geology and production systems that are incorporated into the animals and their products (Kelly et al., 2005; Camin et al., 2016). For example, H and O isotopic ratios in meat are linked to the corresponding ratios of drinking water and water present in the feed of the animals that have geographic variability. Variability in the $^{13}C/^{12}C$ ratio in the meat relates to the botanical origin of the feed based on the different photosynthetic pathways (C3 or C4; derived by the different isotopic discrimination capabilities of the carboxylase enzymes involved in CO_2 fixation; corn is a C4 plant with a higher ^{13}C content). The levels of the $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios in the meat are mainly related to the diets of the animals, which in turn derive from different factors of the production area. SIRs are usually determined with the continuous-flow isotope ratio mass spectrometer (CF-IRMS, having sample preparation online, smaller sample size, faster and easier analysis, more cost effectiveness, and the ability to interface with other preparative techniques) and dual-input isotope ratio mass spectrometer (DI-IRMS), which has sampling preparation offline but more accurate measurements (Danezis et al., 2016).

Useful geographic fingerprints are also provided by trace elements that vary in different regions mainly for pedological and geological factors. Selenium, among several other elements, has been one of the most frequently evaluated in beef cattle (Hintze et al., 2002). The combination of both SIR and trace elements has been largely used to improve the determination of the geographic origin of meat of different species (Kelly et al., 2005; Heaton et al., 2008).

In general, more sound geographic attribution of meat samples using SIR and trace elements can be obtained comparing products coming from distant geographic regions, whereas correct attribution is usually limited if production regions are geographically close. As the whole methodology relies on the availability of large data sets obtained from samples of different origin, it is important to construct large reference databases to improve reliability of the assignment statistical approaches. To reduce the cost of sampling, Liu et al. (2013) have proposed to simplify the collection of biological materials from different areas by analyzing tail hairs from many cattle, considering the high correlation between SIR measured both from these specimens and meat of the same animals.

Geographic origin can be determined by adding indirectly information from feeding regimes of the animals that could derive from a more intense use of cereal feed ingredients such as "corn-fed" in some areas, extrapolated from

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios (Heaton et al., 2008; Vinci et al., 2013; Camin et al., 2016). Feeding regimes of the animals retrospectively evaluated by $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios analyzed in the meat has been considered as a monitoring approach of farming methods (Monahan et al., 2012), like poultry labeled as corn-fed for special marketing terms of retail poultry meat products or for application to organic production systems (Rhodes et al., 2010; Zhao et al., 2016).

Other biomarkers have been used to infer feeding regimes of the animals and to detect meat produced from animals raised in organic systems, compared to that obtained from animals raised in conventional ways. Feeding signature in the meat can be derived from a few biomolecules that are directly deposited in the animal tissues with or without any metabolic transformation. In particular, fatty acid composition of the meat (measured by a few systems but in particular using gas chromatography) and the level of carotenoids (considered as xenobiotics: xanthophylls and carotenes, like β-carotene and lutein) and α-tocopherol isomers have been used to infer feeding practices of the animals that, in turn, are related to different production systems. A general higher ratio of polyunsaturated fatty acids versus saturated fatty acids indicates that beef cattle are fed on grass instead of using concentrates or hay (French et al., 2000). An official method to detect cured hams produced from free ranged Iberian pigs, mainly fed with natural resources (i.e., grass and acorns) is based on the higher content of unsaturated fatty acids in subcutaneous fat of these hams than that of the fat of pigs raised in conventional intensive farming regimes (BOE, 2004). The detection of vitamin E (α-tocopherol) stereoisomers in the meat indicates that the beef cattle have received a concentrate supplementation including synthetic vitamin E (Monahan et al., 2012).

Analytical authentication methods for organic meat are based on differences that the products could acquire from being derived by animals raised following organic rules compared to those produced by animals raised in conventional systems (Średnicka-Tober et al., 2016). Methods and biomarkers for organic products can be based on the detection (and comparative evaluation against nonorganic production systems) of SIR, trace elements, fatty acid composition, xenobiotics, α-tocopherol, and permitted or nonpermitted use of tetracycline antibiotics (Kelly et al., 2006), using different methods and in different combinations, together with appropriate chemometrics approaches to analyze high-dimensional data sets with many variables (Capuano et al., 2013). Heterogeneity of the organic production systems, environmental differences, and the use of different breeds complicate in most cases the correct allocation of the meat, organic vs conventional (Średnicka-Tober et al., 2016).

18.3.2 Treatments and processing procedures

Many different treatments and processing procedures could be applied to meat products. Some of them are under specific national or international regulations and require specific labeling of the products. Others are part of the production

process. In this case, it could be needed to evaluate if they are appropriately applied. Some of these treatments modify physical-chemical characteristics of the meat that can be used to verify what is claimed. Curing and smoking processes can be applied to improve preservation. Additives could be also added for the same purpose or to improve palatability or to mask undesired flavors. Environmental conditions in which treatments and raw materials are originated could produce specific microbiota signatures (Belloch et al., 2021) that can be analyzed using next-generation sequencing approaches. Methods and methodologies for the identification of markers related to these production conditions need to be further explored or developed ad hoc. Therefore, the following paragraphs describe only official and unofficial methodologies that are used to detect meat that has been processed using ionizing radiation or that has undergone freezing and then thawing procedures.

18.3.2.1 Irradiated meat

Irradiation is a process mainly aimed to improve preservation and reduce the risk of pathogen transmission by exposing meat to ionizing radiation. This treatment is regulated in different countries (Ehlermann, 2016). At present, fresh meat, poultry, and frog legs are authorized for irradiation in the European Union. Irradiation of fat-containing food produces 2-alkylcyclobutanones and volatile hydrocarbons. In addition, radiation treatments produce radicals that are stable in solid and dry biological components like bones and that can be used to identify irradiated meat with bones. The European Committee for Standardization (CEN) has developed official methods of detection, based on these irradiation-derived modifications, that have been adopted by the Codex Alimentarius Commission as General Methods (European Union, 2022). Analysis of hydrocarbons is based on gas chromatography, and detection of 2-alkylcyclobutanones is obtained by MS after gas chromatographic (GC) separation and irradiated bones can be detected by analyzing the electron spin resonance (ESR) signal, which is attributed to hydroxyapatite (a component of the bones) by ESR spectroscopy.

18.3.2.2 Frozen/thawed meat

Storage of frozen meat is a common preservation method that reduces post mortem enzyme activity, inhibits microbial proliferation, and prolongs the shelf life. Thus, frozen meat is common in international and overseas transports. Frauds can be derived by selling thawed meat as fresh. Therefore, methods that can discover this fraud should be able to distinguish differences between fresh and frozen/thawed meat (Ballin and Lametsch, 2008) or fresh/chilled and frozen/thawed meat, as chilling is commonly used to maintain freshness. The classical method applied for this purpose measures the activity of the mitochondrial enzyme (β-*hydroxyacyl-CoAdehydrogenase,* HADH), which is released in the meat juice when the mitochondrial membranes are damaged during freezing and thawing (Gottesmann and Hamm, 1983). The catalyzed reaction is the

following: acetoacetyl-coenzyme A + NADH + H^+ β-hydroxybutryl-coenzyme A + NAD^+. The enzyme activity is measured using a UV spectrophotometer by following the rate of decrease of NADH, which is proportional to the decrease in the absorption of the extracted juice at 340 nm as measured at a few time points. The HADH activity is measured from a meat on two subsamples of the same specimen: (i) on the intracellular fluid pressed from the subsample as it is received in the lab and (ii) on the fluid of the subsample that has been laboratory frozen and then thawed for the laboratory analysis. The ratio between the two subsamples can be used as an indicator of the thermal history of the sample. An important preliminary step in this analysis is the validation of the cutoff limits of the ratios at 99% confidence interval for declaring that the meat sample has been previously frozen. If the ratio is close to 1, it is likely that the meat has been previously prefrozen and then thawed. The cutoff limits, however, vary between species and have been the matter of cross-laboratory validation as in the case of poultry meat (European Union, 2013). New mathematical approaches have been developed to improve accuracy and cost efficiency of the HADH method by eliminating the need for the additional freezing-thawing step. Instead of 2 absorbance measurements at 0 and 3 min, Boerrigter-Eenling et al. (2017) have proposed the use of continuous absorption data during the enzymatic assay to enhance discrimination and reduce the number of samples to be analyzed considerably. The HADH method cannot be applied in grounded fresh meat, as mitochondrial damage might be also derived by this treatment and is not very effective if meat is not frozen at a temperature below −12°C.

Freezing and then thawing create many other microstructural modifications and alterations of meat components that cannot be clearly distinguished by visual evaluation. Analytical methodologies may capture these differences. NIR has been used to differentiate frozen/thawed beef, pork, and lamb meat (Evans et al., 1998). Spectroscopic technique in different configurations has been also proposed for this aim. For example, NIR and VIS/NIR hyperspectral imaging systems have been applied to differentiate fresh from frozen/thawed porcine muscles with a correct classification ranging from about 90% to 100% of the samples (Douglas et al., 2013), and multispectral imaging and Fourier transform infrared spectroscopy can be applied to correctly classify (>90%) fresh and thawed minced beef (Ropodi et al., 2018).

Górska-Horczyczak et al. (2016) have differentiated fresh from frozen/thawed pork cuts with an electronic nose based on ultrafast gas chromatography supported by supervised artificial neural network for acquired data analysis reaching a correct classification rate of 80%–90% of the samples. Chen et al. (2016) have proposed the use of impedance measurements to distinguish between fresh and frozen-thawed chicken breast muscle with a prediction accuracy ranging from about 85% to 100%, depending by the number of frozen/thawed cycles and by the methods used for data analysis.

Other methods that have been proposed to detect frozen/thawed meat (i.e., DNA analysis by PCR, light-microscopy, electron microscopy, odor, color,

and tenderness evaluations; Ballin and Lametsch, 2008) have not found any practical application. The level of RNA degradation could potentially provide new analytical solutions to evaluate the time from slaughtering and also the level of post mortem treatments of the meat, including freezing and thawing (Fontanesi et al., 2008a).

18.4 Conclusions and future trends

Frauds cause relevant economic damages to many food production chains and increase sanitary risks for the consumers in addition to several other problems. The possibility to guarantee authenticity of meat and meat products is becoming one of the most important prerequisites that the production chains should consider even before a product is conceived and then commercialized. The existence of effective tools and methods that are able to authenticate the products can discourage fraudsters. To identify frauds, it is fundamental to predispose precise, simple, and cheap analytical methodologies. These issues are related to the level of authenticity that should be evaluated. Intrinsic unmodifiable biological characteristics of the animals (i.e., species, breed, individual identity, and sex) can provide information that may solve some of the authentication and traceability problems, mainly using DNA but also proteins and other meat components. These biomarkers have in general a high discriminatory power, derived by the possibility to identify differences or biomarker-specific features that characterize the meat product that is analyzed. In many cases, the complexity of the problems, however, need to be evaluated considering previous assumptions and probabilistic approaches. Other procedures or origins of the meat could differentiate the products not in a very specific way, providing an unclear and not completely distinguishable signature, that might be measured or detected with analytical methods. In these cases (i.e., geographic origin, feeding regimes, organic vs conventional), approaches should consider several sources of information, different markers, and might take into account that the final answer should be considered only as a preliminary indication of a problematic situation.

Novel analytical solutions for meat authenticity have been provided by scientific and technological advancements in the fields of genomics, proteomics, chemometrics, analytical chemistry and biochemistry, and devise engineering. It is expected that some pilot applications and studies, which are now only proof of concepts, could be further developed to fully exploit their potential for the authentication of meat products. New developments are envisaged on the use of real-time methods that could give a preliminary answer without the need of any laboratory analyses that could be anyway subsequently carried out for more sophisticated and precise evaluations. Standardization of methods are also expected for general questions and problems, even if the heterogeneity of the meat, derived by different species, cuts, preparations, and situations, may complicate the definition of standards. In addition, the heterogeneity of the different

questions that are asked and the specificity of many niche products require the identification of ad hoc procedures and methods that should be studied case by case.

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Chapter 19

Meat composition and nutritional value

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19.1 Introduction

Meat, the flesh of animals, has been a central part of the diet of humans for over 1 million years (Klurfeld, 2015). It is widely eaten and enjoyed, and meat dishes are part of the culture of many people around the world. Meat provides many important nutrients in a readily available form: protein, energy, fat, vitamins, and minerals and is commonly viewed as a significant part of a well-balanced diet. However, it is not essential, and both vegetarians and vegans can live healthily without meat. Recently, meat production has come under pressure for environmental, animal welfare, and health reasons, and this has brought into focus the nutritional importance of meat in the diet. This chapter reviews the latest information on the composition and nutritional value of meat in the four main meat species: beef, sheep, pork, and chicken.

19.2 Global meat production and consumption

Meat consumption and production on a global scale have steadily increased since the 1960s. At the time of writing (2020), an outbreak of African Swine Fever has slowed meat production in Asia, but global meat production is still forecast to increase by 40Mt between 2020 and 2029, reaching 366Mt (OECD—FAO, 2020). The bulk of this growth will be in developing regions of the world, and about 50% of it will be chicken, which is now the biggest livestock sector. In 2018, China was the major meat-producing country (90Mt), followed by United States (50Mt) and Brazil (30Mt).

There is a close association between per capita meat consumption and per capita income in the different countries of the world (Sans and Combris, 2015). Recent predictions suggest that global meat consumption will increase by 12% to 2029, but per capita consumption by only 1%, because of slowing income growth in developing countries and a leveling off of intake in high-income countries. The latter is explained by changes in consumer attitudes to meat

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eating, linked to concerns over health aspects, the welfare of animals in meat production systems, and the environmental cost of meat production (OECD—FAO, 2020). Vegetarianism is increasing in many countries. It was 0.2% in the United Kingdom in the 1940s and 3%–7% in 2000 according to Phillips (2005). It is now accepted that vegetarians can meet recommendations for all the major nutrients if they combine foods carefully (Craig and Mangels, 2009).

19.3 Composition of meat. Roles of nutrients in metabolism and recommended intakes

19.3.1 Macronutrients

The nutrients in foods are carbohydrate, protein, fat, sugar, fiber, water, vitamins, and minerals. Meat contains no fiber and very small amounts of carbohydrate, such as glycogen in the muscle and liver. The nutrients present in large amounts are termed macronutrients and include protein, fat (often subdivided into saturated, monounsaturated, and polyunsaturated fat), and water (see Chapter 20). The amounts of these per 100 g (an amount taken to represent a daily serving) in the raw muscle (lean) of beef, sheep, pork, chicken, turkey, and liver of beef and sheep are in Table 19.1. Muscle has been separated from surrounding adipose tissue by dissection, as in someone separating lean from fat on the plate. Liver is listed because of its high nutrient density arising from its central roles in nutrient metabolism and storage. Cholesterol is included because of its importance as a component of cell membranes and a precursor of steroid hormones and its role in heart health. It is transported in lipoproteins in the blood where a high ratio of low-density lipoproteins to total blood cholesterol can lead to plaque formation and coronary heart disease (CHD) (Institute of Medicine, 2005). Cholesterol is obtained from the diet and synthesized from saturated fatty acids (SFA) in the liver: a high intake of SFA is a risk factor for CHD. Various databases for meat nutrients exist, the data in Table 19.1 being published by UK Food Standards Agency (FSA, 2002). The data on raw liver are from Purchas et al. (2014), the data in FSA (2002) being for the cooked liver, which has a lower water content and correspondingly higher amounts of the other nutrients than raw liver.

19.3.1.1 Protein

The amounts of nutrients listed in Table 19.1 can be compared with amounts recommended for a healthy lifestyle by national authorities. In European Union (EU), dietary reference values (RV) are given for different groups in the population (EFSA, 2017), and reference intakes (RI) are single values representing all the groups that are used in labeling to show how the nutrient content of the food matches the recommended amount (EU, 2011). All the muscle and liver samples in Table 19.1 could be labeled as "high in protein" because more than 20% of the energy is provided by protein (values ranged from 54% to 87%). The RI for protein is 50 g so these 100 g samples would provide more than 40% of

TABLE 19.1 Water, protein, fat, fatty acid, energy, and cholesterol content (per 100 g) of raw lean of beef, sheep, pork, chicken, and turkey (FSA, 2002) and of raw liver of beef and sheep (Purchas et al., 2014).

	Beef[a]	Sheep[b]	Pork[b]	Chicken[c]	Turkey[c]	Liver[d]	
						Beef	Sheep
Water (g)	71.9	70.6	74.0	75.1	75.3	70.4	70.8
Protein (g)	22.5	20.2	21.8	22.3	22.6	20.5	20.7
Fat (g)	4.3	8.0	4.0	2.1	1.6	4.1	4.9
SFA[e] (g)	1.7	3.5	1.4	0.6	0.5		
MUFA[f] (g)	1.9	3.1	1.5	1.0	0.6		
PUFA[g] (g)	0.2	0.5	0.7	0.4	0.4		
Energy (kJ)	542	639	519	457	443	494	529
Cholesterol (mg)	58	74	63	90	70	254	386

[a] *Average of 10 different cuts.*
[b] *Average of eight different cuts.*
[c] *Combination of white and dark meat.*
[d] *Average of 9 beef and 10 sheep (lamb) livers.*
[e] *Saturated fat.*
[f] *Monounsaturated fat.*
[g] *Polyunsaturated fat.*

the recommended daily intake. In United States, the daily value (DV) is equivalent to the RI in Europe and is also 50 g for protein, with foods containing more than 20% of the DV such as the muscle samples in Table 19.1 also qualifying as high in protein (FDA, 2020).

The protein in meat has a high biological value because the amino acids and their proportions are similar to those in the body of humans. Amino acids serve many functions, as constituents of cell membranes, enzymes, hormones, and muscle. There are nine essential amino acids that cannot be synthesized in the body, and meat contains all of these. Purchas et al. (2014) showed that 100 g of lean meat from beef and sheep (lamb) provides 80%–110% of the recommended daily intake of most amino acids but only 52%–64% of the recommended intake of the branched-chain amino acids isoleucine, leucine, and valine.

Vegetarian diets provide adequate protein and a good balance of amino acids provided that a variety of foods is consumed (Millward, 1999).

19.3.1.2 Fat

Fat is an important nutrient in food, being a source of energy, essential fatty acids, and fat-soluble vitamins. However, it is also implicated in various health conditions including CHD, cardiovascular disease (CVD), and diabetes. Fat content has to be declared on packed food in many countries and compared with the recommended daily amount. In EU, the RI for total fat is 70 g. To be labeled as "low in fat," the food must contain less than 3 g/100 g, and if it contains more than 17.5 g/100 g, it must be labeled "high in fat" (EU European Union, 2011). In United Kingdom, the lettering of values on the food package that are low in fat is in green and that of high-fat values is in red, a way of presenting a simple health message to the consumer. The samples of chicken and turkey muscle in Table 19.1 could be labeled as low in fat, and none of the samples is high in fat. In United States, the DV for total fat is 78 g, and samples with less than 3.9 g can be labeled "low in fat" (5% of the DV) (FDA, 2020). These amounts are slightly higher than the EU definitions, but the same conclusions apply: only the chicken and turkey muscle samples could be labeled "low in fat."

Lean muscle is relatively low in fat compared with cuts of meat that also contain adipose tissue. FSA (2002) lists sheep (lamb) loin chops and pork loin chops as having 23.0 and 21.7 g/100 g fat, respectively. Pork sausages contained 25 g fat/100 g. The energy content of meat is directly linked to its fat content as shown in Table 19.1 where lamb muscle with the highest fat content has the highest energy value and vice versa for turkey muscle.

19.3.1.3 Fatty acids

Like total fat, saturated fat (termed saturated fatty acids, SFA) is also required to be declared on prepacked food because SFA intake is a risk factor for CVD linked to its effects on raising the blood cholesterol level (Calder, 2015). In both EU (EU, 2011) and United States (FDA, 2020), the RI/DV amount is 20 g, and

the definition of "low in saturated fat" is 1.5 g (EU) or 1 g/100 g (United States). In Table 19.1, only chicken and turkey muscle could be labeled as being low in saturated fat.

Table 19.1 shows the amounts of SFA (fatty acids with no double bonds), monounsaturated fat (monounsaturated fatty acids, MUFA, with one double bond), and polyunsaturated fat (polyunsaturated fatty acids, PUFA, with two or more double bonds) in muscle. The main individual fatty acids, which make up these groups, are shown in Table 19.2, taken from Wood and Scollan (2021). The table lists results for the fatty acid composition of *longissimus* total lipid (TL), consisting of triacylglycerol and phospholipid, in 22 references in which grain-based (concentrate) diets were fed to all the species (along with some forage in beef and sheep) to minimize the effects of diet on fatty acid composition. The ruminant species are relatively high in SFA, particularly 16:0 (palmitic acid), which has a high cholesterol-raising effect (along with 14:0, myristic acid), compared with 18:0 (stearic acid). Beef and sheep muscles are low in PUFA as the result of rumen biohydrogenation, which saturates dietary PUFA, producing SFA and also MUFA and PUFA with *trans* double bonds rather than the usual *cis* double bonds (Scollan et al., 2017). The intake of *trans* fatty acids is also a risk factor in heart disease, similar to SFA, although the rumen-derived *trans* fatty acids are less harmful than those that are produced industrially (Wang and Hu, 2017). There has been great interest in the *trans* isomers of 18:2n-6 (linoleic acid, LA) termed conjugated linoleic acids (CLA), especially *c*9,*t*11-18:2 (rumenic acid) because of possible anticancer and anti-CVD effects (Gebauer et al., 2011), but health authorities such as EFSA (2017) have not endorsed these potential health benefits.

Pork and chicken have higher PUFA contents than beef and sheep, especially of the main PUFA, LA (Wood et al., 2004). LA is an essential fatty acid, being required in metabolism but not synthesized in the body. It has to be provided in the diet—oils in soya and grains, the main sources of nutrients used in animal diets, are high in LA. The other essential fatty acid is 18:3n-3 (α-linolenic acid, ALA), which is a major fatty acid in the leaves of grasses and plants fed to ruminants. Although about 90% of ALA in the ruminant diet is degraded by biohydrogenation (Shingfield et al., 2013), the large amounts of foragefed mean that beef and sheep muscles have relatively high percentages of ALA. Both LA and ALA are converted in the body to their long-chain derivatives: 20:4n-6 (arachidonic acid, AA) from LA and 20:5n-3 (eicosapentaenoic acid, EPA), 22:5n-3 (docosapentaenoic acid, DPA) and 22:6n-3 (docosahexaenoic acid, DHA) from ALA. The de novo production of the long-chain n-3 PUFA is very low, so dietary provision is important. The 20 carbon fatty acids 20:4n-6 and 20:5n-3 are substrates for eicosanoids, which affect inflammation. The eicosanoids from AA are more inflammatory than those from EPA, leading to a proinflammatory state, which predisposes toward conditions such as CVD (Calder, 2015). Simopoulos (2002) has shown how the ratio of n-6:n-3 PUFA in the diet of man has increased over thousands of years, from around 2.0 to 12.0 as LA, a major component of carbohydrate foods, has greatly increased in the diet.

TABLE 19.2 Fatty acid composition of muscle (*longissimus* except where indicated in beef, sheep, and pork, and *pectoralis* in chicken) (% of TL).

	14:0	16:0	*c*9–16:1	18:0	*c*9–18:1	*c*11–18:1	*t*11–18:1	18:2n-6	*c*9*t*11–18:2	20:4n-6	18:3n-3	20:5n-3	22:6n-3	TL[a]
Beef														
Kraft et al. 2008[b]	1.35	19.49	2.02	13.72	28.97	1.50	0.47	8.91	0.29	1.83	0.64	0.31	0.05	1100
Jiang et al., 2010	2.50	27.90	4.00	12.40	37.50	3.10	1.10	3.50	0.30	1.30	1.20	0.30	0.07	3230
Herdmann et al., 2010	2.60	26.30	3.70	14.60	37.10	NR	0.60	5.30	NR	1.40	0.60	0.20	0.05	2367
Rodriguez-Herrera et al., 2018	2.12	22.97	3.35	12.25	36.45	NR	1.99	2.99	0.30	1.16	0.15	0.50	0.31	3359
Vahmani et al., 2017	2.44	23.10	2.13	16.40	31.00	1.01	4.98	2.09	0.48	0.26	1.13	0.15	0.02	5170
Sheep														
Noci et al., 2011	1.81	24.14	1.69	15.65	39.01	0.53	2.85	3.45	0.82	0.60	0.50	0.07	0.04	3524
Urrutia et al., 2015	4.11	27.86	1.62	14.02	30.01	1.80	4.69	7.10	0.08	1.84	0.47	0.11	0.05	3740
Schiavon et al., 2017	2.22	21.38	1.18	17.85	30.97	NR	2.94	4.07	0.76	1.48	0.60	0.08	NR	4480
Natalello et al., 2019	2.17	22.68	1.34	16.16	36.59	1.12	0.73	5.37	0.35	1.42	0.37	0.13	0.09	1880
Parente et al., 2020	1.76	22.90	1.92	15.30	44.50	1.12	0.37	4.43	0.19	2.11	0.28	0.26	0.10	2457
Li et al., 2020	3.16	24.20	2.87	13.35	31.98	1.95	3.36	7.43	0.28	2.61	0.37	0.15	0.16	8736

Pork														
Enser et al., 2000[c]	1.03	21.7	2.60	11.70	29.60	3.3	NR	17.50	NR	4.10	0.84	0.42	0.43	1093
Musella et al., 2009[d]	1.27	23.59	3.26	11.13	47.55	NR	NR	11.19	NR	0.04	0.55	0.12	0.14	NR
Alonso et al., 2012	1.13	22.88	3.36	11.02	41.01	4.49	NR	8.97	NR	2.00	0.35	0.11	0.14	2300
Tous et al., 2013	1.24	21.70	2.76	11.20	33.80	3.51	NR	16.30	NR	3.63	0.29	0.06	0.06	2060
Minelli et al., 2019[e]	1.40	27.99	3.08	14.43	42.53	3.83	NR	4.18	NR	0.31	0.19	NR	0.01	3970
Zappaterra et al., 2020	1.36	23.48	2.93	11.81	40.92	3.90	NR	10.62	NR	1.75	0.37	0.01	0.03	2054
Chicken														
Betti et al., 2009	0.37	17.42	2.68	5.42	39.04	3.52	NR	24.50	NR	1.30	4.18	0.20	0.28	1756
Gatrell et al., 2015	0.59	23.00	3.13	8.73	30.9	NR	NR	29.10	NR	1.44	0.94	NR	0.01	1319
Boschetti et al., 2016[f]	0.36	18.99	2.99	9.87	33.67	NR	NR	23.79	NR	4.07	1.25	0.06	0.68	2230
Li et al., 2017	0.65	23.38	3.21	7.84	32.12	3.20	NR	16.02	NR	2.95	0.92	0.27	0.53	656
Long et al., 2018	0.47	21.80	4.18	8.40	31.90	NR	NR	25.60	NR	2.46	2.02	0.12	0.30	NR

Results for control concentrate rations (Wood and Scollan, 2021).
NR, not recorded/detected.
[a] *mg/100 g muscle.*
[b] *Results for Limousin.*
[c] *Female pigs.*
[d] *Semimembranosus.*
[e] *Immunocastrated male pigs.*
[f] *Fast-growing strain.*

Although health authorities recognize the beneficial effects of PUFA generally in reducing the risks of various diseases, they have not set an RI or DV. However, several bodies have published recommendations for intakes of the long-chain n-3 PUFA, EPA + DHA, recognizing that intakes are insufficient for optimal health (Aranceta and Perez-Rodrigo, 2012). For example, in Europe, EFSA (2017) recommends a daily intake of 250 mg EPA + DHA. A serving (100 g) of 40 mg is considered a "source" and 80 mg a "good source." Similar levels have been set in Australia and New Zealand (FSANZ, 2016). In Canada, ALA is included along with EPA + DHA in the recommendation of 300 mg/100 g n-3 PUFA (CFIA, 2016). There is no DV for EPA + DHA in United States but for ALA, 160 mg/100 g counts as a "source" and 320 mg/100 g is "high in" (Institute of Medicine, 2005).

People can reach the targets for EPA + DHA by consuming two portions of oily fish per week (Aranceta and Perez-Rodrigo, 2012). However, oily fish are not popular with consumers, so introducing these fatty acids naturally into more popular foods such as meat is seen as a viable alternative (Givens and Gibbs, 2008). Feeding linseed/flaxseed, a source of ALA to animals to encourage the synthesis of EPA and DHA, leads to higher levels but not high enough to make a health claim. However, the feeding of preformed EPA and DHA as marine algae has been more successful in achieving the targets (Wood and Scollan, 2021). The highest levels of incorporation have been observed in chicken (Rymer et al., 2010), but high levels were also observed by Cooper et al. (2004) in sheep. The long-chain PUFAs are usually found in muscle as constituents of phospholipid in membranes, but when high amounts are fed, they are also seen in adipose tissue triacylglycerols.

Attempts to improve the balance between SFA and PUFA in muscle and adipose tissue via the diet have been more successful with PUFA than SFA. Reducing the levels of SFA has been difficult to achieve in ruminants because the rumen saturates dietary fatty acids (Shingfield et al., 2013). In all species, changes in SFA and MUFA concentrations are limited by modification of the activities of lipogenic, elongase, and desaturase enzymes, which regulate fatty acid composition to preserve the fluidity of membranes (Maulucci et al., 2016).

A potential problem when incorporating high levels of fatty acids with five and six double bonds into muscle is oxidation postmortem, leading to the production of volatile compounds during cooking with off-odors and flavors (Wood et al., 2004). Vitamin E as α-tocopherol is an effective antioxidant when incorporated into muscle via the diet at a supranutritional level (100–200 mg/kg diet). If a muscle concentration of 3–4 mg/kg is reached, this has prevented oxidation and flavor deterioration in many studies (Rymer and Givens, 2010).

Beef and sheep fed grass-based rather than concentrate-based diets produce higher concentrations of ALA and the long-chain n-3 PUFA in muscle and also higher concentrations of the CLA rumenic acid, although none of these to the levels where health claims can be made (Scollan et al., 2017). Another benefit of grass feeding, especially of freshly grazed grass, is that α-tocopherol

(vitamin E) in grass raises the level in tissues to the important 3–4 mg/kg level. This extends shelf life by preventing oxidation of muscle pigments, thereby retaining the red color of muscle and reducing oxidation of unsaturated fatty acids so flavor deterioration does not occur (Warren et al., 2008). A possible advantage of beef and sheep meats produced organically is higher concentrations of n-3 fatty acids because the meats are likely to have been produced on grass (Angold et al., 2008).

19.3.2 Vitamins

The major vitamins present in the raw muscle of beef, sheep, pork, turkey, and chicken and in beef and chicken liver as listed in FSA (2002) are shown in Table 19.3. The amounts are compared with the amounts defined as a "source" or a "good source" of the vitamin, that is, 15% or 30%, respectively, of the RI in a 100 g sample. The concentrations are similar to those published by USDA (2014) and USDA (2017–2018), the latter being for cooked samples. Choline and vitamin K are not listed in FSA (2002) and are from the US National Nutrient Database (USDA, 2017–2018).

Meat is a "source" or "good source" of many of the water-soluble B-complex vitamins, particularly vitamin B3 (niacin), B6 (pyridoxine), and B12 (cobalamin). Liver is the main storage organ and has the highest concentrations. The B vitamins are required as cofactors in many reactions involved in energy production and in amino acid and fatty acid synthesis. Vitamin B12 is only found in animal-sourced foods, and vegetarians are at risk from deficiency, which causes megaloblastic anemia (Craig, 2009). Vitamin B12 is a structurally complex vitamin with a central cobalt atom linked to six ligands. It is synthesized by bacteria that inhabit the gastrointestinal tract and is required in the body as a coenzyme in propionate metabolism and the conversion of homocysteine to methionine.

Choline is a water-soluble compound, not always classified as a vitamin, present in muscle at "source" levels. Choline is a constituent of the phospholipid phosphatidylcholine and the neurotransmitter acetylcholine. Like vitamin B12 and choline, vitamin B9 (folic acid) is required for methyl group transfer in amino acid metabolism. It is also required for RNA and DNA synthesis and is an important nutrient for pregnant women. Folate must be provided in the diet, usually as folic acid and amounts declared on the label of packaged food. Liver is a good source of folic acid.

The fat-soluble vitamins, A, D, E, and K, are not present at high concentrations in muscle, but very large concentrations of vitamin A are present in liver (the values in Table 19.3 are higher than those listed in USDA (2017–2018), which were 9349 μg/100 g in beef liver and 3945 μg/100 g in chicken liver). Men and women in the United Kingdom derive 34% and 22%, respectively, of their vitamin A from meat and meat products as retinol, which is needed for the maintenance of skin growth and vision (Henderson et al., 2003), so it appears that liver and organ meats make an important contribution. Concentrations of

TABLE 19.3 Vitamin content (per 100 g) of raw lean of beef, sheep, pork, chicken, and turkey and of liver of beef and chicken (FSA, 2002).

	Beef[a]	Sheep[a]	Pork[a]	Chicken[a]	Turkey[a]	Liver[b]	
						Beef	Chicken
B1 (thiamine) (mg)	0.10	0.09	**0.98**	0.14	0.07	**0.61**	**0.63**
B2 (riboflavin) (mg)	*0.21*	0.20	*0.24*	0.18	*0.22*	**2.89**	**2.72**
B3 (niacin) (mg)	**5.0**	**5.4**	**6.9**	**7.8**	**8.0**	**13.6**	**12.9**
B5 (pantothenic acid) (mg)	0.75	*0.92*	*1.46*	*1.16*	0.70	**4.10**	**5.90**
B6 (pyridoxine) (mg)	**0.53**	*0.30*	**0.54**	*0.38*	**0.61**	**0.89**	**0.55**
B7 (biotin) (μg)	1	2	2	2	2	**50**	**216**
B9 (folic acid) (μg)	19	6	3	19	17	**110**	**1350**
B12 (cobalamin) (μg)	**2**	**2**	**1**	Tr	**2**	**58**	**45**
Choline (mg)[c]	*93*	*107*	*77*	*66*	*85*	**422**	**287**
A (retinol) (μg)	Tr	6	Tr	11	Tr	**25,200**	**10,500**
C (ascorbic acid) (mg)	0	0	0	0	0	*19*	*23*
D (cholecalciferol) (μg)	0.5	0.4	0.5	0.1	0.3	0.3	Tr
E (α-tocopherol) (mg)	0.13	0.09	0.05	0.15	0.01	0.50	0.34
K (menaquinone)[c]	1.5	3.9	0	0	0	3.3	0

Values in bold indicate the food is a rich source of the vitamin. Values italicized indicate the food is a source of the vitamin (EU, 2011 or FDA, 2020). Tr trace.

[a] *Same sources as in Table 19.1*

[b] *Livers from beef (calf) and chicken were fried in corn oil.*

[c] *Data from USDA (2017–2018).*

vitamin D in the muscle listed in Table 19.3 are close to the "source" level of 0.75 μg/100 g (EU, 2011), and meat makes an important contribution to intakes, as cholecalciferol, 24% in men and 18% in women (Henderson et al., 2003). Vegetarians and especially vegans are at risk from low intakes of vitamin D, which is needed for calcium absorption and bone health (Craig, 2009). Vitamin E is an important antioxidant in the body, protecting against the free radicals produced in oxidation reactions during growth and especially in the postmortem period when fatty acid oxidation can produce changes in muscle color and meat flavor (Wood et al., 2004). The muscle concentration of vitamin E required to protect against these changes is 3–4 mg/kg, much higher than the values in Table 19.3 (around 1 mg/kg) (Arnold et al., 1993). These high levels can be achieved by supplementing the diet with α-tocopherol or feeding it naturally in fresh grass (Warren et al., 2008). Vitamin K is a coenzyme for vitamin-K-dependent carboxylase, an enzyme required for the synthesis of proteins involved in blood clotting and bone metabolism. The animal-derived form is menaquinone, produced by bacteria in the intestine (Conly and Stein, 1992).

19.3.3 Minerals

Concentrations of the major minerals present in raw muscle samples of the five species and in beef and chicken liver are listed in Table 19.4. The values are from FSA (2002) and are compared with those defined as a "source" or "good source" of the mineral as in Table 19.3. In most cases, the values are similar to those in USDA (2014) and USDA (2017–2018) except that the values for sodium are much higher in USDA (2017–2018) at 340–460 mg/100 g. This is possibly because the latter database lists cooked samples (as eaten), and it is likely that salt had been added during processing, cooking, or at the table. In FSA (2002), the sodium content of cured bacon is listed as 1140 mg/100 g compared with 63 mg/100 g for raw pork muscle (Table 19.4). The RI for sodium in EU (2011) is 2362 mg/day, giving a "source" level of 354 mg/100 g, close to the USDA (2017–2018) amounts. The RI and DV values for sodium are levels not to be exceeded based on the harmful effects of salt on blood pressure and heart disease. Meat industries around the world are working to reduce salt levels (Matthews and Strong, 2005).

Calcium and magnesium are not present in high amounts in meat but potassium, required for the maintenance of water balance, and phosphorus, with many roles in the body including energy production (ATP), are present in significant amounts. Meat and meat products provide 14% of dietary potassium and 21% of phosphorus to the UK diet (Henderson et al., 2003).

Iron in the body is needed for oxygen transport and electron transfer, and meat is an important source so that vegetarians and vegans are at risk of deficiency (Craig, 2009). About 90% of dietary iron is nonheme iron in plants and 10% is heme iron in hemoglobin and myoglobin in meat, but heme iron is much better absorbed and contributes 40% or more of the total iron

TABLE 19.4 Mineral content (per 100 g) of raw lean of beef, sheep, pork, chicken, and turkey and of liver of beef and chicken (FSA, 2002).

						Liver[b]	
	Beef[a]	Sheep[a]	Pork[a]	Chicken[a]	Turkey[a]	Beef	Chicken
Sodium (mg)	63	70	63	*77*	68	70	79
Potassium (mg)	*350*	*330*	*380*	*380*	*340*	*350*	*300*
Calcium (mg)	5	12	7	6	5	8	9
Magnesium (mg)	22	22	24	26	25	24	23
Phosphorus (mg)	*200*	*190*	*190*	*160*	**220**	**380**	**350**
Iron (mg)	*2.7*	1.4	0.7	0.7	0.6	**12.2**	**11.3**
Copper (mg)	0.03	0.08	0.05	0.03	0.05	**23.86**	*0.52*
Zinc (mg)	**4.1**	**3.3**	*2.1*	1.2	*1.9*	**15.9**	**3.8**
Selenium (μg)	*7*	4	*13*	*13*	*13*	**27**	NA

Values in bold indicate the food is a rich source of the mineral. Values italicized indicate the food is a source of the mineral (EU, 2011). NA, not available.
[a] *Same sources as in Table 19.1*
[b] *Livers from beef (calf) and chicken were fried in corn oil.*

absorbed (Young et al., 2018). Lombardi-Boccia et al. (2002) showed that beef and sheep (lamb) have a high content of heme iron in muscle (1.72 and 1.68 mg/100 g, respectively), compared with pork and chicken (0.20 and 0.12 mg/100 g, respectively). The amounts of iron and heme iron are higher in red oxidative muscles than white glycolytic muscles (Lombardi-Boccia et al., 2002; Purchas and Busboom, 2005). Women have a higher requirement (RV) for iron than men and whereas intakes in UK men are higher than the RV, in women they are lower (Henderson et al., 2003).

Zinc is a trace element, which has higher concentrations in the diet of omnivores than vegetarians and vegans who risk deficiency (Craig, 2009). In United Kingdom, 33% of zinc intake is provided by meat and meat products (Henderson et al., 2003). Zinc is a catalyst in many enzyme systems in the body and is required for DNA synthesis. Like iron, meat sources of zinc are better absorbed than plant sources (EFSA, 2017). Copper is present at levels below the "source" amount in muscle but is high in liver, which is important in maintaining body homeostasis. Copper is a component of several enzymes in the body, including those involved in neurotransmitter synthesis, energy metabolism, and elastin cross-linking. Selenium is an antioxidant, a constituent of several selenoproteins including glutathione peroxidases. Soil and plant levels of selenium, which determine levels in meat, differ between regions of the world. In the dataset published by USDA (2017–2018), selenium concentrations in all the species are higher than those published by FSA (2002). For example, beef and sheep selenium is listed as 33.7 μg/100 g in USDA (2017–2018) compared with 7 μg/100 g in the UK database of FSA (2002). Lawler et al. (2004) showed that muscle and liver concentrations of selenium were raised in cattle using sodium selenite or hay and wheat high in organic selenium (mainly selenomethionine). However, O'Grady et al. (2001) found that the muscle concentration of selenium was not raised using an organic source, possibly because the basal diet was already high in selenium.

19.3.4 Bioactive compounds

Bioactive compounds are "extranutritional" food constituents that typically occur in small quantities in foods and may have health benefits. Interest in them was sparked by the finding that plant extracts had protective effects on CVD and cancer (Kris-Etherton et al., 2002). Examples of bioactive compounds in plants are flavonoids and phytoestrogens. Unlike the major nutrients discussed so far, bioactive compounds are not currently endorsed or listed by government agencies. In animal-derived foods, they have been studied more in dairy products, eggs, and fish than in meat (Lafarga and Hayes, 2014).

Arihara (2006) listed several bioactive compounds in meat and suggested that these show the potential for meat products to be classed as functional foods. Bioactive compounds include creatine (an energy store in muscle as phosphocreatine), taurine (an amino sulfonic acid involved in bile salt formation and

muscle function), carnosine and anserine (histidyl dipeptides with antioxidant effects), carnitine (transports fatty acids into the mitochondria for energy production), glutathione (an antioxidant), and coenzyme Q10 (ubiquinone-10, an antioxidant and component of the electron transport chain).

Meat is also a potential source of bioactive peptides produced by proteolysis, which have a range of biological effects, including acting as angiotensin 1-converting enzyme (ACE) inhibitors. These inhibit the conversion of angiotensin 1 to angiotensin 11 and thereby reduce blood pressure (Decker and Park, 2010). Bioactive peptides may also have antimicrobial, antioxidative, antithrombotic, and anticarcinogenic effects and may have a positive impact on mental health (Lafarga and Hayes, 2014).

Although there is interest in these bioactive compounds present in meat and some are available as supplements, there is at present no reliable, science-based information on their health benefits.

19.4 Meat as part of a healthy diet

Although an omnivorous diet provides several nutrients, which may be lacking in the diets of vegans and vegetarians (vitamin B12, vitamin D, calcium, iron, and zinc), some studies have concluded that a vegetarian diet lowers the risk of chronic disease by providing a low intake of saturated fat and cholesterol and a high intake of dietary fiber and health-promoting phytochemicals from plants (Craig, 2009). However, a very large study conducted within the European Prospective Investigation into Cancer and Nutrition (EPIC) of 500,000 healthy people from 10 European countries concluded that there was no significant association between meat consumption and mortality from chronic disease (Rohrmann et al., 2013). For processed meat, however, there was a "moderate positive association" with mortality, due to CVD and cancer. Other studies have concluded that processed meat is a risk factor for cancer, particularly colon cancer (Bouvard et al., 2015). Carcinogenic compounds that have been implicated are N-nitroso compounds mediated by heme iron and heterocyclic aromatic amines formed when meat is heated to high temperatures (De Smet and Vossen, 2016). Low mortality among vegetarians compared with meat eaters in some surveys has been ascribed to a healthier lifestyle generally, for example not smoking and taking more exercise.

Interest over many years in the association between meat intake and chronic disease has centered on the role of SFA and heart disease (see Chapter 20). The case against SFA was challenged some years ago by the findings of metaanalyses, which concluded that SFA intake was not linked to mortality (e.g., De Souza et al., 2015), but this conclusion has been challenged, and the current view is that the risk of CVD and type 2 diabetes is reduced when SFA in the diet is replaced by PUFA rather than carbohydrate (Hooper et al., 2015). Throughout, health bodies around the world, for example, American Heart Association and National Health Service in the United Kingdom, continue to advocate reductions in meat intake so as to reduce SFA intake.

19.5 Effects of cooking on nutrients in meat

When meat is cooked, water is lost due to denaturation of muscle proteins and the loss of their water-holding capacity; and fat is also lost as fat cell membranes are ruptured and fatty acids melt. In a study of nutrient losses during the cooking of sheep/lamb, Kosulwat et al. (2003) found that 40% of water was lost during roasting and grilling and 24% of fat. Most studies show that, in contrast, 90%–100% of proteins and amino acids are retained after cooking (Kosulwat et al., 2003; Wilkinson et al., 2014; Lopes et al., 2015), with losses increasing at higher temperatures and with certain cooking methods. In a study of seven cooking methods for chicken, Kim et al. (2017) found that boiling produced the greatest losses of amino acids.

In Table 19.5, values for the retention of vitamins and minerals in meat after roasting of the different species are presented (USDA, 2007). The data show that some nutrients were very well retained after roasting (iron, zinc, and copper), whereas some (vitamin B1 and vitamin A) were poorly retained. Retention of some nutrients varied between the species (B6, B9, B12, choline, sodium, potassium, and magnesium), with losses tending to be greater for chicken and turkey than for beef, sheep, and pork. Retention of nutrients was increased when drippings (water and fat) were added back to the cooked meat. These conclusions are broadly similar to those of Gerber et al. (2009), who investigated the cooking of beef, pork, and veal by grilling, boiling, and pan frying. They concluded that the fat-soluble vitamins in general were better retained than the water-soluble B vitamins, particularly B1. Several minerals were lost with the water (calcium, sodium, potassium, magnesium, and phosphorus), but iron and zinc were well retained. Boiling produced greater losses than grilling and pan frying.

Some studies have shown that, despite the general loss of fat during cooking, fatty acid composition remains broadly the same (Purchas et al., 2014; Gerber et al., 2009), whereas others have shown that the percentages of PUFA, particularly the long-chain n-3 PUFA, which are particularly liable to oxidation, decline, with a corresponding increase in the percentage of SFA (Duckett and Wagner, 1998; Alfaia et al., 2010; Werenska et al., 2021). In the study of Alfaia et al. (2010), the retention of n-3 PUFA in beef *longissimus* strips was 95% after microwaving, 90% after grilling, and 85% after boiling.

19.6 Conclusions

Meat provides important nutrients to the diet, notably protein with a high biological value, B vitamins including vitamin B12, vitamin D, minerals including iron and zinc, and n-3 fatty acids. It also provides SFAs, high levels of which are a risk factor for CVD and are higher in ruminant than nonruminant meats. Although heme iron is important in protecting against deficiency, it has also been implicated in the development of colon cancer from eating processed meats.

TABLE 19.5 Retention of vitamins and minerals in meat after roasting and in sheep liver after frying (%) (USDA, 2007).

	Beef	Sheep	Pork	Chicken	Turkey	Sheep liver
B1 (thiamine)	55	60	60	70	65	70
B2 (riboflavin)	95	90	95	90	85	90
B3 (niacin)	75	80	85	80	90	80
B6 (pyridoxine)	50	75	85	80	70	60
B9 (folic acid)	95	85	95	60	60	85
B12 (cobalamin)	70	75	80	65	65	80
Choline (mg)	90	90	100	70	70	90
A (retinol)	75	75	75	75	75	75
C (ascorbic acid)	80	80	80	80	80	80
Sodium	85	75	80	80	75	85
Potassium	80	75	80	80	75	85
Calcium	90	100	95	95	100	100
Magnesium	85	80	75	75	80	85
Phosphorus	85	85	85	80	80	90
Iron	100	100	100	90	95	95
Copper	100	80	100	95	70	100
Zinc	100	100	100	100	100	100

Meat eating has come under pressure in recent years on the grounds of health, animal welfare, and the cost to the environment. Attempts to improve the healthiness of meat have centered on raising levels of the n-3 fatty acids by feeding modified diets. Promising results have been obtained in pork and chicken. In beef and sheep, the deintensification of production by changing from a grain-based concentrate diet to one based on grass increases n-3 fatty acids and also the *cis-trans* fatty acid rumenic acid, which may have health benefits, but not to nutritionally significant levels.

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Chapter 20

Meat and health

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20.1 Introduction: Nutrients supplied from meat

Based on historical evidence, such as cave drawings, humans have been omnivorous for more than 40,000 years. Diets provided by hunters and gatherers likely consisted of wild game, native berries, roots, leafy vegetation, and some grains. Over time, agricultural practices and food processing capabilities have contributed to changes in dietary intake, and there has been a shift in meat consumption from wild game to domesticated animals and birds that have been bred and raised specifically for food. The selection of breeds, feeding practices, and manufacturing processes have impacted meat and meat products. While the role of meat in the diet and the understanding of its impact on health and disease have continued to evolve, and are oftentimes disputed, producers, processors, and researchers have continued focusing on meeting consumers' expectations for eating quality and their nutritional needs by providing nutrient-dense meat and meat products that can be incorporated in a healthful and balanced diet.

20.1.1 Macronutrients supplied from meat

Of the three macronutrients—carbohydrates, proteins, and lipids—meat is a source of both protein and lipids. Protein is comprised of amino acids, and it is used by the body to build muscle mass, repair tissues, make enzymes and hormones, as well as help build bones, cartilage, blood, antibodies, keratin, and skin. Proteins are frequently classified based on the completeness of their amino acid profile. Meat, poultry, milk, eggs, and fish are often referred to as "complete" proteins because they provide all nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). According to Bender (1992), meat is a relatively concentrated source of high-quality protein that is highly digestible compared with many plant foods. Raw muscle meat contains around 20–22 g protein/100 g, while cooked red meat contains approximately 26–35 g of protein per 100 g cooked product, the digestibility is around 95%, and the protein contains the essential amino acids (see Chapter 19). Protein digestibility requires proteases and

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peptidases to the hydrolysis of proteins into small peptides and free amino acids, while dietary protein availability is more complex and represents the combination of digestion, absorption, and metabolism of proteins and amino acids. Beach et al. (1943) evaluated the amino acid composition of muscle tissue from beef shank, lamb leg, pork chops, veal, frog legs, and roasting chicken, as well as those from salmon, codfish, and shrimp, and they determined that the protein of one muscle was "as good as" that of another muscle because there were minimal differences in the amino acid patterns. The amino acid composition of muscle tissue is not expected to differ significantly across species or within individual muscles of a species; however, there are differences that could have minor impacts on nutritional composition. Recent research by Wu et al. (2016) looked at the free proteinogenic and polypeptide-bound amino acids and found moderate differences in beef cuts from the round, loin, and chuck. Bailey et al. (2020) reported that the digestible indispensable amino acid scores of different meat products (salami, bologna, beef jerky, raw ground beef, cooked ground beef, and ribeye roasts cooked at three different endpoint temperatures) ranged from 99 to 130, indicating that meat products contain generally high-quality proteins.

Lipids, commonly referred to as fats, in meats are frequently the cause of dietary concerns over meat consumption. Dietary fats are an important macronutrient because they provide concentrated sources of energy, with 1 g of fat providing 9 kcals. In addition to the energy supplied, lipids are also an important structural component of cell membranes, provide essential fatty acids, and facilitate the absorption and transport of fat-soluble vitamins A, D, E, and K.

The percentage of lipid varies among species, among individual animals of the same species, and even among specific cuts within a species. Lipid percentage can be influenced by many different factors including, breed, feeding regimen, sex class, and age of the animal. Along with differences in the percentage of lipids found in meat, there are also different types of lipids found in meat. The main component of most dietary fats is triglyceride, which includes a glycerol backbone with three fatty acids attached to it, and most of the discussion about lipids in meat centers on these fatty acid profiles. Fatty acids are classified as saturated, monounsaturated, and polyunsaturated, based on the number of double bonds between carbon molecules. The ratio of saturated, monounsaturated, and polyunsaturated fatty acids and their location on the glycerol molecule give dietary fats specific physical properties. Fats that are predominately unsaturated are liquid at room temperature, while saturated fats are solid at room temperature. Predominate sources of dietary saturated fatty acids are red meat, poultry, and dairy products, as well as in coconut and palm kernel oils. However, like most other fat-containing foods, the actual fatty acid composition of meat cuts is comprised of all three types of fatty acids. Research has shown that not all fatty acids have the same impact on health; therefore, when looking at the relationship between meat and health, it is important to understand both the total fat content and the actual fatty acid profiles.

20.1.2 Micronutrients supplied from meat

In addition to being a source of protein and providing energy, meat also provides many micronutrients that are beneficial for the healthy growth and development of children and play important physiological roles in adults, too. Meat is an important source of thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), biotin, vitamin B6, vitamin B12, folate, and pantothenic acid, and it also provides many minerals, such as zinc, iron, copper, and manganese, as well as providing phosphorus, potassium, and selenium (see Chapter 19). For some micronutrients, meat may be the only natural dietary source, or it may have greater bioavailability than other food or synthetic sources.

Vitamin B12 is an essential nutrient that is vital for the body's production of healthy red blood cells and is also needed for proper nerve and brain function. Vitamin B12 deficiency may contribute to anemia, fatigue, dizziness, and poor memory or confusion. Vitamin B12 is only found in foods of animal origin; therefore, individuals with limited or no foods of animal origin are encouraged to take Vitamin B12 supplements to prevent a deficiency.

Iron deficiency is a health concern in infants, teenagers, and young women, and it causes anemia, which results in exhaustion and weakness. Iron has a much greater bioavailability in meat as heme iron than does plant-derived nonheme iron. Although the exact mechanism is not fully understood, heme iron from meat has also been shown to increase the absorption of iron from other sources, and this enhancing effect has been called the "meat factor" or "meat effect" (Layrisse et al., 1968; Martínez-Torres and Layrisse, 1971). Bæch et al. (2003) found that adding small amounts of pork increased nonheme-iron absorption from phytate-rich meat that was low in Vitamin C, and research by Cook and Monsen (1976) demonstrated that iron from meat even had a greater absorption than iron from other animal proteins, such as eggs, milk, and cheese.

Meat contains substantial quantities of zinc, which is used for muscle synthesis and proper immune function, and lean meat intake has been shown to improve utilization of both iron and zinc (Johnson and Walker, 1992). Another less frequently discussed micronutrient found in meat is selenium, which serves as an antioxidant, aids in immune function, and helps with the use of iodine for thyroid hormone production. Additionally, offal products, such as liver, can provide vitamin D (Alexandra Schmid and Walther, 2013), which is used in the development of bones and increasing muscle strength. Overall, meat provides a significant source of protein and supplies many easily absorbed micronutrients required for daily functions.

20.2 Meat in healthy nutrition and diet

The role meat plays in an individual's diet varies between consumers in developed and developing countries, as well as between socioeconomic classes within a country. Economic improvements for a country or an individual consumer often relate to an increase in food consumption. The fact that meat is a source

of high-quality protein, provides heme iron, vitamin B12, and many other important minerals and vitamins, make it a valuable dietary component. For individuals who have limited access due to availability or costs, meat, even in small quantities, becomes an important complement to a predominately plant-based diet and helps ensure that essential nutrients are being provided to support growth and development, as well as maintain physiological functions. Overall, the inclusion of lean meats is generally considered as a positive contribution to a well-balanced diet; however, there have been many disputes about the role of meat, especially high-fat meat, in a healthy diet.

20.2.1 Meat and cardiovascular disease

The relationship between meat consumption and the risk of developing cardiovascular disease, a leading cause of death in many industrialized countries, has been a controversial area for many years. Cardiovascular disease includes all diseases that impact the heart and circulatory systems, such as stroke and coronary heart disease. There are many risk factors that have been associated with cardiovascular disease, including genetic predisposition, cigarette/tobacco use, abnormal blood lipid profiles, hypertension, obesity, diabetes, lack of physical activity, and excessive alcohol consumption (Lichtenstein et al., 2006; Rosamond et al., 2007). To help individuals achieve and maintain recommended lipid profiles, many dietary recommendations include reductions in intake of total fat, saturated fat, trans fat, and cholesterol. Bronzato and Durante (2017) reported that recommended consumption levels of red meat, despite the presence of heme iron and carnitine, do not increase cardiovascular risks. Eckel et al. (2014) emphasized the importance of an overall healthy dietary pattern and recommended limited consumption of saturated fat, *trans* fat, sodium, red meat, sweets, and sugar-sweetened beverages, and that when consuming red meat, the leanest available cuts should be selected.

Dietary fat intake influences the risk of cardiovascular disease because it impacts blood cholesterol levels. With the exception of stearic acid, consumption of saturated fatty acids and *trans*-fatty acids has been associated with increases in low-density lipoproteins (LDLs), which increases the risk of cardiovascular disease. Saturated fatty acid intake has been shown to increase the tendency for blood to clot by raising the platelets, but polyunsaturated fatty acids have been shown to have the opposite effect and decrease the tendency for clotting. Therefore, dietary recommendations for reducing the risk of cardiovascular disease frequently include restricting the intake of saturated fatty acids to 10% or less of total caloric intake.

Plasma cholesterol includes three lipoproteins—very LDL (VLDL), LDL, and high-density lipoprotein (HDL). HDLs are generally classified as "good" cholesterol and LDLs are classified as "bad" cholesterol. A high level of LDL combined with a low level of HDL increases the risk of heart disease and atherosclerosis, while a low level of LDL combined with high levels of HDL is

associated with reducing the risk of coronary heart disease. The recommended dietary intake for cholesterol is usually 200 mg/day or less.

Although the nutrient composition across species and among cuts within a species varies, many lean meat cuts meet the nutrient recommendations for reducing cardiovascular risk and can easily be incorporated into a well-balanced diet. In beef, stearic acid makes up approximately one-third of the total saturated fatty acid content, and it has been shown to have a different impact on blood cholesterol levels than other saturated fatty acids. Hunter et al. (2010) conducted a systematic review or metaanalysis to examine the effect of stearic acid on blood lipoprotein files, and the following conclusions were reached:

1. When compared with other saturated fatty acids, stearic acid decreased LDL cholesterol, was neutral with respect to HDL cholesterol, and lowered the ratio of total cholesterol to HDL cholesterol.
2. When compared with unsaturated fatty acids, stearic acid tended to increase LDL cholesterol, decreased HDL cholesterol, and increased the ratio of total cholesterol to HDL cholesterol.
3. When a one-to-one substitution of stearic acid for trans-fatty acid was evaluated, there was an (Kritchevsky et al., 2004) effect on HDL cholesterol, and a decrease in the ratio of total cholesterol to HDL cholesterol.

The consumption of *trans* fats has been shown to increase LDLs and decrease HDLs, which increases the overall risk for developing cardiovascular disease. In the 1990s, research documented the adverse effects of *trans* fats, and steps, including identification of *trans* fats on nutritional labels, were initiated to make consumers aware of the risks associated with consuming *trans* fats. While processed foods containing partially hydrogenated oils were the primary source of *trans* fats, small amounts of *trans* fats are formed by biohydrogenation in ruminants and are naturally present in beef and lamb. These include vaccenic acid and the naturally occurring isomer of conjugated linoleic acid (CLA), *cis*-9, *trans*-11 CLA. Research by Kritchevsky et al. (2004) found that CLA, the *trans* fatty acid found in ruminants, such as beef and lamb, might not increase the risk of cardiovascular disease. Mozaffarian et al. (2006) and Huth (2007) also reported that ruminant *trans* fats found in meat and milk were not associated with increasing the risk of heart disease.

Experimental research (Belury, 2002; Bhattacharya et al., 2006) with rabbits, mice, and hamsters has shown that CLA may reduce the risk of cardiovascular heart disease by lowering total cholesterol and reducing the growth of atherosclerotic lesions. Other research (Arbonés-Mainar et al., 2006) found that CLA promoted atherosclerosis in mice. Mixed results have also been reported in human studies (Arbonés-Mainar et al., 2006; Toomey et al., 2006). Gebauer et al. (2011) concluded that epidemiological studies generally have shown no association or an inverse relationship between ruminant *trans* fats and coronary heart disease, but that the results from clinical studies investigating

the relationship are unclear. Therefore, additional research is needed to better understand the complete and complex relationship between heart disease and *trans* fats from meat.

The relationship between heart disease and meat in the diet is complex, and while some research has cautioned against consuming red meat due to the fat and saturated fat content, other research has shown that lean beef consumption does not contribute to an increased risk of cardiovascular disease. Stanner (2005) reported that elevated levels of homocysteine, which is a risk factor for cardiovascular disease, may be associated with low blood levels of several B vitamins, including vitamin B12, vitamin B6, and folate. Therefore, lean meat consumption provides these micronutrients, which may help decrease the risk for cardiovascular disease.

The Dietary Approaches to Stop Hypertension (DASH) eating pattern showed that including limited amounts of beef (28 g per day) as part of a low-sodium diet helps reduce hypertension, which in turn decreases the risk of cardiovascular disease (Sacks et al., 2001). The eating pattern in the clinical study for the Beef in an Optimal Lean Diet (BOLD) research was similar to the DASH diet, except it increased beef to 113 g of lean beef per day as the primary protein source, while the BOLD-PLUS study increased the protein and lean beef intake to 153 g per day (Roussell et al., 2011). After 5 weeks, the clinical study participants in the BOLD and BOLD-PLUS diets had decreases in both total cholesterol and LDL cholesterol that were similar to those on the DASH diet, thereby supporting the inclusion of lean beef in a heart-healthy eating pattern.

An individual's risk of developing cardiovascular disease is influenced by multiple factors, including genetics, age, gender, smoking, elevated blood pressure, obesity, diabetes, environment, alcohol intake, and dietary intake. Therefore, actions to reduce risks frequently include increasing physical activity, maintaining healthy body weight, controlling blood pressure, properly managing diabetes, stopping smoking, limiting alcohol consumption, and making appropriate dietary choices. The basic dietary recommendations designed to reduce cardiovascular disease focus on balancing caloric intake, restricting total fat, saturated fatty acids, *trans*-fatty acids, and cholesterol. However, this does not mean that consumption of meat should be completely eliminated from a person's diet. Lean meat is a great source of nutrients, and it can be included in a balanced, heart-healthy diet.

20.2.2 Meat and cancer

In 2015, the International Agency for Research on Cancer (IARC) classified the consumption of red meat as probably carcinogenic to humans (Group 2A) and classified processed meat as carcinogenic to humans (Group 1) (World Health Organization, 2015), however, the relationship between meat consumption and cancer remains an area of controversy (North American Meat Institute, 2015). Unfortunately, the specific etiology of most cancers has not been determined;

however, the general consensus is that many factors, such as genetics, environment, tobacco use, alcohol consumption, and dietary intake, are involved. For example, cigarette smoking is commonly attributed as a cause of lung cancer. Some of the most commonly diagnosed cancers include breast cancer in women, prostate cancer in men, followed by lung cancer and colorectal cancer. While the exact relationship between diet and cancer is not fully understood, there is a general agreement that the relationship is significant enough to be a public health issue (Willet, 2006). Some researchers (Doll and Peto, 1981; Willet, 2006) suggest that between 35% and 70% of cancer deaths are potentially attributable to dietary influences. The Working Group of IARC "concluded that there is limited evidence in human beings for the carcinogenicity of the consumption of red meat" and classified the consumption of red meat as "probably carcinogenic to humans," and they "concluded that there is sufficient evidence in human beings for the carcinogenicity of the consumption of processed meat" and classified it as "carcinogenic to humans" (Bouvard et al., 2015). However, IARC (2018) reported there is limited evidence in human beings for the carcinogenicity of the consumption of red meat. While nutritional epidemiological studies have indicated that fat consumption may contribute to causing certain cancers or that high-fiber diets may decrease the risks of certain cancers, the role of individual foods in causing cancer is still not fully understood. Investigating the relationship between diet and cancer is difficult because single foods are rarely consumed in isolation, other factors such as genetic predisposition, environment, body weight, healthcare, and lifestyle confound the associations. Therefore, many questions and much debate surround the issue of meat consumption and specific types of cancer.

20.2.2.1 Meat and colorectal cancer

Colorectal cancer is the third most common cancer in men and second most common in women, and it has been reported as the fourth most common cause of death from cancer. Rates are reported as higher modernized countries; however, some speculate that the lower report rates in underdeveloped countries might be due to limited medical care and failure to diagnose it. Data indicate that environmental factors, such as dietary intake and physical activity, as well as age, are related to the risk of developing colorectal cancer; however, the specific causes of most colorectal cancer cases are unknown.

Due to the colon's role in absorbing water and nutrients from food and storing waste matter before it passes into the rectum, the role of dietary intake has been examined in multiple scientific studies, and the results are variable. Some epidemiological studies have reported that high intakes of garlic and dietary fiber may decrease the risk of colorectal cancer, and that there are fewer cases of colorectal cancers in individuals consuming diets rich in vegetables, while results related to meat consumption and colorectal cancers are inconsistent. The IARC Working Group determined that there was a consistent association between colorectal cancer and red meat consumption (Bouvard et al., 2015). Alexander et al. (2015) reviewed previously published prospective studies and concluded that

the intake-response relationship is not clear, but that the association of colorectal cancer and the consumption of red or processed meats may be greater in men than in women. Hur et al. (2019) reported that there are multiple factors that impact the risk of developing colorectal cancer and that it is "difficult to conclude that dietary red meat is the main cause." As reported by Corpet (2011), one link between red meat and colon cancer could be related to heme iron, and the impact might be mitigated by increasing calcium intake, altering meat processing, or additives.

Concerns have been raised over the formation of heterocyclic amines and polycyclic aromatic hydrocarbons (PAHs) during the cooking process of meat, poultry, and fish, and the formation of *N*-nitroso compounds produced in cured meats (see Chapter 17). The association of these compounds and the role of fat and saturated fat from meat have also been questioned in relation to colorectal cancer. Many of the cohort and case-control studies indicate a link between meat consumption and colorectal cancer. However, Alexander et al. (2015) conducted a systematic quantitative assessment of the literature and concluded that "the state of the epidemiologic science on red meat consumption and colorectal cancer is best described in terms of weak associations, heterogeneity, an inability to disentangle effects from other dietary and lifestyle factors, lack of a clear dose-response effect, and weakening evidence over time."

20.2.2.2 Meat and breast cancer

Breast cancer may occur in both women and men, and it forms in various tissues of the breasts. Multiple factors have been associated with increased risks of developing breast cancer, including family history, inherited genetic mutations, hormone exposure, high body mass index, and physical inactivity. The data related to meat consumption and breast cancer incidence show that countries with high meat consumption have an increased rate of breast cancer than countries with little or no meat consumption, which suggests that eating meat is a potential risk factor. Breastcancer.org (2018) reports that although there have been reports that the consumption of processed meat increases breast cancer, there are still many "unanswered questions" about the relationship. While some epidemiological studies have shown that eating meat may increase the risk of breast cancer due to fat intake, chemicals formed during cooking, or hormone content (Ganmaa and Sato, 2005; Zheng et al., 1998), other studies have not established a relationship between meat consumption and breast cancer (Alexander et al., 2010; Missmer et al., 2002). Therefore, most dietary guidelines focused on reducing the risk of breast cancer include recommendations to limit alcohol consumption; achieve and maintain a healthy weight; eat plenty of vegetables and fruits; limit red meat and processed meats; and limit saturated and *trans* fats.

20.3 Recommended meat intakes

According to Food Agriculture Organization, global consumption of meat proteins is expected to increase by 14% by 2030, with projected increases of 5.9%

for beef, 13.1% for pork, 17.8% for poultry, and 15.7% for sheep (Food and Agriculture Organisation of the United States, 2021). Unfortunately, for many developing countries, an increase in protein consumption is not occurring, and meat is still considered a luxury. In 1997/99, meat consumption in developing countries was 36.4 kg per capita compared with 88.2 kg per capita for industrialized countries. In 2015, the consumption of meat had increased to 41.3 kg per capita for developing countries and 95.7 kg per capita for industrialized countries, and the predicted increase in consumption in 2030 is from 25.5 to 37 kg per capita for developing countries and from 88 to 100 kg per capita for industrialized countries (FAO, 2003). However, other predictions forecast a decrease in per capita consumption of meat in industrialized countries due to an increase in the number of vegetarians. Research has also looked at shifting trends in the relationship between national income levels and fat consumption. Historically wealthy societies consumed fat mostly in the form of milk and meat products, while lower-income countries consumed more vegetables and grains and limited amounts of fat. However, today it appears that the fat intake in lower-income countries is increasing due to consumption of inexpensive vegetable oil, and that while some high-income nations are reducing meat consumption, the fat content remains basically unchanged due to vegetable oil consumption.

Provided energy requirements are met, most individuals in developed countries have protein intakes in excess of their daily requirements. However, protein deficiency is commonly observed in children in underdeveloped countries or in individuals who do not have sufficient dietary intake to fulfill basic energy and nutrient requirements due to conditions such as disease states, extreme stress, and eating disorders. Children with exceptionally low protein intakes may have stunted growth, edema, and skin lesions, while adults with low protein intake frequently exhibit edema, loss of muscle mass, and loss of hair.

Protein requirement is based on two factors: (1) the need for total nitrogen and (2) the need for essential amino acids, and an individual's energy balance also influences the utilization of dietary protein and nitrogen balance. Due to the relationship between energy requirements and protein requirements, specific recommendations for both vary based on many factors including energy needs, protein turnover, body weight, physical activity levels, growth status, which varies with age, and other factors. For adults, the daily protein requirement has historically been estimated as 0.75–0.8 g per kg of body weight. This average daily intake has been determined to fulfill the basic protein needs of nearly all healthy individuals, and additional protein needs for a specific disease or nutritional demands such as bodybuilding would need to be calculated on an individual-by-individual basis. Daily protein requirements have been estimated as 55 g per day for an adult man and 45 g for a woman (FAD/WHO 1985) and 50 g for a 2000 cal diet according to the United States' Food and Drug Administration (FDA). Pedersen et al. (2013) conducted a systematic review of the literature published between 2000 and 2011 to classify the effects of protein intake in healthy adults and determined there was probable evidence from

nitrogen balance studies to suggest an estimated average requirement of 0.66 g good-quality protein/kg body weight/day.

20.4 Functional muscle foods

Some diseases and health conditions, such as coronary heart disease, type 2 diabetes, and some types of cancers, could be reduced, improved, or controlled due to lifestyle changes, including dietary modifications. The increase in consumer knowledge and awareness about the link between food intake and health has triggered an increased demand for foods that will enhance their health and fulfill specific physiological functions. The initial concept of functional food originated in Japan in the mid-1980s and centered on specific ingredients that were known to improve specific bodily functions. Since that time, the functional food area has continued to expand with annual global growth in sales value estimated at around 10%, and the market value of functional foods has been reported to be almost $29 billion (Witwer, 1998). Although there are multiple definitions, the three conditions that define a functional food provided by (Goldberg, 1994) are most often referenced. They are:

1. It is food (not a capsule, tablet, or powder) derived from naturally occurring ingredients.
2. It can and should be consumed as part of the daily diet.
3. It has a particular function when ingested, serving to regulate a particular body process, such as:
   - Enhancement of the biological defense mechanisms.
   - Prevention of a specific disease.
   - Recovery from a specific disease.
   - Control of physical and mental conditions.
   - Slowing the aging process.

While the majority of health-enhancing compounds are associated with foods from plants or with manufactured foods, there are some naturally occurring components found in animal products that have also been shown to have additional health benefits. For example, fish provides omega-3 fatty acids, which contribute to reducing the risk of cardiovascular mortality, while dairy products are exceptional sources of calcium, which helps prevent osteoporosis. Apart from its effect on health through the provision of nutrients, meat is starting to be recognized as a functional food, that is, one that can provide an additional physiological benefit that goes above and beyond meeting the basic nutritional needs of the consumer, thereby, potentially mitigating or preventing disease (Jiménez-Colmenero et al., 2001).

Bharti et al. (2015) noted that meat is a good source of physiological bioactive compounds and that there are opportunities for marketing it as a functional food. Meat has been classified as a functional food because of naturally occurring compounds that improve physiological function (Ferguson, 2010;

Hasler et al., 2004). Lean meat is considered a good source of protein, and the protein provided from red meat has been shown to have additional health benefits, such as contributing to weight loss and reducing blood pressure (McAfee et al., 2010). Specific amino acids found in meat also provide additional physiological benefits and are therefore considered functional components of meat. Taurine has been reported to have positive impacts on eye health and heart disease (Purchas et al., 2006); glutamine aids in metabolic processes and potentially inhibits some diseases (Neu et al., 1996); and other amino acids aid the nervous system (Gaull, 1989). Two of the most abundant antioxidants in beef, pork, and lamb, carnosine and anserine, assist with wound healing, fatigue recovery, and prevention of some stress-related illnesses (Arihara, 2006; Arihara and Ohata, 2008). Some studies also have shown that carnitine may have helped with cardiac function in ischemic heart disease (Ferrari et al., 2004; Lango et al., 2001) while other research reports that L-carnitine may increase the risk of atherosclerosis (Murphy et al., 2013).

Another compound of interest is CLA, which occurs naturally in lamb and beef and is reported to have anticarcinogenic and antiatherogenic properties, to improve cardiovascular health, and to help promote weight loss (Park, 2009; Rainer and Heiss, 2004). The concentrations are highest in fat from ruminant animals, with beef reported as having 1.2–10.0 mg/g and lamb having from 4.3 to 19.0 mg/g (Schmid et al., 2006). Although consumption of meat and meat products contributes 95–440 mg of CLA in Western populations, there is some debate on the actual health impact at this level (Baublits et al., 2007).

In addition to the naturally occurring bioactive compounds found in meat, such as L-carnitine, creatine, and taurine, production and manufacturing modifications may also improve the health benefits of meat. Production modifications to change meat's composition of protein, fat, fatty acid composition, and other nutrients may include genetic selection for specific characteristics, control of animal diets during production, and nutritional supplementation. In addition to altering the composition through production practices, meat processors can reduce the fat content of whole muscle products by trimming intermuscular fat from the primal and retail cuts. Further steps can be taken to formulate processed meat products with reduced fat content, targeted fatty acid profiles, lower cholesterol, and less sodium, as well as including additional functional ingredients, such as fruits, seeds, and nuts, into the processed products. Some products, such as bone broth, now are being marketed as improving a consumer's skin, joints, gastrointestinal tract, lungs, and blood by providing valuable sources of phosphorus, glucosamine, calcium, and magnesium. Additionally, the functional food value of meat products can be enhanced by adding ingredients such as fiber and natural antioxidants, such as herbs (Kausar et al., 2019).

Although still a relatively novel idea, 3D printing of meat could allow production of meat products that would meet specialized needs (Dick et al., 2019). Overall, the role of meat as a functional food may increase as additional

information related to the direct impact of meat and/or individual nutrients within meat or meat products on preventing, treating, or curing health issues is discovered.

20.5 Problematics with toxins and residues

The adventitious presence of nitrate in the salts used to cure meats in early times, and its microbiological reduction to nitrite by halophilic organisms, is responsible for the desirable pink color of the products, for their flavor, and for their reduced risk of *Clostridium botulinum*. Because nitrite can destroy blood pigments and vitamin A (Roberts and Sell, 1963), the residual level in cured meats was restricted to 500 ppm about 35 years ago. The effect of nitrite is very serious for infants since fetal hemoglobin is particularly susceptible to oxidation until they are 3 months old, and the enzyme systems capable of reducing metmyoglobin back to myoglobin are often deficient in the very young.

Awareness that nitrite can react with secondary and tertiary amines to produce carcinogenic nitrosamines, such as *N*-nitrosodimethylamine (Lijinsky and Epstein, 1970), led to a further reduction in the permitted level of residual nitrite to 200 ppm and to attempt to cure meat without nitrite, although this latter possibility cannot be contemplated unless some other means of eliminating *C. botulinum* can be found (see Chapter 9). The most prevalent secondary amines in raw pork appear to be piperidine, diethylamine, pyrrolidine, and dimethylamine (Bellatti and Parolari, 1982). During the maturation of cured pork products, the level of dimethylamine rises from ca 0.1 to 3 ppm. A comparative assessment of the amine content of sausages from Northern and Southern Europe showed that tyramine and phenylethylamine were present at higher concentrations in the latter, possibly due to the decarboxylase activity of certain strains of the starter organisms *Kocuria varians* and *Staphylococcus carnosus* (Ansorena et al., 2002).

It is clearly important, however, to maintain perspective in such problems. Nitric oxide is produced in skeletal muscles (and in other tissues) by nitric oxide synthetase (Brannan and Decker, 2002); and it has been suggested that nitrates, and the nitrites derived from them, enhance the body's defenses against gastroenteritis by suppressing pathogens (Dykhuizen et al., 1996). The quantity of nitrate in vegetables is 10-fold greater than in cured meats, although their relatively high content of ascorbic acid would tend to inhibit nitrosation (Walters, 1973). Moreover, at the low concentrations of residual nitrite in cured products, amines form nitrosamines only with difficulty (Walters, 1973). Random surveys of volatile nitrosamines in meat products from Danish and Belgian markets reported values lower than 1 ppb (Herrmann et al., 2015), although heating of fat, especially at high temperature, increases its concentration (Patterson and Mottram, 1974).

It should be mentioned that *N*-nitrosodimethylamine was found in the blood of 97% of healthy individuals (Lakritz et al., 1980). In those with hypochlorhydria, however, the relatively alkaline conditions in the intestinal tract permit

growth of nitrate-reducing bacteria whereby concentrations of nitrite are enhanced considerably and may become carcinogenic per se (Newberne, 1979). On the other hand, it has been suggested that it is the natural production of nitrite in the human intestinal tract, a capacity developed in early infancy, which usually affords protection against environmental spores of *C. botulinum*, which are ubiquitous (Tannenbaum et al., 1978), and certain unexplained cot deaths have been attributed to the absence of this capacity.

When meat products are smoked, PAHs, including carcinogenic substances such as 3,4-benzpyrene, may precipitate onto surfaces. Racovita et al. (2020) found that PAH levels increased with higher temperatures and longer smoking times (see Chapter 17). It has also been noted that PAHs are more likely to be produced when fat falls onto hot cinders during charcoal grilling of meats.

Various hormones are now administered to enhance the growth of animals, and some of these, such as hexoestrol, are believed to be carcinogenic (Gass et al., 1964). Synthetic estrogens have thus been prohibited in a number of countries. It seems unlikely, however, that residues of these would be present at significant levels in meats. Early studies indicated that there were no detectable residues of the hormones in the flesh of treated cattle (Perry et al., 1955) or pigs (Braude et al., 1950), provided they were used in accordance with instructions. Radioimmune assays have enabled very precise assessments to be made. Attempts have been made to standardize the mode of monitoring meat for residues within the member states of the European Union. In 1988, growth promoters were officially banned in the European Union due to concerns about harmful effects on consumers (European Community, 1988).

It is feasible that meat could be the vehicle for various mycotoxins produced by molds. These could be acquired when animals ate contaminated feeds. They could also arise in such products as mold-fermented sausages. Although selected molds are encouraged to grow on the latter during the maturation period, undesirable species capable of producing toxins could also thrive. Thus the presence of ochratoxins, produced by *Aspergillus ochraceus* (and by various *Penicillium* spp.) and ingested from moldy feed, causes swine disease and carcass condemnations (Krogh, 1977). Aflatoxins are also produced by *Aspergillus* spp. These are believed to be carcinogenic to humans. The significance of ochratoxins for human consumers is still unknown.

There is increasing evidence that fermented meat products, although hitherto considered safe, may cause outbreaks of gastroenteritis involving such microorganisms as *Salmonella* and verocytotoxigenic *Escherichia coli* (Moore, 2004). Since bone tends to concentrate heavy metals, such as lead, barium, and strontium, it might seem that increased use of mechanically recovered meat could be a hazard. However, the overwhelming balance of evidence indicates that these elements are not present in the product at sufficient concentrations to be significant for health (Newman, 1981).

The use of pesticides in agriculture, especially those that are persistent, such as the organochlorine group, could lead to their deposition in the tissues of

animals grazing treated pastures or feeds, and accordingly, various surveys of pesticide residues in meat have been made. Thus, Madarena et al. (1980) assessed meat for residues of 14 organochlorine pesticides. These included BHC isomers, the DDT group, and cyclodienes. Total organochlorine residues in beef, pork, rabbit, and horse were, respectively, 10, 80, 110, and 160 ppb.

20.6 Conclusions

The relationship between diet and health is extremely complex because there are so many confounding factors. Researchers continue searching for dietary factors that influence specific diseases; however, we still have insufficient knowledge to completely distinguish all of the factors, such as genetic predisposition, environmental influences, and lifestyle decisions, from dietary intake. Many dietary recommendations or conclusions about the relationship between diet and health are based on nutritional epidemiological studies that review eating patterns and disease conditions to determine if a relationship exists between intake and disease; however, it has limitations due to poor methods for assessing dietary intake and inability to actually prove cause and effect. Researchers continue to investigate the impact of individual components, such as type of fatty acid or specific amino acid, as well as the overall dietary intake, and it can be expected that dietary guidelines and recommendations will continue to evolve based on new findings. While there might be an absence of absolute proof for some diet and health relationships, there is general acceptance that dietary intake influences health and supports Hippocrates' view of "Let food be thy medicine and medicine be thy food."

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Chapter 21

Sustainability I: Edible by-products

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21.1 Introduction

Animal by-products can be considered as those parts (except carcass) released from a slaughtered animal after dressing, and they can be edible or inedible depending on their use as food. Edible by-products, also known as co-products, can be defined as those products that can be consumed as food by humans (Toldrá et al., 2021). The consideration of edible changes depends on the geographical region or culture; what is considered edible in a country or region may be considered inedible in another. The main uses and applications of all animal by-products are summarized in Fig. 21.1 even though this chapter is primarily focused only on edible by-products (co-products). Most noncarcass material may be edible if properly cleaned, handled, and processed. The usually high nutritional value and low cost make co-products an economical buy, particularly in countries with low incomes (Mullen et al., 2017); therefore, there is a large international trade in these products with many types of by-products exported to those countries with higher demand (Nollet and Toldrá, 2011). However, the large production figures, the low commercial value, and the limited consumption may generate a burden to slaughterhouses so that if not enough uses are given to by-products, they pose a tremendous environmental pollution problem (Przybylski et al., 2020). Fortunately, many cultural groups and areas prepare interesting and delicious variations of the diverse by-products and are often good consumers of these products (Toldrá et al., 2016).

Large variations in the yield of animal by-products are found depending on species, age, animal sex, liveweight, fatness, and methods used for collection. In general, the amounts of total by-products range from 10% to 30% of the animals' liveweight (Ockerman and Basu, 2014). The yield of co-products including blood and organ averages 12% in cattle, 14% in sheep, and 14% in hogs

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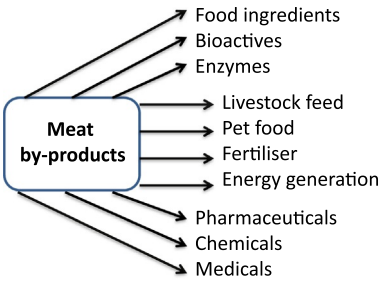

FIG. 21.1 Main uses and applications of all animal by-products. *(Reproduced from Toldrá, F., Mora, L., Reig, M., 2016. New insights into meat by-products utilization. Meat Science 120, 54–59 with permission from Elsevier.)*

when pork rinds are included (Ockerman and Hansen, 2000). A more detailed yield for each organ is shown in Table 21.1.

Co-products are in general more microbial perishable than muscle tissue due to their higher glycogen content and their need for further handling in the collection. Therefore, co-products should be removed on the harvest floor, trimmed, washed, drained under refrigeration, and cooled quickly after slaughter, handled in a hygienic manner, and cooked as soon as possible (Ockerman and Basu, 2014). This chapter is reporting the most usual consumed edible animal by-products (co-products) and briefly discusses the specific products, their yield and further processing, and main uses given by the consumer.

21.2 Main co-products

Most relevant co-products include the liver, heart, kidney, white offal (intestines and stomach), hygienically collected blood, and trimmings. Because of customs, palatability (including flavor and tenderness), and reputation, meat co-products are usually limited to the liver, heart, kidney, tongue, and thymus plus other sweetbreads, brain [limited use in ruminants due to bovine spongiform encephalopathy (BSE)], tripe, and intestines. In addition, pork meat co-products are also excluded in some religions. The approximate average weights of the most relevant co-products are given in Table 21.2, and their main uses are briefly described below.

Liver is perhaps the most consumed co-product worldwide. Liver constitutes a very good source of vitamins A, C, and D; B vitamins; and nutrients such as iron, zinc, and copper (Marti et al., 2011). In fact, liver is used as a special source of vitamins B_{12} and A, and iron. Livers can be vacuum-packaged to extend their shelf-life and maybe also frozen-stored although not so recommended because they become softer. Liver is the main ingredient in the production of paté and liver sausage. Other uses include slicing and cooking by a variety of techniques, or it may be minced and incorporated into many dishes, loaves, spreads, and sausages.

TABLE 21.1 Range of yields (expressed as a percentage of live weight) from beef, pork. and lamb co-products.

By-product	Beef	Hog or pig	Lamb
Carcass + edible products	62–64	75–80	
Blood	2.4–6	2–6	4–9
Brain	0.08–0.12	0.08–0.1	0.26
Cracklings	3.0	2.2	
Edible kill fat (edible fat removed on the slaughter floor)	1–7	1.3–3.5	13–16
Feet	1.9–2.1	1.5–2.2	2.0
Head		5.2	6.7
Head and cheek meat	0.32–0.4	0.54–0.6	
Heart	0.3–0.5	0.15–0.35	0.3–1.1
Intestines		1.8	3.3
Jowl		2.7	
Kidney	0.07–0.24	0.2–0.4	0.3–0.6
Liver	1.0–4.5	1.1–2.4	0.9–2.2
Lungs	0.4–0.8	0.4–0.85	0.7–2.2
Pancreas	0.06	0.1	0.2
Rendered edible fat	2–11	12–16	9
Spleen	0.1–02.7	0.1–0.16	0.1–0.4
Stick trimmings	0.48	0.21	
Tail	0.1–0.25	0.1	
Tongue	0.25–0.5	0.3–0.4	
Tripe (stomach)	0.75	0.6–0.7	2.9–4.6

(Adapted from Gerrard, F., Mallion, F.J., 1977. The Complete Book of Meat. Virtue Press, London, UK; Ockerman, H.W., Hansen, C.L., 1988. Animal by-Product Processing. Ellis Horwood, Chichester, UK; Ockerman, H.W., Hansen, C.L., 2000. Animal by-Product Processing and Utilization. Technomic, Lancaster, PA; Romans, J.R., Costello, W.J., Jones, K.W., Carlson, C.W., Ziegler, P.T., 1985. The Meat we Eat, twelfth ed. Interstate Printers & Publishers, Danville, IL, USA; Ockerman, H.W., Basu, L., 2014. By-products. In: Devine, C., Dikeman, M. (Eds.), Encyclopedia of Meat Sciences, second ed. Elsevier, Oxford, UK, pp. 104–112.)

TABLE 21.2 Proximate average weight of major co-products (expressed in kg per animal) from market-weight animals.

By-product	Beef	Veal	Pig	Lamb
Liver	5.0	1.5	1.4	1.4
Heart	1.4	0.23	0.23	0.11
Tongue	1.7	0.7	0.3	0.2
Kidney	0.5	0.34	0.11	0.06
Brain	0.47	0.12	0.12	0.13

(Data from Ockerman, H.W., Basu, L., 2014. By-products. In: Devine, C., Dikeman, M. (Eds.), Encyclopedia of Meat Sciences, second ed. Elsevier, Oxford, UK, pp. 104–112.)

Heart has been reported to have a lower emulsifying capacity and water-holding capacity than skeletal muscle (Verma et al., 2008). Heart may be diced and added to stews or minced and added to other meats for added flavor and color or used in processed luncheon meat to add high-quality protein and color. Heart cavities may be stuffed with dressing or parsley and then roasted (Ockerman and Basu, 2014).

Tongue is rather tough and requires long-time moist heat cooking. Cooked tongues are usually thinly sliced and can be served hot or cold, often with garnishes or with sweet or sour sauce, horseradish, mustard sauce or other spicy sauces, or dressings. Tongue may also be added to casseroles and salads or used as an ingredient in luncheon meat or maybe pickled, smoked, or canned in an agar solution or used in a perishable jellied product. This canned product can also be manufactured by curing, water cooking, mincing, adding gelatin, seasoning, stuffing or placing in molds, and chilling.

Kidney may be used as an ingredient in meat casseroles, stews, or pies. Beef kidney should be cooked in water or braised. Lamb and veal kidneys are tenderer than beef kidneys and maybe broiled or wrapped in bacon and cooked on a skewer.

Sweetbreads are harvested from calves, lambs, and young cattle and refer to two different organs and three different tissues located in these animals. The thymus consists of two parts, one located in the cervical region in the neck adjacent to the trachea called neck sweetbread and the other in the thorax region. The pancreas is called stomach sweetbread. Sweetbreads may be scrambled (often with eggs), reheated in sauce, breaded and deep-fat fried, or used in salads or coated with butter and broiled.

Brain is less consumed than in the past due to the BSE outbreak, also known as mad cow disease. Cattle brains belong to the "specified risk material," which is legislated in many countries to be removed from bovine carcasses at harvest and incinerated. Brains are tender and are often thinly sliced, dipped in butter or

flour, and deep-fat fried. They may also be broiled, sautéed, braised, cooked in liquid, or broken into pieces and scrambled, often with eggs.

Tripe is obtained by cutting the clean stomach to size and pickling in salt brine or by cooking and pickling in a weak salt and vinegar brine. Tripe may be precooked (usual form) in water, sometimes fully cooked, and maybe packed in vinegar, pickled, or canned.

Large intestines and stomach from pork are collected at a slaughterhouse and cleaned. They are cooked, often with sauce, and are referred to as chitlings in the United States and chitterlings in Europe. The intestines and other areas of the digestive tract of cattle, pigs, or sheep are cleaned, salted, and used as casings for traditional sausages and other meat products (Nollet and Toldrá, 2011).

Fat rendered from fat tissues of pigs is known as lard, and fat rendered from fat tissues of cattle and sheep is known as tallow. Both types of fats have extensive use as food and food ingredients (Baiano, 2014). Pork lard and beef tallow are used for cooking and frying in many countries and contribute to the flavor and more intense consistency of soups and broths (Alfaia et al., 2020).

Blood is the first product obtained in the slaughterhouse, mostly obtained from bovine and porcine sources. Blood from healthy animals is generally free from microorganisms, but it must be obtained under hygienic conditions, stabilized, and collected. The typical composition of bovine blood consists of 80.9% water, 17.3% protein, 0.23% lipid, 0.07% carbohydrate, and 0.62% minerals (Duarte et al., 1999). Blood is a rich source of proteins where hemoglobin, an iron-containing protein, is the most abundant complex (Ofori and Hsieh, 2011). Blood can be separated by centrifugation into the cellular fraction and the plasma fraction (Ofori and Hsieh, 2011). The cellular fraction contains red blood cells, white blood cells, and platelets and can be used as a color enhancer for sausages. However, its real application is quite restricted to particular products because of the dark color of hemoglobin and adverse sensory effects (Ofori and Hsieh, 2011). Red blood cell fractions from sheep, pig, cattle, and red deer have been reported to exert a high antioxidant activity (Bah et al., 2016). Plasma proteins have numerous technological applications in foods (Lynch et al., 2017). So, plasma proteins such as immunoglobulins, fibrinogen, and serum albumin have good gelation and emulsification properties, while other plasma proteins contribute to protein cross-linking, high water-binding capacity, proteins enrichment, or foaming (Del et al., 2008; Ofori and Hsieh, 2014). In view of its high water binding and its ability to form gels on heating, plasma proteins have been used traditionally to bind water and fat to stabilize meat emulsions in ground meat products (Ofori and Hsieh, 2011; Toldrá et al., 2019). Enzymatic hydrolysates of plasma proteins can also exert antioxidant and antimicrobial activity that can be successfully used in meat emulsions and emulsion-type sausages (Jin et al., 2021). Low bitter and high umami protein hydrolysates were successfully prepared from bovine muscle and porcine plasma (Fu et al., 2018). Further, taste enhancers were obtained from protein hydrolysates of porcine he-

moglobin using γ-glutamyltranspeptidase from *Bacillus amyloliquefaciens* (Li et al., 2020). Another by-product obtained from plasma is the enzyme thrombin, a serine proteinase involved in the final step of blood coagulation, which is used as a binder of meat pieces to manufacture restructured meat products. Thrombin has an intense and specific proteolytic activity able to convert fibrinogen into insoluble fibrin, forming fibers by aggregation and creating a 3-D network fibrin clot (Lennon et al., 2010). Blood may be mixed with other meat ingredients, stuffed into natural or artificial casings and water-cooked, chilled and normally cold smoked, and then rechilled. Blood is usually limited to 0.5%–2% in a sausage product because above this level, it has a negative effect on color and flavor. However, some products have a high proportion of whole blood resulting in a product with black color, such as black pudding and blood (black) sausage (Nollet and Toldrá, 2011).

Other co-products such as the spleen may supply protein extracts that can be used as functional ingredients in cooked sausages (Toldrá et al., 2020). Other co-products such as pork jowl, oxtail, pig tail, and pig feet are removed from the carcass, cleaned, and can be combined with other soup ingredients to add taste and texture to soups. They can also be cured like bacon. Testicles are often thinly sliced, dipped in a batter of flour (breaded), and deep fried. Spleen may be fried, used in a pie as melt, or used for flavoring or in blood sausage. Furthermore, skin can be consumed as fried skin tapas, fat is used for cooking in many countries, and bones can be boiled to obtain nutritional and tasty broths (Toldrá et al., 2016; Gallego et al., 2019). Proteins with technological properties can be obtained from brines and trimmings from cooking meat processes (Mullen et al., 2017).

21.3 Nutritional value of co-products

Edible by-products (co-products) are usually different from skeletal tissue in structure, composition, and sensory properties. Co-products such as the blood, liver, lung, heart, kidney, brain, spleen, and tripe constitute part of the diet in different countries worldwide and have a high nutritional value (Honikel, 2011) as reflected by its composition (Table 21.3), especially in its content in minerals and trace elements (Table 21.4) and vitamins (Table 21.5). The values given in such tables reflect approximate values, which are merely indicative for informative purposes since the chemical composition is subjected to many variables for each particular animal such as species, genetics, age, gender, type of feed, and type of breeding. The iron content in edible by-products is higher than in respective meats, while the spleen, liver, tongue, and heart are richer in zinc (see Chapter 19. Liver and kidney are particularly rich in selenium, while the liver, brain, and spleen are rich in phosphorus. In general, all by-products, and especially the liver, constitute a good source of vitamin B and liver and kidney for vitamin A (García-Llatas et al., 2011). A better knowledge of the nutritional value of edible by-products would contribute to an increase in the consumption of these products.

TABLE 21.3 Proximate composition in major constituents per 100 g of beef, pork, and lamb co-products.

Organ	Species	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)
Liver	Beef	130	21	3	5
	Pork	140	22	4.5	3
	Lamb	150	21	5	5
Heart	Beef	115	17	5	0.5
	Pork	115	17	5	0.5
	Lamb	120	17	5.5	0.5
Kidney	Beef	100	16	4	1
	Pork	90	16	3	1
	Lamb	95	17	3	1
Brain	Calf	120	10.5	8.5	<1
	Pork	125	10.5	9	<1
	Lamb	120	10.5	8	<1
Tongue	Beef	185	16.5	13	0.5
	Pork	180	16	13	0.5
Spleen	Beef	110	18	3.5	1
	Pork	105	18	2.5	–
	Lamb	95	17	3	–
Blood	Beef	70	16.5	0.4	0.1
	Pork	70	17	0.4	0.1

(Adapted from Honikel, K.O., 2011. Composition and calories. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, Boca Raton, FL, USA, pp. 105–121; Ockerman, H.W., Basu, L., 2014. By-products. In: Devine, C., Dikeman, M. (Eds.), Encyclopedia of Meat Sciences, second ed. Elsevier, Oxford, UK, pp. 104–112.)

TABLE 21.4 Proximate composition of minerals per 100 g of the raw portion of beef, pork, and lamb co-products.

Organ	Species	Ca (mg)	P (mg)	Fe (mg)	Na (mg)	K (mg)	Mg (mg)	Se (mg)	Zn (mg)
Liver	Beef	7	356	6.7	110	300	35	15	4
	Pork	8	363	20	80	295	30	46	7.5
	Lamb	7.5	250	8.5	85	300	20	55	4.5
Heart	Beef	5	210	4.5	90	250	17	15	1.5
	Pork	4.5	165	4.1	67	200	17	–	6.5
	Lamb	5	210	3.5	140	280	20	2	2
Kidney	Beef	10.5	219	6.5	178	230	20	115	2
	Pork	9.5	240	6.0	160	240	20	190	2.5
	Lamb	8	250	5	150	270	15	–	–
Brain	Beef	10	312	2.3	125	219	15	–	1
	Pork	10	312	2.5	125	219	15	1.5	1.5
	Lamb	10	270	2	110	300	12	–	1.5
Tongue	Beef	7	175	2.5	75	220	18	2	3
	Pork	11	190	4.5	115	255	18	12	2.6
Spleen	Beef	6	360	44	80	320	20	30	4
	Pork	6	370	21	85	320	17	35	7
	Lamb	6	–	42	85	360	20	–	3
Blood	Beef	7	50	50	330	43	3	15	0.5
	Pork	7	75	40	210	170	9	8	0.3

(Adapted from Ockerman, H.W., Hansen, C.L., 2000. Animal by-Product Processing and Utilization. Technomic, Lancaster, PA; Honikel, K.O., 2011. Composition and calories. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, Boca Raton, FL, USA, pp. 105–121.)

Co-products are relatively rich in saturated fatty acids, while the contents of n-3 polyunsaturated fatty acids (PUFAs) are low (Prates et al., 2011; Alfaia et al., 2017) as shown in Table 21.6. There is a large variability in the content of fatty acids among the different by-products. Major fatty acids are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and arachidonic acids (C20:4). However, the brain contains high levels of n-3 PUFA (Alfaia et al., 2017). The amount of conjugated linoleic acid (CLA) in the meat and edible by-products from ruminants is particularly relevant due to its formation by

TABLE 21.5 Proximate composition of vitamins per 100 g of a raw portion of beef, pork, and lamb co-products.

Organ	Species	Vit B1 (mg)	Vit B2 (mg)	Vit B3 (mg)	Vit B5 (mg)	Vit B6 (mg)	Vit B12 (μg)	Vit A (RE μg)	Vit C (mg)	Vit D (μg)	Vit E (mg)
Liver	Beef	0.3	3.5	20	7.5	1.0	100	21,000	30	1.7	0.7
	Pork	0.3	3.0	21	7.0	0.7	40	20,000	25	5.0	0.7
	Lamb	0.35	3.0	14	8.0	0.4	85	50,000	35	0.6	0.4
Heart	Beef	0.2	0.45	35	2.5	0.3	10	6	2	1.0	0.2
	Pork	0.6	0.45	10	2.5	0.45	2.5	5	3	0.7	0.2
	Lamb	0.4	0.99	6	2.6	0.4	10	nil	5	–	–
Kidney	Beef	0.4	2.0	9.5	3.5	0.45	30	800	15	1	0.2
	Pork	0.35	1.7	13.5	3.0	0.6	10	150	12	1	0.2
	Lamb	0.6	2.2	7.5	4.2	0.22	52	316	11	–	–
Brain	Beef	0.15	0.25	4.5	2.5	0.3	12	–	15	–	–
	Pork	0.15	0.30	4.0	1.0	1.0	11	–	15	–	–
	Lamb	0.13	0.30	3.9	0.9	0.3	11	–	14	–	–

Continued

TABLE 21.5 Proximate composition of vitamins per 100 g of a raw portion of beef, pork, and lamb co-products—cont'd

Organ	Species	Vit B1 (mg)	Vit B2 (mg)	Vit B3 (mg)	Vit B5 (mg)	Vit B6 (mg)	Vit B12 (μg)	Vit A (RE μg)	Vit C (mg)	Vit D (μg)	Vit E (mg)
Tongue	Beef	0.1	0.4	6.5	2	0.15	5.0	nil	5.0	Tr	0.1
	Pork	0.3	0.4	8.0	2	0.35	3.5	9	3.5	0.6	0.5
	Lamb	0.1	0.4	4.6	–	0.18	7.2	0	6	–	–
Spleen	Beef	0.15	0.3	8	1.2	0.12	5.5	tr	45	–	–
	Pork	0.15	0.3	6	1.0	0.05	3.5	tr	30	–	–
	Lamb	0.05	0.3	8	–	0.11	5.3	0	23	–	–
Blood	Beef	0.1	0.1	3.5	–	0.01	0.6	30	Nil	0.1	0.4
	Pork	0.1	0.1	3.5	–	0.01	0.6	25	nil	0.1	0.4

(Adapted from Ockerman, H.W., Hansen, C.L., 2000. Animal by-Product Processing and Utilization. Technomic, Lancaster, PA.; Honikel, K.O., 2011. Composition and calories. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, Boca Raton, FL, USA, pp. 105–121; Kim, Y.N., 2011. Vitamins. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, USA, pp. 161–182.)

TABLE 21.6 Range of percentage of fatty acids in fats from beef and pork co-products.

	Liver		Heart		Kidney		Brain		Spleen	
Fatty acid	Beef	Pork	Beef	Pork	Beef	Pork	Beef	Pork	Beef	Pork
$C_{10:0}$	Tr	–	0.1	0.3	0.1	0.1	–	–	–	Tr
$C_{12:0}$	0.2	Tr-0.2	0.1	Tr-0.3	0.2	0.1–0.3	–	Tr	Tr-0.3	Tr-0.4
$C_{13:0}$	Tr	0.1	–	–	–	Tr	–	–	–	–
$C_{13:1}$	Tr	–	–	–	–	–	–	–	Tr	–
$C_{14:R}$	–	–	0.2	–	0.1	0.1	0.2	Tr	–	0.1
$C_{14:0}$	0.8–1	0.5–1.7	0.2–2	0.2–2	2.0	0.5–1.7	0.4–1	0.3–0.8	1–2	1–2
$C_{14:1}$	0.1–0.3	0.2	0.2	0.2–0.3	01–0.34	0.1	0.1–0.2	–	–	Tr
$C_{15:R}$	0.5	–	0.2	–	0.7	–	0.2	–	–	–
$C_{15:0}$	0.7	0.1–0.2	0.3	Tr-0.1	0.8	0.1	2	0.1	0.4	0.1
$C_{15:1}$	2	–	0.3	–	0.4	0.4	–	1	0.7	Tr
$C_{16:R}$	–	0.7	0.6	0.3	–	–	0.2	–	–	–
$C_{16:0}$	12–15	12–16	12–16	14–20	14–22	18–21	12–16	12–16	18–24	18–22
$C_{16:1}$	1–4	0.4–2.8	2–4	0.2–3	1–4	0.5–3.8	1–2	0.8–2.3	3	2–4
$C_{16:2}$	–	–	–	–	–	–	0.8	–	0.8	–
$C_{17:0}$	1	0.4–0.7	0.9	0.2–0.5	0.9	0.3–0.7	0.7	0.3	2	2

Continued

TABLE 21.6 Range of percentage of fatty acids in fats from beef and pork co-products—cont'd

	Liver		Heart		Kidney		Brain		Spleen	
Fatty acid	Beef	Pork	Beef	Pork	Beef	Pork	Beef	Pork	Beef	Pork
$C_{17:1}$	3.7	0.4–0.7	2.3	0.1–0.2	0.9	0.1–0.3	2.8	0.1	1.0	2.3
$C_{18:0}$	15–25	17–27	14–21	12–14	15–25	13–19	10–22	18–23	13–15	13–20
$C_{18:1}$	12–19	13–34	19–29	12–27	18–29	17–40	16–30	21–28	23–31	23–29
$C_{18:2}$	9–10	12–16	7–16	23–35	5–12	7–17	0.2–0.6	0.6–2	7	7–8
$C_{18:3}$	3.2	0.3–1.2	2	0.4–2.4	0.3–2	0.2–0.4	0.1–0.16	Tr-2.3	2	2
$C_{19:0}$	0.3	0.7	0.6	2	2	0.6	0.6	1	1	3
$C_{20:0}$	0.1	Tr-0.1	0.1–0.2	Tr-0.1	0.3–0.6	0.1–0.2	0.2–0.3	0.2–0.3	2	1
$C_{20:1}$	0.1–0.3	0.2–0.3	0.1–0.3	0.2–0.6	0.3–0.6	0.4–0.8	0.2–0.3	1.2–1.8	–	1
$C_{20:2}$	0.2–0.4	0.2–0.4	0.1–0.3	0.6–0.9	0.4–0.7	0.7–0.9	0.1	0–1-	–	2
$C_{20:3}$	Tr-0.1	Tr-0.7	Tr-0.1	Tr-1	Tr-0.3	0.1	0.1–0.2	0.1	–	–
$C_{20:4}$	6–12	3–17	4–14	8–20	11–16	3–19	5–8	9–11	5.2	2.4
$C_{20:5}$	0.3–0.5	0.1–0.5	0.3–0.7	0.3–0.5	0.3–0.6	0.2–0.6	–	0.1	–	0.4
$C_{22:0}$	0.6–1.7	–	0.8–1.7	0.7	0.6–1.7	0.4	0.5–0.6	0.6	–	2
$C_{22:4}$	1.7–3.4	0.3–1.4	0.4–0.7	0.9–1.3	0.6–0.9	0.9–1.8	4.6–5.2	4.2–5.3	1	–
Saturated	37.3–52.0	32.1–46.0	30.0–46.0	26.6–40.9	31.5–56.5	32.00–43.8	23.3–48.4	41.0–46.1	33.3–46.8	33.3–50.6
Unsaturated	48.0–62.7	61.7–54.0	54.0–70.0	59.1–73.4	43.5–68.5	56.2–68.0	51.6–76.7	53.9–59.0	53.2–66.7	49.4–66.7

Tr = trace; R = clockwise rotation around the asymmetric carbon.
(Adapted from Prates, J.A.M., Alfaia, C., Alves, S., Bessa, R., 2011. Fatty acids. In: Nollet, L.M.L., Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, Boca Raton, FL, USA, pp. 137–159; Florek, M., Litwińczuk, Z., Skałecki, P., Kędzierska-Matysek, M., Grodzicki, T., 2012. Chemical composition and inherent properties of offal from calves maintained under two production systems. Meat Sci. 90, 402–409.)

rumen microorganisms (see Chapter 19). CLA is a term defining a group of geometric and positional isomers of linoleic acid that has received attention because of their anticarcinogenic effects as well as other reported effects on the immune system and lipid metabolism (Schmid et al., 2006). The isomer in higher proportion is rumenic acid (*cis*-9, *trans*-11 CLA), which is produced in the rumen through microbial biohydrogenation of dietary linoleic acid and also endogenous formation by delta-9 desaturation of vaccenic acid (Nuernberg et al., 2005). It has been reported that the endogenous synthesis of rumenic acid decreases when the exogenous supply is increased (Palmquist et al., 2004). The concentrations of CLA may be as high as 4.3–19.0 mg/g lipid in lamb and 1.2–10.0 mg/g lipid in beef, but the content varies also considerably from animal to animal and even between different tissues within an animal (Prates and Bessa, 2009). The highest content is in the liver, followed by the tongue, heart, and kidney (Florek et al., 2012). On the other hand, the cholesterol content in co-products is usually several times higher than in muscle tissue (see Chapter 19), as shown in Table 21.7, because it is the major component of cell membranes

TABLE 21.7 Range of cholesterol content expressed as mg/100 g of portion of beef, pork and lamb co-products.

Organ	Species	Raw
Liver	Beef	91–140
	Pork	90–150
	Lamb	371–473
Heart	Beef	192–338
	Pork	214–354
	Lamb	129–140
Kidney	Beef	100–517
	Pork	310–700
	Lamb	315–338
Brain	Beef	1456–3010
	Pork	2195–2550
	Lamb	1352
Tongue	Beef	78–171
	Pork	87–116
	Lamb	132–180

(Adapted from Bragagnolo, N., 2011. Analysis of cholesterol in edible animal by-products. In: Nollet, L.M.L. Toldrá, F. (Eds.), Handbook of Analysis of Edible Animal by-Products. CRC Press, Boca Raton, FL, USA, pp. 43–63.)

and of nerves and is an active metabolite within the cells of organs and glandular meats (Bragagnolo, 2011). This high cholesterol content generates health concerns that may restrict the consumption of edible by-products. The content of essential amino acids in co-products is high with outstanding content of lysine within the range 72–82 mg/g protein, leucine with 80–90 mg/g protein, and valine with 52–62 mg/g protein (Aristoy and Toldrá, 2011).

21.4 Products resulting from co-products

21.4.1 Meat extract

Meat extract may be produced by pressing or by cold water soaking, but the most popular procedure is rapid boiling of meat that is to be canned. The juice from these procedures is concentrated into an extract. When bones or meat is boiled, the juices are combined with meat wash water, and it is often economical to boil new lots of bones or meat two, three, or even more times in the same liquid. The soup is skimmed to remove fat and filtered to remove particles and suspended solids. It is then boiled to coagulate protein, refiltered, concentrated by vacuum evaporation, and then reheated in open pans. Fig. 21.2 shows the procedure. Extracts are also produced in the solid form and are the foundation for various fluid extracts, bouillon cubes, broths, and soups (Ockerman and Basu, 2014).

21.4.2 Trimmings

The meat industry generates trimmings during processing. So, trimmings may be obtained from skeletal muscle deboning but also from by-products such as

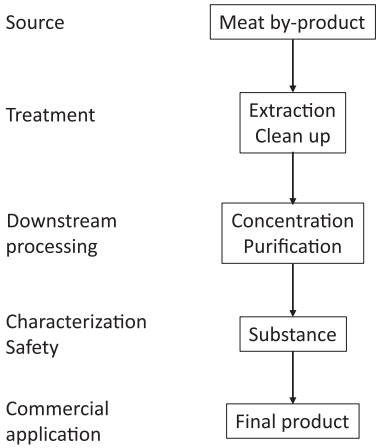

FIG. 21.2 Flow diagram of main routes of applications of substances isolated from meat edible by-products.

the liver, cheek, tongue, and so on. All these trimmings may be collected and used in sausage production and other emulsion-type products.

21.4.3 Soup stock

Soup stock can be produced from cooked or uncooked veal, lamb, pork, or cracked beef bones and stored refrigerated or frozen. This is an economical way to add some nutrition and flavor to cooked products, but bones produce strong flavors, particular for each species so that, for instance, lamb bones should be used in lamb dishes. They can also be used in soups, vegetable dishes, sauces, or gravies (Ockerman and Basu, 2014).

21.4.4 Gelatin

Gelatin is obtained from collagen present in high-collagen sources such as the skin, bones, ears, and so on. Collagen is first cleaned from noncollagen materials and then extracted with hot water and hydrolyzed with acid or alkali (Fig. 21.2). The structure of collagen is broken down forming warm water-soluble collagen, also known as gelatin (Karim and Bhat, 2008). The extent of hydrolysis increases with high temperature and a long period of treatment. Gelatin is rich in glycine, proline, and lysine but poor in tryptophan and methionine. Although its nutritional value is low, gelatin is widely used in the food industry because of its good gel-forming ability, but also for emulsion formation, beverage clarification, stabilization, or as a protective coating, thickening, and texturizing material (Ahmad et al., 2017; Gómez-Guillén et al., 2011). Thus, there is a wide variety of applications such as desserts, candies, bakery, jellied meat products, ice cream, and dairy products, among others. The use of bones for the obtention of gelatin is quite difficult because it requires acid pretreatment followed by hydrolysis with pepsin at 70°C (Cao et al., 2020). The degree of hydrolysis may be increased by adding lipase pretreatment before the enzymatic hydrolysis (Yao et al., 2020).

21.4.5 Haggis

Haggis is made from calf and sheep hearts, lungs, and livers with oatmeal added. It is heavily seasoned and cooked in a sheep's stomach.

21.4.6 Mechanically separated meat

There are several terms for this co-product. Mechanically separated meat (MSM) followed by the name of the species of origin is the most common. Other terms are mechanically recovered/reclaimed meat (MRM) and mechanically deboned meat, but in both cases, they must not give information on species. In view that some small bits of the spinal cord might be present

in MRM and to protect consumers against the spread of BSE, mechanically separated beef was considered inedible by FSIS in 2004 and prohibited its use as human food or as an ingredient in hot dogs or any other processed product. It was also banned in the United Kingdom in 2001. In Europe, the legal requirements for the production of MSM including the definition, the raw materials that can be used, and the production techniques were described in Regulation 853/2004.

MSM looks like a paste that is obtained by forcing bone and attached meat through a sieve using high pressure or by centrifugal force. In this way, the soft tissue containing meat and some calcium dust is separated from the bone and connective tissue. Anyway, the resulting MSM may contain small bone particles, connective tissue, some nerve endings, and pieces of blood vessels (Pearl, 2014). MSM is the most widely used system in the United States. An increased utilization may be obtained through hydrolysis with proteases for partial solubilization (Piazza and García, 2014). MSM pork is mostly used in comminuted meat products, particularly hot dogs at levels below the maximum content of 20% but also in sausages, hams, and culinary products. MSM must be declared in the ingredients statement.

21.5 Added value products obtained from co-products

Several biomolecules of interest can be produced from meat co-products and be used as functional ingredients or as adjuvants in food processing or to produce functional foods (Baiano, 2014). The flow diagram is shown in Fig. 21.3. This is an innovative way to add value to certain co-products and contribute

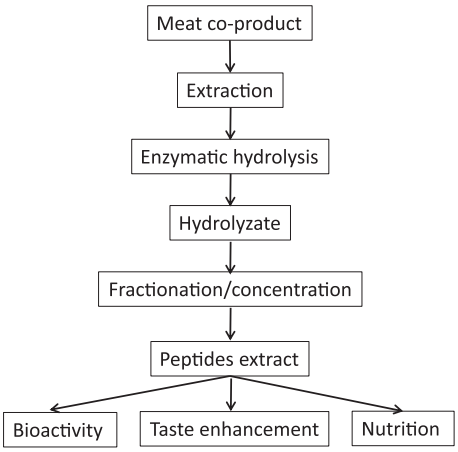

FIG. 21.3 Flow diagram of the enzymatic hydrolysis of meat co-products for the generation of nutritional, bioactive, or taste-enhancing peptides extracts. *(Reproduced from Toldrá, F., Reig, M., Mora, L., 2021. Management of meat by- and co-products for an improved meat processing sustainability. Meat Science 181, 108608.)*

to healthier processed foods (Toldrá and Reig, 2011). For instance, the obtention of heparin (Van der Meer et al., 2017) or the use of protein hydrolysates with taste properties (Toldrá et al., 2021), the obtention of extracts with functional properties (Chernukha et al., 2015), or the generation of bioactive peptides through protein hydrolysis with specific commercial peptidases (Mora et al., 2014; Toldrá et al., 2020). Some of the most used enzymes are pepsin, trypsin, chymotrypsin, corolase PP, papain and also from microbial origin such as neutrase from *B. amyloliquefaciens*, and alcalase from *Bacillus licheniformis* (Ryder et al., 2016; Toldrá et al., 2018). Such bioactive peptides exert an antihypertensive effect because they are able to inhibit the angiotensin I-converting enzyme (ACE-I), an enzyme that participates in the renin-angiotensin system where angiotensin I is converted into angiotensin II that constricts the arteries and therefore increases the blood pressure. But the generated peptides may also exert other biological functions in one or several of the physiological systems in human beings (Xing et al., 2021). Apart from the mentioned effect, hypocholesterolemic, antioxidant, and antithrombotic peptides have been also described, being able to modulate the cardiovascular system, whereas mineral binding and immunomodulatory peptides act in gastrointestinal and immune systems, respectively (Toldrá et al., 2012, 2016). The use of co-products, especially blood and collagen, has been extensively studied during the last years as a source of bioactive peptides through hydrolysis with commercial peptidases (Mora et al., 2019, 2020). Therefore, the generated bioactive peptides that are able to inhibit ACE in vitro and further exert an antihypertensive effect in vivo are very relevant for the development of novel therapeutics and functional foods for preventing hypertension. Of course, the efficacy and safety of bioactive peptides must be proved scientifically supported with human clinical assays, to have regulatory approval (Chalamaiaha et al., 2019).

21.6 Conclusions and future trends

There is a wide variety of edible meat by-products (co-products), most of them are consumed by humans even though its acceptance depends on the country, traditional cuisine, and local culture. What is considered a waste in a certain country may be highly appreciated in other countries. The consumption of co-products also contributes to a better sustainable meat industry by reducing the amount of waste. The meat industry is using science and innovation to add value to animal by-products far beyond its usual profitability based on hides and internal organs. A strategy to those co-products of low cost or poorly appreciated by consumers consists in giving them value addition. This ensures better revenue for the meat industry and saves the costs of disposal of such by-products. So, valorization strategies are being proposed for the hydrolysis of certain by-products to add value by generating active substances such as bioactive peptides with relevant physiological effects (antihypertensive, antioxidant, antidiabetic,

antimicrobial, etc.) all of them having relevant applications in food and pharmaceutical industry.

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Chapter 22

Sustainability II: Sustainable animal production and meat processing

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22.1 Introduction

The demand for meat is projected to increase as both the global population and average incomes increase. This is particularly evident in developing nations that have experienced recent growth in the number of middle-income consumers, who can now afford to purchase meat products. Compounding this market pressure, COVID-19 has prompted governments and other regulative authorities to focus their resources on pandemic management and the repair of affected social and economic systems. This prioritization has introduced unprecedented pressure on animal production systems, meat processors, suppliers, and retailers. It has also highlighted that the supply, delivery, and processing of meat must be more efficient, profitable, and have a positive impact on both society and the environment. Effectively, there is a requirement for sustainable animal production and meat processing.

Meat and other animal-based products constitute a major food group that provides essential and bioavailable nutrients for consumers, young and old. It can be argued that the production of meat is expensive and inefficient, in terms of the resources used to rear animals, process carcasses, and preserve meat until the point of consumption. This is not a true representation of the industry—but there is scope for improvement. This begins with animals that can convert low value feedstuff (with low protein or energy value), that is unsuited for human consumption, into nutrient-rich, palatable, and high-value meat products. Throughout history, animals well suited to this function have been domesticated and reared for the production of meat. These include poultry and pigs, respectively, pseudo- and monogastric animals that forage for feed or are fed low value cereals, pulses, yams, fruits, or protein meals. These also include cattle, sheep,

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goats, deer, yak, and buffalo, being ruminant animals that are used for meat and sometimes for labor, clothing, or recreation. Animal health and wellness and the overall flock or herd performance can be affected by many different factors, such as feed quality and availability, changes in the weather, pest and disease outbreak, human interference, animal husbandry, farm management, and so on. These, in turn, may impact on the productivity of an animal, in terms of the quantity and quality of meat it will produce.

It is apparent that meat production is interconnected around the world. For example, meat produced in one nation may be consumed by the population of another, and the energy resources used for the animal production along the supply chain are shared in a multidynamic manner by many societies. It is also important to look after the health and welfare of dairy animals since their progeny can be utilized for the production of meat or the replacement of flocks and herds for future stock. Ultimately, all dairy herds are used for meat and contribute to the supply of meat animals in many parts of the world. A holistic approach to enhance the sustainability of meat production would be preferable. This is illustrated by Fig. 22.1 that shows the primary sectors of food contribution to the global population and the secondary supply chain cycle of the ecosystem.

Animal production supports the livelihoods of many people. Indeed, poverty and malnutrition in some parts of the world could be alleviated through the careful transition in agriculture-food production sectors and policy making (FAO, 2017). To achieve this outcome, there is a need to collaboratively transform

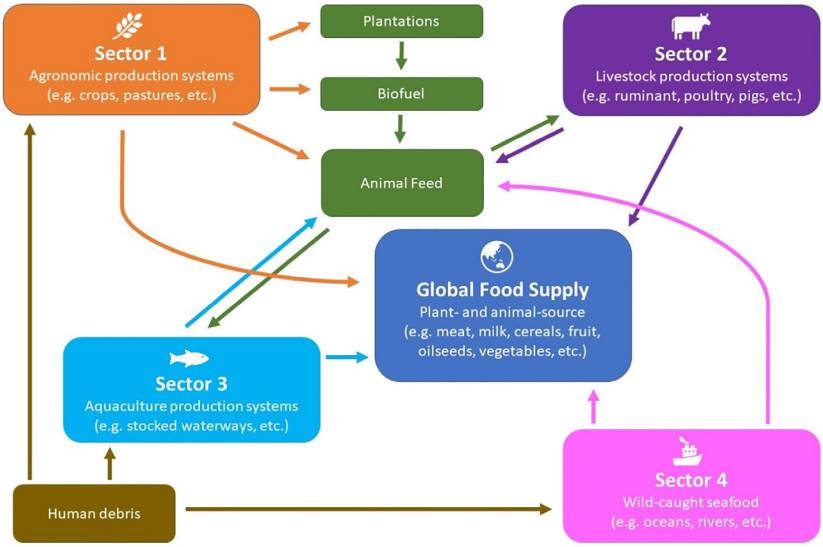

FIG. 22.1 A schematic diagram showing the four main sectors of the food production chain for the human population and the subsections that supply resources to other sectors, in a circular manner, within the ecosystem.

current animal production systems to a level that is sustainable. This involves the implementation of strategies designed to increase production efficiency and to improve the nutritional value, quality and preservation of meat without damaging the ecosystem.

The adoption of animal husbandry practices or technologies that enhance the welfare and health of animals would have perceived and actual benefits to the socio-economic sustainability of meat production. In addition, the meat trade has introduced rapid changes (innovations) to meat processing—within livestock transport (short and long), slaughter procedures, meat packaging, and product handling parameters. The challenges faced by stakeholders using prolonged storage periods or operating within extensive export networks can have a profound effect on the nutritional value and quality of meat and the potential for meat to be wasted and disposed of prior to its consumption. There are a number of ways in which livestock producers utilize available resources from the crop-animal-pasture production systems and extend the shelf-life of meat. Such livestock production and meat processing would maintain environmentally sustainable feeding systems, delivering higher quality and healthier meat products, which are ethical, residue-free, and safe for human consumption.

This chapter will share the importance of animal production systems, management decisions, and processing practices to sustainably meet consumer demands for meat. It will discuss the various approaches to animal production, define sustainable systems, identify technological, and other interventions that can be applied to enhance sustainability and explore the "pinch-points" that should be considered for sustainable animal production and processing of meat. An understanding of sustainable systems for meat production will help meet future demands for high-quality animal-based protein, preserve finite resources, and support innovation to match climatic and consumer constraints. This chapter will mostly cover how production systems can use available resources to sustain the production of ruminants; however, production systems of monogastric animals (swine and poultry) are also covered, in brief.

22.2 Types of animal production systems around the world

Animal production systems can be classified as extensive, intensive, or semi-intensive based on their resource usage, production purpose, and the climatic conditions in which they operate. Primarily, extensive or rangeland systems are used for the production of ruminant meat. This is either with meat as the primary product or with meat as a secondary and ultimate product of wool and milk production. This system is believed to be more economically viable and provide for animal welfare more so than other animal production systems. Nonetheless, each system has its own advantages in terms of their sustainability. Intensive systems are used when the area for animal production is limited and resources (inputs) are not. In this system, animals are fed concentrate-based total mixed

rations and housed in close proximity to one another, in feedlots, factory or battery farms, or alternative types of indoor pens. Advantages to intensive animal production include enhanced feed conversion efficiencies, capacity to monitor individual animal performance and welfare, and the greater production of meat. Semiintensive systems are a combination approach to animal production and are practiced by farmers across the spectrum of agroclimatic regions and socioeconomic status. This involves the supplementation of animals reared under extensive systems with energy- or protein-rich feedstuffs so as to achieve faster growth rates and enhanced meat properties, to that achieved with silage, haylage or forage diets. Forages, grains, and agricultural by-products are a source of energy and protein and can support animal growth, development, and productivity (see Chapter 2). Lipids, vitamins, and minerals are equally important for animal well-being and performance as well as meat quality and shelf-life. The degradation and digestion of forages, grains, by-products, the absorption of dietary nutrients in ruminant animals, and the release of energy and protein fractions have been summarized below.

- **Forages**. These are a major source of nutrients for herbivores around the world. When grazing high-forage diets, ruminants often ruminate or regurgitate ingested forage. This allows them to chew their cud, to reduce particle size, and improve its digestibility. As ruminants are fed diets with increasing levels of concentrate (grain-based or pelletized), they ruminate less. Upon entered the reticulorumen, forage is exposed to a unique population of microbiota that begin to ferment and digest the plant cell wall components (cellulose and semicellulose) into carbohydrates and sugars. Rumen microbiota use carbohydrates along with ammonia and amino acids to grow and proliferate. The microbiota ferment sugars to produce volatile fatty acids (VFA; acetate, propionate, butyrate), methane, hydrogen sulfide, and carbon dioxide. The VFA are absorbed across the rumen wall and transported to the liver, where they are converted into glucose via gluconeogenesis or transported to other peripheral tissues for de novo fatty acid synthesis. The production of VFA is slowed by the resistance of plant cell wall components to digestion. Routine rumination can increase salivary flow, which makes a stable pH environment (pH ~ 6.0).
- **Concentrate feedstuffs**. When ruminants are fed rations rich in grain or concentrates, the digestion process is similar to forage digestion, with a few exceptions. There is less chewing and ruminating, which leads to the production of less salivary and buffering agents. Most grains have a high concentration of readily digestible carbohydrates, unlike the more structural carbohydrates found in forages (roughage). The readily degradable carbohydrate from grains will rapidly digest, resulting in an increase in VFA production. The relative concentrations of individual VFA are also changed, with propionate being produced in the greatest quantity, followed by acetate and butyrate. When compared with forage diets, animals consuming concentrate feedstuffs, that are proportionately high in grain, will liberate less methane and less heat.

- **Protein digestion**. The crude protein in an animal's diet can be categorized as degradable intake protein (DIP) and undegradable intake protein (UIP or rumen bypass protein). Different feedstuffs (e.g., cottonseed meal, soybean hulls, pulses, and annual ryegrass forage) have different proportions of each protein type. There are two sources of protein for a ruminant: (1) protein from feed material and (2) microbial protein synthesized by the microbiota that inhabit the rumen. Ruminant animals have a symbiotic relationship with these microbes. Like other living creatures, these microbes have a requirement for protein and energy to facilitate their growth and reproduction. During digestive contractions, some of these microbiota transit from the rumen into the abomasum, where they are digested accordingly and thereby creating a source of protein for the host animal. Digestion of protein and absorption of amino acids in ruminants occur much the same way as in non-ruminants but, as for lipids, rumen fermentation markedly transforms the form and quality of the protein ingested before it reaches the duodenum.

These types of feedstuffs and the associated animal systems used for meat production have been described with a focus on their sustainability.

22.2.1 Extensive or rangeland animal production systems

Extensive animal production systems can be characterized by their limited use of resources (inputs) and comparatively low production per animal per unit of land (outputs). These systems often use improved pastures or native rangeland as a feed base to support animal growth and meat production. Because of the low cost of extensive animal production systems, they are used to produce meat in a range of agroclimatic regions and by different socio-economic groups around the world—from subsistence farmers to large-scale commercial farming operations (FAO, 2017). Specific to the production and quality of meat, there are factors that contribute to the sustainability of extensive animal production systems (Fig. 22.2).

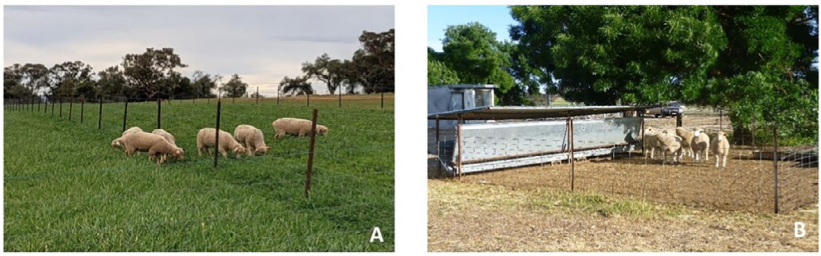

FIG. 22.2 Examples of lambs reared under extensive grazing (A) and feedlot (B) production systems, wherein animals are permitted to graze or are fed a concentrate-based ration, respectively. *(Images courtesy of Agriculture Victoria, Department of Jobs, Precincts and Regions, Victoria, Australia and NSW Department of Primary Industries, Australia.)*

Seasonal variation, climate change, or other forms of human interference can affect the availability and nutritional quality of the feed base in rangeland systems. This may result in the transition of forage mixes to include more drought, waterlogging, salt, or heat-tolerant species. This may also result in the targeted introduction of more adapted forage species into nontraditional agroclimatic regions, such as the introduction of a tropical forage species into traditionally temperate regions. The capacity for these revised feed bases to deliver the dietary energy, protein, minerals, and vitamins required by a grazing animal must be confirmed, as these will impact on animal performance and meat quality. This point is illustrated by Elizalde et al. (2021) finding that lambs grazing alfalfa (a drought-tolerant species) had improved liveweight gains and carcass yield to those grazing the permanent rangeland pastures of Western Patagonia. The carcass and meat quality of Aberdeen Angus steers were reported to be impacted by their grazing of natural, improved-natural, or annual-summer grasslands (Devincenzi et al., 2012). And, Steinshamn et al. (2010) observed that differences to the fatty acid profiles of the meat from sucking calves finished on mountain or cultivated lowland pastures were the result of their varying pasture compositions. Halophytes and other salt-tolerant plants may have application as an animal feed, although they must be grazed within a forage mix to dilute their antinutritive factors (e.g., lignin, oxalates, and nitrates). An agronomic solution would be to breed tolerance into existing, high-quality forage species. Alternatively, the enhancement of feed conversion efficiencies or rumen function could support sustainable meat production in periods of reduced feed availability or when low-quality forage species are dominant within the extensive production system.

Cellulose-rich plant material can be converted into muscle tissue by ruminants because of their unique digestion tract (rumen) and a symbiotic relationship with specialized microbiota. This is of value as it permits nonfoods, plant material that is otherwise indigestible by other animals (monogastrics), to contribute to the production of meat. Feed conversion efficiency refers to the ratio of forage grazed to the amount of tissue deposited by the animal. It is obvious, therefore, that greater feed conversion efficiencies would allow for sustainable meat production in circumstances where an animal consumes low-quality feed, under extensive systems. In addition, feed conversion efficiency can help to diminish the greenhouse gas emissions (primarily methane) and water usage associated with ruminant meat production—as fewer animals are required to deliver the same yield of meat. The bioavailability of nutrients from the grazed forage species, the microbiota of the rumen, and the genetics of the animal will impact on the feed conversion efficiency. For example, Kirschten et al. (2013) found there to be differences in the residual feed intake of lambs sired by Columbia, Suffolk, composite, and Texel rams, when managed within an extensive production system. This supports the selection of animals (or breeds) with greater feed conversion efficiencies as a means to increase farm profitability, reduce greenhouse gas emissions, and more sustainably produce meat within extensive production systems.

The sustainability of extensive animal production systems could be enhanced with the monitoring of animals, although at present these efforts would require

additional inputs and labor to the detriment of overall sustainability. It is fortunate, therefore, that technology has been developed in response to this constraint. McPhee et al. (2017), for example, describe the application of 3-D scans and machine learning algorithms to predict the position 8 (P8) rump fat (mm) and muscle scores of live Angus cattle. Likewise, Nir et al. (2018) found that a 3-D camera installed above a "free-walk" pathway could provide body frame and mass information for Holstein heifers. The potential for automation supports its adoption in extensive production systems, as does the potential to inform management decisions, such as best slaughter time and potential returns from cuts-based grading schemes. Other forms of animal monitoring are important in extensive production systems to ensure the utilization of available forages, avoid damage to critical habitats and water resources, and maintain the consumer perception of sustainable practices. This can include sensors, photogrammetry, animal collars, and other technologies designed to monitor forage, water, and greenhouse gas emissions in the field or via satellite. This information can also provide assurances to the advantages that extensive production systems offer to the "green meat supply chain," wherein the greenhouse gas contributions of the production inputs are included in the calculated environmental impact of meat production—for instance, transportation, feed, and concentrate production and labor. In this respect, the sustainability of extensive animal production can be monetarized with premiums offered for "sustainably" produced meat products.

22.2.2 Intensive animal production systems

Intensive animal production systems support the achievement of higher yields within a smaller area of production. This greater productivity (outputs) is the result of an increased reliance on external resources and precision animal husbandry practices (inputs). The initial "set-up" cost of intensive animal production systems is comparatively expensive, and consequently, it is often large-scale commercial operations that invest in this system for the purpose of meat production. These include feedlots for beef production, although other ruminants are also reared in feedlots; battery systems for chicken and poultry meat; and the factory or housed production of pig meat (Fig. 22.3). An outcome from this practice is that a substantial share of the total meat production within a market is supplied by only a few intensive producers. This represents a risk to sustainable meat production, as an isolated event (being it an economic or natural quandary) could have a significant impact on meat supply. This is demonstrated by the shock that COVID-19 inferred upon the meat supply chain, first as a result of the increased demand for meat from panicked consumers, then as a result of the diminished demand from food-away-from home meat purchases and disruptions to the workforce. It was concluded that the scale and efficiency of larger intensive production systems was an advantage in adapting to this crisis (Hobbs, 2021). Notwithstanding this point, the selection and use of specific inputs by intensive production systems can affect the sustainability of meat production.

Intensive production systems permit individual animal performances to be monitored. This, in turn, allows for the efficient use of feed resources and

FIG. 22.3 Examples of sheep (A) and pigs (B) reared under intensive production systems, wherein animals are housed and fed concentrate-based rations. *(Images courtesy of Agriculture Victoria, Department of Jobs, Precincts and Regions, Victoria, Australia.)*

"precision nutrition," designed to ensure utilization and to deliver specific meat quality properties. Tzanidakis et al. (2021) describe the use of cameras and movement tracking technologies to estimate the liveweight and behavior of pigs reared in intensive production systems. Condotta et al. (2018) reported that images from 3-D cameras, which were captured of individual pigs, could predict the "mass" of Duroc, Landrace, and Yorkshire animals. CCD cameras were used to capture color and NIR images of Japanese black cattle eyes, and ocular changes were used to identify incidences of vitamin A deficiency (Han et al., 2018). This form of precision livestock management promotes animal welfare, health, productivity, and the delivery of quality meat product—with it noted that dark, firm, and dry (DFD) and pale, soft, and exudative (PSE) meat are associated with animal distress prior to slaughter. On this point, the use of precision nutrition has been applied to reduce the susceptibility of an animal to produce a DFD or PSE carcass—albeit to differing levels of success. Precision livestock management also permits animals to be reared and slaughtered at a standardized frame size and mass, to produce carcasses less susceptible to yield losses during slaughter, processing, and fabrication. The standardization of carcasses supports processing efficiencies and the provision of cuts of meat that correspond to the portion requirements of a consumer. As a recent survey of UK consumers found that the meat products contribute to ~7% of all household food waste (Cooper et al., 2018). The rearing of animals to sizes that deliver uniform or more meal-appropriate portions could help limit "at-home" wastage of meat and the sustainability of meat production by intensive systems.

A challenge to sustainable meat production by intensive systems stems from the proximity to which it places humans to animals and the potential for zoonotic disease transfer. Some recent examples of zoonotic diseases include H1N1 (swine flu), avian influenza, and resistant pathogen strains (e.g., methicillin-resistant *Staphylococcus aureus*). The effect of these on the workforce, as well as the general population, should be considered when determining the sustainability

of intensive production systems. Mechanization or automation of tasks could assist in isolating the workforce from the animals. They could also improve economic and operational efficiencies, such as the provision of individualized diets or management interventions without the associated labor costs. Provided these are operated using renewable energy and there is proper due diligent, technology offers a valuable way to improve the sustainability of intensive production systems.

The cost and greenhouse gas emissions associated with the milling and transportation of feedstuffs to intensive systems contribute to the sustainability of this approach to meat production. For this reason, an intensive animal production system should be sited adjacent or within close proximity to the location where its inputs are produced. In practice, this has resulted in various feedstuffs being included into the total mixed ration or feedlot ration for livestock production. These often have the potential to enhance animal productivity and meat quality. Yan and Kim (2013) reported that the provision of microalgae (*Schizochytrium* JB5) to pen raised broiler chicks did not improve growth rates but did result in their meat having increased concentrations of omega-3, docosahexaenoic acid (DHA), and a reduce ratio of omega-6 to omega-3 fatty acids. Forwood et al. (2021) substituted unsalable carrots (horticultural waste) in place of barley grain in the diets of feedlot lambs and found that carrot-fed lambs (45% dry matter, DM) had greater feed conversion efficiencies, dressing percentages, and comparable meat quality. Byrne et al. (2008) reported that boar taint could be reduced in the meat of intact pigs with the inclusion of chicory root into the diet, across a 6-week period, a result that did not impact other sensory properties of the pork. From these examples, it is apparent that intensive systems can repurpose energy- or protein-rich waste products into animal feeds as well as utilize precision nutritional additives to boost meat quality. Further, increased feed conversion efficiencies will reduce the water usage (footprint) of intensive ruminant, pig, and poultry systems.

The secondary outputs from intensive systems that affect sustainable meat production include the potential for nutrient, chemical, or fertilizer run-off into the surrounding environment. There is a requirement for labor. Furthermore, if animals are reared indoors, then there is a requirement for climate control. This latter element of intensive animal production would require substantial resources to operate, but there would be advantages to avoiding thermal stress and its effect on animal performance and meat quality. Animal refuse or debris could contribute to the resourcing of an intensive production system, with efforts applied to generate electricity from the biogas of pig or cattle manure. Collectively, the sustainability of intensive systems for meat production requires a balance between productivity and the input cost for its achievement.

22.2.3 Semiintensive animal production systems

Elements from extensive and intensive systems can be combined into a hybrid approach that is referred to as a "semiintensive" system for livestock production for meat, milk, wool, and offal. In practice, the balance of extensive and intensive elements can be opportunistic and selected to capitalize on market demand

FIG. 22.4 Examples of cattle (A) and lambs (B) reared under semiintensive production systems wherein animals are provided energy and protein rich supplements (oilseed, cereal grain, or silage) while being permitted to graze and forage for feed. *(Images courtesy of Agriculture Victoria, Department of Jobs, Precincts and Regions, Victoria, Australia.)*

or resource availability. Examples of semiintensive systems include the use of supplementation to fill forage "feed-gaps" in extensive animal production systems, with conserved forage, silage, grain, and concentrates; the transfer of animals into an intensive system for a period (finishing) before slaughter; or the in situ utilization of intensive practices, such as providing housing to "free-range" animals when conditions are not conducive to performance (Fig. 22.4). As a result, semiintensive systems are adaptable, accessible to producers from different socio-economic or geographic groups, and provide a sustainable means to produce meat. That is not to say that there is a perfect (1:1) transfer of efficiencies from intensive or extensive systems, and this has resulted in unique factors that affect the sustainability of semiintensive animal production systems.

Semiintensive production systems allow for energy- or protein-rich feedstuffs to be included into the total ration of an animal. This can improve animal performances and introduce precision nutrients that enhance animal productivity and meat quality. Even so, the effect of feedstuff on animal performance and meat quality is not universal, or necessarily positive. Inappropriate supplement may therefore prove detrimental to the sustainability of meat production. The effect of a total ration on animal performance, feed conversion efficiencies, and meat quality have, consequently, been the topic of much research. For instance, Dieters et al. (2021) captured free-range Australian rangeland goats and fed them ad libitum hay and a commercial finisher pellet for 42-days, prior to slaughter. It was found that goats categorized as heavy or light delivered compared dressing percentage and meat quality; demonstrating the value of supplementation to rangeland goats to achieve consistent yield and meat quality, when extensive grazing conditions are not favorable. Yet, any management decision must be made with an understanding of the resource and input costs of supplementation, even if efforts are lesser than for intensive animal production systems. In addition, the effects of supplementation on access to markets should be considered—for example, some feedstuffs (but not all) are permitted to be fed to animals and without affecting access to the premium markets of grass-fed or organic meat.

22.2.4 Advantages and disadvantages of different animal production systems

There are advantages and disadvantages to the different systems for animal production that affect the sustainability of meat production (Fig. 22.5). Understanding these will help in the selection of the most appropriate production system for a given circumstance.

Consumer opinion is that extensive animal production systems operate to a high standard of animal welfare and produce meat that is both healthier and of greater quality to other systems for meat production. They require fewer inputs. This means that labor, feed, energy, and animal husbandry expenses are minimized and that the extensive systems are scalable to match the resources available. High-quality meat is produced in these systems, with a comparison of geese reared under free-range (under a vineyard) or intensive systems finding that the nutritional value of the meat from free-range geese, in terms of omega-3 and vitamin E concentrations, was the highest (Mancinelli et al., 2019). Weaner lambs finished under grazing pasture produced meat with higher concentrations of essential fatty acid and vitamin E concentrations than meat from lambs finished on feedlot ration for 6-weeks (Ponnampalam et al., 2017b). Likewise, pigs finished under extensive production systems (grazing pasture and acorns) produced meat with lower shear force and cooking loss values than their intensively finished counterparts (Almeida et al., 2018). The botanical diversity and composition of rangeland forages may support these findings, as they permit preferential grazing by animals and contain many bioactive compounds that enhance meat shelf-life

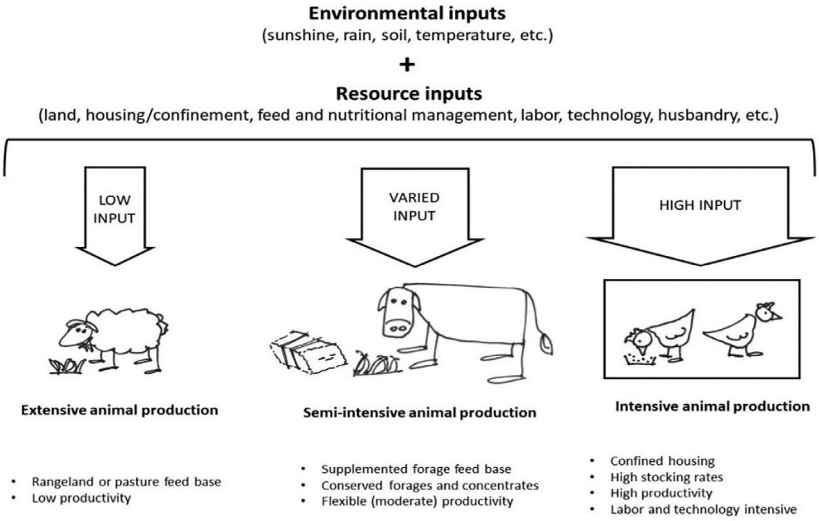

FIG. 22.5 A diagram of the resource inputs and characteristics of different animal production systems.

and sensory quality—for example, polyphenols, tannins, tocopherols, and flavonoids. It is observed, however, that animals reared under extensive systems will grow at slower rates and deliver lower yields. The severity of this is a function of the forage type, its quality, and availability to grazing animals. Changes to these factors can also be to the detriment of meat quality. Extensive animal production systems compete with other land uses, such as urban encroachment, the production of other agri-foods, and environmental reserves. In addition, these systems often compromise their surrounding ecosystems, contribute to water pollution, and compete with native animals for feed or shelter resources.

Intensive systems of animal production for meat are highly specialized and efficient. They require less area of land to produce animals and meat of uniform size and quality. This is the result of the use of standardized concentrate-based diets that also support the consistent production of meat at the level demanded by the market. Intensive animal production systems support individual animal monitoring, to deliver more precise welfare and nutritional interventions. The labor requirements of these systems can help to create employment and social well-being within the surrounding community. Further, intensive animal production systems are positioned to utilize novel organic by-products or food waste to produce meat. However, the cost of intensive animal systems for meat production is high, with true cost linked with the availability of inputs. On this point, intensive meat producers must compete with alternative users of labor, animal feed (cereals, grains, etc.), and foods that may otherwise be consumed by humans. There is a perception of low animal welfare associated with intensive production systems. This could be somewhat be attributed to the contributions to zoonotic diseases, resistance to antibiotics, and both direct and indirect negative effects on the environment—for example, disposal of litter and effluents, odor, energy requirements for feed milling, and resource transportation.

If semiintensive animal production systems are considered to be a hybrid of extensive and intensive systems, it is reasonable to expect comparable, albeit tempered, advantages, and disadvantages. There are, however, some factors specific to semiintensive animal systems for the production of meat. First, they are highly adaptable and allow producers to respond to economic signals, climate variability, and changes to the feed base or availability of feedstuffs that drive animal growth rates, feed conversion efficiencies, and yields. Some feed supplements and semiintensive practices have been shown to improve the nutritional value and sensory quality of meat. For example, the feedlot finishing of cattle is commonplace because it reduces the time necessary to achieve marketable weights, thus allowing for cattle to be slaughtered at a younger age, which increases the tenderness of high connective tissue muscles. However, the reactiveness and variability of semiintensive production systems can result in carcasses and meat of inconsistent quality, depending on the inputs utilized and their interactions with the feed base. In this respect, the management of semiintensive systems is dynamic and continuous.

TABLE 22.1 A summary of the advantages and disadvantages to the different animal production systems within the context of sustainable practice.

	Advantages	Disadvantages
Extensive	• Low labor requirement • Low feed inputs • Perceived and actual positives for animal welfare • Botanical diverse feedstuff allows preferential grazing	• Competition with other land uses • Competition with native animals for feed and water resources • Low productivity per land unit (stocking rate) • Vulnerable to changing climates and seasonal variation • Slow to react to changing economic, climate, or crises
Intensive	• Easy animal monitoring • High productivity per land unit (stocking rate) • High feed use efficiencies • High growth rates • Suited for automation and robotization • Uniform animals, carcasses, and meat products	• High set-up cost • High requirement for labor and management • Proximity of animals and humans • Indirect costs incurred from the production and cartage of feedstuff (economic and environment) • Perceived and actual animal welfare concerns
Semiintensive	• Reactive to changing economics and climates • Opportunistic supplementation and stocking rate • Scalable across different socioeconomic groups	• Variable feedstuffs can impact on animal performance and meat quality • Supplements must align to governing body or product type stipulations or standards

From these observations, which are summarized in Table 22.1, it is apparent that there is not one animal production system that is superior to another. Rather, selection of an animal production system should be made with reference to the circumstances that advocate the sustainable production of meat.

22.3 What is sustainable animal production?

Sustainable animal production systems are designed to maintain the conversion of available and inexpensive feed resources into animal products without diminishing the surrounding environment or wasting natural resources. Agriculture is the world's biggest employer and largest economic sector for many countries. Animal production is one of the major industries and is highly integrated

with crop production, pasture production, food security, and nutritionally dense human food supply. The contributions made by livestock, poultry, and other species of lesser prevalence (e.g., rabbit, deer, horse, etc.) to the supply of food to the global population are complex and multidimensional. For examples, these industries impact the human population by providing quality food, manure and labor for crop production, as well as providing incomes necessary to purchase other commodities and education.

Global meat consumption increased by 58% over the 20 years preceding 2018 to levels of 360 million tons/year (OECD-FAO, 2020). The main driver for this increase is the growth in population and incomes, particularly evident in the burgeoning socioeconomic status of the middle-class in Asia, Africa, and some Middle Eastern countries. Population growth accounted for 54% of this increase and per person consumption growth accounted for the remainder—the latter influenced by changing consumer preferences and income growth (Whitnall and Pitts, 2019). The world population is expected to be 8.5 billion by 2030 and 9.7 billion by 2050. Table 22.2 shows the estimation of population growth in the world by 2030 and 2050. In many high growth regions, resources (including water, fertile land, agriculture technology, and other) are limited, and this may reduce the capacity for efficient and quality animal protein production with

TABLE 22.2 Projected global populations until 2100, categorized by region and income status.

	Population, millions				
	2020	**2030**	**2040**	**2050**	**2100**
Africa	1340	1688	2077	2489	4280
Asia	4641	4974	5189	5290	4720
Europe	748	741	728	711	630
Latin America and the Caribbean	654	706	742	762	680
North America	369	391	410	425	491
Oceania	43	48	53	57	75
World	7795	8549	9199	9735	10,875
High-income countries	1263	1299	1319	1324	1303
Middle-income countries	5753	6252	6648	6933	7082
Low-income countries	776	994	1228	1474	2485
Rest of the World	3	3	4	4	5

Modified from United Nations, Department of Economic and Social Affairs, Population Division, 2019, World Population 2019. (ST/ESA/SER.A/434).

optimum return, and it is essential to sustain our livestock production systems for high-quality meat production.

Recent trend is that natural, clean, and environmentally friendly foods are preferred, and there is a growing market for products from all sustainable farming sectors. It is noted that fast-food (takeaway) sectors and supermarket outlets are marketing animal products from grass fed or free ranged systems more so than products from intensive animal production. Population around the world also prefer meat, milk, and their processed products from production systems where animals are reared free from hormone or growth promoting substances or artificial antibiotic application because of their belief that they affect human health. An animal production system that delivers the best outcome would be environmentally sound, economically viable, and socially acceptable.

The inefficiencies in the livestock production systems may be wasteful or harmful to overall food supply as well as food and nutrition security. Small holder farming to medium scale farming comprise the majority of individual animal production operations in developing countries. Hunger, malnutrition and food security are significant concerns in the developing nations. There are many gaps in the efficiency of animal production supply chain and sustainable animal production is the viable pathway to increase the production efficiency that can improve many livelihoods without causing environmental pollution, resources depletion, social nuisance, and animal welfare issues. Fig. 22.6 illustrates this observation, depicting the integrative components of a sustainable animal production.

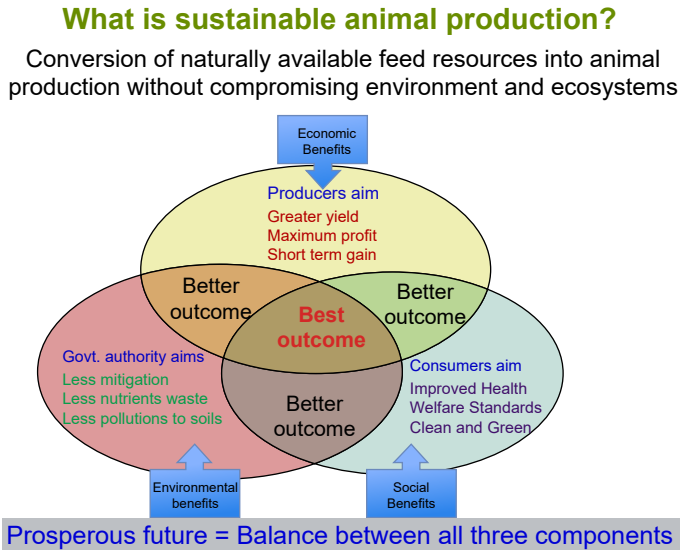

FIG. 22.6 The integrative components of sustainable animal production systems.

22.4 Why it is necessary to maintain sustainable animal production?

The management of our natural resources is vital for the long-term prosperity of future generations, enhancing economic growth, social life, and environmental protection. Sustainable animal production and meat processing will help to build resilient communities around the world by generating income as well as delivering nutrient-rich meat to boost human health, wellness and lifestyle. The world currently faces two major challenges. First, it must feed a growing population, and second it must address environmental problems that include the degradation of natural resources and damage due to climate variation. There is a global preference for animal-based foods from clean and green production systems. World research organizations such as FAO, ILRI, IRRI, UNICEF, IPCC, government authorities, and policy makers are working together to achieve a carbon neutral agriculture and animal production systems by 2030–50. Advisory committees around the world on climate change and resource utilization are encouraging the farming communities and livestock enterprises to follow carbon farming, which is a process of managing soil, vegetation, water, and animals to increase carbon storage and reduce greenhouse gas emissions. For example, Australia's red meat industry has set a target to be carbon neutral by 2030, and consequently, the production systems and processing sectors of Australian beef, lamb, and goat must adapt to maintain productivity without contributing to the net release of greenhouse gas emissions. It could be argued that for low- to middle-income population, livestock production is necessary to earn a salary and fund daily life; while those with high incomes consider animal production a routine food or profit-making enterprise.

Over the past century, there was a deviation in income levels among those earning high- (wealthy), middle- (average), and low incomes (subsistence and poverty). At present, a shift in the economic growth of the developing world is observed, with dramatic changes in the distribution of wealth through a significant increase in those earning a middle income. Such economic changes will bring significant transformations in the pattern of production and distribution of agriculture commodities around the world. It is anticipated that there will be a shift among middle-income people from the consumption of carbohydrate-based products to protein-based food products, such as red meat and dairy. Estimates are that the global production of livestock must double by 2050 so as to deliver upon the increased demand for animal products (FAO, 2013). However, further enhancement to the production of animal products will need to be balanced against their requirements for water, land, and other resources utilized for this increased production. It must also account for the environmental sustainability of meat production (e.g., methane emission from belching or litter, nitrogen runoffs into waterways from fertilizer application or manure, etc.) and competitiveness to plant-based allegories. Irrespective, most projections of the trends for future food production state that sustainable agriculture, animal production, and food security must be key objectives of any international or national strategy.

22.5 Some practices of sustainable animal production systems

The development of sustainable animal production systems should be a priority for all involved. Underpinning nutrition and the maintenance of welfare standards are crucial for optimal animal performance and productivity. This is because the welfare and health of an animal will drive productivity and sustain producer incomes—for instance, in the event of a disease outbreak or environmental disaster that partially or entirely affects a production system and its viability. There are many ways to improve sustainability, but primarily they involve the adoption of feeding systems that are geographically suited and will support animal performance and productivity. A significant portion (~60%) of the land available for agriculture is of poor quality (unproductive) or utilized for the production of another agricultural product to meat. In this context, ruminants are well adapted as they can convert fibrous forages, which cannot be utilized by humans or other monogastric animals, into meat. The rearing of ruminants for meat production can be integrated into cropping and horticultural systems as they can utilize the residues as a feedstuff and prevent their wastage (e.g., stubble, off-cuts, and foliage). In addition, ruminants are often reared for the production of milk and will contribute to the supply of meat at the end of this function (dairy cull cows) or if unsuited (male calves). It is apparent that ruminant meat is a sustainable product, and for this reason, it is the focus for the following sections that describe practices for sustainable animal production.

22.5.1 Use of fresh and conserved grass pastures

The balance of nutrients from pastures and forages is vital to optimize animal performances, because these are the major feed type consumed by grazing herbivores around the world. Ruminant animal production in high rain fall tropical areas mainly rely on C_4 plants such as Napier grass (*Pennisetum purpureum*), Setaria (*Setaria anceps*), Guinea grass (*Panicum maximum*), Buffel grass (*Cenchrus ciliaris*), Digitaria (*Digitaria smutsii*), and Kikuyu grass (*Pennisetum clandestinum*), while temperate areas use C_3 plants, mainly perennial ryegrass, annual ryegrass, Phalaris, Tall fescue, Timothy, Cocksfoot, and more (Devendra and Thomas, 2002). In some parts of New Zealand, United Kingdom, Ireland, and Australia, grass pastures are solely used to produce milk and meat from cattle, goats, and sheep. In these areas, the year-round climatic conditions are well suited for the establishment, growth, and persistency of grass pastures. As a result, farmers can manage their sheep or cattle purely on grazing pasture grasses, in rotation. For example, under New Zealand pastoral dairy farming, nutrients supplied to dairy cows are largely derived from grazed herbage, particularly from swards based on perennial ryegrass (*Lolium perenne* L.) (Macdonald et al., 2008). This is mainly due to ryegrass-based diets offering greater organic matter, water-soluble carbohydrate, and crude protein concentrations, which is adequate for nutrient utilization of dairy cows and lactation performance. At the

same time, another study indicated that perennial ryegrass swards are suitable for sheep meat production, and they are highly productive under high nitrogen input levels, with the potential to grow between 12 and 15 tons of DM/ha (O'Donovan et al., 2011) and are of high nutritional value offering crude protein content of 22%–24% DM and metabolizable energy content of 9.9–10 MJ/kg DM. It should be noted that previous studies with growing cattle consuming fresh forages indoors (Beever et al., 1985) and at pasture (Beever et al., 1986) have shown substantial rumen proteolysis and preduodenal losses of ingested nitrogen when nitrogen content of forages exceeds 25–26 g/kg DM. Such situation should be avoided because it is a waste in terms of sustainability of food production based on digestion of nutrients and animal productivity (anabolisms) since excess dietary nitrogen will be lost through gases or in the form of urea with urine excretion, which can reach waterways.

Optimizing the proportion of grazed herbage used in the system can increase the profitability of sheep, goats, beef cattle, and dairy production due to the lower production cost of grass compared to alternative feeds, such as feedlot diets or roughage plus grain supplements. In the last century, the requirements for higher meat and milk production from ruminant livestock systems are mainly achieved from intensification of agriculture both in plant and animal production sectors. It is reasonable to state that well-managed pasture grazing systems, containing grasses, can fulfill the nutrient requirements of cattle and sheep as a single sward or multiple swards. However, when grasses are alone cultivated, the restoration of intensively managed land generally results in a decline in both land and animal productivity.

Tropical and temperate grasses can produce more harvesting materials and yield more meat or milk when cultivated as mixed crops with other species such as legumes or novel forages. The reasons are (1) both grass species and stage of harvest (grazing) can significantly influence the processes of rumen digestion and nutrient supply to the animal; (2) as the pasture matures with season, the structural carbohydrates and lignin increases, which can cause significant amount of energy loss from the animal on voluntary feed intake, rumination, methane liberation, and digestion process; and (3) grass pastures are more susceptible to worm infestation and worm burden particularly when they are tender and immature because they are low in natural anthelmintic properties. To overcome these, the introduction of other pasture or fodder species in combination in the livestock feeding systems is valuable.

The management of mixed sward systems containing legumes and other novel forages for ruminant production could have positive consequences for agricultural productivity, compared with feeding grass monocultures or mixed grasses alone in the system. There are also many practical advantages of introducing pasture-, fodder-, and tree-legumes into field crops and grass cultivation. The reason behind this is that animals consume ad libitum by preferences that permits them to determine themselves the proportion of each forage they eat. When animals are able to determine themselves the

composition of their diet, they generally select a mixed diet. Nutritional and behavioral factors that determine diet choices are complex and not completely understood. Provenza (2018) suggested that diet choices are the result of maximizing digestive, metabolic, and sensorial comfort by the animals. There are associative effects of using mixed forages in livestock production, for example, it impacts on environment (nitrogen and methane emissions) and animal health (e.g., parasitism) (Niderkorn and Baumont, 2009). Increase intakes were observed for pasture with ryegrass and white clover association, but also with a biculture of two grass species (Cortes et al., 2006). Moreover, increases to feed intake in choice situations were also found with animals fed indoors with the hay of different grass or with a mixture of hay and silage (Baumont and Pomies, 2004).

22.5.2 Use of agri-food and industrial by-products

Growing ruminants under pasture or forage feeding systems are economical, but these do not always produce faster growth rates or heavy weight animals when compared with concentrate mixed ration or feedlot finishing. This is due to an inconsistency (variation) in the nutritional value of the forage they consume, which leads to a decreased rate of digestion and an increase to the heat production that associated with the high fibrous nature of the diet. Feeding studies have indicated that there is a reduction in ruminant size associated with pasture finishing, which is associated with a smaller carcass and the potential for meat quality defects. For example, lower muscle content, decreased juiciness, flavor, and texture (tenderness) scores are found for ruminants that were finished on pasture, these having lower carcass weights and fat depth than ruminants finished on concentrate diets. In these situations, using agricultural, oil and other industry by-products as a supplement to the diet of ruminants could prove a useful pathway to maintain carcass characteristics and meat quality.

There are a wide range of by-products (co-products) available, in abundance, that can be utilized as a feedstuff for animal production (Table 22.3). Often, these by-products would be wasted, and in some cases contribute to economic and environmental issues as a result of their disposal. Ruminants have the unique characteristics of converting low-quality waste and by-products into high-quality foods (meat, milk, and offal), with an additional advantage that inclusion of by-products can help reduce feeding costs. Some examples of agricultural by-products are sugarcane tops, grain legume haulms (e.g., groundnut and cowpea), root crop tops (e.g., cassava and sweet potato), oilseed cakes and meals (e.g., oil palm kernel cake, cottonseed cake, and copra cake), rice bran, grape marc, and bagasse. Care should be taken when feeding co-products in large quantities or proportions with fibrous basal feeds because they may contain antinutritional components. For example, polyphenols in olive cake or caffeine in coffee pulp may interfere with protein digestion. These materials vary in their composition and this, in turn, can affect their shelf-life and nutritional

TABLE 22.3 Some examples of crop residues and other co-products from food grade oil-, fuel-, horticulture, and brewery-industries used as feedstuff for animal production systems around the world.

Industry	Product	Nutritional properties	Regional usage
Biofuel	Corn husks and cobs	Sources of energy	
	Dry and wet distillers' grain		
	Peanut shells		Asia
	Glycerol		Asia, Latin America
Cereals and pulses	Cereal and rice bran	Sources of protein, energy, and fiber, especially for ruminant animals	Asia, Sub-Saharan Africa
	Seed coats (hulls)		East Asia, Sub-Saharan Africa
	Soybean meal		Latin America, Europe, North America
Horticulture	Apple pomace	Sources of energy, fiber, and bioactive compounds for ruminants and monogastric animals	
	Artichoke		
	Banana waste		
	Broccoli		
	Cassava and meal		Thailand, Vietnam, Cambodia
	Citrus peel		
	Pomegranate waste		
	Pumpkin waste		Mexico
	Tomato pomace		
	Root vegetables		
Oil	Canola meal	Sources of energy, protein, and polyunsaturated fatty acids	Canada, Australia, Europe
	Olive cake and pulp		Middle East, Europe
Sugar	Bagasse	Source of energy	Sri Lanka, India
	Molasses		India, South Africa, Australia
	Sugar beet pulp		
Textile	Cottonseed meal	Sources of protein and bioactive compounds	India, Australia
	Flaxseed cake		
Viticulture	Grape marc	Source of energy and bioactive compounds	Australia, Europe

value as a feedstuff. This point is illustrated by citrus peels, grape vines, or grape marc, which is highly susceptible to spoilage, due to their high content of yeast and mold populations that are associated with fermentation. These by-products should be used rapidly or preserved carefully. Horticultural by-products, such as from the vegetable and orchard industries, cannot be ensile alone but should be ensiled in a mixture with dry, fibrous materials, such as straws or broiler litter and fed to ruminants.

Dry and wet distiller's grains are major by-products from the distillation of cereal grains during the production of ethanol, a process used in the distillation of alcohol for biofuel or beverage production. These dry and wet distiller's grains contain protein, lipid, and fiber, which can be valuable feeds for ruminants. In recent years, efforts have been intensified on the use of distiller's by-products as a protein supplement in animal diets. The lipid and highly digestible insoluble fiber contribute to comparable levels of metabolizable energy to that provided from a basal diet containing cereal grain such as barley, sorghum, or corn. By-products with a high carbohydrate or protein content may serve as concentrate supplements in the mixed rations for ruminants that also include fibrous materials as roughage. Examples include soybean meal or canola meal, which serves as a protein source after oil extraction, while wet brewer's grains or dry brewer's grains that serve as energy.

Investigations of nonhuman foodstuff for utilization in animal production systems include Ribeiro et al. (2018b) investigation of the value of palm kernel cake (a residue form biodiesel production) in the total mixed ration of goat kids. It was found that palm kernel cake contributed to reduction to total digestible nutrients and the carcass yields of goat kids. Further study found that inclusion rates of 21% DM palm kernel cake had no detrimental effect on the sensory or nutritional (fatty acid) quality of goat kid meat (Ribeiro et al., 2018a). The replacement of cereals with pomegranate seed cake in the total mixed ration of lambs was shown not to affect animal performance, although it did have a positive effect on the fatty acid profile and antioxidant capacity of the meat and adipose tissues (Kotsampasi et al., 2021). The replacement of a conventional feedstuff with a cost-effective alternative incentivizes this practice. The same is true with the enrichment of the nutritional value of meat (e.g., omega-3 concentrations when oilseed meals from brassica family are fed to ruminants), improvements to shelf-life and quality—provided these properties attract a price differential in the market.

It is observed that by-products may be used to replace cereal grains in the rations for feedlot beef cattle and lactating dairy cows without inferring any adverse effects on animal performance. However, inclusion at higher levels in the diet can suppress feed intake, digestibility, and animal performance. By-products such as brans, sugar cane bagasse, seed coats of beans (soybeans, mung beans, and kidney beans), and cottonseeds are high in fiber and their use in ruminant rations improve rumen microbial function. Alternatively, by-products may contain beneficial nutritional components such as antioxidant properties in

pomegranate peels or vitamins from almond meal or flavonoids from soybean meal. By-products of palm oil industry in Southeast Asian countries (Indonesia, Malaysia, and Philippines) and coconut oil industry in South Asia (Bangladesh, India, Pakistan, and Sri Lanka) include copra and palm kernel meal. These are high in protein and fat that makes them useful supplements for goats, cattle, sheep, and buffalo and supplementation of such feeds to ruminants consuming low-nutritive and high fibrous cereal straw, grass hay, or senesced native pastures improve weight gain as well as meat and milk production.

Fibrous by-products of agricultural industries are produced in large quantities in tropical countries but have limitations for their use due to their low nutritive value, low density, and high moisture content, which enforce technological and economic constraints. It is important to identify specific ways of improving their feeding value suitable for regions within a country. An example is the alkaline treatment of NaOH or NH_4OH to increase the rumen breakdown and digestibility of high fibrous (high cellulose and lignin) straw and cereal residues that are abundantly available in tropical regions. The latter process will improve the digestibility of the structural carbohydrates in the plant cell wall, as a result of the actions of rumen microbes and digestive enzymes. Alkali treatment of high fibrous-low nutritive feed materials can increase the feed intake of ruminants in tropics and help increase growth rates, liveweight, and feed conversion efficiency. Treatment with NaOH is more effective when compared with treatment of NH_4OH. Further, sheep appear to respond better to treated feeds when compared to cattle.

Integration of crop, animal, and agroindustry operations in many tropical regions of the world may be a useful pathway to utilize these materials in small holder to medium-scale farming. By-products from agricultural industries can be used for livestock production when pastures or forage are limited. The use of agricultural by-products as source of protein and energy (fiber and fat) for ruminants improve environmental sustainability. It can also reduce feed prices by using feed materials that are locally and readily available. The same strategy can be used in different agroclimatic regions by incorporating locally available by-products into large-scale animal production, such as feedlot beef production or total mixed ration for dairy cattle production. Agricultural by-products have additional positive effects on ruminants when used as a roughage source, including decreased methane emission in lambs (Yulistiani et al., 2017). Recent study suggested that feeding mixture of 150 g of cowpea hay and 150 g of wheat bran to sheep consuming ad libitum grass hay improved growth performance and carcass traits offering highest meat value with desirable fat as compared with other treatments containing different ratios of hay and supplements (Gebrekidan et al., 2019).

22.5.3 Utilization of low-nutritive high-fibrous roughages

Both crop and animal production generate a range of fibrous materials, which have traditionally been called "residues." These can be classified as crop residues,

fibrous agroindustry residues, and urban fibrous residues. These include the grass hay of tropical and temperate climatic regions, cereal hay, legume hay, and other roughage (straw and chaff) materials. These materials are very low in nutritive characteristics, but valuable sources of feeds as basal diets (energy) to maintain or grow ruminants during dry seasons, when there is no quality pasture or fodder available for feeding. In Asia and Africa, rice straw and grass hay have been used for animal feeding in small holder farming (Devendra and Leng, 2011; Gebrekidan et al., 2019). Cereal straw (e.g., rice, wheat) is a poor source of roughage, of low DM intake and nutrient digestibility. Physical treatments, such as chopping and/or chaffing prior to feeding, can deliver positive responses in terms of increasing intake and digestibility. Nonconventional feed resources are used as traditional animal feedstuffs. These feeds are diverse and include palm oil mill effluent and rubber seed meal (Indonesia and Malaysia), cocoa pod husks (Malaysia), pineapple waste (Philippines and Malaysia), cassava pomace (Malaysia and Thailand), and poultry litter (all countries) and approximately 80% of the total feed available is potentially best suited for feeding to ruminants (Devendra and Thomas, 2002).

If the challenges of environmental effects and resource competition are adequately addressed, livestock production is expected to become the most significant agricultural sector for value added food (high-quality protein food) production. Recent climate variability in many parts of the world has further impacted pasture and crop production, which in turn has resulted in the reduced production of meat, milk, and wool by sheep, cattle, goats, and other ruminants. One example is that the 2018–19 drought in eastern Australia that considerably reduced the persistence, nutritional value, yield, and availability of pasture and fodder crops, which in turn heavily affected livestock productivity and the supply of meat from sheep and cattle for national and international markets during 2018 and 2019 (Hatfield-Dodds et al., 2018). Livestock producers under these circumstances face many challenges and issues and feeding of locally available fibrous residue (roughage materials) or preserved cereal straw from previous seasons is an option to overcome or manage these situation, as shown in Fig. 22.7.

To improve animal performance, the meat or milk productivity of livestock consuming low nutritive characteristics roughages, protein supplements such as whole grain pulses, or whole grain cereals from grain industry are used in many countries worldwide. Alternatively, meals or oil from oil seed industry are used as lipid (energy) supplement in many Mediterranean and temperate regions for fattening of beef cattle and sheep or increasing nutritional value of meat and milk from cattle, sheep, and goats. This include canola meal, sunflower meal, safflower meal, flaxseed, and camelina meal. For ruminants consuming roughage diets of low nutritive value, the effects on carcass traits and meat quality due to cereal grain (energy) supplements are lower than protein supplements (oil seed meals or legume grains) (Ponnampalam et al., 2004). Ramos et al. (2019) reported that the growth performance was improved in lambs receiving protein

FIG. 22.7 Some animal producer reactions to climate and seasonal variation effects on feed availability. *(Images courtesy of Agriculture Victoria, Department of Jobs, Precincts and Regions, Victoria, Australia and Philippa (Pip) Farquharson, New South Wales Farmers Association, Australia.)*

supplements at 12%, 16%, or 20% crude protein compared with lambs fed no supplemental protein, when grazing low-quality forages.

Middle Eastern countries are often a densely populated and situated within a semiarid climate, and there is almost no pasture for grazing. The diets of ruminants under grazing are composed mainly of low-quality forages mostly throughout the year. This leads to the elimination of grazing areas and the reduction of grazing performance. Young sheep (lambs) average daily gains finished in forage is usually lower than the lambs finished on high grain diets (Ponnampalam et al., 2002). The use of supplemental protein can satisfy animal daily nutrient requirements and increase the growth rates during consumption periods of low-quality forages. These conditions dictate intensive feeding regimes of ruminants, and under these scenarios most concentrate feed (e.g., cereal grains and soybean meals) is imported and fed with locally grown roughages such as silages of whole crop wheat or maize and some wheat hay along with by-products.

The supplementation of lambs grazing alfalfa with barley was reported to provide no improvements to animal growth, carcass yield, meat color, or its sensory quality, with the exception of supplementation providing for a firmer subcutaneous fat cover (Devincenzi et al., 2019). Smith et al. (2017) observed the performance of grazing alpacas to be enhanced with grain supplementation (mix of whole oats, rolled barley, cracked lupins, cracked corn, and black sunflower seeds), contributing to increased carcass yields, but no effect on the IMF or concentration of fatty acids in the meat. The authors suggest an alternative outcome would have been observed and had the pasture been of lower quality. Furthermore, Coelho et al. (2020) reported that carcass weights improved, and

meat quality was sustained in lambs finished on biologically diverse rangeland pastures and supplemented with grain concentrate at 1% liveweight as well as being dosed daily with 6 mL of 600 mg/mL mesquite pods (*Prosopis juliflora*) as a natural phytogenic additive. This form of precision nutrition was shown to increase the nutrient intake, digestibility, and yield of salable meat, even if it did reduce the fat content in sheep meat. It is noted that the supplementation of concentrates to animals does subtract from foodstuffs that could be directly consumed by humans. However, the cereals and legume meals offered to animals are often not of high quality and would otherwise be subject to discount if directed for human consumption. Nonetheless, the sustainability of meat production should be considered within the holistic context of food production.

In Asia, Africa, Central and North America, cattle, mithun, buffalo, yak, baruwal sheep, and Sinhal goats are part of life. Animal traction can improve the quality and timeliness of farming operations, thus raising crop yields and incomes. The transfer of nutrients from grazing lands to croplands through manure contributes considerably to the maintenance of soil fertility and the sustainability of the farming systems. Livestock provide a low-cost, labor-efficient route to intensification through their role in nutrient cycling. Therefore, keeping animals on the farm provides a use for other resources such as crop residues, horticulture sector leftovers, and household wastes, which might be wasted in the absence of animals.

In developing countries, rice straw, native pasture hay or tropical pastures, field crop residues, and foliage of large trees, while in developed countries hay from temperate grasses and cereal straw from oat, wheat, and barley are abundantly available. The major limitation with these roughage diets is high in lignin, cellulose and low in crude protein, making these feed materials underutilized for milk or meat production. Low-quality forages are defined as those forages that are deficient in crude protein, low in soluble sugars and starches, and are comprised mostly of native pastures, hay from improved grass or cereal crop residues. It has been reported that ruminant feeds should contain minimum of 7%–8% crude protein for the optimal microbial activity in the rumen for digestion of feeds and absorption of dietary nutrients into the body (Gebrekidan et al., 2019). The consumption of low-quality forage by ruminants has been found to be enhanced by supplementation with rumen degradable protein. Furthermore, the addition of crude protein to the low-nutritive roughage offered to ruminant animals can reduce feeding expenses while enhancing liveweight gain and carcass traits through increased intake and digestibility of nutrients, therefore increased meat quality.

22.5.4 Fodder and tree legumes for integrated mixed farming

Increased environmental temperatures and low rainfall in temperate and Mediterranean regions from late spring to autumn reduces the density, yield, and nutrient value of pasture, which in turn reduces the performance of lambs

grazing such pastures (Ponnampalam et al., 2017a). When grass pastures and fodders are insufficient for ruminant animal production in terms of quantity and quality, supplementation of diets with concentrate by-products, cereal grains, pulses, or oilseed meals enables faster animal growth rates, increased carcass weights, and enhanced meat quality or nutritional value. The application of concentrate diets high in cereal grains or feedlot diets can alter carcass composition and meat preservative (shelf-life) aspects compared with traditional grazing systems (Ponnampalam et al., 2012). Supplementation strategies are also costly although it help to maintain the health and wellness of ruminants during periods of insufficient pasture availability and prolong drought seasons. In this context, integration of pasture-, fodder-, or tree-legumes into crop and livestock production systems has many advantages, but recent climate variability also impacts upon the survival and persistence of some legume cultivars across many temperate and tropical livestock production regions.

The associative effects of legumes with other grass or crop residue mixtures carried out in vitro and in vivo experiments showed improvements in intake, digestion, and productivity at the whole farm scale. These outcomes are likely the result of fast digestion of the soluble fraction of legumes and a higher rate of particle breakdown and passage through the rumen; increased digestion when a low-quality forage is supplemented by a high nitrogen content legume, which can be explained by stimulation of the microbial activity; modification of digestive processes in the rumen, including degradation and hydrolysis of some plant lipids (polyunsaturated fatty acids, PUFA), which can interfere bypass nutrients for the host animal use; and/or the modification of digestive processes in the rumen involved in improved gut health and lower methane emission when certain bioactive secondary metabolites (e.g., tannins, saponins) are present. However, appropriate levels of legumes should be included in ruminant feeds based on animal species and legume cultivar type, otherwise the effects can be unfavorable on animal productivity and product quality due to rumen bloat or oversupply of dietary protein (Niderkorn and Baumont, 2009).

Alfalfa as a forage legume that can enhance animal performance and lead to increased meat and milk productivity. For example, sheep grazing perennial pasture that contained mainly senesced alfalfa were found to have greater liveweight gain and carcass weight than those grazing senesced annual ryegrass pasture supplemented with cereal grains (Burnett et al., 2012). A study of weaned lambs rotationally grazing alfalfa pasture for 6-weeks produced premium quality carcasses, equivalent to lambs fed a feedlot concentrate diet (Ponnampalam et al., 2017a). Furthermore, Ponnampalam et al. (2020a) reported that the productivity of lambs fed a low-energy diet, containing a higher proportion of alfalfa chaff (~50%), was equivalent to a high energy diet having a higher proportion of cereal grain (~50%) based on liveweight and carcass traits at slaughter.

Increasing the health enhancing omega-3 fatty acid concentration in meat and milk from livestock is the focus of many feeding studies, and while

challenging it is achievable. The *longissimus* muscle of Merino and crossbred sheep fed alfalfa diets had significantly greater concentrations of health enhancing fatty acids (α-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and DHA). Another aspect to note is the mixing of clover, medic, or vetch in ruminant feeding systems has many advantages when compared with feeding animals with single or mixed stand grass pastures. Research conducted in temperate regions found that feeding dairy cattle, beef cattle, or sheep with mixed pasture stand containing legume-based and grass/legume-based systems delivered greater yield and quality of milk and meat compared with feeding livestock on grass pasture systems as single stand or mixed stand (Grace et al., 2019; Hammond et al., 2014). It should be noted that these legume pastures should be harvested at the right stage and preserved as silage for future purposes if surplus, otherwise they will lose their nutritive value as the weather become drier at the end of spring or early summer stage based on the demographic location and climatic conditions. Silage is the final product when forage of sufficient moisture (>~50%) is conserved and stored anaerobically (oxygen-free), under conditions that encourage fermentation of sugars to organic acids.

Livestock production under tropical climates utilize legume fodders and tree legumes used as cover crops and by-products of legume grains (seed coats, hulls) cultivated for human consumption. The introduction of improved legume species for ruminants can promote the sustainability of cropping- and livestock production systems. In addition to their feeding value, legumes can make an important contribution to erosion control by providing cover and to increased soil fertility by enhancing nutrient and organic matter levels. Options include (1) the undersowing of food crops such as rice with annual or perennial herbaceous legumes as intercrops or relay crops; (2) the introduction of leguminous cover crops in perennial tree crop plantations; and (3) the development of agroforestry systems that include multipurpose trees, such as alley farming and the three-strata forage system. Supplementation of chopped straw with small amounts, ~300–400 g DM/day, of protein sources, such as leucaena or gliricidia leaf, can deliver a significant improvement in terms of increasing intake, nutrient digestibility, nitrogen retention, and animal weight gain under tropical regions.

In many south east Asian countries, nonproductive weed species in plantations can be replaced with productive improved legume species. In rubber and oil palm cultivation, leguminous cover crops have been planted to control less-desirable weed species and contribute to the early growth of the trees through nitrogen accretion. However, it is imperative that the introduction of forages and grazing animals into plantations does not interfere substantially with the management of the trees and reduce their yields. Legumes are less competitive than grasses, although there is variation between grass species in their competitive behavior. The grazing animals also produce manure and promote the recycling of nutrients to improve tree yields. Examples of ruminant-plantation crop

combinations include cattle under coconut, oil palm, and mango; sheep under coconut, rubber, oil palm, and durian; and goats under coconut (Devendra and Thomas, 2002). In the subtropical areas of Bhutan and Sri Lanka, legumes of the genus *Centrosema*, *Desmodium*, and *Stylosanthes* have been used for cattle and goat production for enhanced nutrient absorption result in increased milk and meat production.

In arid regions, the optimum livestock productivity is largely depending on the efficiency of utilization of locally available feed resources. Concentrate feeds, especially grains, are expensive and mainly used for human consumption. Therefore, it is imperative to look for other inexpensive feedstuffs to sustain livestock productivity. For example, indigenous to Ethiopia is Desho grass (*Pennisetum pedicellatum*), and it is widely cultivated due to its rapid growth and drought tolerance. However, Desho grass can only be used as a basal diet because of its low protein content. High-quality feed for ruminants in developing countries can be achievable through intensive utilization of forage legumes that have a better nutritional quality nearly equivalent to grain-based concentrates. In this regard, the use of leguminous forage crops such as vetch (*Vicia villosa*) is very valuable to improve the quality of herbage yield. Legumes in general and vetch, in particular, are excellent sources of nitrogen for livestock feed and the importance of forage legumes in livestock and crop production is well recognized.

Legume-based grassland-livestock systems offer important opportunities for tackling future agriculture challenges. It was found that grass-clover mixtures containing 40%–60% clover and receiving 50 or 150 kg of nitrogen per hectare achieved the same yield as grass monocultures fertilized with 450 kg of nitrogen per hectare per year (Nyfeler et al., 2011). It is suggested that botanically rich forage mixes are more resource efficient than monocultures, with the ability to be niche complimentary, and have positive interspecific interactions. They also enable symbiotic fixation of atmospheric nitrogen and thus a reduced industrial nitrogen fertilizer dependency. The development of legume-based systems of grassland husbandry undoubtedly offers opportunities for more sustainable and competitive ruminant production systems. Therefore, it is reasonable to expect that legumes will become more important in the future for sustainable animal production.

There are many advantages to the soil, crop, and grazing animal that result from introducing legumes in the integrated agriculture system and they include (1) increased forage production; (2) "greenhouse gas neutral" and "energy neutral" nitrogen input into grasslands via symbiotic nitrogen fixation; (3) support of nonnitrogen-fixing plants in the grassland through transfer of symbiotically fixed nitrogen; (4) higher nutritive value and voluntary intake of the forage with a less-marked decline of quality with advancing maturity than grasses, leading to higher livestock performance; (5) bioactive plant secondary metabolites of legumes can enhance the efficiency of protein digestion by ruminants; and (6) benefit animal health through reducing the need for worm control. Their

advantages are most pronounced in mixed stands containing with 30%–50% of legume species.

22.5.5 Matching welfare and nutrition to genetic potential

Balanced nutrition, being the supply of nutrients based on the physiological conditions of the animal and demand for metabolic homeostasis and growth, contributes to animal welfare and productivity. For example, Ponnampalam et al. (2020b) showed an alternative option to deliver 1 million lambs, each weighing 22 kg carcass by extending the feeding length of currently available 11 million animals for 3 additional weeks rather than producing 1 million additional new lambs through mating new ewes followed by feeding for 5-months before slaughter. This strategy is environmental and animal welfare friendly in terms of methane gas emission and use of veterinary (expenses) requirements for ewes and newly born lambs. Devendra and Leng (2011) stated that 2 billion tons of straw are produced worldwide and considering feed conversion efficiency of 10:1 potential exists to produce 200 million tons of live animal annually approximately delivering 100 million tons of meat allowing for 50% dressing. By utilizing the available straw, they could support 4 billion people provided with 25 kg meat per year. Another study on the effects of supplementation of a low-quality pasture hay with cottonseed meal (CSM), barley, or sorghum grain to young cattle (McLennan et al., 1995) showed that the efficiency of conversion of the supplement to liveweight gain with increasing amounts of CSM was approximately fourfold greater when compared with the efficiency of conversion with the use of barley or sorghum cereal grain. However, in the smallholder to medium-sized cattle (dairy and beef), sheep, goat operations, it may be a challenge to provide a balanced diet. The feeding of unbalanced diets is widespread as many livestock producers. Feeds are either not available or farmers lack in knowledge in preparing balanced diets for their flocks or herds. This will result in low animal productivity as dietary nutrients are not utilized efficiently to support an animal achieve its potential.

Generally, indigenous sheep had a potential for the multipurpose role to generate income under small-holder farming. They are relatively drought-tolerant, small in size, easily manageable, and are saleable resources that the family can use for ready cash and can rear in areas characterized by high rural human population pressure, fragmented land holdings, and scrubland. Even so, these animals generally underperform and have a low overall productivity for instance, the average carcass weight of Ethiopian sheep and goats is 10 kg on an average, which is the second lowest in sub-Saharan Africa (Hirpa and Abebe, 2008). Fluctuations and inadequate feed supply in both terms of quantity and quality and the low attention given to small ruminants compared to large ruminants. Whereas under Australian production systems, animal grazing senesced annual pasture supplemented with cereal grains or oil seed delivered carcasses weighing 18–22 kg weight (Burnett et al., 2012), indicating gaps and inefficiencies in the production systems around the world.

With the use of molecular biology and genomic selection tools for growth traits, performance, and ultimate productivity, producers can select different breeds, species, and genetics for their production systems. For example, the selection of fast-growing genetics to achieve heavy body weight animals at slaughter requires a diet with balanced nutrients for maximum liveweight gain and optimal carcass composition for producer and consumer requirement. Imbalanced animal nutrition or malnutrition also causes metabolic disorders and behavioral stress in livestock of many species. This is inefficient nor sustainable for the production system. Farmers often find it difficult to adopt practices that promote animal welfare without having sound information on the impact of such practices on animal health, animal productivity, and product quality.

Another scenario is that production systems in the tropics mostly employ animals that are not heavily selected for a given production trait but are suited to the immediate production environment. This inevitably eases the burden of disease and production risks for small to medium holder farmers, but the policy and planning of the responsible authorities of respective countries should be prepared to make policy directives to foster such healthy and sustainable systems. If not, farmers in developing countries can lose profit due to disease outbreak, which has the potential to completely wipe out their livestock or lead to marginal productivity losses due to various environmental stresses including subclinical levels of disease. On this point, in these agroclimatic regions, there is the potential for production-limiting and trade-preventing diseases that arise from zoonotic and food-borne sources. Government bodies and research organizations should regularly provide updated information and training to farmers and producers so that farm productivity can be enhanced through best animal breed selections and management practices, which in turn can increase producer income and profitability of production systems. Such support is essential for smallholder to medium range farmers in developing countries. For instance, countries such as Thailand and Vietnam have improved cattle and buffalo production with existing animals, which are well adapted to the given environment in the crop-livestock production system by simply improving the production of fodder and by intensification (Lambertz et al., 2012; Stür et al., 2013) resulting in desired sustainability, improved production, and enhanced product quality.

In general, a profitable production of meat and milk from pasture-based systems is dependent on high levels of pasture production being efficiently harvested by grazing ruminants of high genetic merit. A better herd productivity can be achieved by using appropriate stocking rates, lambing and calving at the right seasonal and the judicious conservation, and supplementation of feeds to match the nutrition to genetic potential or product output potential. In all pasture systems, an optimum stocking rate ensures high utilization of pasture without large amounts of pasture conservation but avoids the need to purchase expensive supplements during times of the year when feed demand exceeds current pasture growth. The advantages of these feeding systems center around the low cost of pasture compared with systems based on concentrate and forage that may maximize farm profitability.

The ability to identify specific limiting nutrients, and understand the biological reasons for these limitations, can allow meat and milk production from high-quality pasture to be increased. For animals with large nutrient requirements and high genetic potential, most of this production increase is associated with supplementary feed and is simply a result of increased DM and thereby increasing metabolizable energy intakes. The predicted benefits of providing a balanced supply of nutrients arise principally from the reduction in energy costs associated with urea excretion and avoiding the nutrient deficits induced when a large amount of supplement is fed. Animal genotypes that are better suited to diets of different types (e.g., pastures, silage, mixed rations, and grain feedlot) and stage of maturity of pasture (e.g., prebloom, postbloom, senesced, and hay) could be used to improve the whole-farm profitability of livestock under grazing systems. In real time, the on-farm technologies using spatial assessments that may predict nutrient composition of herbage and appropriate harvesting or grazing time would be an important factor in large-scale production systems so that livestock producers can rotate their stocks at the right time for grazing or harvest them for silage preparation for future use without loss of the herbage and its nutrient value.

22.5.6 Perennialized grazing cereals and multipurpose crops

The development of perennial, multipurpose crops will help diversify mixed farming systems, improve resource-use efficiencies, and provide a variety of environmental benefits when compared to the intensive cropping of annual species. Indeed, a recent example of the investigation of perennial wheat as a dual-purpose crop found it suitable to be grazed by ruminants and then harvested for grain (Newell et al., 2020). Preliminary results demonstrate that lambs grazing perennial wheat deliver comparable growth rates, carcass yields, and meat quality to those grazing annual wheat (Holman et al., 2021). There are agronomic advantages to sowing wheat with a companion legume (to permit the fixation of atmospheric nitrogen) as well as a natural solution of potential nutrient deficiencies of a cereal monoculture diet. Consequently, the holistic effects of the forage mix on animal performance and meat quality must be considered. The perennialization of other types of cereal grains (e.g., corn, barley, oats, triticale, sorghum, and teff) could likewise prove a viable ruminant feedstuff and offer producers the flexibility to respond to market demands (harvest for grain when grain prices are high or graze for meat production when meat prices are high). This would facilitate the efficient use of available resources to sustainably produce meat.

22.6 Other technologies or strategies to improve sustainability of animal production

22.6.1 Strategies to minimize diet-related subclinical diseases

The ruminant digestive system is uniquely qualified to efficiently use high roughage feedstuffs from forages, field crops residues, and agroindustry waste.

Green pastures, senesced hay, and cereal supplements are a major source of nutrients for ruminant animal production systems. However, the presence of some constituents in the feedstuff or their overconsumption may adversely affect feed intake and digestibility, leading to subclinical disease or ill health. This can result in lower overall productivity of the herd or flock and, unless rectified in a timely manner, will compromise the sustainability of a system. Several common maladies that result from the dietary management of ruminants have been discussed.

22.6.1.1 Bloat

Bloat occurs when rate at which gas is generated within the rumen exceeds the capacity of the animal to discharge this gas, either by eructation or belching. Bloat is therefore the result of complex interactions between the type of feed, intake level, ruminal microbiota, and the host animal. Factors that can play a role in the severity of bloat include the basal diet, soluble protein levels of the feed, the rate of feed degradation and fermentation in the rumen, bacterial slime production, and digesta passage rates. For example, the fermentation rate of legume forages, such as alfalfa, gliricidia, and leucaena as well as fabacean grains (lupins, peas), can contribute to the rapid generation of substantial quantities of gas in the rumen. This gas remains trapped in the rumen fluid as bubbles and the frothy content can inflate the rumen, compress the lungs to inhibit inhalation, and in severe cases the bloated ruminant animal may die. Legume forages and pulse grain supplements should therefore be offered in appropriate amounts, based on the species of ruminant, its body weight, the type of legume cultivars, and the total protein content of the diet. Legumes can be included in ruminant diets at a rate of 20%–60%. Processing grain to an optimal particle size, inclusion of ionophores in the diet, and allowing a period of adaptation during the transition from forage to grain diets can help to lower incidences of bloat or acidosis in animals fed with feedlot diets.

22.6.1.2 Acidosis

Acidosis is caused by the rapid ruminal fermentation of carbohydrates that are highly digestible and ingested in excessive amounts. Common sources for these carbohydrates include feedstuffs based on oats, wheat, and barley grains. It is noted that cereal grains have a small particle size that contributes to their rapid fermentation by ruminal microbiota and acidosis. This has given rise to the term "grain overload" being used to describe acidosis. Other feedstuffs such as breads, candy, apples, and other fruits, beets, and potatoes have also been implicated as sources of the excess dietary carbohydrate and can cause acidosis. Rumen acidosis usually occurs in animals that have been fed predominantly forage-based rations and are suddenly given access to large amounts of highly fermentable concentrates.

Acidosis can cause a shift in the population of ruminal microbiota, by decreasing the prevalence of forage-using-microbiota to potentially result in a decline in digestibility of forages. Lactic acid is a by-product of starch

fermentation. As highly digestible carbohydrates are fermented, rumen pH drops, and *Lactobacillus* species, which are lactic acid producing microbiota, proliferate in the acidic rumen environment, and further lower rumen pH (~5.5). As the rumen pH drops, the number of rumen protozoa and many of the lactate using microbiota will dwindle. Lactic acid production causes the osmotic pressure in the rumen to increase. Fluid is drawn from the systemic circulation into the rumen, resulting in dehydration and possibly hypovolemic shock. Lactate concentrations increase in the blood, potentially leading to systemic lactic acidosis. Animals from mild acidosis will recover without treatment. Animals facing severe cases with a metabolic acidosis should be treated immediately. Intravenous infusions of sodium bicarbonate or the evacuation of rumen contents (esophageal flushing) are easy interventions. Prevention is based on dietary management. Ruminants fed cereals ad libitum should always have access to fibrous feedstuff, such as straw or roughage. Inclusion of cereal grains at 20%–40% in the ration is ideal, based on the species and metabolic status of animal. Supplementation of cereal grain can go up to 60% if highly fibrous ingredients or feed are added in the ration.

Excessive concentrations of lactic acid in the rumen can be toxic to the rumen epithelium. The acidic environment leads to tissue damage within the rumen and can lead to ulcerations of the rumen wall. Damage to the epithelium can result in leakage of bacteria and toxins into the portal and systemic circulation. Chronic sequelae to rumen acidosis include fungal rumenitis and occasionally formation of liver abscesses. Liver abscesses are more common in cattle than in sheep and goats. Laminitis typically will occur more in sheep than in goats. The severity of the disease depends on the composition of the feed, particle size, amount of cereal grain consumed, and the period of adaptation to the diet.

22.6.1.3 Ammonia toxicity

Chemical treatment with alkalis, ammonia, and other oxidants can increase intake and nutrient digestibility of low-quality cereal straw. Straw treatment with ammonia or alkalis as calcium oxide or calcium hydroxide has potential applicability in tropical regions. However, when these treatments are put into practice at the small farm holder level, it is important to minimize ammonia escape during the treatment period.

Urea utilization in ruminant feeding was promoted due to a critical shortage of plant-based protein supplements for livestock production. The current situation of rising global demand for vegetable protein for livestock feed may reignite interest in utilizing urea in ruminant feeding. Nonprotein nitrogen (NPN) compounds are relatively cheap compared to protein feed sources like soybean meal or canola meal. They are affordable and alternative to natural plant protein sources. Of the NPN compounds, urea has found to have a wide application in ruminant rations. A single unit of urea will substitute 5 units of soybean meal in a ruminant diet (Khan et al., 2015). However, the use of urea as an NPN source is limited because of the rate at which urea is converted to ammonia sometimes

exceeds the capacity of rumen microbiota to utilize rumen ammonia-nitrogen, resulting in a loss of nitrogen for microbial protein synthesis. Various options have been evaluated over the years to resolve this constraint and reduce the risk of urea toxicity in ruminants, such as developing slow-release nitrogen sources that provide a steady supply of nitrogen to improve microbial protein production and utilization performance.

Rumen microbiota break down dietary protein into ammonia, amino acids, and peptides. They also break down carbohydrates into VFA. In general, protein and energy from feeds are used by the microbiota for growth and reproduction. Excess ammonia is absorbed via the rumen wall and converted into urea in the liver, where it returns in the blood to the saliva or is excreted by the body. Urea toxicity comes from overfeeding with urea supplements or from the consumption of high-protein diet by ruminants. Ingested urea is immediately degraded to ammonia in the rumen. When the ammonia production is more than the energy production available for building protein from the nitrogen supply, the excess ammonia is absorbed through the rumen wall. Toxicity occurs when the excess ammonia overwhelms the liver's ability to detoxify it into urea. High level of ammonia production can be lethal to the animal. However, with sufficient energy, rumen microbiota can use ammonia and amino acids to grow and proliferation. The rumen does not degrade the UIP component of feedstuffs. The UIP can bypasses the rumen and makes its way from the omasum to the abomasum. In the abomasum, the ruminant animal uses UIP along with microbiota washed out of the rumen as a protein source for body build-up.

22.6.1.4 Antinutritional factors

Animal feeds and human foods may contain poorly digestible proteins from less refined cereals and grain legumes, high levels of insoluble fiber, and high concentrations of antinutritional factors. Antinutritional factors may occur endogenously or may be formed during heat/alkaline processing of proteins. Examples of naturally occurring antinutritional factors are trypsin inhibitors and hemagglutinins in legumes; tannins in legumes and cereals; phytates in cereals and oilseeds; glucosinolates in mustard and canola protein products; and gossypol in cottonseed products. Phytate, with its abundance of negatively charged phosphate groups, is best known to chelate several nutritionally essential nutrients in the gastrointestinal tract of humans and animals, making them less bioavailable. Phytate interferes with zinc homeostasis and also negatively impacts on the bioavailability of other nutrients, including proteins. Phytate can negatively influence the activity of digestive enzymes, such as carboxypeptidases and aminopeptidases, by the chelation of mineral cofactors or interaction with the protein.

Some food crops, such as sorghum, millet, and various types of beans and peas, may contain substantial amounts of tannins (up to 72 g/kg). These food crops are staple sources of nutrients for the people of the Asian and African continents. The prevalence of protein malnutrition in these regions can be further aggravated by the negative effects of tannins on protein and amino acid

digestibility. Although processing treatments such as dehulling, soaking in water or alkaline solutions, addition of chemicals with a high affinity for tannins. and germination have been used to reduce tannin contents of sorghum and fabacean. Recent breeding, genetic, and genomic improvements have enabled the production of field crops, vegetable crops, grains, legumes, fruits with significantly reduced contents of antinutritional factors. Ruminant animals can better handle this issue than monogastric animals (swine, chicken, and human) due to their digestive tract and presence of microbiota that can degrade and inactivate many of these antinutritional factors, allowing them to utilize as feeds.

22.6.1.5 Mycotoxins

Mycotoxins are a toxin produced by molds (fungi) that contaminate food or bedding and are harmful to many animals. Warm and moist environments make a perfect condition for mold reproduction. Goats are more resistant to mycotoxin than horses. Pregnant animals and young animals are more susceptible. Some general signs of poisoning are appetite loss, weight loss, respiratory issues, increased susceptibility to infectious diseases (poor immune function), and poor growth rate. Prevention is necessary to avoid serious health issues and productivity loss. Prevention of mycotoxin poisoning can be achieved by assurances that animal feed, grain, and hay storage areas are clean, dry and cool; efforts to keep food storage areas protected from vermin that can damage food bags, increasing the likelihood of grain being exposed to damp conditions; feeding always fresh and dry feed; the cleaning of storage areas, bins, or feeders regularly; and the regular testing of grains for the presence of mycotoxins before formulating the feed ration.

22.6.1.6 Ketosis

Ketones arise from butyrate that is produced in the rumen and from the mobilization of body fat reserve. A large proportion of butyrate produced by rumen fermentation of the diet is converted to β-hydroxybutyrate (BHB) in the rumen epithelium and is absorbed into circulatory systems. Free fatty acids produced from the mobilization of fat are transported to the liver and oxidized to produce acetyl-CoA and NADH. Acetyl-CoA may be oxidized via the tricarboxylic acid (TCA) cycle or metabolized to acetoacetyl-CoA. Complete oxidation of acetyl-CoA via the TCA cycle depends on an adequate supply of oxaloacetate from the precursor propionate. If propionate, or oxaloacetate, is deficient, oxidation of acetyl-CoA via the TCA cycle is limited, and acetyl-CoA is metabolized to acetoacetyl CoA and subsequently to acetoacetate and BHB. The ketones, BHB, and acetoacetate can be utilized as energy sources. They are normally present in the plasma and serum of ruminant animal, and their concentration is a result of the balance between production in the liver and utilization by the peripheral tissues. Acetoacetate can be converted to acetone and diffusion of acetone across the rumen epithelium into the rumen and some of them can be eructated.

22.6.2 Strategies to mitigate methane gas emission

Global warming has thus far been linked to an increased concentration of greenhouse gases in the atmosphere, among them carbon dioxide, methane, and nitrous oxide. Methane is an important greenhouse gas since its effect has been reported to be 21-fold greater than that of carbon dioxide in terms of global warming (Li et al., 2010). It was predicted that by 2050, we would need twice as much grain as we produce today but half of that would be used by the livestock sector and the environmental footprint of livestock will grow further (FAO, 2013). Data from the United States have clearly shown that while the carbon footprint per animal has increased, the footprint per unit of output has declined, significantly (see Fig. 22.8) and due to the achievement of higher productivity (FAO, 2013). This offers a great opportunity for the developing world because in the future there is hope for improvement in ruminant animal production in developing countries while reducing its environmental footprint.

22.6.2.1 Use of lipid supplements

The addition of supplementary lipids (fats or oils) to the diet of ruminants can effectively reduce methane production. The effect of supplementary lipids appears to be twofold: either through inhibition of the activity/viability of the cellulolytic microbiota in the rumen or as a consumer/binder of hydrogen. As a consequence, lipid supplementation results in a reduction in the digestibility of cell wall carbohydrates, leading to decreased production of acetate and an increased ratio of propionate to acetate plus butyrate, which in turn decreases the production of hydrogen and thereby methane.

Experiments show that different dietary lipids can reduce methane emission to different levels. Increasing the level of dietary coconut oil resulted in a linear reduction in methane production in beef cattle, where supplementation of 250 and 375 g/day induced in a reduction of 18%–21% and 39%, respectively (Yuan et al., 2007). This same study found that, supplementation with coconut oil at 7% of DM in the diet of lambs causes a reduction in methane production of some 38%. Feeding coconut oil at 375 g/day decreased the digestibility and DM intake, whereas supplementation with lower levels at 250 g/day has no influence on digestibility or DM intake. In support of which, Machmüller et al. (2000) reported a methane reduction of 26% in lambs without affecting digestibility. This study observed treatment to decrease the population of protozoa as well as the proportion of acetate and butyrate, whereas rumen microbiota and the proportion of VFAs in cattle remained unchanged. The effect of sunflower bean, linseed oil, and rapeseed on methane production in cows and lambs has been studied by Beauchemin et al. (2007) and Machmüller et al. (2000), respectively. Supplementation of sunflower bean to the diet decreased methane production in lactating cows and lambs by 10% and 27%, respectively, while supplementation with linseed oil resulted in a reduction of 18% in cows and 10% in lambs. Consequently, in both cases, dietary supplementation had a negative effect on digestibility, reflecting a direct inhibition of the cellulolytic microbiota.

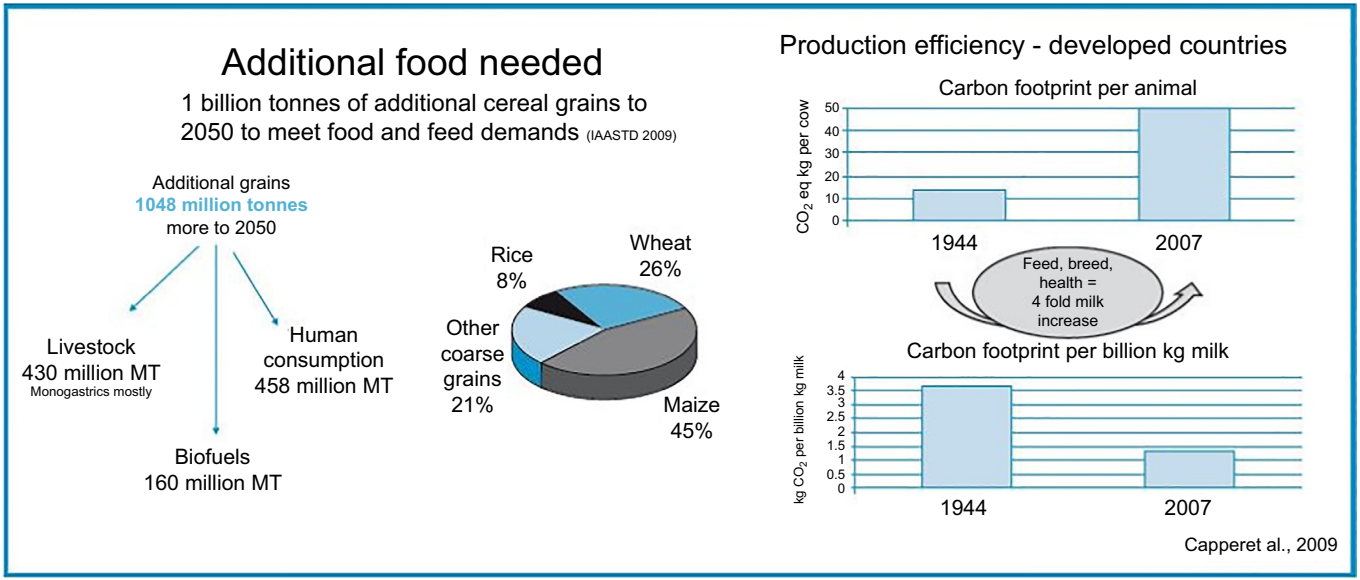

FIG. 22.8 A comparison of the environmental effect of milk production in 1944 and 2007 as well as the projected increases in demand for cereal and legume grains by 2050. *(Courtesy of the author: Gimmy Smith, Director General, International Livestock Research Institute, Nairobi, Kenya. From FAO, 2013. Asian Livestock: Challenges, Opportunities and the Response. Retrieved from Rome, ITA: http://www.fao.org/3/i3166e/i3166e00.pdf.)*

A decline in methane production in cows can be further attributed to a decrease in DM intakes. Supplementation with rapeseed reduced methane production by 16% in cows without affecting digestibility, whereas a methane reduction of 19% in lambs resulted in a decreased digestibility. The population of protozoa was uniquely decreased upon supplementation with sunflower bean and rapeseed, whereas this occurred only with cows given linseed oil. Rapeseed has a high content of monounsaturated fatty acids (C18:1), whereas sunflower bean and linseed oil are both rich in PUFA yet differ in that sunflower bean is rich in C18:2 while linseed oil is rich in C18:3, which may explain in part some of the variation in terms of their effects in reducing methane production in ruminants. In support of which, Dohme et al. (2001) showed a methane reduction in vitro upon incubation of C18:2 with rumen fluid of some 25%, which is consistent with a reduction in methane production of 27% in vivo in lambs given sunflower bean (Machmüller et al., 2000). Martin et al. (2008) observed a reduction in methane levels of 12%–64% upon addition of different types of linseed oil in cows. The linseed oil types differed in their form of processing, which indicates that heat treatment, pelleting, and other processing steps may further complicate/influence the efficacy of these natural lipid methane inhibitors. Dietary lipids, given to ruminants, inhibit methane production, with lauric or myristic fatty acids (saturated fatty acids) as well as linoleic or especially linolenic acid fatty acids (PUFA) having a significant effect. Likewise, adding seaweed or marine algae rich in PUFA to sheep and cattle diets have much potential in reducing methane emission while improving animal health and nutritional value of meat and milk.

22.6.2.2 Use of polyphenols

The introduction of legumes and forages containing secondary metabolites is important because it could provide a positive impact on both animal-human health, food preservation, and the environment protection. Ruminants fed legumes or forages containing secondary plant compounds have been shown to emit less methane compared to ryegrass-based forages. Studies with cattle using the SF6 technique reported average methane yields of 23.4 vs 29.6 g/kg DM intake when feeding trefoil versus ryegrass (Woodward et al., 2004) and 19.5 vs 24.2 g/kg DM intake when feeding sulla vs ryegrass (Woodward et al., 2002). There was a decrease in methane emissions when percentages of white clover (*Trifolium repens*) in pasture mix increased from 15% to 30% to 60% and the values were 20.9, 18.6, and 18.1 g/kg DM, respectively (Lee et al., 2004). Hammond et al. (2014) reported reductions in the methane emissions of heifers fed a botanically rich ryegrass pasture that contained different flowering plants, compared to a perennial ryegrass control, and the values were 19.5 and 25.6 g/kg DM intake, respectively. Reynolds et al. (2020) reported that the reduction of methane production from ruminants fed diets containing condensed tannins might be beneficial for reducing greenhouse gas emission. When cattle and sheep are fed with diets containing excess organic matter and protein, it may

lead to large quantity of organic matter and nitrogen excretion. This can cause a potential manure methane emission. Nitrogen (e.g., ammonia, nitrate) excretion negatively impacts air, soil, and water quality and leads to nitric oxide, which is the second most abundant greenhouse gas emission from cattle. Grass silage obtained from different maturity stages substantially affected dietary fiber and crude protein contents and increased dietary fiber and decreased crude protein resulted in greater methane emission, lower organic matter and neutral detergent fiber degradability, and lower nitrogen excretion.

22.6.2.3 Use of cereal grains

The influence of diet is greater than genetics in terms of their effect on the sensory quality of meat. For instance, cattle fed concentrates (grains) generally rank higher than forage (grass) fed cattle for meat attributes of flavor and tenderness. However, improved productivity and good meat-eating quality can also be achievable from pasture finishing systems of cattle and sheep via management and application of strategic dietary supplementation. In Australia and Europe, feedlot supplementation or grain supplementation toward finishing is a common practice when a pasture finishing system is not optimal for growth rate and carcass production. There is clear indication that supplementation is required to optimize nutrient utilization and carcass productivity when finishing diets of ruminants are suboptimal. This will allow producers to achieve, at relatively low cost, a carcass weight appropriate to market specification. In North America, feedlot finishing of cattle is commonplace specifically because it reduces greenhouse gas emission and the number of "rearing" days until the animal can be sold or slaughtered. It is noted that an appropriate supplementary feeding strategy within the grazing system is essential, otherwise consumption of cereal grains at a greater level by dominant animals can cause acidosis. Such cases can be costly and affect average flock performance and welfare of animals.

22.6.2.4 Use of ionophores

Ionophores are feed additives used in ruminant diets to increase feed efficiency and liveweight. They are compounds that alter rumen fermentation patterns. Ionophores have been used widely in the beef and poultry industries to improve feed conversion efficiencies and/or to control coccidiosis. Similar to many other feed additives, ionophores are fed in very small amounts and supplied via another feedstuff as carrier for intake. Ionophores function by selecting against or negatively affecting the metabolism of gram-positive bacteria and protozoa in the rumen. This result in decreased rumen digestion and the energy supplied from the ruminal digestion. By controlling certain protozoa and bacteria in the rumen, methane generation can be reduced. The shift in ruminal bacteria population and metabolism allows beneficial bacteria to be more efficient through an increase in the amount of propionic acid and a decrease in the production of acetic acid and lactic acid. This increases the overall energy status and use feed resources more efficiently.

22.6.3 Sources of secondary metabolites and bioactive compounds for better animal health

It should be noted that there are benefits from novel forages containing secondary plant compounds (e.g., condensed tannins, lignans, and polyphenols) that may help in enhancing gut health, oxidative stress, and mitigation of greenhouse gas emissions that collectively result in improved productivity.

22.6.3.1 Secondary metabolites

Grace et al. (2019) concluded that emergence of resistance to chemical anthelmintic will force farmers to seek alternative and more sustainable parasitic control methods. There was a reduced requirement for anthelmintic usage in the herb containing 6 and 9 swards mix pasture compared with lamb grazing perennial ryegrass and white clover sward or perennial ryegrass sward, based on the botanical composition and this was supported by reductions in fecal egg counts in lambs grazing chicory and plantain. This effect might be due to chicory and plantain containing secondary metabolites, for example, condensed tannins, which are proposed to have anthelmintic properties. It was also proposed that improved mineral and trace element status of the animals due to the elevated trace element concentrations of herbs, which may contribute to reduced parasitic burden or improved immunity against helminth parasites (Grace et al., 2019).

The ability of condensed tannins on protein binding in the rumen has some advantages to the ruminant animal (host) due to formation of complex materials with DIP and amino acids, preventing their loss (degradation) in the rumen and help bypassing them to the small intestine for absorption by the host animal. There were studies reporting when condensed tannins are included in the ruminant diets at appropriate levels, it increased the animal growth performance by increasing the nitrogen retention of diet. Another potential benefit of condensed tannins is anthelmintic properties due to their ability to inhibit egg hatching and larval motility of gastrointestinal nematode parasites. In the meantime, condensed tannins can cause some antinutritional problems to ruminants, when fed at higher levels, for example, more than 60 g/kg DM due to their astringent properties, which in turn can reduce feed intake and animal growth rate.

Plants and plant products containing lignans consumed by animals and humans have many advantages, not only improving the gut microbial population and gut health but also provides a range of health benefits. Lignans can improve the antioxidant status of tissue systems and whole body such as improving the immune status of individuals by providing defense against infectious diseases, prevention of cancer by limiting the proliferation of cells via anticarcinogenic effects, and can act as pro-inflammatory compounds alleviating oxidative stress result in reduced metabolic syndrome or disease risk in animals and humans. Increased total antioxidant capacity in the blood was observed when sheep grazing senesced annual ryegrass pasture was supplemented with whole flaxseed

or flaxseed meal for 8 weeks when compared with oat grain supplementation (Burnett et al., 2012), and flaxseed is known to have lignan properties, an anticancer substance.

The cinnamon essential oil may also improve nutrient uptake by protecting intestinal gut morphology and integrity. Supplementation of cinnamon essential oil in broiler chicken diet increased the villus height in the duodenum and jejunum with associated increased villus surface area and the efficiency of absorption and digestion of nutrients (Ali et al. 2021). A greater villus height means greater mucosal digestive enzyme activity, which ultimately improves the digestibility of nutrient. In addition, the digestive process liberates reactive oxygen species that act on intestinal mucosa and shorten the intestinal villi, but antioxidant enzymes bind the reactive oxygen species. The essential oil acts as hydrogen donor and exhibits antioxidant activity which protect the intestinal villi from oxidative damage by stimulating the activity of antioxidant enzymes.

22.6.3.2 Polyunsaturated fatty acids

There is substantive evidence to support the regular consumption of omega-3 PUFA, as these are beneficial for growth, development, health, and the welfare of humans and animals (Ponnampalam et al., 2021). Specifically, α-linolenic acid (C18:3n-3) and the long chain derivatives EPA (C20:5n-3), DPA (C22:5n-3), and DHA (C22:6n-3) have each been reported to play a role in the prevention of cardiovascular disease, diabetes, hypertension, inflammation, allergies, cancer, renal disorders, neural function and improve immune response. All fish are rich in long-chain omega-3 PUFA, especially EPA and DHA, but this is especially true for oily fish such as salmon and mackerel. The levels of these same PUFA are comparatively moderate in red meat sourced from pasture grazed ruminants, these having levels similar to many white fish that are low in fat such as snapper, leatherjacket, and flounder.

Certain plant species (Asteraceae, Apiaceae, Rosaceae, and Cyperaceae) have been positively correlated with PUFA concentrations in milk of grazing animals. Botanically diverse pasture systems have been associated with enhanced PUFA concentrations in the meat of lambs. Lourenço et al. (2008) reported increased concentrations of DHA in the intramuscular fat of lambs grazing a biodiverse pasture compared with an intensive ryegrass pasture. For example, red clover (*Trifolium pratense*) and certain other perennial forage species contain enhanced levels of polyphenol oxidase, which can prevent lipolysis and subsequent rumen biohydrogenation of PUFA from plants to result in increased PUFA absorption. The application of grains or some feedlot rations within livestock industries to increase animal growth rates can lower the level of omega-3 PUFA in red meat. In addition, climate change has led to prolonged drought in some parts of the world, which diminishes the availability of feedstuff that is rich in omega-3 PUFA and an increased reliance on concentrate and commercial feeds that are often rich in omega-6 PUFA. Omega-6 PUFA supports in human health, but when consumed at high levels, are potentially

harmful. Research shows that current consumption of omega-6 PUFA by the human population is high due to their meal choices and the supplied food types (Ponnampalam et al., 2021).

Plants containing condensed tannins also protect dietary PUFA from biohydrogenation, and thus enhance PUFA concentration in ruminant food products (e.g., meat and milk). However, the degree of PUFA protection can be influenced by the concentration, chemical structure, and degree of polymerization of the condensed tannins, which can vary both within and between plant species. This may explain why certain tannin containing species appear to affect fatty acid profile to a greater extent than others. Girard et al. (2016) observed that sainfoin (*Onobrychis viciifolia*) raised PUFA concentration in cheese to a greater extent than bird's-foot trefoil (*Lotus corniculatus*), with both providing similar amounts of α-linolenic acid to the animal. Collectively, research show that animal grazing diets high in essential FA and vitamins have better metabolic conditions and oxidative stress status than those consuming diets of low nutritive value, contributing to improved wellness and lower veterinary care.

The *semimembranosus* muscle contains a higher concentration of phospholipids, which have a higher PUFA content. The differences in muscle fatty acid concentrations suggest an increased availability of both C18:2n-6 and α-linolenic acid for tissue incorporation. This may reflect a reduction in rumen biohydrogenation of these dietary fatty acids for the lambs grazing botanically diverse pasture mixes. The lower trans-11 C18:1 concentration further illustrates this point, as this is a key intermediate of the biohydrogenation of both PUFA. There are several possible explanations for this, including (1) inhibition of initial lipolysis of plant lipids prior to rumen biohydrogenation may have contributed to this effect; (2) inhibition of biohydrogenation before the hydrogenation step that synthesizes trans-11 C18:1; and/or (3) increased rate of passage for animals consuming botanically diverse pasture mixes may have resulted in greater amounts of PUFA escaping rumen biohydrogenation.

Animals and humans have a multidynamic defense mechanism regulated by enzymatic and nonenzymatic antioxidant activities in order to protect their tissues, organelles, and individuals from oxidative stress and metabolic disorders. The latter components have the capability to chelate, scavenge, or decompose the damage causing hydroperoxides, transition metals, and other free radical forming substances or prooxidants and therefore protecting the body from adverse changes. Glutathione peroxidase (GPX) and superoxide dismutase (SOD) have been reported as the major enzymatic antioxidants involved in defense mechanism in tissue systems of living organisms. Using feedlot feeding as a control, the GPX1 and SOD2 enzyme activities were higher in alfalfa-fed animals, and these differences in activity were reflected in the mRNA abundance (Ponnampalam et al., 2019). In this same study, alfalfa treatment substantially increased muscle α-linolenic acid, whereas a feedlot diet substantially increased muscle linoleic acid concentrations. It indicates that diet-induced changes in muscle PUFA can alter expression and activity of SOD and (or) GPX antioxidant enzymes in the

muscle tissue system result in lower oxidative stress. Previous studies conducted in mice and rats showed that feeding fish oil rich in EPA and DHA enhanced GPX and SOD enzyme activities in spleen and liver tissues, respectively.

22.6.3.3 Antioxidants

In general, ruminants consume 80%–85% of diet as forage (fibrous materials), such as green pastures, fodders, silage, and other roughage materials. From these diets, they ingest adequate amounts of antioxidants, such as vitamins, minerals, and carotenoids. Monogastric animals grown under intensive systems consume 80%–85% concentrated diets, and they receive carotenoids and antioxidants from the ingredients of cereal grains, pulses, and oilseeds. Carotenoids, such as carotenes and xanthophylls, are pigments present in leaves, seeds, fruits, and animal products, including blood, meat, and milk. Carotenoids have the ability to act as antioxidants as they are quenchers of singlet oxygen and other reactive oxygen species or substances that causes oxidative damage in the body from the cellular to tissue level. The biological roles of carotenoids and polyphenols in the ruminant digestive system and their metabolism are not yet fully understood. It is speculated that increased level of antioxidant potential in the circulatory and tissue systems can protect the oxidation of omega-3 long chain PUFA from the tissues. This improves the health and well-being of individuals. Previous research reports the relationship among diets, antioxidants, essential fatty acids, and lipid oxidation in meat and milk in cattle, sheep, and goats (Ponnampalam et al., 2017a,b; Reynolds et al., 2020).

22.7 Meat processing

There is an expectation to produce high-quality meat using less resources and having fewer negative effects on society and the environment. This has driven innovation toward sustainable meat processing methods.

22.7.1 Transport and lairage management

The movement and holding of animals prior to their slaughter is pivotal to animal welfare and sustainable meat production. The duration, stocking rate, trailer design, and conditions in which animals are transported represents an expense, but when considered in isolation, these factors do not define the effectiveness of transportation. That is because these factors affect the carcass and meat quality of the transported animal. For instance, PSE incidences were observed to increase when pigs were transported short distances (of only 2-h) or under low stocking densities, with PSE incidences observed to increase when there was more than 0.4 m^2/100 kg of pig (Gajana et al., 2013). Mixing groups of animals from different farms or locations immediately prior to or during transportation can cause stress and be detrimental to meat quality. A survey of poultry processors found that heavier birds and feed withdrawal periods of > 6-h prior to

loading were both risk factors for bird mortality during transportation (dead on arrival) (Cockram et al., 2018). María et al. (2003) reported that transportation time affected the compression values and color of beef, although shear force and pH were not affected by these same short (30-min), medium (3-h), and long (6-h) transportation times. Collectively, these examples demonstrate that animal losses, discounted carcass value, and reduced meat quality can result from unsustainable transportation practices.

Lairage time refers to the interim between unloading at the abattoir and slaughter, during which animals are held for a period to recover from transport stressors or to facilitate the preslaughter processes. If well-designed, there are benefits to animal welfare and the quality of meat. Driessen et al. (2020) reported that sufficient lairage time can help to improve the meat quality of pigs, specifically pH, color, and water-holding properties. Biffin et al. (2020) found that drip and purge losses (yield) were higher in the meat from alpacas held in lairage for 7-days than in the meat from alpacas slaughtered direct upon unloading. Teke et al. (2014) compared lairage times for Simmental bulls that had long (30-h) transport times and recommended that 72-h lairage was necessary to mitigate the effect of transport stress on beef quality. From these studies, it is evident that lairage is necessary for sustainable meat production—but its application should be specific to the animal species and transport time. They further demonstrate that effective lairage requires space, resources, and time; inputs that contribute to the cost and disposal of the total waste generated by meat production (e.g., odor, blood, skin, and animal refuse). These outputs detract from sustainable practice. In addition, and unless appropriately managed, any improvements to carcass or meat quality that result from sustainable transportation and lairage may be undone by poor preslaughter handling.

Short-term stressors that affect meat quality can result from poor human-animal relationships and practices of rough handling in the lead up to slaughter. Examples of rough handling include the use of sticks and electric prods; twisting tails, hitting and pushing; using dogs to move animals and; noises and other unnecessary interference with an animal. Not only does rough handling compromises the efficiencies of meat processing, but it also undermines animal welfare, the social license to produce meat, and a consumer's willingness to purchase. The perceived and actual handling of animals prior to slaughter is therefore of consequence to the sustainability of meat production. Animal handlers should be trained in low-stress methods and animal behavior, as this knowledge will support animal welfare and movement through the preslaughter processes. Mechanical installations to foster animal movement offer a secondary tool to maintain animal calmness. These can include the shielding of animals from abattoir workers; lighting to eliminate dark areas or reflections that can distress animals; conveyor-belts to facilitate passive animal movement; and false floors to avoid animals becoming startled by "visual cliff effect" (Grandin, 2020). It is important to supervise the implementation of these low-stress handling methods to ensure adoption and sustainable practice.

22.7.2 Slaughter

The slaughter of animals is unavoidable in the production of meat. There are ethical, legislative, and religious factors that will contribute to the sustainability of animal stunning and slaughter. These can impact on the social license to operate, market access, a consumer's willingness to pay, technical efficiencies, and more. Rather, this section will describe the effects of stunning, eviscerating, and carcass inspection on the quality and sustainability of the meat produced for consumption.

The restraint, act of slaughter (severing of the jugular), and the experience of extinguish are causes of distress to the animal. It is common, therefore, for animals to be stunned prior to slaughter so as to desensitize them to these processes. Electric, gas, and captive bolt stunning are effective in this function, but their use can affect meat quality and other processing efficiencies. Captive bolt stunning employs a physical trauma to achieve desensitization, a method that is potentially hazardous to the operator directly and indirectly—for example, the distribution of cerebral material as a route of exposure to bovine spongiform encephalopathy. Electrical stunning causes heart fibrillation to achieve desensitization and has been associated with ecchymoses (blood splash and spots) in ruminant and poultry meats. The management of electrical frequencies (voltage) may reduce the incidence of blood splash—although if over applied there is a potential to impact on carcass pH decline and associated meat quality properties. Gas stunning utilizes hypoxia to desensitize animals by exposing them to specific concentrations of gas (CO_2, N_2, O_2, etc.). The gases used will affect rate of gasping and the time before unconsciousness. This method is often used for the slaughter of pigs, as it can help to reduce animal stress and therefore the incidences of PSE meat. It is noted that, in small groups (~5 animals), the order in which pigs and sheep are stunned is reported to have no effect on their stress levels (Schaeperkoetter et al., 2021). Other research has, however, observed that in larger groups, stunning order can affect the rate of postmortem glycolysis in lamb carcasses (Stewart et al., 2018) (see Chapter 5).

The evisceration of a carcass will dictate the yield and efficiency by which an animal is converted into meat. The amount of trimming (yield loss) will depend on the requirements for safe meat production—being the removal of tissues or primal cuts contaminated (e.g., gut content, microbiota) or damage (e.g., due to bruising). Workplace hygiene and carcass sanitization can help to reduce the proliferation of spoilage microbiota, but in some situations, additional trimming or condemnation of the whole carcass is the better solution. For example, a recent survey found bacterial arthritis in 0.7% of Australian lamb carcasses (total surveyed: 63,287) and that for every carcass condemned, another 140 were subjected to additional trimming at an average loss of 0.7 kg to carcass weight (Lloyd et al., 2019). The amount of trimming will also depend on the composition of a carcass—with excessively fatty carcasses often requiring additional trimming that represents a cost to the

processor, lower yields of salable meat products, and poor animal production efficiency. Differential pricing and the inspection (grading) of carcass quality may help to incentives the production of more sustainable carcasses, without superfluous fat coverage and that match market specifications. There are different carcass grading schemes around the world, but as a general observation they each include the inspection of a carcass for markers of quality and safety. The time of inspection and parameters assessed can impact on its validity, such as for the identification of DFD beef carcasses. Nonetheless, grading and inspection data can be used to inform the distribution of carcasses to "best-fit" market, reducing the potential for waste and contributing to a more sustainable partitioning of meat resources.

22.7.3 Carcass processing

Here, processing is defined by the conversion of a raw product (carcass, input) into a secondary product (primal or cut of meat, output) that can either be retailed, stored, or value-added with additional tertiary processing.

22.7.3.1 Hot boning

Hot boning refers to the fabrication of meat portions or cuts from a warm carcass before *rigor mortis*. This is practiced by subsistence farmers as well as among small to medium farming communities where there is lack of resources available for commercial slaughter and preservation. In these cases, animals are slaughtered at a domestic dwelling (backyard) or a common retail place to be immediately sold. Commercial scale processors also utilize hot boning as this type of fabrication results in higher yields, lower energy, and labor costs, and an increased rate at which a carcass can be processed and on-sold. These are some advantages to hot boning, but there are also disadvantages. There is the potential for muscle slippage within a primal cut, which is of lower visual appeal to consumers. The proliferation of microbiota has been reported to be higher for meat from hot boned carcasses. There is also an increased susceptibility to cold shortening in cuts prepared from hot boned carcasses. Ithurralde et al. (2020) demonstrate this outcome, finding that hot boned lamb carcasses produced tougher meat (higher shear force, tenderness scores, and shorter sarcomere lengths) to the meat from cold boned carcasses. On this point, Crownover et al. (2017) found that the negative effects of hot boning on quality were muscle specific, with the *longissimus thoracis* and *psoas major* muscles from hot boned beef carcasses reported to have a reduced tenderness and overall liking by consumers, whereas there was a positive or neutral effect of hot boning on the palatability of other beef muscles. This latter finding could recommend a hybrid approach to carcass processing. Hot boning could initially be used to remove specific cuts from a carcass, which is then chilled and cold boned. The benefits to sustainable meat processing of a hybrid approach must be confirmed.

22.7.3.2 Cold boning

Cold boning refers to the fabrication of meat portions or cuts from a chilled carcass, after *rigor mortis*. This point of fabrication is often used as it permits a carcass to be graded within current systems. and it avoids the risk of cold shortening, to the detriment of meat quality. Cold boned primal cuts may be less susceptible to microbial and "blown pack" spoilage than hot boned primal cuts. Anecdotally, it has been said that cold boning permits abattoir staff to more easily grip and handle carcasses when fabricating cuts, an outcome from which is a lesser chance of injury. In these respects, there are advantages to cold boning when considering the sustainability of meat processing. This has resulted in its being the primary means for carcass processing when cuts are to be preserved and distributed to different end-users within or between countries. Nonetheless, the expense and space required to chill carcasses prior to fabrication does represent a challenge to the sustainability of cold boning (Fig. 22.9)—specifically because of the associated energy requirements and contribution to greenhouse gas emission. This should be the focus of future innovation as regulatory groups are introducing carbon neutral policies to the agricultural sector and its supply chain.

FIG. 22.9 Examples of sheep (A and B), Achilles hung beef (C), pig (D), and tender-stretched beef (E) carcasses held chilled in Australian abattoirs prior to fabrication via cold boning. *(Images courtesy of Agriculture Victoria, Department of Jobs, Precincts and Regions, Victoria, Australia and NSW Department of Primary Industries, Australia.)*

22.7.4 Automated and on-site meat processing

The sustainability of conventional, on-site meat processing is constrained by the availability of labor, the risk of injury to employees, and increasingly stringent food hygiene standards. Recent innovation in the automation and robotization of meat processing could be used to address these concerns.

The use of automated carcass sorting, within abattoir chillers, has been used to eliminate labor inputs, the risk of injury, as well as the potential for between carcass contamination from the "carcass-pushers." The robotization of stunning, evisceration, giblet harvesting, and carcass cutting has increased the rate at which broilers can be processed—with estimates suggesting a threefold increase in the number of birds able to be processed per hour currently, when compared to rate at which birds were processed in the 1970s (Barbut, 2014). A result of any increase to the rate of processing is that comparable levels of productivity can be achieved within a shorter timeframe, therefore fewer resources are required and sustainably is improved. Automated cutting technology has been installed into beef, lamb, and pork abattoirs to improve the precision and reproducibility of carcass fabrication. Real-time X-ray imaging and laser scanning technologies are used to define the cutting lines and support the use of these technologies—recent examples include the Meat Factory Cell, SCOTT Automated Boning Room, SRCViand, GRIBBOT, and Frontamatec AiRA Robots (de Medeiros Esper et al., 2021). Sustainable practice is improved by these forms of automation because of increases to dressing percentages and reductions to processing wastage. The elimination or hybridization of human involvement in high-risk jobs is another positive for industrial sustainability.

Automation of meat processing is not limited to the replacement of physical tasks. The assessment of carcasses for quality and welfare indicators has been automated to reliability partition premium products to premium markets. This point has been explored with the use of a camera-based system to automatically assess pig carcasses for ear and tail lesions (Blömke et al., 2020). This system would replace the need for a human observer and identify individual carcasses at risk of associated quality and hygiene defects. Technologies, such as spectroscopic devices, have been applied as tools to confirm the production system in which beef cattle were reared (Logan et al., 2021) as well as the sensorial properties of the meat from lamb carcasses (Fowler et al., 2018). These devices offer a means to replace farm audits and protect the monetized elements of sustainable animal production for meat (e.g., origin, residue concentrations, and growth hormone status). Yet, at present, these devices still require an operator, validation and are therefore a somewhat hybrid robotization of on-site meat processing.

Abattoirs are often wet, humid, and skilled so that mechanical failure is inevitable. The resultant mechanical downtime, due to breakdown or repair, can interrupt entire meat processing (e.g. boning or packaging sytems) and the supply of meat. This is true if the automated task is critical to the processing and fabrication of a carcass. There may be other (unforeseen) hazards that arise

with the introduction of automated technologies, as these may emit radiation or use lasers in their operation. The scalability of automation is important, as the initial cost for these technologies would prohibit their use by small meat processing plants. The potential cost savings from the use of automation should be calculated with reference to the costs of additional training and service the technology, liberation of greenhouse gases, as well as the social costs from fewer employees and community involvement. Further, policies must be in place that define the end of service use or disposal of any technology (e-waste). These are some of the factors that define the effect of automation on the sustainability of meat processing in long-term application.

22.7.5 Waste management

The production of waste is unavoidable in the production of meat. That said, there are strategies to minimize or repurpose this waste so as to achieve greater sustainability. Waste from meat production includes unutilized nonedible and low-value components of a carcass. The extensive use of water in meat processing results in wastewater that contains animal residues (from lairage), by-products of slaughter and processing, and chemical contaminants from cleaning, and other operational procedures. Loss of this waste represents an environmental and economic failure. Particularly as, if captured and appropriately treated, waste can be converted into high-quality bionutrients for use as crop fertilizers, aquaculture feed, pet foods, and as niche consumer products. Technology has been applied to improve the efficiency of waste capture, although it should be noted that there are cultural considerations and legislative restrictions to the use of meat processing waste. Alternatively, bio-digestors have been widely investigated to convert meat processing waste into methane and in doing so, provide another source of energy that can be traded or used in-house. Irrespective, it is essential that any waste from meat processing is correctly captured and treated prior to its disposal. To do otherwise is to risk the environmental, societal, and economic sustainability of the meat industry.

22.7.6 The preservation of processed meat

Meat deterioration in the interim between slaughter and consumption represents a significant loss that destabilizes a sustainable meat industry. Indeed, it has been reported that ~20% of meat is wasted during animal production, slaughter, processing, distribution, and consumption (FAO, 2011). The sustainability of different preservation methods for meat is explored below.

22.7.6.1 Immediate consumption or retail display

The preparation of meat for immediate consumption or retail is limited to skilled individuals and small or specialized retail outlets (butchers). Frequently, these groups will purchase an entire carcass and undertake the labor-intensive task of fabrication themselves. The outcome from this is the minimal wastage

of edible carcass tissue, although there is limited value adding to the secondary, nonedible bone, blood and trim. The balance of these products will dictate the sustainability of these enterprises. The slower processing speeds may reduce the chance of worker injury; however, this would depend on the skill, training, and equipment of the individual. Likewise, individual packaging and cold storage capacities will dictate the preservation of butchered meat. It is noted that sustainable advantages from economies of scale do not apply in these practices.

22.7.6.2 Wet aging to preserve meat

The tenderness of meat is improved with wet aging, a process wherein packaged meat is refrigerated for a period that allows for proteolytic, lysosomal, and other "tenderizing" reactions to ensue (see Chapter 9). The temperature and duration of wet aging will affect the extent of protein degradation, denaturation, and tenderization. These and the initial microbiota populations will determine the freshness or spoilage of a meat product. The selection of packaging material can help to extend the shelf-life of wet aged meat, with various types of anaerobic and active packaging designed for this purpose. There are yield losses associated with long-term wet aging of meat. Reductions to the amount of salable product does diminish the sustainability of wet aging. Yet, wet aging has proven effective with research having demonstrated that vacuum packaged beef held at −1°C (superchilled) remained of acceptable freshness for up to 140-days, in terms of microbiota and total volatile basic nitrogen concentrations (Frank et al., 2020). The concentration of health claimable fatty acids in beef held at 1.5°C remained preserved across wet aging periods of up to 12-weeks (Holman et al., 2019). Vacuum packaged pork, wet aged for 43-days at −1.7°C was reported to have comparable sensory and physicochemical quality to pork that was wet aged for 5-days at 3.1°C (Ngapo et al., 2012). Moreover, Bellés et al. (2017) demonstrated that vacuum skin packaging prevented lamb from oxidizing, when it was wet aged at 4°C or −1°C (superchilled), the latter found to preserve the microbiota and shelf-life of lamb for 28-days, postslaughter. While these extended periods of preservation are beneficial to a sustainable industry, in terms of market access and the reduction of waste, there are disadvantages that should be considered (Table 22.4).

Meat packaging has become increasingly complex (see Chapter 10), with films now designed to infer antioxidant and antimicrobial properties, be robust, and have retail-appeal to the purchasers. The paradox to these material advantages is their restricted capacity to be recycled and the resources necessary for their manufacture. An option with obvious advantages in sustainability is the use of biodegradable or edible types of packaging. To be practical, however, these types of packaging must provide a cost-effective barrier against hazardous contaminants that simultaneously contributes to the longevity of product quality and enhancement of consumer appeal. The potential to preserve wet-aged meat for long durations may be compromised with these alternative packaging types, unless tested. The wet aging of meat within retail-ready packaging would avoid

TABLE 22.4 The advantages and disadvantages to different aging methods and frozen storage within the context of sustainable meat preservation.

	Dry aging	Wet aging	Frozen storage
Description	Unpackaged meat is held under controlled humidity, air flow and temperature within specialized chambers	Vacuum packaged meat is held refrigerated (−0.5°C to 4°C)	Packaged or unpackaged meat is held frozen at subzero temperatures
Advantages	• Improved meat quality • Premium value	• Low trim loss and shrinkage • Improved meat quality • High value	• Long-term preservation • Easy to manage
Disadvantages	• High trim loss and shrinkage • High cost to maintain climate controlled chambered	• Purge and water losses • Packaging integrity necessary • Microbiota proliferation will continue	• Static meat quality (no enhancement) • Freezing and thawing methods impact on quality • Low value

the unsustainable need for repackaging. To support this, it would be valuable to adopt a form of "in-pack" tracking to assure the quality and integrity of the packaged meat. These could include nondestructive spectroscopic scanning or intelligent packaging devices.

There is an expense associated with holding meat for prolonged periods under refrigeration, with warmer temperatures more cost effective as well as inferring a faster rate of tenderization. Sustainable wet aging would therefore benefit from knowledge of the total storage duration, beforehand, as this could inform the selection of holding temperature and packaging requirements. There is some flexibility in this regard, with research showing aging prior to freezing (or vice versa) can help to preserve the color and quality of beef and lamb meat, to differing degrees (Coombs et al., 2017). Irrespective of the means to improve the sustainability of wet aging, legislative guidelines and standards for maximum allowable aging durations, temperatures, and packaging materials must be consulted. This is of particular importance as these policies are often market and meat product specific.

22.7.6.3 Dry aging to preserve meat

Dry aging is the process by which unpackaged meat is preserved using airflow, humidity and temperature controls (see Chapter 9). This process promotes the

tenderization of meat as well as the intensification of its organoleptic properties. The act of dry aging will result in some shrinkage and the requirement for a meat product to be trimmed, whereby the discolored and crusty meat is removed. These contribute to a lower yield of salable product and the requirement for additional labor inputs, when compared to wet aging. Nonetheless, the high demand and value of dry aged meat offers some compensation to these factors and their effect on sustainable practice. Witte et al. (2020) reported that the trimmings from dry aged beef could be salvaged, processed and incorporated into fermented sausages. This is an example that could reduce wastage and enhance the sustainability of dry aging. That said, there is variation in the effectiveness of dry aging that results from the "ripening chamber" settings and the selection of an appropriate cut of meat to preserve.

Sustainable dry aging refers to the effective preservation of meat quality until the point of its consumption. Within this context, the duration of dry aging is a function of the temperature, humidity and airflow in which the meat is held. The specific settings used will therefore dictate the duration for which dry aging is effective. Smaldone et al. (2019) reported that dry aging (at 0°C and 70% humidity) preserved the shelf-life of beef for up to 290-days but this was at the expense of salable yield. Terjung et al. (2021) concluded that a dry aging time of 40-days (at 2°C, <85% humidity, and 0.5 m/s airflow) is best practice for beef. And, Kim et al. (2016) advised that beef should be dry aged for 21 -days (at 3°C, 49% humidity and 0.2 m/s airflow). This latter study also reported there to be no differences in the drip or cook losses of wet and dry aged beef, both achieving comparable juiciness scores when tasted by a sensory panel. It has been reported that meat with higher marbling and intramuscular fat concentrations are more responsive to dry aging. This is likely due to the VFA generated from lipid peroxidation that infer the unique flavor profile to dry aged meat. Further, there could be potential to utilize packaging when dry aging meat. It was found that beef which is dry aged in a bag (permeable packaging) is scored by consumers to be of equal or greater acceptability to nonbagged product (Terjung et al., 2021). The avoidance of packaging may be considered an advantage, however its reliance on strict climate control in the "ripening chambers" requires sophisticated and intensive inputs (Table 22.4). Another alternative involves the stepwise (combination) aging of meat, wherein dry aged meat is packaged and subsequently wet aged. This has been reported as effective at reducing yield and trim losses and delivering beef that is of equal or better quality to dry aging and wet aging, only (Kim et al., 2018). Still, it is evident that knowledge of the total duration and meat product to be preserved is important for the sustainable use of dry aging.

22.7.6.4 Frozen storage to preserve meat

Frozen storage is used to preserve meat across extended periods (see Chapter 7). This is because frozen storage temperatures will inhibit the activities of endogenous enzymes and the microbiota that are associated with the spoilage of meat.

The required temperature for frozen storage is often defined by legislation or market specifications, although it is noted that at −33°C there is still an unfrozen, liquid water fraction within meat that can facilitate spoilage (Rahelić et al., 1985). In terms of energy usage, it would be more sustainable to hold meat at "warmer" frozen storage temperatures. Research has shown that −12°C offers comparable preservation of red meat quality across a 12-month holding period to −18°C (Holman et al., 2017). Indeed, the benefits from "warmer" frozen storage are compounded as the period of frozen storage increases. Viable long-term frozen storage can support consistent meat supply, buffering against production gluts and shortages. It offers flexibility when operating within a changing market. Further, long-term frozen storage allows for meat to "slow-shipped," a mode of transportation that requires less fuel, has a lower environmental impact, and provides economical delivery of meat. The adoption of "slow-shipping" is advisable for meat because many meat exporting nations are geographically isolated from the majority of consumers, and frozen storage has proven effective at maintaining acceptable lamb meat quality for up to 21-months (Muela et al., 2015). That said, the quality of meat will deteriorate as a result of its freezing and thawing.

There is a risk that frozen meat will be undesirable to consumers, attract a lower market value, and its disposal will contribute to food waste (Table 22.4). Technology has been applied to limit the physical disruptions caused with ice-crystal formation within frozen meat and to help preserve its sensory properties. Examples include the methods for high pressure, electro-magnetic, ultrasound-assisted, and cryo-freezing. While these will help sustainable frozen storage, it should be remembered that prolonged or unnecessary frozen storage does not reflect sustainable practice. Indefinite frozen storage requires substantial inputs. Furthermore, frozen storage can contribute to losses of essential nutrients and water (thaw loss) from meat to the detriment of its quality and yield. To avoid these outcomes, it is important to understand the purpose, end-use, destination, and timeline for meat that is held frozen.

22.8 Benefits of sustainable animal production and meat processing

The cost and benefit of animal production and meat processing can be interpreted in many ways. This is because livestock producers and meat processors seek profitability, government authorities consider productivity, as well as social and environmental consequences, and the wider population consider animal welfare and the safety of meat for consumption. Animal production in the modern era should consider approaches that deliver economic benefits to primary producers, social benefits to the public through improved health and well-being, and environmental benefits with minimum mitigation and efficient use of natural resources. Ideally, animal production systems should address all three of these elements. While economic sustainability is essential, consumers are demanding

that meat is sourced from animal friendly systems that are "clean and green" and offer improved human health benefits. If precaution is not taken to use our natural resources now, it may be difficult and costly to remedy the opportunity costs in the future. There are many reasons for maintaining sustainable animal production and meat processing. These encompass many stakeholders at multifaceted levels along the supply chain from the production point (on-farm) through meat processing sectors (off-farm) to the point of consumption (dining).

22.8.1 Economic benefits

Farmers, livestock producers, and meat processors often focus their efforts on the achievement of economic profits, their maximization, and in turn may sometimes overlook the credence values desired by the general public and government authorities. With an increase in population growth and income generation, the demand for cereal grains and pulses for human consumption is expected to increase. Further, to satisfy the growing demand for animal-based foods (meat) as a source of dietary protein, it is expected that poultry and pig production will intensify to result in parallel increases in their competition with humans for cereal grains and legume grains. Moreover, the demand for brewed beverages and liquors is projected to increase into the future, and distillers and brewers use large quantities of grains, yams, and fruits. This competition will again diminish the availability of these feedstuffs for human and animal consumption—driving increases to their relative costs. To remain cost effective, animal producers must explore alternative feedstuffs that provide sufficient dietary protein and energy (fat) to maintain and accelerate productivity and profits. In this case, oilseed meals, by-products of brewery, novel forages (alfalfa, moringa, gliricidia, leucaena), or conserved pastures (silage) may be explored as future alternatives to cereal and legume grains.

22.8.2 Social benefits

The trend for food choice among general populations is for meat from high welfare systems that are safe to eat, of increased nutrient value (e.g., rich in antioxidants, minerals, and essential fatty acids), and free of hormones, additives, and artificial preservatives. "Good health provides good life" and thus it is one of the major reasons for consumers choosing safe, clean and environmentally sustainable foods. Consequently, "fast" food outlets such as Hungry Jacks and food retailers such as Coles Ltd. in Australia and A&W, Loblaws and Sobeys in Canada are moving toward offering meat and meat products with no added hormones and artificial preservatives. In recent years, there has also been increased preference for meat products from animals grown extensively or in free-range systems because consumers are now well informed on food security, food quality, animal welfare, and environmental sustainability. The general belief is that meat from extensively grown animals is better than that of intensively grown animals.

The antibiotic and pesticide usage in agriculture is reducing globally. This is due to government regulations that limit both artificial antibiotic use and chemical treatments for parasite control implemented for growth promotion. There is also increased desire for meat supplemented with natural antioxidants and bioactive compounds as opposed to synthetic antioxidants and synthetic additives. It is expected that the application of synthetic additives and hormones in meat production will be minimized or ceased due to continued high demand for meat products that are perceived as "natural." Formulation or production of animal diets with optimal nutrients, natural antioxidant potential, and natural control for parasites and disease will be influenced by the demands of people of high and middle incomes, a demographic that is expected to grow significantly.

Another aspect to replacing grain with sustainably derived feedstuff, such as oilseed meals or aquatic algae (seaweed), which act as the source of protein and energy for farm animals, may be the opportunity to improve public health and decrease environmental impact (e.g. methane mitigation). These feeds are rich in lipids containing omega-3 fatty acids, which can offer value-added benefits for animal health as well as to the nutritive value of meat produced (Ponnampalam et al., 2021). It is well known that humans and animals cannot synthesize essential parent (precursor) fatty acids of LA and ALA de novo and therefore these fatty acids must come from the diet. These parent fatty acids are the source for the formation of their products of long-chain omega-3 fatty acids of EPA, DPA, and DHA as well as omega-6 arachidonic acid (C20:4), through desaturation and elongation processes. These PUFA offer many health benefits and are important for the growth and development of individuals. Previous studies showed that adding flaxseed meal at 10% or algae at 2% DM to the diets of sheep significantly increased long-chain omega-3 fatty acids in meat without impacting the feed intake or carcass weight (Hopkins et al., 2014; Ponnampalam et al., 2016). Application of cereal grains to ruminants is costly and may cause some inefficiencies in the production systems. For example, hulls and husks are high in lignin and cellulose that may cause low digestibility of nutrients. On the other hand, rapidly degradable grains can cause acidity leading to subclinical disease to animals. In addition, application of grain in ruminant diets can elevate omega-6 PUFA in meat, which may not be beneficial for the health of animals as well as those who consume animal products, when consumed at higher concentrations, as these omega-6 PUFA are proinflammatory.

22.8.3 Environmental benefits

A significant challenge to animal production for meat is the need to reduce its impact on the environment. Promoting sustainable agricultural production systems that consider a holistic (ecosystem) approach to farming that including the adoption of integrated pest and weed management as well as minimize artificial fertilizer application to pasture and crop cultivation.

For example, part of Australia's current response to climate change is the aim to facilitate "carbon farming" to sequester more carbon and reduce greenhouse gas emissions. Agriculture is a significant emitter of greenhouse gases and also has a significant opportunity to reduce emissions and sequester carbon. In Australia, direct livestock emissions account for about 70% of greenhouse gas emissions by the agricultural sector and 11% of total national greenhouse gas emissions. This makes Australia's livestock the third largest source of greenhouse gas emissions after the energy and transport sectors. Livestock are the dominant source of methane and nitrous oxide, accounting for 56% and 73%, respectively, of Australia's total emissions.

22.8.4 Welfare benefits

Animals reared under extensive systems often face extreme weather conditions such as heat waves or severe drought. Such conditions can impact on animal performance and the quality of meat and milk produced as a result of metabolic disorders associated with reduced feed intake, physiological changes, immune suppression, and weight loss. Rearing livestock with feeds of low nutritive value (e.g., high fibrous grass hay or cereal straw) will result in their malnutrition—which is a cruelty, unproductive, and also can contribute to higher methane emission. Animal health and welfare issues associated with prolong period of high temperatures and low precipitation leading to long-term drought are significant concerns in some parts of developed countries, where farmers can lose their entire flock due to low availability of forage feeds or another unavoidable impetus to slaughter them for meat. For example, a prolonged heat wave in the mid-central United States of America during the summer months of 1995 severely impacted livestock. Likewise, in the central and southern regions of Australia, during late 2017 to early 2019, prolong drought led to culling or euthanasian of thousands of beef cattle, sheep, and dairy cattle (see Fig. 22.7). To avoid these and other similar circumstances, farmers and livestock producers must be proactive in allocating adequate funds and feeds.

Minimizing or avoiding disease outbreaks in livestock as a result of the contamination from wild birds and animals is also an important animal welfare issue. There are many examples to quote, including the mad cow disease outbreak in United Kingdom and Europe in the 1980s and the recent outbreak on avian influenza that spread from wild ducks to chicken, turkey, and emu in Australia caused massive issues to animal ethics, animal liberation, and animal welfare groups as well as loss to many poultry producers. Unless appropriate measures and cautions are taken, these events can contribute to animal cruelty and penalties, including litigation and the offending livestock producers losing their license for production. It is important to introduce on farm measures or techniques that can maintain an animal disease surveillance system to reduce or prevent such outbreaks, which is animal welfare friendly.

22.9 Future opportunities and perspectives

Global meat production is projected to expand by nearly 40 Mt by 2029, reaching 366 Mt. This will be achieved with the intensification and growth in beef, sheep meat, poultry, and pork production, across all systems and regions (see chapter 2). Overall, the bulk of meat production growth is attributed to developing regions, which will account for 80% of the additional output (Fig. 22.10).

In the short term, the supply response of the various meat types remains influenced by African Swine Fever outbreaks in Asia, as well as reductions of beef cattle herds and sheep flock numbers in Australia and other countries due to climate variability and drought. Inherent differences in the production system imply that favorable meat-to-feed ratios are more beneficial to poultry and pig meat production, whereas beef producers have more flexibility in the intensity of feed use. Sheep meat production is mostly pasture-based, and producers benefit less from lower meat-to-feed price ratios. Due to these reasons, beef and sheep meat prices have increased over recent years (Fig. 22.11). Not only that, sheep meat and beef are rich in iron, essential fatty acids, and B vitamins when compared with chicken and pork, and in the future, it is expected to grow more demand with the boost in economic status of middle-income consumers in developing countries, who can afford to buy high quality red meat.

The FAO (2013) estimated that, by 2050, the world will require twice as much grain as was produced in 2012, but half of that would be utilized in the livestock sector and the environmental footprint of livestock will grow further.

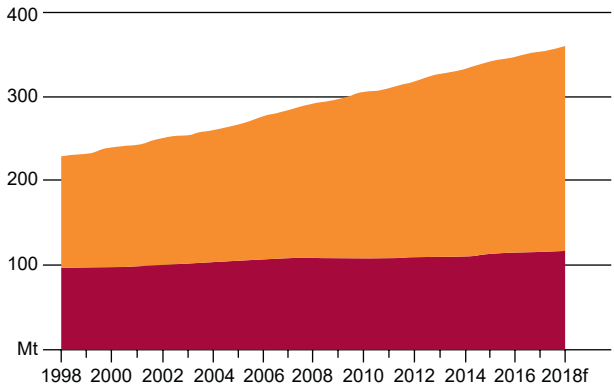

FIG. 22.10 Global trends in meat consumption grouped by consumers of developed *(red)* and developing *(orange)* nations (**f**—OECD forecast). *(From Whitnall, T., Pitts, N., 2019. ABARES, Agricultural Commodities: March quarter 2019, Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra, December. CC BY 4.0. doi:10.25814/5c635953223b1.)*

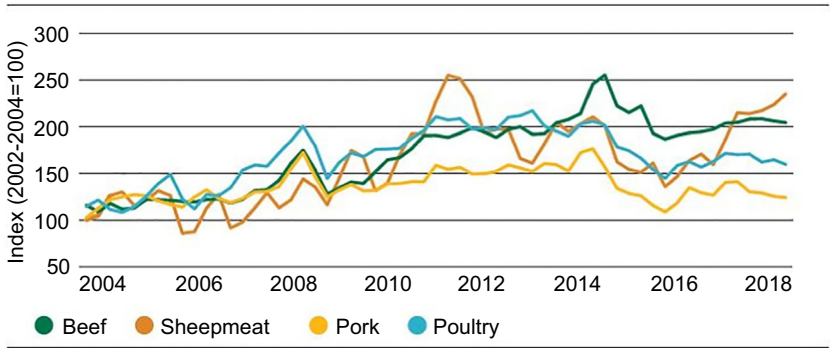

FIG. 22.11 The trends in world meat price indices from 2004 until 2018. *(Courtesy of Meat & Livestock Australia Limited—www.mla.com.au. From MLA, 2020. Global Snapshot, Sheep Meat, January 2020 (1).)*

It was also proposed that most of the grain yield will be used in developing countries for livestock production, mainly due to the population growth and improved middle income socioeconomic status. It can already be observed that the world is demanding high-quality animal protein, and there is competition for grains for human consumption as well as for monogastric animal feed industry (pigs and poultry), biofuel industry, and brewery (beverages) industry. Can we sustain the animal production around the world with minimum use or without the use of cereals grains since there are many limitations and issues?

It would be argued that government incentives should be offered to farmers and livestock producers of all countries to increase pasture and fodder preservation and efficient utilization for livestock production. They must be provided with training and technology to assess the stage of maturity and nutritive characteristics of all types of forages. This will give some directions to allow animals to graze at the right time or harvesting the surplus herbage for silage or haylage production. For example, using drawn facilities for spatial evaluation and harvest of feed material for better forage utilization. Most importantly, harvesting the surplus feed materials at the right time and preserving them for out of season usage may increase the production yield in the future (Fig. 22.12). This would decrease reliance on purchasing feed ingredients, mainly grains from other regions or even importing feeds from other countries, where resources are limited. The cost per unit of protein and energy supply from fodders or forages is often lower than from concentrates. Consequently, it might be time to re-evaluate the use of cereal grains in livestock production systems and the sustainability since there are other by-products of biofuels and distiller's as well as protein meals from oilseed industry available in the markets due to introduction and expansion of other industries in recent years.

FIG. 22.12 Conservation of grass pasture as bales during spring, in a region of temperate climate, when production is beyond animal consumption (grazing) requirements, due to favorable seasonal conditions for pasture growth and establishment.

22.9.1 Potential of legumes as ruminant feeds

Legumes that can be used in mixed feeds for ruminants and partially or fully replace pasture grasses or grain supplements. Alfalfa is one potential legume cultivated in Australia and some European countries as it is best suited to temperate and Mediterranean climates. France, Canada, Spain, and United States are the leading producers of alfalfa hay. Alfalfa, also called lucerne, is an adaptive perennial flowering plant of the legume family which is native to warmer temperate climates and has productive stand life of about 5–7 years. It is a valuable feed for sheep, beef cattle, dairy cattle, horse, and goats. Alfalfa can be also used as an animal feed in dry regions and during drought as hay, silage, chaff for sheep, beef cattle, and dairy mixing up with grass hay, cereal hay, and by-products (Ponnampalam et al., 2020a). Alfalfa provides high-quality supplements as compared to other pastures used as a fodder. Alfalfa pasture or hay provides vitamin A and E as well as magnesium and calcium, which are important for the antioxidant potential in the body and preservative aspects of meat (and milk) at retail display (Ponnampalam et al., 2017b). Alfalfa leaves contribute to a high-protein diet, therefore grazing it as a monoculture may be inefficient as excess protein will be excreted as urea or ammonia.

Leucaena, gliricidia, and moringa leaf are protein sources often used in the rations of animals reared in the tropical regions of Asia and South America. Supplementation of chaff or chopped cereal straw with small amounts ~300–400 g DM/day of these legume leaves (or 20%–50% daily DM intake) offer

an excellent improvement on intake, nutrient digestibility, and nitrogen retention in sheep and goats, to result in increased liveweight gain and meat yield. Leucaena can be useful under Mediterranean climate (Europe and Australia) for beef cattle production and, in this context, 8- to 10-fold feed consumption on DM basis is ideal for improved growth and milk or meat productivity. Vetch is another legume that can be grown as a forage crop, fodder crop, cover crop, and green manure. Supplementation with forage legumes to livestock can enhance the utilization of roughages of low-nutritive value in smallholder mixed farming systems for better animal growth and carcass yield. Legumes can contribute to better utilization of tropical grasses due to their enrichment in proteins and minerals. However, the amount of forage legumes needed to provide effective supplementation could vary with the quality of the basal diet, the quality of the supplement, metabolic status of animal, and the level of animal production expected.

22.9.2 Potential of lipids as ruminant feed supplement

Supplementation of lipids (oils and fats) in ruminant diets have many advantages. They may be used to increase the nutrient availability of diets when the nutrient composition of basal pasture or forage diet is below the requirement for optimum productivity. Ruminants are accustomed to harvest forages and roughage of 1%–2% lipids on DM basis. However, when oilseed, oilseed meals, or oils are supplemented with a basal roughage diet, the lipid concentration can rise up to 4%. Dietary levels >6% lipids on DM basis can negatively interfere with feed intake and digestion, which can affect carcass weight and meat fatty acid composition. Lipids in the diets can be effective at lower concentration because they contribute approximately 2.5 times more energy than carbohydrates (fiber) and produce less heat than carbohydrates and proteins during digestion and absorption. This is due to the fact that fat has a much lower heat increment in the rumen when compared with starch and fiber; and it is associated with reduced metabolic heat production per unit of energy consumed from diet.

Supplementation of oilseeds and meals from sunflower, safflower, corn, and cottonseed to ruminant diets could elevate omega-6 PUFA, while flax, canola, chia, and camelina can increase the concentration of omega-3 PUFA within meat and milk. Lipids rich in omega-3 PUFA can also cause a reduction in methane production accompanied by decreased acetate and increased propionate in the rumen, which can improve the efficiency of growth and muscle (or milk) productivity. Producing foods that are environmentally sustainable and have healthy nutritive attributes, for the consumer, is essential to maintaining market share. If the consumption of omega-3 PUFA from land- and marine-based foods improves human health, it is likely that these same food types can improve the health and wellbeing of livestock (farm animals) by likewise enhancing the levels of the omega-3 PUFA in their blood and tissue systems.

22.9.3 Potential of antioxidants in livestock feeds

In vivo studies revealed the efficacy of dietary antioxidants for combating oxidative stress in livestock and other animals reared for meat production. The effect of antioxidants is vital to enhance animal health, well-being, and meat or milk productivity. Similarly, research has found that dietary antioxidants can improve the nutritional, organoleptic, and shelf-life properties of animal products. For these reasons, dietary antioxidants have traditionally been used by feed manufacturers and livestock producers. However, when supplemented in excess some antioxidants could act as prooxidants and exert detrimental effects on animal well-being and product quality. Antioxidants present in the diet can influence the metabolic activities of an animal's body (e.g., total antioxidant potential or oxidative stress) that in turn influences the energy and protein utilization by the tissues for the production of meat and milk.

Ruminants evolved to consume a major proportion of their diet as forage (fibrous materials) such as green pastures, fodders, silage, and other roughage materials. Animals ingest adequate amounts of antioxidants, such as vitamins, minerals, and carotenoids from these diets. Monogastric animals grown under intensive systems consume substantial amounts of concentrated (grain) diets, and they receive carotenoids and antioxidants from the ingredients of cereal grains, protein meal, and oilseeds. Carotenoids, such as carotenes and xanthophylls, are pigments present in leaves, seeds, fruits, and animal products of blood, meat, and milk. Carotenoids have the ability to act as antioxidants, as they are quenchers of singlet oxygen and other reactive oxygen species (ROS) that causes oxidative damage in the body from cell to tissue level. The biological roles of carotenoids and polyphenols in the ruminant digestive system and their metabolic actions are not yet fully understood. It is speculated that an increased level of antioxidant potential in the circulatory and tissue systems can protect against the oxidation of omega-3 PUFA from milk and meat from ruminants. This improves the health and well-being of individuals who consume these products.

Synthetic antioxidants used in the meat industry to preserve the product quality at retail display or postmortem storage have fallen under scrutiny due to potential toxicological effects in recent years. In the meantime, lipid oxidation in meat and milk can cause quality deterioration because it can negatively affect sensory attributes such as color, texture, odor, and flavor as well as the nutritional value of the product. These issues urge the producers and meat industry look for effective natural antioxidants that can replace synthetic antioxidants without negatively affecting the quality of products and consumer perceptions.

22.9.4 Potential of feeds containing secondary metabolites and bioactive compounds

Animal feeds contain a vast range of secondary metabolites called phytonutrients. Pastures, fodder crops, shrubs, and higher plant species produce (secrete) these phytonutrients in their body for their protection, survival, and establishment

against disease and pest and harsh climate under various conditions. They also produce these compounds to attract insects for pollination. These phytonutrients may include health enhancing compounds that prompt animals to selectively consume these pastures, fodders, and other by-products of field crops to support their good health and well-being. Phytonutrients are classified as alkaloids, polyphenols, organosulfur compounds, and so on. It is likely that animal feeds containing some types of polyphenols, such as tannins, phenolic acids, and flavonoids, can protect dietary PUFA from the hydrolysis and biohydrogenation in the rumen, resulting in beneficial effects. Hence, increased omega-3 PUFA would be available for absorption across enterocytes and, therefore, have increased deposition within tissue and meat. This is possibly due to these phytonutrients having low bioavailability and long retention times within the rumen, causing a slow degradation of fibrous diets by the microflora and allowing the PUFA and other nutrients present in the diet to bypass the rumen and be available for intestinal absorption by host animals.

Forages containing condense tannin have the potential to reduce pasture infection with parasite eggs and doing so, reduce adverse effects of worms to the grazing animals. Feeds or plant extracts from forages have been shown to reduce the production of eggs and one of the possible explanations for reduced fecal egg count may be related to reduced fecundity of the adult worm population. However, at higher levels, some phytonutrients can negate animal growth and ultimate meat productivity. For example, high concentrations of condensed tannins in sheep diets (>60 g/kg DM) may affect ruminal microbial activity and digestive efficiency by inhibiting enzymatic activity and intestinal permeability, which consequently decreases nutrient absorption and liveweight gain.

22.9.5 Potential of matching nutrition to genetics

The rapid advancement in gene technology and molecular biology has provided producers with the opportunity to select high-performance and high-yielding animals, plants, and pastures. It is equally important to optimize aspects of the production system, such as nutrition, to maximize productivity and support the achievement of animal genetic potential, regardless of geographic location. In Tanzania, for example, the low nutrient concentration in the feeds and delay in slaughtering animals could be the major factors that affect poor quality beef production from tropical cattle breeds (Shorthorn Zebu) (Asimwe et al., 2016). The selection of an animal breed or species that will perform under the available resources and diet is a simple approach to sustainably match nutrition to genetics. Alternatively, fetal programming is being explored to understand its effects on meat yield and quality. This is demonstrated in findings from the progeny of bulls and heifers selected for their high residual feed intakes, with these animals reported to have lower carcass yields when reared on low-nutrient diets (Meale et al., 2021). As a result of this and other epigenetic investigations, it is increasingly evident that genetic-nutrition interactions are complicated and require additional understanding beyond the

simple Mendelian principles. The development of CRISPR gene editing technology merits consideration because of its capacity to manipulate the genetic potential of livestock to better utilize available feedstuffs and achieve more sustainable production of meat. The ethical dimensions to this approach will impact on its practicality—but it is expected that advancement in human genetic techniques, such as occurring in the fields of medical research, will offer parallel advancements to the expression of genetic potential in livestock reared for meat production.

22.9.6 Potential for animal transport and slaughter

Based on the effort and inputs necessitated to rear animals for meat production, it is counterintuitive to risk the quality and safety of meat before it can be purchased and consumed. Many countries around the world have defined approaches and strategies for animal transport and slaughter that adhere to stringent guidelines that protect animals and their humane treatment, as well as to support the production of quality meat. That said, this approach is not universal. In some systems, there is an obvious lack of knowledge and inefficiencies in the animal transport, lairage, and slaughter sectors that must be improved. Education to improve animal-human interactions and financial incentives for humane transportation and slaughter would help to sustain the meat processing. Consumer pressure is expected to affirm this requirement—whether perceived or actual. This is already evident from a changing demand for live animal export markets, wherein animal disease status and welfare must be documented during transportation. In-direct financial incentives are applied from the association between best-practice transportation and slaughter methods to premium meat products with longer shelf-life. The combination of these social and economic factors will help to drive industry sustainably.

22.9.7 Potential of packaging technology for better meat processing and preservation

Innovation in smart packaging for meat products has proven advantageous in addressing many challenges to meat quality and shelf-life (see Chapter 10). Modified atmospheric packaging has been applied to recover the color parameters of DFD beef to better match customer expectations; active packaging films have been loaded with antimicrobial and antioxidant agents to inhibit these effects on meat integrity; intelligent packaging devices have been proposed to inform consumers and retailers of in-pack product status; packaging has been developed to be retail-ready and resilient to tampering and damage; and biodegradable alternatives to conventional plastics have been developed. These outcomes would help meat transit the interim between animal slaughter, carcass fabrication, and the point of its consumption. However, as is common to emerging technologies, the associated costs and degree of consumer-interactivity must

be considered against the advantages gained in terms of market access and assurances of quality and safety. Plus, there is likely to be a governance element to the adoption of any novel packaging technology or requirement. This observation is expected to include the requirement for advanced food safety (biosecurity) across the supply chain. Indeed, food regulatory authorities are projected to enforce the labeling of nutritional information on food products sold in restaurants, fast food outlets, and supermarkets through a formal labeling system. Correct identification of the meat's origin or location of production is further likely to be mandated for inclusion onto any packaging. The importance of this information is to assure that the customer is informed of the packaged meat—and base their decision to purchase on its healthiness, food miles or carbon footprint, method for production, etc. Essentially, using the appropriate processing and packaging technology for each food sector and purpose of production, can allow producers to maintain the integrity and safety of the food as well as satisfying the consumer requirements or desires.

22.10 Conclusions

The world faces many challenges to fulfill the demands for food that are placed upon crop and livestock production systems. To respond, two major problems must be addressed. First, food and water capacity are required to support a rapidly growing population; and second, food and water capacity are required to support the increased amount of meat being consumed. It is reasonable to think that the welfare systems and policies of developing countries will try to improve the living standards of their people to match with the standards of the people in developed nations, in terms of high-quality food consumption, health care, and human welfare. To achieve the latter, in the space of livestock production, the following question is raised: Can the world offer animal-based food products to sustain the demand of the growing population in developing and developed countries, without placing significant stresses on land, water, and other natural resources?

Animal-based protein is necessary for the physical and mental development of children and young adults as well as the longevity of all. There are, however, consequences to the expansion of agriculture production, particularly animal-based food production in many parts of the world. Greater reliance on grain feeding; high fertilizer application to field crops and pasture production; soil erosion and pollution to natural waterways by grazing and intensive animal production; and increased greenhouse gas emissions are some issues that animal producers (farmers) must tackle in the coming years. Climate variability is another issue that must be accounted for while trying to increase crop and livestock productivity. This is a challenge as climate variability can diminish the availability and quality of animal feeds for livestock production by reducing both the yield and nutritive characteristics of pasture and fodder crops. This may impact on the livelihoods of many rural- and regional communities,

who are often responsible for producing the foods consumed by high-density suburban and city-living populations. Unexpected disease outbreak is another concern. Consequently, a holistic approach of international research organizations, such as FAO, ILRI, IRRI, ACIAR, IUCN, WSPA, UNICEF, and IPCC, that work in collaboration with national research institutions and government authorities is essential. Collectively, these groups must prepare (and respond) to emerging challenges that affect the sustainability of animal production and meat processing.

The world population is projected to increase, exponentially. For this reason, crop-animal-food production systems should be robust and sustainable. Much of growth in population, and associated demand for food, is from South/South East Asia, Sub-Saharan Africa, and Middle East/North Africa regions, yet present resources and technology are inadequate to fulfill this future demand. In addition, the majority of the lower socioeconomic population, in developing countries, live in rural areas and under-resourced agricultural regions that are divided into small farm holdings. In these regions, greater efficiency and associated growth in agricultural (livestock and crops) outputs would be the most effective way to increase the health and well-being of these people. Currently, the Asia Pacific region contributes most to global agricultural production, accounting for almost half of global output. Europe and Central Asia and the United States are responsible for another 45% (OECD-FAO, 2020). It is projected that, within the next two decades, most of the livestock and crop production will come from the Asia Pacific, Latin America, and Caribbean regions due to the resource availability, population growth, and increases to economic status (Fig. 22.13).

Although there are other minor constraints, it is evident that sustainable animal production and meat processing can be achieved. This outcome is facilitated by (1) using the resources strategically and efficiently to feed the animals at the right time for optimum productivity because feed is the major cost of meat

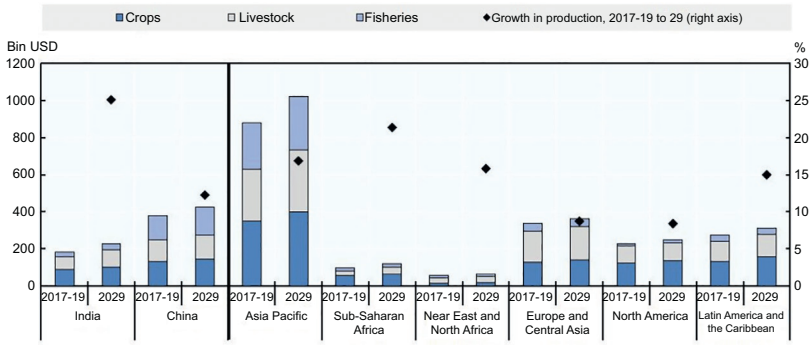

FIG. 22.13 The estimated net value of the production of agricultural and fish commodities from 2017 to 2019 until 2029. *(From OECD-FAO, 2020. OECD-FAO Agricultural Outlook 2020–2029. OECD Publishing/FAO, Paris/Rome. https://doi.org/10.1787/1112c23b-en.)*

production; (2) harvest and conserve the surplus production of forages, crop residues, by-products, and organic waste for future use; (3) select farm animals for suitable agro-climates and production purposes (meat, dairy, wool, feather, and pharmaceuticals), efficient growth, and that are harvestable for meat at the right time; and (4) processing and preserving meat appropriately from the production precinct until the point of its consumption (i.e., farm to fork). These strategies will contribute to the sustainable production of meat, in terms of inferring neutral or positive benefits on the livelihoods and wellbeing of all people, as a result of satisfying the requirements of environmental, economic, welfare, and social sectors.

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Chapter 23

Future meat market

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23.1 Introduction

With its high bioavailable nutritional content in macronutrients and micronutrients, and unique and attractive taste and flavor profiles, meat has become an indispensable food resource for human beings. It is evident that meat provides the majority of the nutrients required for human health. However, with the increase of population and the improvement of living standards, meat and meat products are gradually in short supply. The demand for foods in worldwide is driven by the growing world population growth that is expected to reach 9.3 billion in 2050 (UN, 2012). Recent findings have revealed that, with the individuals being more educated, healthier, and living longer, the higher levels of demand will occur for agricultural products such as wheat, fruits, dairy, and meat over the coming decades. Particularly, the global consumption for high-quality animal products processed from red meat will be increased approximately 200% by 2050 among which the porcine meat will increase by 158% in the next two or three decades (Alexandratos and Bruinsma, 2012). However, the more greenhouse gases (e.g., methane) are expected to be released into the environment by animals from grazing or rearing systems due to its inefficient use of the fodder. On the other hand, large amounts of arable land, water, and raw materials are consumed to satisfy these high energy-consuming agriculture (De Boer and Aiking, 2018). Therefore, the research community is targeting on the development of innovative technologies in the future that are expected to meet the demand for the low production cost while enhancing animal welfare standards and environmental sustainability.

Meat alternatives are the substitutable product that can partly address these concerns in meat production and consumption in the future. In a narrow sense, the term "meat alternatives" indicating the meat analogs that are manufactured by plant-based proteins, which are closely similar to the animal whole-muscle meat in appearance, texture, and flavor. In most cases, meat alternatives can be classified as plant-based (soy, wheat, pea, etc.) and cell-based (synthetic or cultured meat) products (Sha and Xiong, 2020). In the last two decades, the

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plant-based meat replacements or alternatives have occupied a major market share in this rapidly evolving industry because plant proteins can be utilized directly to construct meat alternatives (Fig. 23.1). In addition, the formation of filamentous protein is the advantage of proteins that are cultured in vitro (Zhang et al., 2020). The cultured meat is considered as safer and healthier as well as the sustainable and eco-friendly means for meat producing. However, there still have technical challenges and social or ethical constraints to produce cultured meat due to the high production costs, food safety regulation and other factors.

Furthermore, recent advantages in the field also include other protein sources such as insect proteins (Onwezen et al., 2021). Edible insects have been considered as a good source as alternative meat and are able to contribute to the increasing demand of animal protein in an environmentally sustainable means (Fig. 23.1). At present, it is estimated that about two billion people regularly take insects as foods and that is relatively prevalent in China, India, and other Asian or African countries (Oonincx et al., 2011). Nevertheless, eating insects is still uncommon in Western countries where people meet with feelings of food neophobia. In addition, developing organic meat and meat products by improving feeding and management methods is also effective to meet the increasing demand for eco-friendly meat, while reducing the cost of activity and the consumption of agricultural resources. More importantly, this can be recognized as a powerful strategy for differentiation and then raise the competitiveness of the products in the global market (Akaichi et al., 2019). The history of meat alternatives is shown in Fig. 23.1.

In this chapter, the ingredients, the principles of processing or construction methods, and the technology aspects in the currently plant- and insect-based meat analogs and cell-cultured meat are discussed. The future prospects for organic meat products are also presented. The environmental benefits, the market prospect, and their future remarks are discussed. Finally, in case of a future pandemia resulting in the disruption of meat supply chains, the strategies and planning are provided.

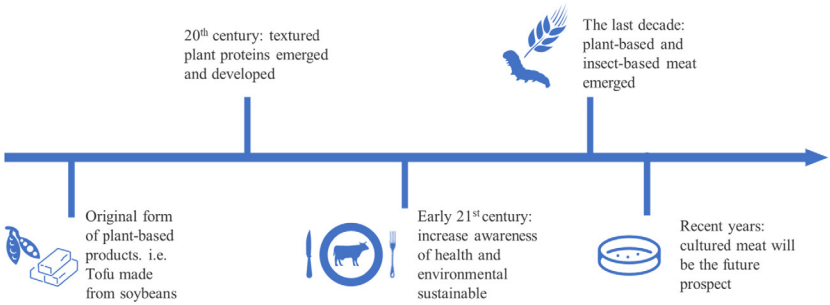

FIG. 23.1 History of meat analogs. *(Modified from Ismail, I., Hwang, Y.-H., and Joo, S.-T., 2020. Meat analog as future food: a review. J. Anim. Sci. Technol. 62, 111–120.* https://doi.org/10.*5187/jast.2020.62.2.111.)*

23.2 Trends in plant-protein-based meat analogs

23.2.1 Plant protein structure and functionality

Meat analogs resembling the texture of meat such as pork, beef, or chicken are created from plant proteins by processing through a cooking extruder or other novel technologies to create long fibrous strands of protein (Arntfield and Maskus, 2011). Almost all plant proteins can be selected as candidates for the preparation of meat analogs. However, considering the availability, cost, and processing functionality, the proteins from pea (legume seeds), wheat gluten, and soy are the ingredients most widely used as the fundamental food matrix to produce alternative meat products (Table 23.1). In addition, mung bean proteins

TABLE 23.1 Proteins used in commercial plant-based meat alternatives (Sha and Xiong, 2020).

Proteins	Composition and structural characteristics	Functions
Soy protein	Isolate and concentrate; globulins mainly constructed by glycinin (11S, hexamer, 320–380 kDa) and β-conglycinin (7S, trimer, 150–220 kDa); basic and acidic subunits in glycinin linked by disulfide bonds	Aggregation, gelation, and fiber formation through heating and extrusion; oil-binding and emulsification
Pea protein	Isolate and concentrate; predominant globulins: legumin (11S, hexamer, 320–380 kDa), vicilin (7S, trimer, 150–170 kDa, contains one cysteine residues; minor fraction convicilin (290 kDa)	Functionality similar to soy proteins
Wheat protein	Gluten in an elongated structure with two components: gliadin (alcohol-soluble, polypeptides with 25–100 kDa linked by intramolecular disulfide bonds) and glutenin (alkali-soluble, polymeric large subunits, 66–88 kDa and small subunits 32–45 kDa linked into polymers of 150–1500 kDa)	Used together with legume proteins to provide fibrous texture, elasticity, and extensibility
Mung bean protein	Globulins (60%, vicilin-type 8S with 26–60 kDa), albumins (25%, 24 kDa); other globulins including basic-type 7S and legumin-type11S	Good gelling potential to aid in particle binding and water holding
Rice protein	Glutelin (alkali-soluble, 80%, 60–600 kDa) with subunits linked by disulfide bonds, globulin (salt-soluble, 12%, 12–20 kDa), albumin (water-soluble, 5%), and prolamin (alcohol-soluble, 3%) fractions	Texture formation; nutrition
Potato protein	Classified into three groups: patatins (40%–60%, 40–43 kDa), protease inhibitors (20%–30%, 16–25 kDa), and other high-molecular-weight proteins	Used to complement legume proteins to improve textural characteristics

and rice proteins are often supplemented into common legume proteins in order to balance the amino acid profile of the products (Sha and Xiong, 2020).

Plant proteins are a kind of high-quality protein resources. Compared to the meat and meat products, protein extracted from plant obtains the advantages of very low or no content of cholesterol, hormones, and antibiotics. In addition, the high content of essential amino acids is more in line with the requirements for a healthy diet of human. In terms of the composition of amino acids, the essential amino acids in plant protein are close to the proportion of human body. Moreover, plant protein has good processing characteristics due to their excellent functional properties such as solubility, water and oil absorption, foaming stability, emulsification stability, and gel formation. These functional advantages lay the foundation for the application of plant proteins into the promising meat analogs industry. However, the native globular structure of plant proteins is not suitable for building the meat-like fibrous texture (Fig. 23.2). Therefore, it is necessary to transform the native globules into interactive fibers or filamentous aggregates through disruptive processes such as fiber spinning or thermal extrusion.

In practice, due to the low cost and abundant availability, soy proteins are the mainly sources that are utilized in food formulation ingredient for meat analogs manufacture. The globulin fraction and the albumin fraction are the most common structures in proteins. According to their sedimentation coefficients, the isolated globulin fraction is made up of β-conglycinin (7S globulin) and glycinin (11S globulin). Glycinin consists of one acidic polypeptide (38 kDa) and one basic polypeptide (20 kDa), which are linked by a disulfide bond (Fig. 23.2A). Glycinin can form the structure of hexamers (11S, 300–360 kDa) at ambient temperatures and pH 7.6. When adjusting pH to 3.8, glycinin is present as

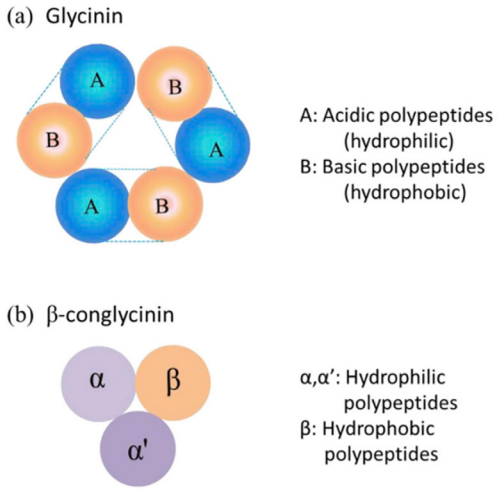

FIG. 23.2 Schematic structures of legume 7S (A) and 11S (B) globulins (Sha and Xiong, 2020).

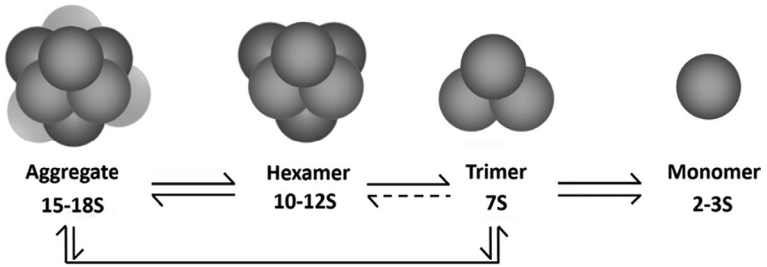

FIG. 23.3 Schematic model for the quaternary structure association-dissociation phenomena of legumin- and vicilin-like globulins. *(From González-Pérez, S., Arellano, J.B., 2009. Chapter 15—Vegetable protein isolates. In: Phillips, G.O., Williams, P.A. (Eds.), Handbook of Hydrocolloids, second ed. Woodhead Publishing.)*

trimers (β-conglycinin, 7S, 180 kDa). Moreover, the dissociation of 11S glycinin into 7S glycinin can be appeared at neutral pH when lowering the ionic strength from 0.5 to 0.1 M (Fig. 23.3) (González-Pérez and Arellano, 2009). β-Conglycinin (7S) is a trimeric glycoprotein consisting of three types of subunits including α (57–68 kDa), α′ (57–72 kDa), and β (45–52 kDa) (Table 23.1, Fig. 23.2B). These subunits are combined via hydrogen bond and hydrophobic interactions without the disulfide bonds. At pH > 5 and ionic strength < 0.1 M, β-conglycinin is exhibited as the form of hexamer, whereas a trimer structure appears at the ionic strength equal to 0.5 M. In addition, when ionic strength is lower than 0.1 M, β-conglycinin dissociates into a 5-6S and 2-3S fraction reversibly at pH 2.5.

As for the pea proteins, the globulin fractions include the legumin, vicilin, and convicilin (Table 23.1). The structure of legumin is similar to that of the glycinin in soy proteins. Vicilin (7S pea protein) is also found to have a structure with trimer with each subunit having a molar mass of around 50 kDa. The minor fraction of pea convicilin belongs to a 7-8S trimeric structure with a molecular mass about 290 kDa. Contrary to the other 7S globulins, convicilin contains one additional cysteine residue.

Wheat protein is another plant protein that is most commonly utilized in meat and meat products. About 80% of wheat proteins is gluten, while the minor fraction of wheat proteins includes albumins and globulins (20%). According to their solubility properties in solvents, wheat proteins can be classified as globulin soluble in salt solution, albumin soluble in water, glutenin soluble in dilute acid or alkali, and gliadin soluble in 70% aqueous ethanol (Uthayakumaran and Wrigley, 2017). Gluten mainly consists of two groups of proteins. Gliadins are the sulfur-poor monomer proteins that can interact with the glutenin in the network, while glutenins are the sulfur-rich proteins that are able to form a polymeric network by chemical bonds. Adding wheat gluten to products can provide the meat-like chewiness due to its elongated structure. The contents of glutenin

and gliadin are related to the elasticity and extensibility properties of products. Several other plant proteins including mung bean protein, potato protein, and rice protein have been applied to form the specific textural characteristics of meat analogs by the physical interaction with the main proteins (Brishti et al., 2021). In practice, the compounds of raw material systems (soy protein-wheat protein, soy protein-starch, soy protein-edible glue, etc.) are mostly utilized for the production of nonmeat products that can contribute to convert the orbicular structure of the proteins into fibrous structures (Zhang et al., 2019).

23.2.2 Processing of plant-protein-based meat analogs

A typical meat analog contains water (50%–80%), flavorings (3%–10%), fat (0%–15%), nontextured proteins (4%–20%), textured plant proteins (10%–25%), coloring agents (0%–0.5%), and binging agents (1%–5%). Meat analogs can be obtained with the accepted sensory attributes of "real meat" when combining such ingredients. Meat replacement by using texturized proteins can occur via two routes. One is based on the complete replacement of meat by texturized proteins to form fully vegetarian products, while another one is based on blending the texturized proteins with meat.

The essential technical concerns in producing meat analogs are to form the physical structure of "texturization or texturing" of plant proteins, which can provide a sensation of eating meat. Until now, the more relatively mature techniques to produce plant-based texturization products can be classified in two categories. The first approach is similar to that used for the production of synthetic fibers for the textile industry by a "spinning" process. The second one is based in converting the plant material into a hydratable, laminar, and chewy mass without true fibers by screw extruder.

The spun-fiber-based texturization for soy protein was first described in a 1954 patent issued by Boyer. A concentrated protein solution is prepared by adding alkali to the slurry to adjust pH to 12–13. The solution, containing approximately 20% protein, is to permit unfolding the protein molecules until its viscosity reaches 50–100 Pa s. This viscous protein solution is technically known as "dope". Second, the "dope" is transformed into stretched fibers (spinning) by coagulating fine jets in an acid solution (phosphoric acid and salts, pH 2.5) through a spinneret. These fibers are then stretched to increase fiber strength and enhance molecular orientation. Finally, the fibers are subjected to washing to remove the excess salts (Fig. 23.4). After mixed with fats, binders, flavorings, and coloring additives, soy protein fibers are to form a meat-like structure (Boulet et al., 1982). At present, the main shortcoming of the spun fiber type texturized products is the cost aspect. The starting material such as isolated soybean protein is expensive and the processing itself including initial capital investment and running expenses is also costly. In addition, the washing step and the coagulation bath are essential but produce large volume of waste-

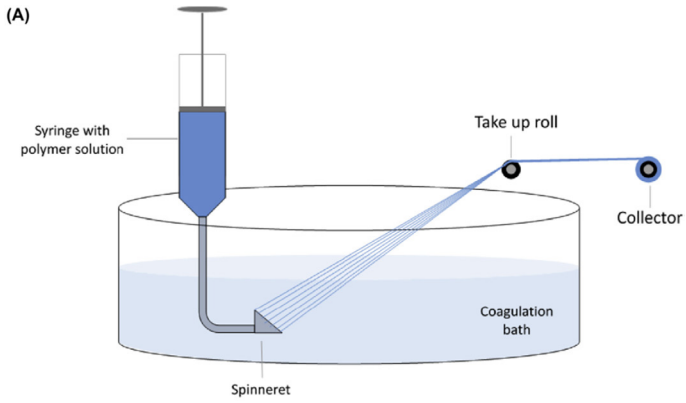

FIG. 23.4 Wet spinning processes for the production of fibrils (Kyriakopoulou et al., 2019).

water. Therefore, these products are facing the competition of the less expensive extrusion texturization technique.

Thermoextrusion is a most studied and successful approach that is widely utilized for the manufacture of meat analogs (Dekkers et al., 2018). It was first invented by Anelly in 1964 with single screw extruder and applied for patent in United States. Studies have confirmed that the thermal and shear effects are mainly responsible for the modification of proteins during extrusion cooking. Under the influence of high moisture and temperature, the native plant proteins unfold their structure and adsorb the water. Similar to starch gelatinization, protein at lower moisture content can produce a high viscosity melt due to denaturation. Texturization is believed to be the effect of unidirectional shear force that leading the melted proteins to be re-orientated near the "die" (Fig. 23.5). The protein-protein intermolecular cross-linking (mainly the formation of disulfide bonds) is thought to be responsible for the stabilization of the fiber texture. On the other hand, the opposite results that do not increase the number of disulfide bonds are often observed through the analysis of soy extrudates (Jin et al., 1995).

An extruder is a drag flow device with forward pumping action of a twin or single rotating screw (Figs. 23.5 and 23.6). According to the moisture content of raw materials, the extrusion technology can be divided into low-moisture and high-moisture extrusion. The product with an aggregated and expanded conformation and the textured vegetable protein preparation usually adopt the technology of low-moisture extrusion (20%–40% moisture). As the protein-water mixture advances along the barrel, it is rapidly heated by the energy supplied by the heating elements around the barrel as well as the action of extrusion and friction. The high temperature attained through the compression mechanism is permitted up to 150–180°C, which can lead proteins to undergo the extensive heat denaturation. Meanwhile, the directional shear forces cause the alignment of high molecular weight component. A porous and laminar structure can appear due to the instant evaporation of the water and "puffing" of the paste when

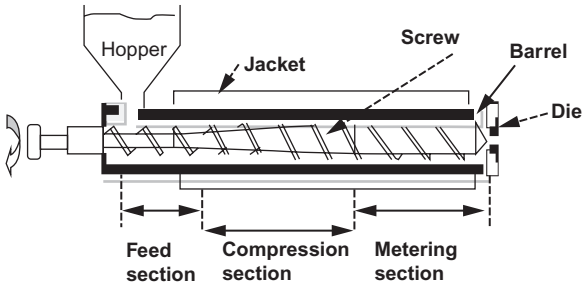

FIG. 23.5 Basic structure of a single-screw extruder (Berk, 2018).

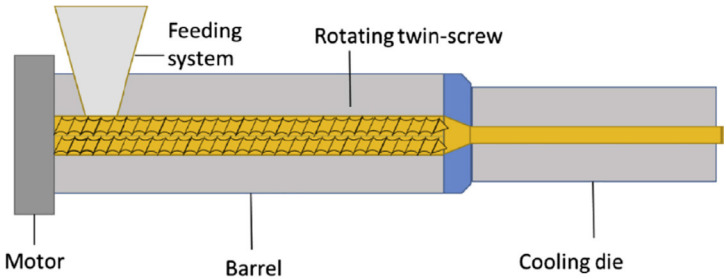

FIG. 23.6 Scheme of a twin-screw extruder for high-moisture extrusion of proteinaceous materials into fibrous meat analogs (Kyriakopoulou et al., 2019).

the sudden release of pressure near the "die". Although the extrudate obtains a meat-like fiber structure, it is difficult to regard it as "meat" according to its spongy appearance. The products are usually needed to be rehydrated before eating (Brishti et al., 2021).

The high-moisture extrusion or wet extrusion (20%–40% moisture) has been widely used to produce meat-like fibrous structure from plant proteins. Like the low-moisture extrusion, the process of extrusion is involved with mixing, hydration, shearing, homogenization, compression, heating, shaping, and expansion (Fig. 23.6). However, the products do not need to be rehydrated before eating due to the formed fibrous structure and texture characteristics to meat and thus it is suitable for whole-muscle meat analogs (Akdogan, 1999). In terms of the equipment, a long cooling tube with the temperature below 75°C should be installed at the exit of the die in order to allow the protein to form a dense fibrous structure and better textural properties (Fig. 23.6). Nevertheless, the exact mechanism for the fibrous structure formation under the high moisture extrusion has still no consensus. Studies have indicated that the slit and the low temperature of the cooling die keep the mixture in a laminar flow, which is a key factor in the formation of the fiber structure of plant-based protein products (Sandoval et al., 2019; Fig. 23.7). It is worth noting that although folded protein molecules can easily be orientated to form fibers, the fibers are anisotropic and do not resemble natural muscle tissue structurally.

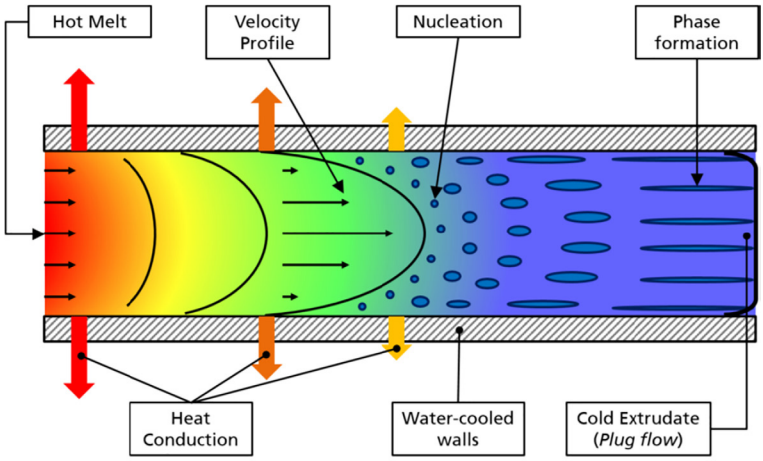

FIG. 23.7 Texture formation with laminar flow in the cooling die (Sandoval et al., 2019).

Several other methods also have been introduced to produce meat analogs such as electrospinning, conical shear, and 3D printing. The operation principles, structural, and morphological properties of the fibers fabricated by these methods have been described in detail by Dekkers et al. (2018).

Electrospinning is the most attractive method that is frequently reported for fabricating the fibers (Nieuwland et al., 2014). The high voltage is applied over the concentrated protein solution producing fibrils with a nanometer length scale. The solution by fabricating fibers should obtain the high solubility, the viscosity, the surface tension, and the ability of the components to entangle. After that, the protein solution can form a Taylor cone, and it is attracted to a metal collector electrically. The solvent of protein solution evaporates and creates the entangled polymers by these thin fibers (Fig. 23.8). This technology presents the advantage that does not leave large volumes of waste solutions compared to the wet spinning processes.

High-temperature conical shear cell (HTSC) technology is a relatively new technique based on the principle of flow-induced structuring to prepare plant-based meat analogs (Krintiras et al., 2016). The shear cell device is designed as a cone-in-cone structure, and the top cone remains stationary while the bottom cone rotates. The processing temperature (95–140°C) can be controlled by both cones with the steam or heating bath. A well-oriented protein fibrous structure can be formed with the combination of simple heat and shear due to the interspace inside the device that is kept constant during processing (Fig. 23.9). Moreover, the structure of fibers can be obtained from homogeneous, layered, or fibrous depending on the process conditions. Studies have revealed that the use of high temperatures could induce the higher flexibility of polymers, lower the viscosity of solutions, and unfold the proteins (Schreuders et al., 2019). In

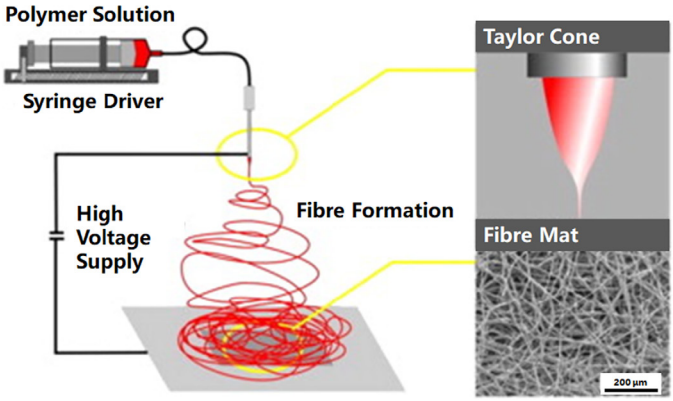

FIG. 23.8 Electrospinning processes for the production of fibrils. *(From Nieuwland, M., Geerdink, P., Brier, P., Van Den Eijnden, P., Henket, J.T.M.M., Langelaan, M.L.P., Stroeks, N., Van Deventer, H.C., Martin, A.H., 2014. Reprint of "food-grade electrospinning of proteins." Innovative Food Sci. Emerg. Technol. 24, 138–144.)*

FIG. 23.9 Conical shear cell device (Kyriakopoulou et al., 2019).

addition, the shear rate and the processing time are also important parameters for the shear-induced fiber structure.

3D printing (or additive manufacturing) is an emerging technology that offers the potential opportunity for creating muscle-like products with improved sensory profile and nutritional value through precise control of plant protein (Portanguen et al., 2019). The technology involves a layer-by-layer deposition with predetermined thickness to form complex three-dimensional structures by using plant proteins as "inks," and it is necessary to add food additives to consolidate the shape of printed muscle-like fibers. Typically, transglutaminase, agar, xanthan gum, or other ingredients are essential in a printing system that is applied for meat-like products. In practice, by using plant-derived ingredients, a commercial inverter NovaMeat has successfully been used to produce beef steaks that are similar to the taste, texture, appearance, and nutritional properties of real meat products by using 3D technology (Sha and Xiong, 2020).

23.2.3 Challenges and opportunities

23.2.3.1 Sensory aspects

The delicate and complex sensory profile of real meat products is difficult to reproduce due to natural differences between plant and muscle proteins. Traditional and new processing methods such as shear cell and thermomechanical extrusion have achieved some degree of success. The desired sensory property of a plant-based product requires creating the type of highly water-binding capacity and organized texture of products. However, the three-dimensional structure like a muscle fiber is difficult to be microscopically formed if the plant proteins are highly aggregated and denatured. To solve these deficiencies, various texture-enhancing, water-binding, and thickening agents are included in plant protein-based products. Unfortunately, the common drawbacks like low juiciness in cooked products are still observed (Sha and Xiong, 2020).

The lack of meat flavor is another major noticeable challenge to these alternative products. Although the spices, herbs, and meat-like flavors are generally added into those products, the off-flavors naturally existing in plant like beany odor can still be detected in many plant-based products. In addition, the bitter or astringent tastes caused by isoflavones and saponins affect the acceptance of customers (Boatright and Lu, 2007). These defects may hinder the application of plant protein as the basic materials for meat alternatives. Thus, the successful simulation and retention of meat flavor while removing the off flavors in plant proteins is essential for the final consumption. Further research can be engaged in enhancing the interaction of meat flavor and plant protein, while minimizing the impact of undesirable flavors by modifying plant proteins and improving the preparation of plant protein isolate or concentrate.

Color is the essential attribute of meat. However, the lack of fresh meat (distinct red) or processed meat color (pinkish) is another constraint to plant-based

products. Currently, despite the stability and coloring effect of pigment for meat analogs is not always of high quality, some heat stable coloring ingredients have been used in plant-based alternative products such as caramel colors, annatto, leghemoglobin, and carotene. Furthermore, in order to control the color migration from the dyed analog products to outside, color retention aids such as hydrated alginate and maltodextrin are usually used along with the coloring agents (Kyriakopoulou et al., 2019). Most importantly, the economy feasibility needs to be further evaluated.

23.2.3.2 Nutrition, health and safety aspects

The main purpose of meat from a nutritional point of view is to provide high-quality proteins. A meat analog should provide a similar nutritional value in case this product fully replaces the meat product. It has been proved that eating plant proteins has some nutrition and health benefits, but some essential amino acids could be missed (Nakata et al., 2017). However, the final plant-based products will lose some of the nutrients inevitably either added as supplements or naturally present due to the vigorous processing conditions. In general, meat analogs are often designed to contain more salt than the corresponding meat products which presenting a challenge for the consensus of reducing sodium intake. Apart from these concerns, the limited essential amino acids and the trace elements present in plant protein ingredients are a high challenge to create the nutritional meat alternatives. The added ingredients, either extracted from natural resources or chemically synthesized, raise a discussion among consumers with the awareness of environmental protection.

In terms of plant proteins isolated from legumes, the influence of allergen and certain antinutritional factors present in materials should be considered. Although the nutritional inhibitors (protease inhibitors, trypsin inhibitors, and IgE-binding G2 glycinin) are generally heat sensitive, most of the inhibitors at moderate processing temperatures cannot be destructed because the extrusion time is too short. Ultrahigh temperature pretreatment is considered as one of the promising methods to reduce the content of the nutritional inhibitors in legume protein materials. For example, after heat treatment at 121°C for 45 s, more than 75% of the activities of trypsin inhibitor in soymilk were lost (Kwok et al., 1993). In addition, if the meat analog containing wheat proteins, the potential risk of allergy or sensitivity of gluten must be carefully evaluated in susceptible populations (Sha and Xiong, 2020).

Apart from health aspects, the large number of required additives including colorants, stabilizers, and preservatives that do not commonly appear in meat products, and the addition of high salt and saturated fat that to simulate the tastes and flavors of meat products, also raise the safety concerns of plant-based alternatives. Furthermore, it has been revealed that meat proteins undergoing high-temperature processing could produce carcinogens and toxicants such as benzopyrene and heterocyclic aromatic amines (Jiang and Xiong, 2016). Whether the toxicant could be formed in plant-based meat alternatives with high

contents of proteins and additives under high temperature processing should be further investigated.

23.2.3.3 Market and consumption prospects

Recent efforts for developing meat analogs lead to the booming of these market as increasing numbers of consumers seek sustainable food and protein alternatives. Currently, European countries have the top consumers in meat analogs accounting for about 40% of the global meat-analog sales. Asian countries could also be the promising market in the future in exporting meat analog products (Kyriakopoulou et al., 2019). However, numerous costs, consumerism, and technological challenges are presented in the development of meat analogs. First, the higher prices of meat alternative products constitute an obvious disadvantage in contrast with regular meat products. Further research and technological innovations shall be carried out including optimizing processing conditions, developing high-capacity production methods, seeking for low-cost proteins and inexpensive functional ingredients to reduce the cost while maintaining the quality of products. Next to that, the naming and the ingredient labeling of plant-based meat analogs raise the challenges of regulations and standards. The use of words or phrases commonly applied in general meat products to describe the meat analogs can only confuse the consumers. The FDA and some of the states in the United States have passed some restrictive regulations to regulate the labeling of meat alternatives. In addition, the plant-based meat analogs can adopt the carbon footprint labels to demonstrate the eco-friendly and sustainability aspects of the products.

Finally, it should be noticed that the demand for regular meat and poultry meat remains strong worldwide, and the plant-based meat analogs can be selected as one of the dietary options for humans. However, in the foreseeable future, they are unlikely to completely replace animal products. Therefore, the efforts that intend to completely substitute or replace animal meat by meat alternatives are unrealistic and imprudent. The demand of meat market and the ever-increasing global population urge food processors and scientists to develop nutritious and organoleptic qualities of food from sustainable plant proteins to meet the concerns related to environmental issues.

23.3 Trends in insect proteins to be used in meat products

Similar to plant proteins, edible insects have the potential to be received as an alternative source of proteins for meat analogs due to its high content of fat, protein, and micronutrients (Rumpold and Schlüter, 2013). The lower levels of water consumption and greenhouse-gas emissions and the higher efficient feed conversion into protein or calories than conventional livestock have attracted the attention to these novel proteins. A survey from Rumpold and Schlüter (2013) indicated that more than 2000 species of insects could be adopted as food

sources across 119 countries and regions in the world. However, the limited consumer acceptability in some western countries such as the United States and the United Kingdom is particularly a prevalent issue. Currently, the attitudes of consumers in the developed countries are already starting to change in sign. Like the issues faced by plant-based meat analogs, the successful simulation in taste and physical properties to animal meat product is also a great challenge both in technological and scientific ways.

At present, there are few studies employing insect proteins for completely replacing the animal meat to produce the meat alternatives. A recent report from Bessa et al. (2019) taking black soldier fly larvae as an ingredient in Vienna sausage formulations revealed that the texture profile and the proximate analysis of sausages made with a 28% of insect protein were similarly to that of control groups. Smetana et al. (2018) found that the mixture of protein concentrates by 40% of *Alphitobius diaperinus* and 60% of soy dry matter could obtain a product with a texture similar to meat products by twin screw high-moisture extrusion. Current research is engaged in increasing the utilization of insects as meat protein source and broadening potential markets.

23.3.1 Nutritional aspects and risks of insect consumption

23.3.1.1 Nutritional aspects

A lot of literature has been published addressing the nutrient composition and nutritive value of various insects (Williams et al., 2016). In general, edible insects are good sources of fat, proteins, minerals, and vitamins (Rumpold and Schlüter, 2013). For example, the nutrition levels of three silkworm pupae are equal to one chicken egg (Rumpold and Schlüter, 2013). The consumption of 100 g of caterpillars can provide almost 76% of proteins and 100% of the vitamins daily required by humans (Agbidye et al., 2009). A systematic summary of the nutrient composition as well as mineral and vitamin contents in selected insect species are given in Tables 23.2–23.5.

According to Table 23.2, whole insects with high fat content are generally also accompanied with a low moisture content. Insects intended for human diet should contain less water content than raw insects. Furthermore, due to the protein-rich exoskeleton, the highest concentration of nutrients in insects is usually the protein (based on dry matter). As for fat, data from Lepidoptera suggest that larvae fed fresh plant material are lower in fat than those fed artificial diets. Processing methods such as roasting and frying of insects can dramatically affect the fat contents. Frying treatment can increase fat content, while roasting can remove fat from the insect. As expected, ash accounts for the small amount ingredient in most insects due to their lack of internal calcified skeleton that exists in most vertebrates. In addition, the removal of specific parts of insects during the processing can affect the overall nutrient composition.

Insects are a good source of regular and essential amino acids for human beings (Azzollini et al., 2019; Table 23.3). However, part of the amino acids

TABLE 23.2 Summary of the nutrient composition for selected insect species with high commercial values.

Food insects	Common name	Preparation	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash
Lepidoptera						
Bombyx mori (larva fed artificial diet)	Silkworm	Whole, raw, not fasted	82.7	53.8	8.1	6.4
Callosamia promethea (larva)	Silk moth	Whole raw, freeze-dried	4.5	51.7	10.5	7.2
Galleria mellonella (larva)	Waxworm	Whole raw, fasted	58.5	34.0	60.0	1.4
Manduca sexta (larva fed fresh plant material)	Carolina sphynx moth	Whole raw, freeze-dried	4.7	60.7	17.3	8.5
Spodoptera eridania (larva)	Fall army worm	Whole raw, freeze-dried	4.5	57.3	14.6	10.3
Pseudaletia unipuncta (larva)	Army worm	Whole raw, freeze-dried	2.0	55.5	15.2	7.0
Coleptera						
Oileus rimator (larva)	Beetle	Whole raw, not fasted	26.0	36.0	–	3.0
Rhyncophorus palmarum (larva)	Red palm weevil	Whole raw, not fasted	71.7	25.8	38.5	2.1
Tenebrio molitor (larva)	Mealworm beetle worm	Whole raw, fasted	61.9	49.1	35.0	2.4
Zophobas morio (larva)	Darkling beetle	Whole raw, fasted	57.9	46.8	42.0	2.4
Orthoptera						
Acheta domesticus (adult)	House cricket	Whole raw, fasted	69.2	66.6	22.1	3.6
Brachytrupes sp.	Cricket	Fresh: blanched, inedible parts removed	73.3	47.9	21.3	9.4
Zonocerus sp.	Grasshoppers	Whole raw, not fasted	62.7	71.8	10.2	3.2

Continued

TABLE 23.2 Summary of the nutrient composition for selected insect species with high commercial values—cont'd

Food insects	Common name	Preparation	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash
Isoptera						
Cortaritermes silvestri (worker)	South American termites	Whole raw, not fasted	77.8	48.6	6.9	8.5
Macrotermes bellicosus (alate)	African termites	Dewinged, raw	6.0	34.8	46.1	10.2
Nasutitermes corniger (soldier)	Central American tree termite	Whole raw, not fasted	69.6	58.0	11.2	3.7
Hymenoptera						
Apis mellifera (larva)	European honeybee	Whole raw, not fasted	76.8	40.5	20.3	3.4
Oecophylla smaragdina	Weaver ant	Fresh: blanched, inedible parts removed	74.0	53.5	13.5	6.5
Polybia sp. (adult)	Wasp	Whole raw, not fasted	63.0	13.0	–	6.0
Atta mexicana (reproductive adult)	Leaf-cutter ant	Whole raw, not fasted	46.0	39.0	–	4.0
Diptera						
Drosophila melanogaster (adult)	Common fruit fly	Whole raw, not fasted	67.1	56.3	17.9	5.2
Hermetia illucens (larva)	Black soldier fly	Dried, ground, not fasted	3.8	47.0	32.6	8.6
Hemiptera						
Pachilis gigas (nymphs and adults)	–	Whole raw, not fasted	64.0	22.5	–	3.5
Hoplophorion monograma (nymphs and adults)	Treehopper	Whole raw, not fasted	64.0	14.0	–	3.0

Data from Williams, J.P., Williams, J.R., Kirabo, A., Chester, D., Peterson, M., 2016. Chapter 3—Nutrient content and health benefits of insects. In: Dossey, A.T., Morales-Ramos, J.A., Rojas, M.G. (Eds.), Insects as Sustainable Food Ingredients. Academic Press, San Diego, CA.

TABLE 23.3 Essential amino acid content for selected insect species (mg/100 g dry matter).

Food insect	ILe	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Limiting AAA	Amino acid score
Lepidoptera														
Caterpillar, *Nadurelia oyemensis*	25.6	82.7	79.8	23.5	19.7	58.6	75.7	44.5	16.0	96	63.5	18.1	ILe	91
Caterpillar, *Imbrasia truncata*	24.2	73.1	78.9	22.2	16.5	62.2	76.5	46.9	16.5	102	55.5	17.4	ILe	86
Gusano rojo de maguey, *Cossus retenbachi*	51	79	49	8	13	40	53	47	6	61	60	16	Trp	55
Caterpillar meal *Bombycomorpha* sp.	46.1	62.1	64.5	17.9	29.9	59.8	81.4	42.9	13.0	60.5	41.8	8.31	Leu	94
African silkworm larvae Trp (*Anaphe venata*)	21.4	13.12	8.8	–	–	21.4	24.9	3.8	–	17.6	3.20	7.8	Trp	–
Orthoptera														
Chapulín, *Sphenarium histrio*	53	87	57	7	13	44	73	40	6	51	66	11	Trp	55
"Chapulín," *Sphenarium purpurascens6*	42	89	57	25	18	103	63	38	6.5	57	60	22	Trp	59
Mexican "Chapulines"	46	64	52	8	–	36	32	49	10	54	42	21	Lys	90
Blatta lateralis (nymphs)	7.73	12	12.8	3.35	1.44	7.67	14.3	7.89	1.66	12.3	14	5.49	–	–
Brachytrupes sp.	3.1	5.5	4.8	1.9	1	2.9	3.9	2.75	–	4.42	3.7	1.94	–	–
Isoptera														
Termites, *Macrotermes bellicosus*	51.1	78.3	54.2	7.5	18.7	43.8	30.2	27.5	14.3	73.3	69.4	51.4	Thr	81
Termites, mature alates, *Macrotermes subhyalinus*	37.1	79.7	35.4	12.9	9.0	43.1	36.8	41.9	7.7	51.4	–	–	Lys	61

Continued

TABLE 23.3 Essential amino acid content for selected insect species (mg/100 g dry matter)—cont'd

Food insect	ILe	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	His	Limiting AAA	Amino acid score
Hymenoptera														
Ants, *Atta mexicana*	53	80	49	19	15	41	47	43	6	64	47	25	Trp	55
Escamol, *Liometopum apiculatum*	49	76	58	18	14	39	68	42	8	60	50	29	Trp	73
Apis mellifera	41	66	60	25	9	70	41	44	7	59	64	33	Trp	64
Brachygastra mellifica	44	78	36	18	20	40	75	44	7	54	57	36	–	–
Diptera														
Copestyla anna and *Copestyla haggi*	40	74	55	19	18	54	66	49	7	61	63	29	Trp	64
Hemiptera														
"Ahuahutle" or Mexican caviar, eggs of water bugs (Corixidae)	50	80	35	15	–	34	111	40	11	60	77	33	Lys	60
"Axayácatl," adults and nymphs of water bugs (Corixidae and notonectidae)	59	80	43	16	–	32	45	44	16	55	55	24	Lys	74
"Jumiles," nymphs of several species of Pentatomidae	45	62	38	15	–	25	40	28	15	48	29	30	Lys	66
Edessa petersii	40	71	40	28	10	67	115	45	5	64	45	23	Trp	45

Data from Williams, J.P., Williams, J.R., Kirabo, A., Chester, D., Peterson, M., 2016. Chapter 3—Nutrient content and health benefits of insects. In: Dossey, A.T., Morales-Ramos, J.A., Rojas, M.G. (Eds.), Insects as Sustainable Food Ingredients. Academic Press, San Diego, CA.

TABLE 23.4 Vitamin content of selected insect species (mg/100 g dry weight unless otherwise noted).

Food insect	Retinol (A)	Thiamin (B1)	Riboflavin (B2)	Niacin	Pyridoxine (B6)	Folic acid	Pantothenic acid	Biotin	Cyanocobalamin (B12)
Lepidoptera									
Bombyx mori	1580 IU/kg	0.33	0.94	2.63	0.16	0.071	2.16	0.025	<1.2 μg/kg
Galleria mellonella	<1000 IU/kg	0.23	0.73	3.75	0.13	0.044	2.02	0.029	<1.2 μg/kg
Nudaurelia oyemensis	32 μg	0.21	3.4	10.1	54 μg	21.5 μg	9.5	32.0	0.015 μg
Imbrasia truncata	33 μg	0.32	5.5	11.8	151 μg	40.0 μg	11.0	48.5 μg	0.027 μg
Chilecomadia moorei (larva)	<300 μg	<0.01	64.5	33.6	3.29	0.83	26.5	0.46	–
Coleptera									
Zophobas morio larvae	<1000 IU/kg	0.06	0.75	3.23	0.32	0.0066	1.94	0.035	0.42
Tenebrio molitor (giant larvae)	<1000 IU/kg	0.12	1.61	4.13	0.58	0.117	1.45	0.0037	0.13
Tenebrio molitor (adult)	<1000 IU/kg	0.10	0.85	5.64	0.81	0.139	2.40	0.028	0.56
Tenebrio molitor (larvae)	<1000 IU/kg	0.24	0.81	4.07	0.81	0.157	2.62	0.030	0.47
Orthoptera									
Acheta domesticus (Adult)	<1000 IU/kg	0.04	3.41	3.84	0.23	1.50	2.30	0.017	5.37
Acheta domesticus (nymphs)	<1000 IU/kg	0.02	0.95	3.28	0.17	0.145	2.63	0.005	8.72
Blatta lateralis (nymphs)	<300 μg/kg	0.9	15.6	43.8	3.10	1.11	37	0.37	–

Continued

TABLE 23.4 Vitamin content of selected insect species (mg/100 g dry weight unless otherwise noted)—cont'd

Food insect	Retinol (A)	Thiamin (B1)	Riboflavin (B2)	Niacin	Pyridoxine (B6)	Folic acid	Pantothenic acid	Biotin	Cyanocobalamin (B12)
Isoptera									
Termes sp. Dried	–	0.03	6.07	5.9	–	–	–	–	–
Termes sp. Fried	–	0.14	3.79	9.81	–	–	–	–	–
Hymenoptera									
Oecophylla sp.	–	0.44	0.98	–	–	–	–	–	–
Vespa singulata (canned)	–	0.70	1.08	11.3	–	–	–	–	–
Diptera									
Chaoborus sp.	–	1.5	4.1	21.7	–	–	–	–	–
Hermetica illucens	<300 μg/kg	7.77	16.2	71	6.01	2.7	38.5	0.35	–
Musca domestica	<300 μg/kg	11.3	77.2	90.5	1.72	1.82	45.3	0.68	–

Data from Williams, J.P., Williams, J.R., Kirabo, A., Chester, D., Peterson, M., 2016. Chapter 3—Nutrient content and health benefits of insects. In: Dossey, A.T., Morales-Ramos, J.A., Rojas, M.G. (Eds.), Insects as Sustainable Food Ingredients. Academic Press, San Diego, CA.

TABLE 23.5 Fatty acid composition of selected insect species.

Food insect	C16:0	C18:0	Total SFA	C16:1	C18:1	Total MUFA	C18:2	C18:3	Total PUFA
Lepidoptera									
Smoked caterpillar *Nudaurelia oyemensis*	21.8	23.1	45.3	0.6	5.6	6.2	5.7	35.6	43.4
Smoked caterpillar *Imbrasia trúncala*	24.6	21.7	46.5	0.2	7.4	7.6	7.6	36.8	44.4
Smoked caterpillar *Imbrasia epimethea*	23.2	22.1	46.1	0.6	8.4	9.0	7.0	35.1	42.5
Caterpillar *Imbrasia ertli*	22.0	0.4	–	22.0	2.0	–	20.0	11.0	–
Waxworms, *Galleria mellonella*	79.6	3.4	–	5.1	124.0	–	15.2	1.1	–
Witchetty grub, *Xyleutes* sp.	29.4	3.1	32.5	–	67.1	67.1	0.4	–	0.4
Spent silkworm pupae, *Bombyx mori*	26.2	7.0	33.2	–	36.9	36.9	4.2	25.7	29.9
Tebo worms, *Chilecomadia moorei*	69.3	2.19	–	14.7	149	–	6.99	0.45	–
Coleoptera									
Mealworm (larvae), *Tenebrio molitor*	22.9	3.9	–	3.5	53. 9	–	34.8	1.4	–
Mealworm (adult), *Tenebrio molitor*	8.5	2.6	–	0.6	17.9	–	13.7	0.4	–
Palm worm (entire larvae), *Rhynchophorus phoenicis*	38.0	4.5	44.3	2.5	46.2	48.7	5.0	1.5	6.5
Superworms (larvae), *Zophobas morio*	52.8	12.6	–	0.7	66.0	–	32.9	1.1	–

Continued

TABLE 23.5 Fatty acid composition of selected insect species—cont'd

Food insect	C16:0	C18:0	Total SFA	C16:1	C18:1	Total MUFA	C18:2	C18:3	Total PUFA
Orthoptera									
Crickets (a1.21dult), *Acheta domesticus*	15.6	2.9	–	0.9	15.4	–	22.9	0.6	–
Crickets (nymph), *Acheta domesticus*	6.1	2.9	–	0.3	6.4	–	11.0	0.4	–
Turkestan cockroaches, *Blatta lateralis*	17.4	4.22	–	1.21	40.9	–	21.6	0.71	–
Isoptera									
Termites, *Macrotermes bellicosus*	46.54	–	46.7	2.09	12.84	14.9	34.42	3.85	38.3
Termites, mature alates, *Macrotermes subhyalinus*	33.0	1.4	–	33.0	9.5	–	43.1	3.0	–
Termites, boiled	28.0	8.5	37.8	3.4	48.0	51.4	9.5	1.4	10.9
Diptera									
Soldier Fly, *Hermetica illucens*	16.1	2.45	–	4.96	15.6	–	16.9	0.65	–
House Fly, *Musca domestica*	3.72	0.40	–	1.96	2.89	–	4.15	0.45	–

Data from Williams, J.P., Williams, J.R., Kirabo, A., Chester, D., Peterson, M., 2016. Chapter 3—Nutrient content and health benefits of insects. In: Dossey, A.T., Morales-Ramos, J.A., Rojas, M.G. (Eds.), Insects as Sustainable Food Ingredients. Academic Press, San Diego, CA.

is sclerotized in the exoskeleton, and they may not be readily available when consumed. Thus, further innovative technology should be involved to maximize the rate of extraction of amino acids from edible insects. In addition, Defoliart (1992) showed that the digestibility of insect proteins was higher than many plant-based proteins while slightly lower than that of animal protein materials.

Insects can provide some vitamins, but limited analysis data are available for insects. In general, because most of the vitamin A is concentrated in the eye of insect, which represents very low levels of vitamin A in whole body (Table 23.4). A study conducted by Feltwell and Rothschild (1974) analyzed the level of retinol of bees by using high-pressure liquid chromatography, and they found only low levels of vitamin A (850–930 μg retinol/kg) in adult bees and no retinol in honey bee pupae or larvae. Nevertheless, several species of lepidopteran larvae and termites contain significant quantities of preformed vitamin A. Regarding the data from Schabel (2010), bee was rich in vitamins A and D, and caterpillars were especially rich in Vitamins B1, B2, and B6. As for B-vitamins in insects, most of the data are reflected as the niacin, riboflavin, and thiamin (Table 23.4). It is reported that almost all insects contain substantial quantities of choline. Owing to part of the B-vitamins are not heat stable, insects undergoing boiling or roasting processing are likely to result in a degradation of these vitamins.

Regarding the composition of fatty acids, essential fatty acids such as oleic acid and linoleic acid are present with significant quantities in almost all insects (Table 23.5). However, there seems to be no consistence in fatty acid composition patterns across a variety of insect species. For example, it has been determined that high levels of palmitoleic acid (C16:1) and myristic acid (C14:0) existed in Diptera and Hemiptera, respectively, while linolenic acid (18:3) and linoleic acid (18:2) were virtually absent in *Dictyoptera* (Williams et al., 2016).

Apart from the insects collected in the wild, it should be emphasized that the nutrient composition of insects is also highly dependent on the feed materials. It has been reported that the nutrient compositions in mealworm larvae were different after being fed with diets containing organic wastes (Ramos-Elorduy et al., 2002). Therefore, the nutrient profile of insects industrially farmed with organic waste should be evaluated again. In addition to the nutrient aspects, the presence of potentially hazard ingredients such as toxics, allergens, microbial safety, and inorganic contamination should be investigated to ensure safe insect-based meat alternatives.

23.3.1.2 Risks of consumption

After reared in domesticated setting or being harvested in wild, insects can be killed by sun-drying, freeze-drying, or boiling. They can be processed and consumed in three ways including whole insects, ground or paste form, and as an extract of protein, fat, or chitin for food additives. Especially, insects

undergoing grinding (milling) or protein extraction are suitable for producing meat analogs.

In societies where consumers are not used to eat whole insects, paste or granular forms may be better accepted. For example, in Thailand and the Lao People's Democratic Republic, chili paste with ground *Lethocerus indicus* as a main ingredient is popular. In addition, isolating and extracting insect protein for food processing is carried out to satisfy the industry market and increase the acceptability of consumers. At present, the cost of protein extraction is expensive. However, as another nonmeat protein resource that can be adopted to process the insect-based meat alternatives, insect protein is still facing the challenges or issues that are related to food safety and consumer confidence.

(1) Microbial safety

The microorganisms including bacteria, fungi, protozoa, and others that are associated with insects can influence food safety. The present studies on microbial safety of edible insects mostly focus on traditional aspects of insect harvesting and consumption while seldom on deciphering the causal sources of infestation for insects. The fundamental solution for mitigating potential microbiological hazards is to allow greater control over hygienic aspects and safe feed sources for insects. The contamination of fungus on feeds used for insect farming is a particular safety issue. The most frequent fungal isolates are species of *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Phycomycetes* with mycotoxin production such as aflatoxins (Huis, 2013). Aflatoxins are stable, immunosuppressive, and cancer-causing mycotoxins that are associated with reduced weight gain and rapid death. The maximum safe level set by FAO is 20 μg/kg. A report from Mpuchane et al. (1996) indicated the levels of aflatoxins from *Imbrasia belina* in Botswana were varying from 0 to 50 μg/kg of insect-based product. Another research from Kachapulula et al. (2018) revealed that the average aflatoxin concentrations in dried moth *Gynanisa maja*, dried moth *Gonimbrasia zambesina*, and dried termite *Macrotermes falciger* were 11 μg/kg, 12 μg/kg, and 24 μg/kg, respectively. Meanwhile, when samples were subjected to the environment with high humidity, aflatoxins were increased significantly to unsafe levels in caterpillars (4800 μg/kg). Feeding with clean fodder, quickly drying after harvesting and processing, and storing the insect products in a cool and dry place are essential to maintain the best sanitary quality. Therefore, it is necessary to evaluate the hazard of human exposure to aflatoxins and other mycotoxins in insect-based meat analogs before consumption.

In case of the hazard of bacteria in insect-based foods, Banjo et al. (2006) found that three rhinoceros beetle species of the genus *Oryctus* including *O. monoceros*, *O. owariensis*, and *O. boas* can carry the *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Bacillus cereus*, which may pose a risk to consumers during consumption. Generally, insects undergo heating or boiling for a few minutes that can eliminate most of the pathogenic bacteria, but spores may survive during this process and germinate in the final

products at favorable conditions that may cause food spoilage. It is recommended that insects should undergo cooking or pasteurization procedure to render inactive or reduce their microbial content so as to maintain the microbial safety of products.

(2) Toxicity aspect

Some insect species are considered toxic; however, after taking precautionary measures, they can be eaten and prospected for industrial processing. For instance, the Tessaratomidae *Encosternum delegorguei* in South Africa and Zimbabwe can excrete a pungent fluid that can induce temporary blindness and severe pain if it contacts with the eyes. After the removal of fluid by squeezing the thorax and placing the bug in tepid water, the insect can be consumed. In addition, the caterpillars with hairs containing dangerous toxic substances must be burned off before consuming the bugs.

There are limited reports about the adverse reactions during insect consumption. In southwest Nigeria, a literature reported an ataxia syndrome that was related to the consumption of the seasonal silkworm *Anaphe venata*. Nevertheless, the reaction was most probably related to a structural undernourishment of thiamine deficiency in the consumers. On one hand, the species of edible insects have been artificially selected and farmed and most of the hazard bugs are naturally excluded out of the human diet. On the other hand, most toxic substances can be destroyed during the subsequent processing such as thermal treatment and extraction.

(3) Organic and inorganic contamination

The organic contamination is usually referred to the pesticide residues in edible insects. The wild harvested edible insects are particularly in great concern to the presence and accumulation of pesticide residues because the migration of insects is not controlled as well as feed on the pesticide-sprayed plant or crops (Imathiu, 2020). According to a survey from Thailand, despite to the disinfection procedure, the insects sold in the market still contained pesticide contaminant that was hazardous to human's health (Defoliart, 1999). Moreover, in order to control the pest, the locust in market in Kuwait was reported to contain the pesticides residue of organochlorine and organophosphorus with the content being as high as 49.2 μg/kg and 740.6 μg/kg, respectively (Saeed et al., 1993). Other literatures have also reported the pesticide contamination including metalaxyl (Gao et al., 2014), dioxins, polychlorinated biphenyls, and polyaromatic hydrocarbons (Poma et al., 2017) in edible insects. However, the chemical levels in edible insects are determined lower than those in common animal meat products. More importantly, with the standardization and controlled feeding of edible insect, it is possible to produce pesticide residue-free insect-based products.

Heavy metals are usually considered the inorganic toxicants from the environment including arsenic, cadmium, lead, and mercury, which are capable of toxicity at low levels of exposure. They have been found bioaccumulated in several insect body parts such as the fat, integument (exo-

skeleton), reproductive organs, and digestive tracts. Consumption of foods that are contaminated by heavy metals can induce acute and chronic disease in humans and animals. It has been shown that the growth phase and the feed substrate are the possible way of heavy metal accumulation in edible insects (Imathiu, 2020). Among these, cadmium is the greatest concern due to its potential to accumulate in the common edible insect such as yellow mealworm (larvae and adult) and black soldier fly (Van Der Fels-Klerx et al., 2018). Most studies point out that the wild collected insects may increase the risk of insect-based food safety; however, the challenge can be mitigated by controlled production, processing, and storage of edible insect.

(4) Allergies

Like most protein-containing foods, it is possible that some insects and insect-derived foods can induce the allergic reaction in sensitive persons. It is estimated that in Belgium, 19% of population are sensitized by skin prick tests prepared with grilled *Tenebrio molitor* and *Acheta domesticus* insects (Francis et al., 2019). It has been reported that insect food produced from mealworm, caterpillars, bee, grasshopper, cicada, silkworm, sago worms, locust, as well as the silkworm pupa in China and mopane caterpillars in Africa can cause allergic reactions (De Gier and Verhoeckx, 2018). Some types of proteins considered as allergens exist in edible insects including venom, arginine kinase, α-amylase, tropomyosin, and even fecal matter of insects (Murefu et al., 2019). In several studies, chitin existed in crustaceans is the potential allergen to cause food-related allergies (Muzzarelli, 2010). In addition, the insect-derived food additive carmine that is obtained from female cochineal insects is implicated in triggering an allergic reaction (De Gier and Verhoeckx, 2018).

23.3.2 Consumer acceptance and future research

It is safe to say that the negative perceptions surrounding insects are fully entrenched in Western societies. Studies carried out in some European countries revealed that only 19% of people were ready to eat insects as a protein source and only a limited people were likely to eat insects as alternative for meat (12.8% for males and 6.3% for females) (Verbeke, 2015). However, insects have long been a significant dietary food in the poorer regions of the world. From the nutritional point of view, common prejudice against eating insects is not justified. A more recent study revealed that consumers from Belgian could accept the processed edible insects other than eating whole insects (Megido et al., 2014). The factors influencing consumer consumption of edible insects include convenience, interest in the environment, and food neophobia (Gere et al., 2017).

In order to encourage insect farming, processing and eating, strategies can be developed to improve the added value of insect-based products that are more

attractive and competitive. Insects can be consumed indirectly through changes in edible form or added as a part of food ingredient, which offers an avenue to alter the perceptions of people for insect products (Megido et al., 2014). The insect-based meat alternatives, which are produced by insect powder or isolated insect proteins, have the potential promotion prospect to increase consumer willingness to eat insects. This should be encouraged especially disseminating and educating the consumers as well as the food manufacturers on the nutritional benefits of consuming edible insects. More importantly, the advantages such as the high nutritional value of insects and their low-risk nature, low environmental impact, and future palatability improvement may also contribute to a shift in the perception to consumers.

Insect-based meat alternatives are promising industries, but more research is needed. Further research should be centered on developing the process and render it applicable and profitable for meat industry use. Following are some aspects for future research of insect-based meat alternatives:

(1) Further research should be engaged in the means of the large-scale production of insects. The popularizing for farming insects, including species and strain collection and training in insect farming, is recommended to fit in the potential growth for insect-based meat analogs. As previously mentioned, insects harvested in wild may experience the challenges including pathogenic bacteria, pesticide residues, and heavy metals contamination. The characters of intensivism, the scale-production, and the management standardization of farming enterprises own the advantage to avoid these issues. Apart from these, farming the selected insects can exclude the toxic species of the bugs. Therefore, farmed insects will provide the clean and safety resources for the production of insect-based meat alternatives.

(2) At present, most of the researches related to insect allergy focus on the insect venom allergy and inhalation insect allergy while little effort toward allergenicity in terms of the safety of insect-based meat. Although eating and/or exposure to insects do not pose significant risk of causing allergenic reactions for the great majority of people, the mitigation measures are needed to safeguard consumer health. These measures include proper product labeling to inform consumers that foods contain edible insects or insect ingredients and develop innovative processing technologies such as ultrasound, irradiation, and others to destroy the allergenic components.

(3) Limited studies have focused on the production of insect-based meat alternatives. Similar to plant-based meat alternatives, the successful simulation for sensory, flavor, color, and meat-like structure to animal meat product is still the challenge to the insect-based product. The innovative processing technology including wet spinning process, electrospinning processes, and twin-screw extruding may be introduced and optimized for the production of insect-based meat analogs. In addition, seeking the proper flavor- or texture-enhancing, water-binding, and thickening agents to enhance the quality of insect-based products is still important.

(4) Lack of the strict regulation frameworks in and out of the countries is obviously one of the barriers to utilize edible insects to develop meat alternatives. Parts of the insect-based foods are currently considered novel foods based on Regulation (EU) No 2015/2283; however, none of the edible insect product had been included in the European Union novel food list until November 2018. Although substantial scientific research data could support the safety of novel food, the requirements of applications for the authorization of a novel food are stringent. Nevertheless, with more data being obtained in the support of edible insects as novel foods, the acceptance and the market prospect of insect-based meat alternatives are bound to change in the very near future.

23.4 Cell-cultured meat and future market opportunities

The term "cell-cultured meat" (also in vitro, lab-grown, or synthetic meat) refers to edible meat based on collecting cells from living animals and then produced in a bioreactor with cell engineering technology (Zhang et al., 2020). In contrast to conventional meat, this novel process can obtain meat without going through the process of raising livestock that promises to address public health issues, resource shortages, animal welfare ethics, environmental, and financial concerns (Stephens et al., 2018).

The idea of cell-cultured meat can be originally found in the book *Thoughts and Adventures* written by Winston Churchill in 1932 (Arshad et al., 2017). In the early 2000s, the National Aeronautics and Space Administration (NASA) first designed to investigate the cultured meat with the aim of long-term space flights and inhabitants of space stations (Benjaminson et al., 2002). The advances of cell and tissue engineering in medicine areas have paved the way for the research and production of cultured meat. With the financial support from Google co-founder Sergey Brin, Professor Mark Post of Maastricht University was the pioneer in the production of the world's first cultured beef burger in 2013 with the similar appearance to that of the conventional meat (Post, 2014). However, the time to produce a 5-oz meat patty in laboratory required about 3 months, and the cost was more than $330,000. At present, the limiting factors such as inefficient tissue culture technology and high costs block its commercialization and application and thus the cultured meat is remaining relatively nascent in their industry development. In addition, the sufficiently mimic taste, texture, and appearance to livestock meat, which are related to consumer acceptance is currently difficult to achieve at the present stage (Moritz et al., 2015).

23.4.1 Benefits and current developments

23.4.1.1 Benefits of cultured meat

In recent years, the concerns about animal welfare, environmental burden, and sustainable development have motivated the production of cultured meat for replacing the conventional animal meat. There are at least three benefits to promote the exploration of cultured meat to substitute the livestock meat production.

(1) Environment and economic aspect
In the conventional animal husbandry, the conversion rate to edible meat is low for meat production. A series of challenges are emerged with the considerable portion of greenhouse gas emission, water and energy consumption, and land usage in livestock meat production (Bhat et al., 2017). The contribution of livestock to the emission of the greenhouse gases of methane, carbon dioxide, and nitrous oxide is 39%, 9%, and 65%, respectively. However, compared to the traditional meat livestock system in Europe, it has been reported that cultured meat could reduce approximately 78%–96% of greenhouse gas emissions, 82%–96% of water usage, and 99% of land use (Tuomisto and Teixeira De Mattos, 2011). It can be concluded that cultured meat is potentially an eco-friendly and sustainable means in comparison to those of conventionally produced meat. Nevertheless, according to a report from Smetana et al. (2015), the cultured meat could have more environmental impact than plant-based proteins and chicken but less than beef due to the lack of resource-efficient and cost-effective methods. When considering the indirect costs and environmental benefits, the overall energy saving could tip in favor of cultured meat while retaining significant gains in land use. It can be prospected that cultured meat will reduce the energy requirements below those reported in literature once the technology is advanced adequately.

Although the precise economic value of cultured meat has not been estimated, the potential to obtain large number of cells from the donating animals gives rise to higher economy returns than traditional agriculture. Thus, owing to its potential profitability, cultured meat could provide an alternative option to intensive farming systems. On the other hand, when considering the amount of by-products in traditional meat industry, cultured meat provides a new opportunity to produce the customization of meat for processing or consumption rather than the whole carcass. Another advantage of culture meat is to create the own version of product for each producer (much like farmhouse cheesemakers and charcuterie producers). If the development in such way is realized, the majority of waste products from conventional livestock and cultured meat production can be cyclically utilized. The circular economy can be achieved by combining the traditional agriculture with new technologies (Stephens et al., 2018).

(2) Animal welfare

Another motivation for developing cultured meat is the concern about animal welfare. The general consensus agrees that animal suffering should be avoided during slaughtering. As a competitive meat production system, cultured meat can mitigate the slaughter of millions of animals while promising to supply meat (protein) in vitro to meet global demands (Bhat et al., 2017). Culturing muscle cell is based on the ability of proliferation of parent cell and thus live animal can be used as a source for the initial cells. This innovative technology does not involve animal slaughter and therefore it provides a promising approach to relieve animal suffering.

From the animal protection perspective, cultured meat could appeal to omnivores, vegetarians, and vegans in reducing meat intake (Hopkins and Dacey, 2008). As shown by Tonsor and Olynk (2011), even nonvegetarians have decreased the consumption of meat due to the concerns about animal welfare in public media. Therefore, the consumer behavior can be affected by public attitude toward animal welfare thereby prompting the meat industry to continuously explore new alternative practices including cultured meat in respond to that concern. It can be believed that people have the moral obligation to support the development of cultured meat. In addition, animal welfare groups are generally in favor of the production of cultured meat because cultured cells do not have nervous system and animals cannot feel pain (Van Der Weele, 2014).

(3) Health and safety

The present interest in food safety has been increasing by concerns about food related hazards on human health. With many negative reports about animal disease, epidemics, and antibiotic misuse, the public must increase the scrutiny of the conventional meat industry as well as the possibility to an increased interest in the potential benefits of cultured meat. The safety and quality of cultured meat during the production process can be guaranteed by using moderate concentrations of safe preservatives, applying online monitoring systems and standardizing production methods to protect the growing meat (Seman et al., 2008). Moreover, the quality of cultured meat can be further optimized through controlled culture system and processing technology.

23.4.1.2 Current developments for producing cultured meat

Cultured meat can be obtained from the cultivation of stem cells or myosatellite cells by using a bioreactor. The advantage of this innovative production is the ability to control differentiation and cell growth (Datar and Betti, 2010). The basic method to produce cultured meat is the scaffolding technique. After isolating the satellite cells or embryonic myoblasts from the adult skeletal muscle, these cells are then introduced into a bioreactor containing substances such as nutrients, growth factors, and energy sources. The stem cells can differentiate and fuse into myotubes, and then further differentiate into myofibers and grow on the preplaced scaffold. When the highest cell density is achieved, transgluta-

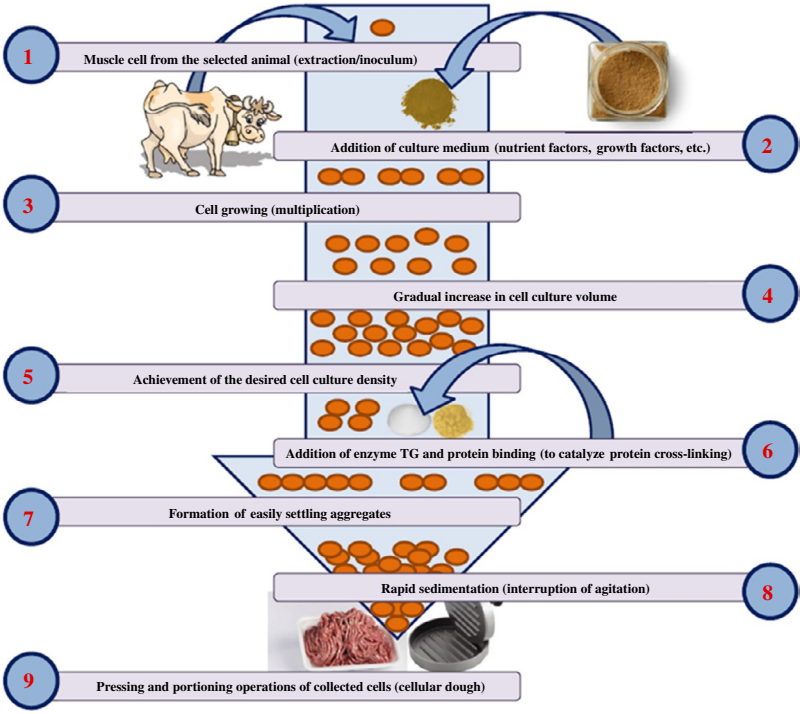

FIG. 23.10 Production flow chart of cultured meat (Alfieri, 2019).

minase enzyme and binding proteins (generally soybean proteins) are added to catalyze the cross-linking of animal and plant proteins in vitro. The aggregates are formed and can easily settle to the bottom through constantly stirring the culture solution. Finally, the collected cellular mass can be processed and consumed as a ground meat (Alfieri, 2019; Fig. 23.10).

Another alternative method to produce cultured meat is the self-organizing techniques by using explanted animal muscle tissues (Bhat et al., 2015). It has been reported that the highly structured meat could be obtained by the proliferation of existing muscle tissue in vitro. Benjaminson et al. (2002) successfully obtained the tissue pellets by culturing and centrifuging the minced red fish tissue in Petri dishes containing specific nutritive medium after 7 days. However, due to the lack of blood circulation in these explants, the tissue growth is very difficult and the necrotic cells are frequently detected. Currently, the recapitulation of the muscle-growing environment in bioreactor and the production in large-scale in the laboratory or factory are the main challenges for the cultured meat. Many reviews and studies have discussed in detail about the technical development and the challenges of producing cultured meat that are summarized as follows:

(1) Cell sources

The selection of the appropriate stem cell for animal tissue culture is one of the key challenges for cultured meat. During the last two decades, a greatly advanced research of identification, selection, and modification of stem cells has been achieved. At present, the cells commonly applied for tissue culture include four stem cell types:

(a) Primary cells or cell lines. After cell is isolated from the original animal tissue, genetic engineering, or chemical methods are induced to result in unlimited cell proliferation (Ramboer et al., 2014). Another option is to select spontaneous mutation cells that can proliferate infinitely and then culture the resulting population (Stephens et al., 2018). These immortalized cells can increase the speed of proliferation and differentiation while decrease the dependency on fresh tissue samples. However, the genetic instability and the phenotypic drift, together with the misidentification and continuous astatic evolution, are challenges during cell culture. Furthermore, it has been argued that these proliferated cells are not always similar to the primary cell and thus the harvested cells should be evaluated with caution (Stephens et al., 2018).

(b) Stem cells isolated from animal tissues. These cells generally include the muscle stem cells, embryonic stem cells (satellite cells or myosatellite cells), and mesenchymal stem cells. Myosatellite cells are the most relevant candidate cell sources and can differentiate into specific cells through biological, mechanical, or chemical stimulation during the process of proliferation (Post, 2014; Ding et al., 2018). Since 2010, due to the higher proliferation capacity and ability to grow in serum-free media, the mesenchymal stem cells have gained extensive attention (Stern-Straeter et al., 2014). Embryonic stem cells are reported to be an alternative starting source for cultured meat with their pluripotent capacity and infinite proliferation (Telugu et al., 2010). However, directing toward a muscle cell line is more difficult, and it has not yet been achieved from several species such as bovine and porcine. Moreover, whether the embryonic stem cell generated from those species can fully differentiate into myofibrils is still to be determined. On the other hand, the amplification ability of stem cells can be affected by the accumulation of mutations during the infinite proliferation process thus leading to the termination of cell growing or aging (Ding et al., 2017).

(c) Induced pluripotent stem cells (iPSC). iPSC are dedifferentiated cells, and the rendered pluripotent embryonic gene expression programs are driven by the transfection with a set of specific transcription factors to the somatic cell (Takahashi and Yamanaka, 2006). It should be stressed that, before incorporating iPSC into cultured meat production, the proliferative capacity must be improved as well as establishing the methods to guide the differentiation, dedifferentiation, and

trans-differentiation of iPSC to form myosatellite cells (Kadim et al., 2015). Recently, a relatively promising but challenging technology is proposed to generate safe iPSC without any genomic modification. This procedure is involved in reprogramming methods, variations of the reprogramming genes, and selecting the type of somatic cells for reprogramming (Zhang et al., 2020).

(2) Culture media

Serum-based media can provide nutrition and growth-promoting factor for a range of mammalian cell lines in vitro. Generally, fetal calf serum is added with a final concentration of 5%–20% in the medium. Cyanobacteria, which can be easily cultured for the necessary biomass, can be used as a potential food source for cell growth in meat culture (Arshad et al., 2017). Chicken embryo extract is also used as an additional nutrition to some culture media (Stephens et al., 2018). Moreover, the availability of attachment factors, trace elements, growth factors, hormones, amino acids, vitamins, and lipids are essential factors required for rapid cell growth. The cell species origin largely determines the direction of medium optimization. However, the risk of contamination with prions or viruses is the main challenge for the serum-based media. Particularly for long-term cultures, the common practice is to add antibiotic or antimitotic to cell cultures to prevent the infection. In addition, providing essential growth factors is important for the proper growth and development of cells in culture.

In recent years, the serum-free medium has been invented for the primary cultures as well as for mammalian cell lines (Zhang et al., 2020). A serum-free medium usually comprises of medium supplements and basal medium. Vitamins, glucose, amino acids, inorganic salts, and additional chemical components or growth factors are the essential factors for cell metabolism and growth in the basal medium (Brunner et al., 2010). The medium supplements can be generally divided into special and necessary supplements. The special factors are generally referred to the hormones, binding proteins, and adherent factors, while the necessary factors include the required substances that all cell lines grow in serum-free medium such as transferrin and insulin (Miki and Takagi, 2015). In practice, the serum is gradually replaced by growth factors or essential nutrients under which the cells could be adapted to the serum-free media. This operation is a promising method for the expected large-scale production of cultured meat under safe conditions. However, the current serum-free media still needs a time-consuming search for appropriate medium formulations, and it shows poorer performance in cell growth promotion. Nowadays, the synthetic biology and the computer-aided design have been proposed as efficient approaches to build chemically composed media.

(3) Scaffold in cultured meat

Appropriate scaffold design may promote the production of extracellular matrix molecules that comprise connective tissue or enhance matura-

tion and myofiber alignment, thus recapitulating the textural properties in cultured meat (Ma et al., 2021). It has been reported that nonanimal and animal-derived biomaterials are suitable for utilizing in scaffold manufacture. The majority of successful cultured meat is obtained from the scaffolds made by collagen. It was observed that the myogenic cells preferred to attach to the animal-derived materials because these materials were more closely similar with their natural physiological state (Snyman et al., 2013). To date, achieving self-tissue formation on synthetic biomaterial scaffold has been proven to be problematic. Therefore, food-grade animal or nonanimal derived biomaterials that are employed for the production of scaffold need to be further investigated.

Apart from the tissue growing on the scaffolds, the perfusion with 3D scaffolds has been adopted in tissue engineering as the alternative technique for cultured meat production (Specht et al., 2018). If the ultimate form of cultured meat is to mimic the large cuts rather than ground, the 3D scaffolding seems to be a reasonable choice to form a highly structured product. However, the heat and mass transfer efficiency is limited during perfusion due to the micro-porous structure of scaffolds. In most cases, the thickness of scaffold applied in tissue formation is less than a few millimeters and the perfusion velocity is around 1 cm/min or less. Recently, MacQueen et al. (2019) successfully obtained the tissue of rabbit skeletal muscle and bovine aortic smooth muscle with 2×3 cm area and 1.5 mm thickness through the cross-linked gelatin fiber scaffolds. Nevertheless, the current form of 3D scaffolding is unsuitable for large-scale application due to the inherent limitations of mass transfer and mixing. If there is a sufficient demand for the highly structured cell-cultured product, other techniques may emerge but substantial research is needed.

(4) Large-scale production systems

The aim of a large-scale cell production facility is to generate a large number of cells within a short period with the minimal handling and smallest possible amount of resources. For this purpose, bioreactors are required to achieve the large-scale cultivation of stem cells for foods. Generally, three alternatives can be applied to obtain high-density cultures in suspensions: (1) cultivation on microcarriers; (2) packed bed bioreactor, and (3) cultivation in aggregated cells (Moritz et al., 2015). The basic overview of the three large-scale production systems can be seen in Fig. 23.11.

Microcarriers are the beads where cells can adhere and grow by apposition. The beads (diameter 100–200 μm) that are made of polystyrene can float in the medium. To ensure anchor-dependent mixture of nutrients and gases as well as the myoblast cells, the suspended culture must be agitated by the rotation of bioreactor, gas flow, or impeller (Mered et al., 1980). The cultured tissues can be harvested from the microcarriers by intensive agitation. The current challenge for the microcarriers is searching for the edible or biodegradable materials. Ideally, the downstream separation step will be

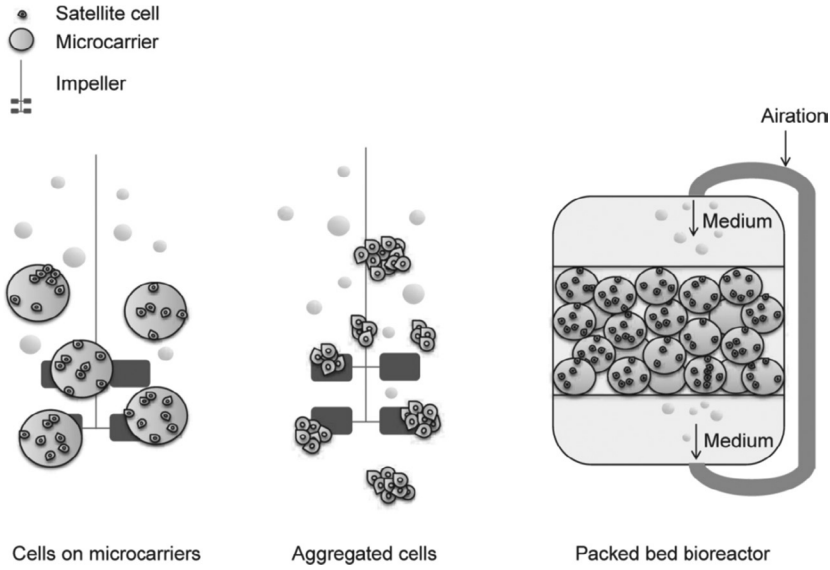

FIG. 23.11 Overview of three possible large-scale systems for cultured meat (Moritz et al., 2015).

eliminated because these materials can be integrated into the final product. Currently, the cross-linked pectin such as pectin-thiopropionylamide (PTP) and RGD-containing polypeptide such as thiolated cardosin A are the suitable materials for this purpose (Zhang et al., 2020).

Packed bed bioreactor (PBR) is another system that applies immobilized microcarriers with a flowing media in the bioreactor (Fig. 23.11). The advantage of this type of reactor is the evenly distribution of nutrients and gases around the beads through the radially across flow of growth medium. Another one is PBR that can serve as a scaffold and combine the following tissue development in one system. Finally, the culture medium can be recycled through the replenishment of utilized nutrients and the removal of waste products to reuse part of the medium (Liu et al., 2019). Therefore, the PBR system has shown a high efficiency in cell proliferation and differentiation. PBR has been proven to achieve high cell densities in mammalian cell culture but is not common for large volumes. Furthermore, the reuse of microcarriers without affecting cell growth is important such as the washed cytodex-3 that is reusable for mammalian cell culture (Wang and Ouyang, 1999).

As for the cell aggregates in suspension, the cell density, the composition of medium, the size of the aggregates, and the parameter of mixing through agitation are the important determinants of a successful culture. In general, a high initial cell density is preferable to the metabolic activities and assures the colonization of cells. Growth media formulation can change the properties of cell cultures. The addition of Rho-associated protein kinase-inhibitor

(ROCKi) is commonly used to increase the proliferation and inhibits apoptosis in cell aggregates (Watanabe et al., 2007). The large aggregates of cells can induce a heterogeneous size distribution. By adopting the passaging methods to collect the cell aggregates at regular intervals as well as changing the agitation rate of the medium, size could be contained to decrease the occurrence of large cells aggregates (Chen et al., 2012).

After cell is proliferated, a second bioreactor system is needed for cell differentiation and tissue generation. The common method is to add a scaffold wherein the cells can organize and mature, while another option may apply the microcarriers for further tissue development. No matter whether changing the medium and adding microcarriers, the cells could stay in the same bioreactor because the aggregated cells can attach to the microcarriers (Bradford et al., 2021). The advantages of cell aggregation on microcarriers are protecting cells from stress and decreasing the lag phase during bead-to-bead transfer, making it an option for the expansion to large-scale production (Kino-Oka et al., 2013).

(5) Anticipated texture or appearance in cultured meat

Currently, a small amount of cultured meat can be produced in a laboratory with the rapid development of stem cell for cultured meat, while the high cost and low market acceptance make it a long way for a real commercial production. The main reason is that the current cultured meat products do not simulate the color, nutrition, aroma, and taste of real meat. The present development for synthetic essential nutrient additives that are applied for cultured meat has been systematically reviewed by Zhang et al. (2020). The commercial food additives, enzymes, and other compounds that have been designed by synthetic biology and produced by the microorganisms lay the foundation to endow the artificial meat unique textural and flavor properties of real meat.

23.4.2 Attitudes and acceptance of cell-cultured meat

The current social issues so far related to cultured meat are the ethics and consumer acceptance. Existing studies have been published from Finland (Vinnari and Tapio, 2009), Netherlands (Van Der Weele and Driessen, 2013), Belgium, Portugal, and the United Kingdom (Verbeke et al., 2015), China, Ethiopians, and Dutch (Bekker et al., 2017), Italy (Mancini and Antonioli, 2019), Germany (DuPont and Fiebelkorn, 2020), and other selected countries including the Australia, France, South Africa, Mexico, Sweden, Spain, and the United States (Siegrist and Hartmann, 2020). However, it is too early to accurately assess the readiness with which consumers will accept cultured meat products. The real challenge will be emerged when cultured meat products are available on the market.

With respect to the ethics of cell-cultured meat, the academic literature especially adopting a philosophically orientated approach generally reports positive arguments for cultured meat. For instance, the development of cultured meat should be supported from both rights-based and utilitarian viewpoints (Pluhar, 2010). Schaefer and Savulescu (2014) argued that, even from vegetarian perspec-

tives, the development of cultured meat was permissible and worth promoting. In addition, Chauvet (2018) believed that producing cultured meat did not violate the dignity of animals. In summary, the evidences of animal welfare and environmental benefits for a successful cultured meat system can evoke an explicit supportive attitude toward the artificial meat. Nevertheless, some authors have adopted negative positions from different perspectives. Cole and Morgan (2013) argued that cultured meat continued the existing fetishization of meat from a critical animal study perspective. Meanwhile, a nonmeat-eating consumer can feel nonguilt at the expense of the less well-off due to the high cost of cultured meat.

A second important area is the attitude of the public to cultured meat. Those in the field sometimes expressed as the acceptance of consumer and the likely purchasing decisions. Moreover, the ambivalences and uncertainties about the societal impact of cultured meat and the broader political and personal convictions are also the wider scope of this issue (Stephens et al., 2018). Although the present studies on perceptions of cultured meat vary in methodology, many commonalities are found in different opinions from the negative to the supportive. For example, according to the comments on news articles and the social media, the unnaturalness of cultured meat can be recognized as a challenge for the acceptance of customer (Laestadius, 2015). Lack of familiarity may be responsible for many negative perceptions. Some evidences suggested that the acceptance was increased with the familiarity to cultured meat (Bekker et al., 2017). Studies have shown that (1) anchoring cultured meat to more familiar concept of technologies (such as genetically modified organisms and cloning) and (2) defining cultured meat in terms of its differences and similarities to conventional meat are beneficial for the commercial success of cultured meat. Conversely, the ambivalence of participants will appear when revealing dilemmas for cultured meat, weighing up the costs and benefits, analyzing the pragmatic reasons, and reflecting on the process of public acculturation to new technologies. However, a study conducted by Siegrist et al. (2018) argued that cultured meat with nontechnical description emphasizing the final product rather than the production methods could cause a significantly higher acceptance rate of this novel food.

Meanwhile, the acceptance of cultured meat is sensitive to the additional provided information even not related to the cultured meat. Comparing to the groups just providing basic information, when given additional information about the benefits for the public health and environment, the participants were reported to have the willingness to purchase and pay more for cultured meat (Verbeke et al., 2015). Assuming the lower price and the higher prospective market share of cultured meat, the preference for cultured meat could be significantly higher than conventional animal meat (Slade, 2018).

Food safety and healthiness aspects are other common concerns related to cultured meat. The proliferation of cells may cause the instability of genes and lead to cancer cells. Although these cancer cells will be inactivated before consumption and then digested by intestines; however, this is still a sensitive issue for consumers. In addition, a survey revealed that the participants

generally believed that conventional meat would be healthier than cultured meat. Nevertheless, when considering its lower fat content, some consumers were open to perceiving health benefits of cultured meat (Verbeke et al., 2015).

Finally, it should be pointed out that, the existing studies that aim to analyze the public perception and consumer acceptance for cultured meat are still not enough. More investigation and survey are needed and new conclusions might be obtained. With the pace of technological innovation, the potential change for perceptions should be recognized.

23.5 Trends in organic meat products

Organic farming is an emerging area for crop and livestock production that has attracted attention from all over the world. The large progress in organic agriculture has been made in developed countries; however, in some developing countries, especially the Asian and Africa countries, it is still staying in the stage of conception. Fortunately, some Asian countries have begun to focus and be engaged in the development of organic animal products.

Organic farming is a production system largely excluding or even avoiding the synthetic fertilizers and pesticides to maintain the agricultural productivity (Lampkin, 1990). In contrast, although conventional farming systems are able to furnish low-cost food and productive, they are heavily dependent on the use of synthetic pesticides and fertilizers that bring a variety of environmental effects such as soil erosion, water depletion, pesticide pollution and biodiversity reduction. Therefore, the sustainability of modern agrochemically based agriculture is increasingly questioned by farmers, scientists, and the public (Epule, 2019). Comparing to the organic crops, organic meat is referred to meat obtained from animals raising in organic systems. It is based on the natural growth of the animals with being allowed to exercise and grow in open spaces/outdoors, and the health and vitality being maintained through the proper nutritional management. Another sustainable meat system such as grass-fed meat and natural meat is normally considered healthy and rich in nutrients. A comparison of conventional meat, organic meat, natural meat, and grass-fed meat in different parameters is shown in Table 23.6.

23.5.1 Production system for organic meat and meat products

In order to be sold as organic products in the countries, all agricultural products including imported and domestic meat products must comply with some regulations. According to the successful implemented acts and regulations from the United States Department of Agriculture (USDA) such as the National Organic Program (NOP), Section 7 of the Code of Federal Regulations (CFR), Part 205 (NOP Final Rule), and the Organic Foods Production Act of 1990, any livestock that is to be slaughtered and sold or labeled as organically produced shall be

TABLE 23.6 Characteristics of different types of meat.

	Conventional meat	Organic meat	Natural meat	Grass fed meat
Usage of hormones	Yes	No	Vary	Vary
Antibiotics	Yes	No	Vary	Vary
Feed grown with chemical pesticides and fertilizers	Yes	No	Vary	Vary
Necessary to allow the cattle to graze on pastures?	No	Yes	No standards (normally farmers allow cattle to graze)	No standards (normally farmers allow cattle to graze)
Animal confinement	Yes	No (outdoor access not necessary, if weather is not favorable)	No standards (normally farmers provide outdoor access)	No standards (normally farmers provide outdoor access)
Animal by-products feed	Yes	No	No standards (normally animal by-products are not fed)	Vary
Presence of GMOs in feed	Yes	No	No	Vary
Meat irradiation carried out	Vary	No	No	Vary
Usage of preservatives	Vary	No	No	Vary

Data from: What is Organic Meat, https://www.organicfacts.net/organic-products/organic-food/organic-meat.html.

raised using organic management practices and that organically raised livestock must be separated from their conventional counterparts.

Like other organic products, organic meat must be:

(a) Produced without sewage sludge, ionizing radiation, or genetic engineering.

(b) Managed in a manner that conserves biodiversity and natural resources.
(c) Raised according to the National List of Allowed and Prohibited Substances.
(d) Meeting all USDA organic regulations, overseen by a USDA National Organic Program authorized certifying agent.

For this purpose, organic producers and handlers must follow some common practice and adopt the organic livestock production system to ensure the integrity and operation sustainability of organic meat. The introduction of quoting from USDA regulations (the NOP, Section 7 of the CFR, Part 205 and the Organic Foods Production Act of 1990) for the production system of organic meat and meat products are as follows:

23.5.1.1 Organic livestock production practice

(1) Livestock living conditions and facilities
Organic livestock producers should provide living areas that encourage the health and natural behavior of animals (Vukina et al., 2014). Organic practices reflect the concerns for animal welfare and a desire to balance productivity with both animal welfare and environmental quality. Organic livestock must have access to outdoor areas, shade, shelter, space for exercise, fresh air, clean drinking water, and direct sunlight. Livestock shelters should give animal protection from extreme temperatures, adequate air circulation and ventilation, and space to exercise. The shelters are usually climate-controlled and are energy-intensive; however, sources of renewable energy such as solar heating and natural ventilation can reduce energy from fossil fuels.

(2) Grazing
Organic producers must give ruminant animals (cattle, sheep, and goats) access to pasture during the grazing season. Livestock should not be continuously confined, while temporary confinement is allowed under specific circumstances, mostly regarding the health and safety of the animal. By providing access to the outdoors, organic livestock producers convert forage, legumes, and grasses into meat, milk, wool, and other products. Grazing livestock also provides producers with manure, a very important source of fertility in organic farming systems, and an excellent means of recycling nutrients. Rotational grazing may improve forage quantity and quality while preventing overgrazing (Oliveira et al., 2018).

(3) Animal health
Organic animal health, like organic crop health, relies on preventative practices and systems. Good genetics are important as organic livestock producers should select breeds that are well adapted to their particular environment. Balanced nutrition, exercise, and a low-stress environment also contribute to building strong immune systems in animals. Vaccination and other preventative measures are common and antibiot-

ics and growth hormones are prohibited (Iannetti et al., 2020). Organic livestock producers work to manage exposure to disease and parasites through grazing management, proper sanitation, and preventing the introduction of disease agents.

(4) Organic feed

The type of feed has an important role in sustainability and organic livestock must eat certified organic feed which must be grown and processed by certified organic operations (Fanatico et al., 2016). Similarly, any pastures, forages, and plant-based bedding (such as hay) accessible to livestock must be certified as organically grown and processed. In practice, manure from the livestock can be used to fertilize feed crops, especially when raised on the same farm as the animals. Or livestock can be fed byproducts such as fruit pulp or cull crops to increase nutrient cycling. Insects raised on crop and food residues can also provide sustainable feeds for the animals. Certain additives such as vitamins and minerals that are not produced organically can be fed to organic livestock in trace amounts, but others including hormones used to promote growth are strictly prohibited.

(5) Animal origin

Organic livestock generally must be raised organically since the last third of gestation. Birds used for poultry or egg production may come from any source, but they must be raised organically beginning the second day of life. In addition, a variety of hybrids and breeds including standard breeds helps increase biodiversity and can be bred to adapt to the local conditions.

23.5.1.2 Organic livestock product processing practices

(1) Organic ingredients

Under USDA organic regulations, organic processors must use certified organic ingredients (for a minimum of 95% of the product) and only approved nonorganic ingredient in products that are labeled organic. Products labeled as made with organic specified ingredients may include up to 30% nonorganic agricultural ingredients, but all other additives must be approved for organic use. No ingredients or products may be produced using genetic engineering, sewage sludge, or ionizing radiation.

(2) Commingling and contact

To preserve the integrity of organic ingredients and products, organic processors must prevent commingling (i.e., mixing) with nonorganic ingredients and products throughout processing. Moreover, the contact between organic ingredients and nonorganic substances including prohibited sanitizers must also be prevented. Finally, when changing from nonorganic to organic products, the processing equipment must be cleaned and sanitized. The processors shall run organic meat products first after their cleaning with approved materials.

(3) Energy consumptions

Processing and slaughter processes tend to use a lot of energy and water. Increasingly, processing facilities should focus efforts on renewable energy and energy efficiency to reduce the impact on the environment. Waste from production or processing can be integrated in sustainable systems such as composting for organic matter.

(4) Managing pests
Similar to pest management on organic farms, organic processing facilities must emphasize prevention over treatment. Organic processors may use approved synthetic substances if all other approaches have failed but must ensure that these substances do not come in contact with the organic products they handle.

23.5.1.3 Organic livestock verification and certification

The organic meat must be verified, certificated, and labeled in line with various standards developed from the government. According to the USDA National Organic Program, organic certification is designed to certify every step of the organic chain in strict accordance with the guidelines. The job of certifying agents is to verify that organic integrity is maintained.

23.5.2 Quality difference between organic and conventional meat products

Over the last 20 years, the demand for organic meat products has increased steadily. The major reason for the increase is the perception of consumers tending to believe that organic meat products are healthier due to its content in higher level nutritionally compounds (Dangour et al., 2009). Most of the studies tend to support that food including meat under the organic production standards could result in positive changes in food nutrition and quality (Średnicka-Tober et al., 2016). However, some research considered that, compared to conventional meat products, there still has scientific uncertainty over whether and to what extent to the quality changes in organic meat (Smith-Spangler et al., 2012). The fact to reduce the probability of exposure to antibiotic-resistant bacteria and pesticide residues, livestock under organic production systems are only fed with grass and organic feeds that are free of animal by-products or pesticides (Fossler et al., 2004). A radical view from Rosen (2010) claimed that any consumers who bought organic food due to the trust that it contained more healthful nutrients than conventional food were wasting their money. Through comparing the data from critical literature, the author find that some organic meat proponents intentionally omit data that do not support their views and stretch the truth of benefit of organic meat.

When nutrition and manage method are similar and adequate, animals under organic and conventional production system can be expected in the similar quality performance. The only thing that could be different to conventional reared system is the better management of preslaughter for organically raised livestock

due to the more outdoor activity and diverse stimuli during life. Evidences of the lower final pH value and slower pH decline in organically reared Krškopolje pigs muscle indicated the higher resistant to preslaughter handling and the more glycolytic potential of these pigs (Tomažin et al., 2019). As the nature of pH decline is related to color parameters and water holding capacity in meat, the water holding capacity in organic pigs is slightly lower, while the objective color parameters are higher than conventional pigs (Huff-Lonergan and Lonergan, 2005). Meanwhile, Olsson et al. (2003) believed that though the alternative system resulted in satisfactory meat quality, some of the technological quality problems may appear such as the increased shear force and the decreased water holding capacity. The influence of outdoor rearing and organic production system on meat quality is also controversial. In addition, Enfält et al. (1997) reported a higher drip and lower final pH and tenderness in organic pig meat. Nevertheless, the defects of technological meat quality are of little significance to eating quality in some cases, therefore the consumers generally cannot discriminate between conventional and organic meat (Jonsäll et al., 2000).

As for the chemical and nutritional analyses of muscle, livestock reared in organic production system can obtain a higher protein content and higher degree of unsaturated fatty acid (Olsson et al., 2003). Although a high content of unsaturated fatty acid in meat is beneficial for human health, it may induce the potential for higher level lipid oxidation during storage with negative effects on organoleptic quality. With respect to the vitamin content in meats, early studies found no difference between organic and conventional meat such as vitamin E in pork (Hansen et al., 2006) and vitamin A and vitamin E in beef (Walshe et al., 2006). However, Tomažin et al. (2019) recently reported a lower vitamin E and a higher vitamin A concentration in backfat of organic reared pigs. The former is mainly related to the antioxidant protection of unsaturated fatty acids, while the latter can be ascribed to the high level of carotenes in feed which are converted to vitamin A in the pig intestine and absorbed. In summary, regardless of the rearing system, the diet, the breed, the sex, and the level of adiposity are the contributing factors mainly influencing nutrition composition of muscle tissues.

Finally, it should be mentioned that animals reared in organic system are still in the risk of contamination with pathogenic microorganisms. After comparing the literature on organic and conventional animal products, Smith-Spangler et al. (2012) found no difference in the probability of *Salmonella* and *Campylobacter* contamination from meat products. However, owing to the using animal waste for fertilization during growing of feed plant, organic meat products had a higher risk for contamination with *Escherichia coli* (Mukherjee et al., 2007). These findings need to be further confirmed with additional research.

23.5.3 Future prospects for organic meat products

Despite the current trend toward the reduction of meat consumption, the growing concern for animal welfare, environmental sustainability, and human health

indicates a promising future prospect for organic livestock production. To a large extent, the future of organic meat products will depend on the consumer demand. Therefore, many papers have attempted to identify the determinants for the organic meat consumption in the last three decades. It has reached a consensus that labeling, information, taste, product design, packaging and design, and attitudes of consumers are related to organic meat consumption (Schleenbecker and Hamm, 2013). In addition, the use of health claims and animal-welfare can increase the competitive power for organic meat. For instance, when informing that bacon is made from animal friendly meat, consumers are willing to pay their price premium for organic products (Akaichi et al., 2019). This premium is between 15% and 200% for organic meat and about 30% for natural meat. Therefore, the development of organic meat products is not only dependent on the production systems but also on the perception of organic foods from consumers. The former can guarantee the production of qualified organic products, while the latter is mostly related to the proper marketing for organic meat products.

Nevertheless, it should be mentioned that nonorganic meat is not lower to organic meat on all aspects of animal welfare. The tendency of reducing the application of veterinary drug in organic systems means that the sick or injured livestock may compromise for health-related aspects of welfare. Therefore, if aspiring to expand the market for organic meat, the organic meat products should provide the additional information to buyers. For example, sellers can explain the exact measures that are taken to be more friendly to animals (more space or more outdoor access) on the product's package. This information is expected to make the claim of animal welfare less misleading and more consistent.

All in all, the future of organic meat consumption could be improved not only by promoting its advantages but also by selling its superiority in terms of environment benefit and sustainability. Therefore, if the marketers and producers of organic meat products want to improve its demand as well as its competitive power, they should fully realize the potential of the products and be ready to exploit all its advantages.

23.5.4 Anticipated markets

The organic livestock has gained importance in recent years. The increasing consumer preference and the awareness for health and environmental benefits associated with the consumption of organic products objectively promote the ever-increasing demand for these products (Siegrist and Hartmann, 2019). Though organic meat products currently only take a small part of entire meat industry around the world, the increasing demand has attracted the attention of everyone. Since reports of United States and Canadian livestock being affected by bovine spongiform encephalopathy in 2003, the sales of organic beef have increased dramatically, nearly doubling each year. Recently, owing to the COVID-19 driven consumer awareness about health and nutrition, the coronavi-

rus pandemic has led to a surge in the demand for organic and sustainable foods. However, the challenge is to maintain the supply with such a surge in demand. Thus, the organic meat product market is prone to supply-demand swings and the market growth is not as significant as expected. The market in global is expected to reach $20.39 billion in 2023 (Company, 2020). Europe is the largest region in the global organic meat products market in 2019. The production of organic meat has grown by 42.96% over the last 6 years, while the production of organic lamb meat has increased by 11.81% (Rabadán et al., 2020). In addition, the Asian Pacific is expected to be the fastest-growing region in the forecast period.

However, the relatively higher price of organic meat products is likely to hinder the growth of the market. The organic meat production process is labor-intensive and time-consuming. The nonuse of any antibiotics, growth hormones, or synthetic chemicals in an organic system leads to higher prices involved in raising the livestock. Moreover, the organic meat to a certain extent is an "on-demand" product, which are usually imposed the price premiums by producers to gain the competitive advantage. However, due to the higher price of organic meat as well as the decreased affordability of products, the market growth may be hindered by the price competition (Hermansen et al., 2014).

23.6 Pandemic planning for the meat industry

The emergence of novel infectious diseases such as severe acute respiratory syndrome (SARS), pandemic influenza, HIV/AIDS, and COVID-19 (SARS-CoV-2) has shown the vulnerability of human beings and animals to new zoonotic health threats. The rapid pandemic spread of SARS coronavirus in 2003, a new triple-reassortant H1N1 influenza in 2009, and the outbreak of the novel coronavirus COVID-19 in 2019 resulted in substantial economic loss as well as in some instances the lockdown of the global travel and trade networks. These vulnerabilities emphasize the need for a systematic and preemptive planning that aims to prevent the initial emergence and spread of the pandemics in order to protect the production and trade of animal products for food and other uses (Morse et al., 2012).

Zoonoses are infectious diseases caused by bacterial, viral, parasitic, or unconventional agents that can jump from a nonhuman animal to humans. They can be spread to humans through direct contact or through food, water, or an intermediate host like insects. Some zoonoses, such as Ebola virus disease and salmonellosis, can cause recurring disease outbreaks. Other diseases such as HIV begin as a zoonosis but later mutate into human-only strains. Recently, the COVID-19 has caused the global pandemics among human beings. Due to the close relationship between human and animals in the natural environment and in agriculture, the emerging zoonoses represent a major public health challenge around the world. In addition, with the strengthening of quarantine of imported food in various countries, the outbreak of infectious diseases in local areas will

also lead to the economic loss of exporter and the disruption of animal product supply chains.

The recent outbreak of human coronavirus disease COVID-19 pandemic has generated massive disruptions of social and economic life globally (Reina, 2020). Until now, apparent short-run impacts on initial food supply chains by the pandemic have appeared. During the early stages, food supply chains are subjected to a set of exogenous demand and supply shocks as various countries adopted lockdowns to slow the spread of the virus. One view holds that the large firms are likely to be affected by short-run shocks. However, some regional and local food suppliers are flexible and able to turn to new buyers and market channels during the pandemic (Thilmany et al., 2021). Nevertheless, it is hard to prospect the potential implications of pandemic in longer-term for agro-food supply chains and the uncertainties of pandemic such as the duration and severity may cause the certain negative effects for food businesses. The other major uncertainty for food supply chains is the resurgence of COVID-19 infections and the subsequent renewed lockdowns and regulatory interventions in some areas. The timing and the duration of these measures remain affecting the stabilization of food supply chains. Therefore, the digital technology such as contactless electronic transactions and blockchain technology within food supply chains may be accelerated as a result of the renewed attention to supply chain during the pandemic (Hobbs, 2021; Zhao et al., 2019). Moreover, the application of blockchain in the pandemic can improve the logistics and collaboration of supply chain in the face of disruption. However, this technology is difficult to address other potential supply chain disruptions such as labor shortages.

Apart from these, other strategies and planning that can induce positive effects for socioeconomic, ethical, environmental, and food supply chains during the pandemic disease are raised as follows:

(1) Limitation to wildlife trade. The most profitable illegal activities of trafficking of wildlife within countries and across borders are widespread and its annual value is estimated over 20 billion USD (Van Uhm and Wong, 2019). However, the fact that the frequent transmission of viruses from wildlife to humans makes the consequences of wildlife trade be undesirable and harmful (Cucinotta and Vanelli, 2020). The illegal smuggled animals are not usually tested for pathogens in advance and the following transport may favor and spread the infection of pathogens. After analyzing numerous cases of transmission of zoonotic viruses to humans, most of the species related to zoonosis revolving the wildlife trade are chordates (approximately 18,203 species) and birds (Halabowski and Rzymski, 2021).

Therefore, identifying all animal species (from terrestrial mammals to other terrestrial vertebrates and aquatic animals) traded in illegal markets around the world is the priority affair. All possible pathogens (especially viruses) associated with these species should be identified and then assessed their transmission ability to humans. In addition, it is necessary to

study and evaluate the sanitary transport of wildlife. Finally, the ultimate goal is to take actions to limit or ban the trade in wild animals in terms of possible transmission of viruses. In addition, the protection of endangered and rare animals is also the side goals in these actions.

(2) Decreasing hunting activities. Hunting is a significant factor that is associated with a higher risk of transmission of zoonotic pathogens to humans as well as with the decrease of biodiversity (Johnson et al., 2020). The intense hunting of wild animals is also one of the reasons for the rampant illegal wild animal trade. It has been revealed that the legal and illegal hunting is the major way for the spread of pathogens (especially viruses) due to the close contact between people and the hunted animals (Kreuder Johnson et al., 2015). The contact activities with potentially infected animals include tracking, capturing, carrying, wounds from contact with wild animals, handling, and transporting slaughtered animals. Among these, the highest risk is the slaughtering of hunted animals, where it may contact directly with blood and result in infection. Moreover, once the virus from wild animals enters human cells, the evolution of a new strain of virus may appear and therefore lead to its wide spread in the human population (Wolfe et al., 2004).

In view of the current health and economic situation in various countries, the priority measurement to prevent the pandemic is the detection of pathogens (especially viruses) in hunted animals. In addition, the assessment of the potential transmission of pathogens to humans is also essential to human health. The next future step will be to limit or prohibit legal or illegal animal hunting (Halabowski and Rzymski, 2021). However, as for indigenous peoples in some countries, hunting wildlife is essential activity for their survival. Therefore, in order to minimize the potential risk of virus transmission to humans, all steps should be taken to compensate the possible losses of these people due to the implementation of bans (Wolfe et al., 2007).

(3) Changes to meat production. Meat and meat products represent one of the significant vehicles for food-borne diseases. Although the risk of transmission during the production process has been positively decreased by the strengthened sanitary regulation, the significant health issue is still possibly posed to certain meat-borne pathogens. For instance, the virus diseases such as avian influenza, African swine fever, and SARS, prion diseases such as variant Creutzfeldt-Jakob disease, and bacterial contamination such as *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter* spp., and *Yersinia enterocolitica*. Contrary to wild meat, meat from the farm system is generally not considered as the source of viral pathogens of humans. However, it is estimated that zoonotic viruses may be passed to the public and lead to the raised potential risk of an epidemic through veterinarians and workers exposed routinely to livestock (Rock, 2017). Therefore, the recent outbreaks of zoonosis and global COVID-19 pandemic are the reminder that the unexpected emergence and transmission of

viral disease in livestock can easily destabilize the meat production chains. As for the COVID-19 pandemic, the infection among workers in meat industry and there from the market disruptions caused by the reduced operation or closures of meat processing plants are responsible for the decreased consumption of meat (Middleton et al., 2020). Moreover, the closure of restaurants and schools is also a contributing factor (Attwood and Hajat, 2020). Nevertheless, a recent report identified that COVID-19 virus could be spread from frozen meat and packaging surface of seafood (Liu et al., 2020), which is likely to change the dietary choices of some individuals regarding meat originating from livestock farming.

Considering the trends in meat demand and the forecasted steady population increase, the limitation of meat consumption is impractical to control future zoonotic pandemic. Therefore, the pursuing of research and development for alternative meat products is important in the future. The greatest hopes for solving the associated problems rest on the above-mentioned plant-based meat, insect-based meat, and cell-cultured meat. In addition, adopting eco-friendly production systems such as organic livestock farming system is also beneficial for reducing the potential risk of pandemic outbreaks.

(4) Temporary relaxation of regulations. In order to help meat processing plants in face of the unprecedented disruption induced by COVID-19 and the recovery of food supply chains, the temporary relaxation of regulations in some countries has been proposed and implemented. Although relaxing these measures related to food safety indeed assist in boosting food or meat supply in the early stages of the pandemic, the downside risks including the increased infection of COVID-19 for employees in meat processing plants and may result in a weakening of consumer confidence on meat safety (Kecinski et al., 2020). More importantly, a new evidence indicated that COVID-19 from seafood packaging surface could re-infect humans and possibly cause the outbreaks through cold-chain transportation (Liu et al., 2020). Fundamentally, food safety regulations are aimed to protect firms and consumers from any relaxation of the rule risks undermining the development of meat supply chains. Therefore, in the long run, the strict regulatory environment during pandemic is essential for maintaining the adaptability and sound development of meat supply chains in future.

Finally, an important finding from the pandemic is the interdependencies of the meat supply chains. The effective communication and the higher degree of collaboration are an advantage of the supply chains and tend to be more adaptable in the face of uncertainty. After the end of COVID-19 pandemic, meat industry should be better prepared to deal with major disruptions of supply chains when identifying the main risk source of supply chain and enacting strategies to mitigate these risks.

23.7 Conclusion and remarks

Meat and meat products are rich in many nutrients. The future enhancement of meat quality and composition such as increasing protein content while reducing fatness should be engaged in a manner that does not deteriorate the texture, taste, and appearance of meat. It is estimated that the consumption of red meat will increase over the next 2–3 decades in developing countries; however; the processing, production; and consumption patterns of red meat in the developed countries may be changed due to the increasing environmental awareness of the people. The current trends indicate that the future consumption of meat and meat products will be partly replaced by the plant-based, insect-based; and cultured meat to decrease the risk of disease deriving from meat consumption or to improve the health status of humans. In addition, the demand for reduced environmental footprint and improved animal welfare during meat production will be increased with the gradual application of newly or organic livestock farming systems. The current COVID-19 pandemic reminds us that a systematic and preemptive planning that aims to prevent the initial emergence and spread of the pandemics is essential to protect the production and trade of animal products. Nevertheless, innovating meat processing methods and developing meat alternatives are better ways to cope with the disruption of supply chain in case of the outbreak of pandemic diseases. More importantly, except for the acceptability, consistent quality, reliable production, and cost-effectiveness toward the newly products, the safety of meat analogs or alternatives is the top priority to be considered. Therefore, the regulatory frameworks need to be developed along with promoting the health development of future meat market.

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Index

Note: Page numbers followed by *f* indicate figures and *t* indicate tables.

A

Abnormal postmortem metabolism, 173–176
  acid meat, 175
  dark, firm, dry meat (DFD), 176
  pale, soft, exudative meat (PSE), 173–175
Acceptable daily intake (ADI), 606
Acidosis, 758–759
Adenosine diphosphate (ADP), 160
Adenosine monophosphate (AMP), 163–164
Adenosine monophosphate deaminase (AMPD), 163
Adenosine triphosphate (ATP), 159
Adipose tissue, 35–36, 52–53, 86–88, 118–119, 292*f*, 672
Aging, proteolysis, 186–189
  calpain system, 187
  caspase system, 188, 226
  cathepsin, proteasome systems, 189
AGPs. *See* Antibiotic growth promoters (AGPs)
Aminoacetonitrile derivative (AAD), 596
Amino acids, 668
Aminoglycosides, 592–593
Ammonia toxicity, 759–760
AMP-activated protein kinase (AMPK), 147
Amphenicols, 593
Anabolic steroids, 598
Angiotensin 1-converting enzyme (ACE) inhibitors, 678
Animal
  blood, 711–712
  brain, 710–711
  by-products, 707, 708*f*, 709*t*
  heart, 710
  kidney, 710
  large intestines, 711
  liver, 708
  pancreas/stomach sweetbread, 710
  skin, 712
  spleen, 712
  sweetbreads, 710
  thymus/neck sweetbread, 710
  tongue, 710
  tripe, 711
  welfare, 11–12, 12*t*, 14–15, 146–148, 733–734, 769–770, 846–847
Animal co-products/edible by-products, 708–712, 710*t*
  added value products, 722–723, 722*f*
  gelatin, 721
  haggis, 721
  meat extract, 720, 720*f*
  mechanically separated meat (MSM), 721–722
  nutritional value, 712–720, 713–719*t*
  soup stock, 721
  trimmings, 720–721
Animal influences, 24–31
  gender, 30
  genetics, 25–30
    breed differences, 28–29
    breeds, 25–28, 26*t*
    crossbreeding, 28
    efficiency, growth, 29–30
    modification, 30–31
  physiological age/stage, maturity, 24–25
Animal, nonanimal influences, 39–44
  growth, utero, 39–41
    epigenetics, 40–41
    fetal developmental programming, 39–40
  postnatal growth, 41–44
    compensatory growth, 42, 43*t*
    plane, nutrition, 41–42
    prenatal, postnatal growth, 43–44
Animal welfare, 729–730
Anomalies meat color, defects, 382–385
  cooked meat, 384–385
  fresh meat, 382–384
Anthelmintic resistance, 596
Antibiotic growth promoters (AGPs), 36
Antinutritional factors, 760–761
Antioxidants, 769
Apollo-Soyuz Test Project (ASTP), 246
Arginine-glycine-aspartic acid (RGD), 86

Aroma
pre, post slaughter, 435–440
breed, sex, aging, 435–436
cooking, 439–440
farming, feeding practices, 436–439, 437*t*
taste compounds, 421–422
Assay
DNA, 635
enzymatic, 656–657
LAMP, 644
quantitative real-time PCR, 643
TaqMan probe, 643–644
Atmospheric pressure chemical ionization (APCI), 617–618
Atmospheric pressure ionization tandem mass spectrometry (API-MS/MS), 617–618
ATP-ase, 70–71, 71*f*, 170, 223–224
Authenticity, 628, 630*t*

B

Bacon, 246, 284, 307–309, 445–447
Bacterial spoilage monitoring, 210
Barcode Of Life Data (BOLD) Systems, 640–641
Beef in an Optimal Lean Diet (BOLD), 692
Beta-agonists, 35, 93–95, 597
β-lactams, 593
Biological meatborne
hazards, 550–551, 552–553*t*
issues, 576–579
biogenic amines, 577–579
prions, 576–577
Biomarkers
molecular, 632–633
species-specific, 645
Bloat, 758
Blown-pack spoilage, 209–210
Boar taint, 38, 442–443, 735
Body/carcass composition, measurement, 22–24
BOLD. *See* Beef in an Optimal Lean Diet (BOLD)
Bovine spongiform encephalopathy (BSE), 576–577, 649, 708
Breast cancer, 694
Breeds, 25–28, 26*t*
BSE. *See* Bovine spongiform encephalopathy (BSE)
B vitamins, 673
By-products, 707–712, 708*f*, 709–710*t*, 738, 745–748, 746*t*

C

Calcium, 675
Calpains, 187
Calpastatin, 126, 187, 226, 408–409
Canned meat, 235
Carbohydrates, 687–688
Carbon dioxide, 125, 229–230, 328, 762
Carbon monoxide, 255, 259–260, 328–329, 368–369
Carbonyl compounds, 296, 424
Carboxymyoglobin, 380
Cardiovascular disease (CVD), 668
Caspases, 95, 125, 188, 226, 409
Catalase, 305
Cathepsins, 95, 126, 189, 226, 409–410
Cell-cultured meat, 826–835
benefits, 826–828
consumer acceptance, 834–835
production, developments, 828–834, 829*f*, 833*f*
Cellular prion protein (PrP^C), 576
Cereal grains, 765
CHD. *See* Coronary heart disease (CHD)
Chemical composition, meat, 105–120, 106–107*t*
carbohydrates, 114–115, 114*f*
intramuscular fat, 115–120, 116–118*f*, 116*t*
muscle proteins, 105–111
connective tissue, 110–111, 110–111*f*
myofibrillar proteins, 105–108
sarcoplasmic proteins, 108–110, 109*f*
water, 111–114, 113*f*
Chemical contaminants, 592–605
analytical methods, 606–618
instrumental analysis, 617–618
sample preparation techniques, 607–617, 609–615*t*
heavy metal, 603–604
mycotoxins, 604–605
persistent organic pollutants (POPs), 599–603
dioxins, polychlorinated biphenyls (PCBs), 600–602
pesticides, 599–600
polycyclic aromatic hydrocarbons (PAHs), 602–603
risk assessment, 605–606
veterinary drug, 592–599
anthelmintics, 596
antibacterials, 592–596
β-agonists, 597
coccidiostats, 597

hormones, 598
nonsteroidal antiinflammatory drugs (NSAIDs), 597–598
tranquilizers, 599
Chemical ionization (CI), 617–618
Chilling, meat, 220–230
chill storage, 229–230
fiber shortening, prevention, 221–225, 224*f*
meat tenderness, 225–226
oxidative changes, 227–228
processes, 220–221, 221*f*
superchilling, 228–229
Cholesterol, 666
Choline, 673
CJD. *See* Creutzfeldt-Jacob disease (CJD)
CLA. *See* Conjugated linoleic acids (CLA)
Clostridium botulinum/botulism, 557–558
Clostridium difficile, 558–559
Clostridium perfringens, 557
Clustered regularly interspaced short palindromic repeat (CRISPR), 30–31
Cold shortening, 220
Collagen, 75–81, 76*f*, 78*t*, 80*f*
Color, 180–181, 269–270, 369–378, 371–372*t*, 382–385
Colorectal cancer, 693
Common-primer-multiplex PCR (CP-M-PCR), 639–640
Conjugated linoleic acids (CLA), 117–118, 669, 691
Consumer, 259, 540–545, 824–826, 834–835
Cooked ham, 305–307, 305*f*
Corned beef, 282
Coronary heart disease (CHD), 666
Corticosteroids, 598
COVID-19, meat supply chain, 572–573, 733, 843
Creutzfeldt-Jacob disease (CJD), 576–577
Cured meat pigments, 378–380, 379*t*
Curing, 282–290
CVD. *See* Cardiovascular disease (CVD)
Cytoskeleton, 69

D

Daily value (DV), 666–668
DALY. *See* Disability-adjusted life year (DALY)
Dapsone (diamino-diphenyl sulfone), 596
Dark, cutting, 176, 383
Dark, firm, and dry (DFD) meat, 733–734
DASH. *See* Dietary approaches to stop hypertension (DASH)
Dehydration, 270–274, 271*f*, 293–295, 294*f*
Deoxymyoglobin (DeoxyMb), 369–370
Descriptive testing, 527–531
DFD. *See* Dark, firm, dry (DFD)
Diaminopyrimidines, 595
Dielectric heating, 238–239
Dietary approaches to stop hypertension (DASH), 692
Dietary reference values (RVs), 666–668
Diffuse adherent *E. coli* (DAEC), 559–562
Digestible crude protein (DCP), 32
Digital PCR (dPCR), 643–644
DI-IRMS. *See* Dual-input isotope ratio mass spectrometer (DI-IRMS)
Disability-adjusted life year (DALY), 574
Discoloration, 228, 254–255, 375
Domestication, livestock, 3–6
cattle, 4–5
sheep, 6
swine, 5
Droplet digital PCR (ddPCR), 643–644
Drying. *See* Dehydration
Dual-energy X-ray absorptiometry (DXA), 23–24
Dual-input isotope ratio mass spectrometer (DI-IRMS), 653–654

E

Eating quality, 413
Edible by-product, 708–712, 710*t*
ECDC. *See* European Centre for Disease Prevention and Control (ECDC)
ECMs. *See* Extracellular matrices (ECMs)
Ecosystems, 737–738
EFSA. *See* European Food Safety Authority (EFSA)
Electrical stimulation, 185, 465–467
Electron impact ionization (EI), 617–618
Electron spin resonance (ESR) signal, 656
Electron transport chain (ETC), 168
Electrophoretic techniques, 632
Electrospinning, 807
Electrospray ionization (ESI), 617–618
ELISA. *See* Enzyme-linked immunosorbent assay (ELISA)
Embryogenesis, 52
Emerging/underutilized sensory techniques, 545–547, 546*f*
Enteroaggregative *E. coli* (EAEC), 559–562
Enterohemorrhagic *E. coli* (EHEC), 559–562
Enteroinvasive *E. coli* (EIEC), 559–562
Enteropathogenic *E. coli* (EPEC), 559–562

Enterotoxigenic *E. coli* (ETEC), 559–562
Environmental contaminants, 591
Enzyme-linked immunosorbent assay (ELISA), 644
EPIC. *See* European Prospective Investigation into Cancer and Nutrition (EPIC)
European Centre for Disease Prevention and Control (ECDC), 555
European Committee for Standardization (CEN), 656
European Food Safety Authority (EFSA), 555, 605
European Prospective Investigation into Cancer and Nutrition (EPIC), 678
European Union (EU), 551, 666–668
Evaporation, 131–132, 230–231, 271–272, 298–299, 720
Extended spectrum β-lactamase (ESBL), 563
Extracellular matrices (ECMs), 75
Extrinsic characteristics, meat, 653–658
  origin, feeding regimes, 653–655
  treatments, 655–658
    frozen/thawed meat, 656–658
    irradiated meat, 656
Extruder, drag flow device, 805–806
Exudation, 112–114, 224–225
Exudative, 130, 173, 224–225, 270, 401–402, 442, 464

F

FAO. *See* Food and Agriculture Organization (FAO)
Farm animals, 23–24, 72–73, 566–567
Fast-twitch glycolytic (FG)/fast-twitch oxidative-glycolytic (FOG), 70–71
Fat, 51
Fat-soluble vitamins, 688
Fatty acid, 655
FDA. *See* Food and Drug Administration (FDA)
Feed conversion efficiency (FCE), 29–30
Feed conversion ratio (FCR), 29–30
Fermentation, 290–293, 291–292*t*, 292*f*
Fermented sausages, 301–305, 301*f*, 303–304*t*
Fiber, muscle, 70–73, 71*f*, 72*t*
Filaments, muscle, 108, 268–269, 400–401
Flavor, 440–449, 535–536
Fetal programming, 40
Food and Agriculture Organization (FAO), 53, 605, 694–695
Food and Drug Administration (FDA), 695–696
Foodborne pathogens, 550
Foodborne zoonosis, 559–562
Free radicals, 228, 246–248, 380
Freeze dehydration, 270–274, 271*f*
  histological aspects, 271–272, 272*f*
  organoleptic aspects, 273
  physical, biochemical aspects, 272–273, 272*f*
Freezing, 230–234
  effects, muscular tissue, 231–234, 232–233*f*
  process, 230–231

G

Gas chromatography (GC), 606–607
Gastrointestinal infection/nontyphoidal salmonellosis, 567–568
Genes, 77
Genetically modified organisms (GMOs), 15–16
Genome-wide association studies (GWAS), 93
Gerstmann-Sträussler-Scheinker syndrome, 576–577
Glycogen debranching enzyme (GDE), 165
Glycolysis, 164–168, 166*f*, 400–403, 403*f*
GMOs. *See* Genetically modified organisms (GMOs)
Graphitized black carbon (GBC), 617
Green meat supply chain, 732–733
Growth, 29–44, 88–96

H

Halal laws, 13–14
Ham, 250*t*
Hazard an alysis and critical control point (HACCP) systems, 581
Health, 599, 605
Heating, meat, 234–238
  heat treatments, 234–235
  pasteurization, 235–236
  sterilization, 237–238
Heat shock proteins (HSPs), 147
High density lipoprotein (HDL), 690–691
High-performance liquid chromatography (HPLC), 633
High pressure, preserve food, 261–270
  antimicrobial aspects, 264–265, 266–267*t*
    microbial inactivation, 265–268
  antimicrobial mode of action, 264

chemical, biochemical aspects, 264–270
color, 269–270
lipid oxidation, 269
meat proteins, 268–269
high-pressure processing (HPP), 261–264, 262–263*t*
High resolution mass analyzers (HRMS), 618
High-temperature conical shear cell (HTSC) technology, 807
Hormones, 34–35
HPLC. *See* High-performance liquid chromatography (HPLC)
HRMS. *See* High resolution mass analyzers (HRMS)
HSPs. *See* Heat shock proteins (HSPs)
Hydrophilic-lipophilic balance (HLB), 617
Hygiene, 284, 597, 771–772
Hypertrophy, muscular, 27, 30–31, 81

I

IARC. *See* International Agency for Research on Cancer (IARC)
Immunoglobulins (IgG), 632–633
Inosine monophosphate (IMP), 163–164
Insect proteins, 811–826
consumer acceptance, 824–826
consumption risk, 821–824
nutritional aspects, 812–821, 813–820*t*
Institute of Food Technologists (IFT), 509
International Agency for Research on Cancer (IARC), 618–619, 692–693
International Assessment of Agricultural Knowledge, Science, and Technology for Development (IAASTD), 591–592
Intramuscular connective tissue (IMCT), 73–86, 78*t*, 111*f*
Intramuscular fat (IMF), 89
Ionizing radiation, preserve food, 246–260
antimicrobial aspects, 249–251, 250*t*
chemical, biochemical aspects, 252–256
color changes, 254–256
lipid oxidation, 252
off-odor production, 252–254, 254*f*
texture change, 256
consumer acceptance, irradiated meat, 259
detection, 259–260
food irradiation, 246, 247*t*, 260
irradiation process, 246–248, 248*t*
mode of action, 248–249
organoleptic aspects, irradiated meat, 256–259, 258*t*
Ionophores, 765
Iron, 675–677
Irradiation, 246–249, 252, 256
Isoelectric focusing (IEF) electrophoresis, 632

J

Joint FAO/WHO Expert Committee on Food Additives (JECFA), 603–604
Juiciness, 481–483, 483*f*

K

Ketosis, 761
Kosher laws, 14

L

Lactic acid, 128, 208–209, 316
Lactic acid bacteria (LAB), 196
LAESI-MS. *See* Laser-ablation electrospray ionization mass spectrometry (LAESI-MS)
Lard/pig fat, 711
Laser-ablation electrospray ionization mass spectrometry (LAESI-MS), 645
Lateral flow device (LFD) assay/lateral flow immunochromatographic assays, 632–633
Lateral flow immunoassays (LFIA), 632–633
Lateral flow tests (LFT)/rapid immune strip test, 632–633
Limit of detection (LOD), 631
Lipid oxidation, 219
Lipids/fats, 688
Lipid supplements, 762–764
Liquid chromatography (LC), 606–607, 633
Loop-mediated isothermal amplification (LAMP), 644
Low density lipoprotein (LDL), 690
Low-quality forages, 751
Low-temperature long-time cooking (LTLT), 481
Lysosome, 126, 189

M

Macrolides, lincosamides, 594
Mad cow disease, 710–711
Magnesium, 675
Maillard reactions, 422–427, 434, 439
Mass spectrometry (MS), 606–607
Matrix metalloprotease (MMP), 81
Matrix solid-phase dispersion (MSPD) approach, 616

Maximum residue limits (MRLs), 592
Meat, 591–592
analogs/meat alternatives, 799–800, 800*f*
authentication, traceability, 628–629, 629*f*, 630*t*
functional muscle foods, 696–698
global production, 665–666, 783–790, 783–785*f*
animal transport and slaughter, 789
antioxidants in livestock feeds, 787
legumes as ruminant feeds, 785–786
lipids as ruminant feed supplement, 786
nutrition to genetics, 788–789
packaging technology, 789–790
secondary metabolites and bioactive compounds, 787–788
healthy nutrition and diet, 678, 689–694
cancer, 692–694
cardiovascular disease, 690–692
industry, pandemic planning, 843–846
intakes, 694–696
mechanically deboned, 721–722
microbiology, spoilage, 196–205
microbiota, meat products, 196–202, 198–200*t*
new genomes, 202–205, 203–204*t*
other animals, 2–3
preservation, 240
nutrients, cooking effect, 679, 680*t*
product flavor, 444–449
dry-cured meat, 446–447*t*, 448
fermented dry-cured meat products, 448–449
wet-cured meat products, 444–448
quality attributes, 178–182, 729–730
color, 180–181
texture, 181–182
water-holding capacity, 178–180, 179*f*
safety challenges, 579–582
supplied nutrients, 687–689
macronutrients, 687–688
micronutrients, 689
3D printing/additive manufacturing, 697–698, 809
toxins and residues problem, 698–700
Meatborne bacterial pathogens and toxins, 554–569
Campylobacter spp., 554–556, 560*t*
Clostridium spp., 556–559
enterohemorrhagic *Escherichia coli*, 559–563, 561*t*
Listeria monocytogenes, 563–566
other parasites, 575–576
other pathogens, 569
Salmonella enterica, 566–568
Yersinia enterocolitica, 568–569
Meatborne parasites, 573–576
Taenia spp., 574
Toxoplasma gondii, 574–575
Trichinella spp., 575
Meatborne viruses, 570–573
hepatitis, 570–571
noroviruses (NoV), 571–572
other viruses, 572–573
Meat color, 369–378, 371–372*t*
cooked, 380–382, 381*t*
fresh, myoglobin redox stability
endogenous factors, 373–376, 373*f*
exogenous factors, 376–378
myoglobin oxidation, reduction, 369–370, 370*f*
measurement, 385–386
Meat composition, nutrients intake, 666–678
bioactive compounds, 677–678
macronutrients, 666–673, 667*t*
fat, 668
fatty acids, 668–673, 670–671*t*
protein, 666–668
minerals, 675–677, 676*t*
vitamins, 673–675, 674*t*
Meat, hierarchical structures, 53–88
adipose tissues, 86–88, 87*f*
anatomy, individual muscles, 58–61, 60*f*
skeletal muscle structure, 61, 61*f*
carcass level, meat cuts, 53–58, 54–55*f*
fat, lean muscle, 53–58, 56–57*f*, 59*t*
individual intramuscular connective tissues (IMCTs), 73–86, 74*f*
cell–matrix connectors, 83–86, 85*f*
collagen crosslinking, 79–81, 80*f*
collagens, muscle, 75–79, 76*f*, 78*t*
composition, 75
glycoproteins, proteoglycans, 82–83, 84*f*
microfilaments, elastin, 81–82
turnover, remodeling, 81
variations, 75
striated (skeletal) muscle cell, 62–73
cytoskeletal protein structures, 66–70, 68*f*
muscle fiber types, 70–73, 71*f*, 72*t*
myofibrillar proteins, 64–66, 67*f*
structures, cell, 62–64, 63*f*, 65*f*
Meat identification, intrinsic characteristics, 629–653
animals
genetically modified, 652–653
sex, 651–652

traceability, 649–651
specie's origin, 629–646
DNA analysis, techniques, 634–644, 636*t*
fat, metabolite analysis/physicochemical components, 644–646
proteins, peptides analysis, 631–634
subspecies/breed origin, 646–649
Meat processing, 281–282, 282*f*, 769–779
automated, on-site, 774–775
carcass processing, 772–773
cold boning, 773, 773*f*
hot boning, 772
curing, 282–290
antimicrobial effect, 285–286
antioxidant effect, 286
color development, 286–287
nitrate and nitrite chemistry, 284–285
nitrosamines formation, 287–289
production, without nitrite, 289–290
salting, 283–284
dehydration, 293–295, 294*f*
fermentation, 290–293, 291–292*t*, 292*f*
preservation, 775–779
dry aging, 777–778, 777*t*
frozen storage, 778–779
immediate consumption/retail display, 775–776
wet aging, 776–777, 777*t*
processing technologies, cured meat, 297–309
bacon, wiltshire cure, 307–309
cooked ham, 305–307, 305*f*
dry-cured ham, 297–300, 298*f*, 300*f*
fermented sausages, 301–305, 301*f*, 303–304*t*
slaughter, 771–772
smoking, 296–297
transport, lairage management, 769–770
waste management, 775
Mechanically recovered/reclaimed meat (MRM), 721–722
Metmyoglobin, 370
Microbial contaminants, meat spoilage, 205–210, 206*f*
bacteria, 206–210
Brochothrix thermosphacta, 209
Clostridium sp., 209–210
lactic acid bacteria, 208–209
Pseudomonas sp., 206–208
microbial interactions, spoilage, 210
yeasts, molds, 205–206
Microwave-assisted extraction (MAE), 607
Minerals, 689
Mitochondria, 73
Modified atmosphere packaging (MAP), 229–230
MRFs. *See* Myogenic regulatory factors (MRFs)
MRM. *See* Mechanically recovered/reclaimed meat (MRM)
MSM. *See* Mechanically separated meat (MSM)
Multidrug-resistant (MDR) phenotypes, 580
Multiresidue analysis, meat products, 609–615*t*, 617
Muscle, adipose tissue, 21–22
Muscle development, growth, 88–96
adipogenesis, fibrillogenesis, 91
embryogenesis, prenatal growth, 88–91, 89*f*, 90*t*
fetal development, 91–92
muscle cells, satellite cells, 96, 96*f*
postnatal muscle growth, 92–95, 94*f*
Muscle function, meat quality, 135–152
age, 143–146
animal welfare, stress, 146–148
conversion to meat, 159, 178
diet, plane of nutrition, 148–151, 149–150*f*
interanimal variability, 151–152, 152*f*
muscle fibre type, anatomical location, 135–140, 136*t*, 137*f*, 138*t*
sex, 143
species, breed, 140–143, 141*t*, 143–144*t*
training, exercise, 146
Muscle metabolism, biochemical aspects, 120–135
muscle function in vivo, 120–127, 121*f*, 124*f*
postmortem muscle function, 127–135, 127*f*, 129–133*f*
Mycotoxins, 699, 761
Myofibrillar contraction, 402–403
Myofibrillar proteins, 64–66, 105–108, 181
Myogenic regulatory factors (MRFs), 89–90
Myoglobin
concentration, 364–366, 365*t*
structure, 366–369, 367–369*f*
Myostatin (*MSTN*) gene, 647

N

Nanoflow-liquid chromatography/high-resolution mass spectrometry (nLC-HRMS), 634
National Aeronautics and Space Administration (NASA), 246, 826
National Organic Program (NOP), 836
Natural resources, 742
Next-generation sequencing (NGS), 641

Nicotinamide adenine dinucleotide (NADH), 165
Nisin, 318–319
Nitrate, 289, 305
Nitrite, 284–287, 378–380
Nitrofurans, 594
Nitrosamines, 287–289, 698
Nitrosomyoglobin, 286–287
nLC-HRMS. *See* Nanoflow-liquid chromatography/high-resolution mass spectrometry (nLC-HRMS)
NMR. *See* Nuclear magnetic resonance (NMR)
Nonanimal influences, growth, 31–39
  environmental influences, 38–39
  exogenous agents, 34–38
    β2 agonists, 35
    gut-active compounds, 36–38, 37*t*
    somatotropin, 35–36
    steroid hormones, 34–35
  immunological approaches, 38
  nutrient supply, 31–33
    energy feeding systems, 31–32
    other nutrients, 33
    protein feeding systems, 32–33, 34*t*
No observed adverse effect level (NOAEL), 605–606
Novel organic by-products/food waste, 738
Novel thermal procedures, 238–240
  dielectric heating, 238–239
  ohmic heating, 238, 239*f*
  radiant heating, 240
NSAIDs. *See* Nonsteroidal antiinflammatory drugs (NSAIDs)
Nuclear magnetic resonance (NMR), 644–645
Nutritional value, 728–729

O

Odors, 512
Offal, 51, 603–604, 689, 745–747
Off-flavors, 440–444
  boar taint, 442–443
  irradiation odors, 442
  microbial odors, 443–444, 445*f*
  warmed over flavor, 440, 441*t*
Off-odors, 208–209
Ohmic heating, 238
Olfactometry, 432
Organic Foods Production Act (1990), 836
Organic meat products, 836–843, 837*t*
  anticipated markets, 842–843
  future prospect, 841–842
  production system, livestock, 836–840
  quality difference, conventional, 840–841
Organochlorine pesticides (OCPs), 600
Organophosphorus pesticides (OPPs), 600
Oxidation, 370, 371–372*t*, 379*t*
Oxidative reactions, 318–319, 422–424
Oxygen, 181, 201–202, 332, 353*f*
Oxidative status, 147–148
Oxymyoglobin, 369–370

P

PAACO. *See* Professional Animal Auditor Certification Organization (PAACO)
Packaging technologies, 322, 368–369
Packed bed bioreactor (PBR), 832–833
Palatability, 181–182, 510, 824–825
Parasites, 573–576, 766
Pasterurization, 211–212, 235–236
Pathogens, 552–553*t*, 558–559
Pale, soft, and exudative (PSE) meat, 733–734
PCA. *See* Principal component analysis (PCA)
Peptides, 595
Persistent organic pollutants, 599–603
Pest control, 599–600
Pesticides, 599–600, 609–615*t*
Plant-protein, meat analogs, 801–811
  challenges, opportunities, 809–811
    market, consumption prospect, 811
    nutrition, safety aspects, 810
    sensory aspects, 809–810
  processing, 804–809, 805–808*f*
  structure, functionality, 801–804, 801*t*, 802–803*f*
Plasma cholesterol, 690–691
Pleuromutilin, 595
Polybrominated diphenyl ethers (PBDEs), 603
Polychlorinated dibenzofurans (PCDFs), 600–601
Polychlorinated dibenzo-*p*-dioxins (PCDDs), 600–601
Polychlorinated naphthalenes (PCNs), 603
Polycyclic aromatic hydrocarbons (PAHs), 694
Polyfluorinated alkylated substances (PFAS), 603
Polymerase chain reaction (PCR), 635–638
  analyses coupled, simple post-PCR methods, 639–641
  chip analysis, 642
  digital, 643–644
  next-generation sequencing, 641
  non-PCR systems, 644
  real-time, 642–643
Polyphenols, 764–765
Polyunsaturated fatty acids (PUFAs), 115, 252, 714–720, 767–769
POPs. *See* Persistent organic pollutants (POPs)
Postmortem handling, meat quality, 182–186

accelerated processing, 185–186
actomyosin toughening, sarcomere shortening, 182–183
electrical stimulation, 185
muscle temperature, 183–185
Postmortem metabolism, 162–170
ATP homeostasis, 162–163
factors controlling
extent, 171–173, 171*f*
rate, 170–171
glycogenolysis, glycolysis, 164–168, 166*f*
mitochondria, 168–170
phosphagen system, 163–164, 164*f*
Postmortem pH decline on meat quality, 160–162, 160*f*
Presalughter stress, 176–178
fasting, 177–178
stunning, 178
transport, lairage, 177
Pressurized liquid extraction (PLE), 607
Principal component analysis (PCA), 645
Professional Animal Auditor Certification Organization (PAACO), 12
Protease, 395–396
Protected denomination of origin (PDO), 646
Protected geographical indication (PGI), 653–654
Protein, 687–688
Protein posttranslational modifications (PTMs), 147
Proteolysis, 224
Proteomics, 297, 501, 633–634
Provisional tolerable monthly intake (PTMI), 603–604
PrP^C. *See* Cellular prion protein (PrP^C)
PSE. *See* Pale, soft, exudative (PSE)

Q

QTL. *See* Quantitative trait loci (QTL)
Quantitative PCR (qPCR), 642–643
Quantitative trait loci (QTL), 88
QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), approaches, 606–607
Quinolones, 594
Quinoxalines, 596

R

Radiation, 246–260, 319–321
Rancidity, 228, 286, 440
Ready-to-eat (RTE), 234–235, 549–550
Reference dose (RfD), 606
Reference intakes (RI), 666–668
Refrigeration, 219–220
Residual feed intake (RFI), 30
Residues, 79, 164, 367*f*, 592–599, 698–700
Rho-associated protein kinase-inhibitor (ROCKi), 833
Rigor mortis, 160–162, 161–162*f*
Risk assessment, 605–606
ROCKi. *See* Rho-associated protein kinase-inhibitor (ROCKi)
Ruminants, 36

S

Sarcomere(s), 69–70, 395
Sarcoplasmic proteins, 70
Sarcoplasmic reticulum (SR), 62
SARS-CoV. *See* Severe acute respiratory syndrome coronavirus (SARS-CoV)
Saturated fatty acids (SFA), 666
Sausage, 301–305, 484
Secondary metabolites, 766–767
Sensory characteristics, 248–249
Sensory controls, meat, 521–527, 522*t*
environmental controls, 525–526
panelist controls, 526–527, 528–529*t*
product controls, 523–525
Sensory evaluation meat, 510–511
perceived, sensory attributes, 511–521, 514–520*t*
Sensory techniques, 527–545, 530*f*
consumer analysis, 540–545
ballot development, use, 543–545, 544*f*
central location tests (CLT), 542–543
home use tests (HUT), 543
descriptive analysis, 534–540
flavor, texture profile, 535–536
quantitative descriptive attribute analysis, 539–540, 541*f*
sensory evaluation, 536–537, 537–538*f*
spectrum descriptive attribute analysis, 537–539, 540*f*
discriminative analysis, 531–534, 533*f*
Severe acute respiratory syndrome coronavirus (SARS-CoV), 572–573, 843
Shiga toxin *E. coli* (STEC), 559–562
Shiga toxins, 562
Single nucleotide polymorphisms (SNPs), 52, 203–205, 648–649
Skeletal muscle, 58
Slime, 208–209
Slow-twitch oxidative (SO), 70–71
Small leucine-rich proteoglycan (SLRP), 83
Smoking, 282, 296–297, 602
Solid phase extraction (SPE), 606–607
Solvent extraction (SE), 606–607

Species identification, 631–635, 636*t*
Spices, 121–122, 260, 318–319, 449
Spores, 249–251, 325, 698–699
Spoilage manifestations, 211
Stable isotope ratios (SIR), 653–654
Sterilization, 234–235, 237–238
Steroid hormones, 34
Storage, preservation, meat, 315–316
  extend product shelf-life, 317–329
    chemical preservatives, 318–319, 320–321*t*
    chilling, 317
    heat-shrink wrapping, 324–326, 325–326*f*
    ionizing radiation, 319–321
    modified atmosphere packaging, 327–329, 328–330*f*
    meat developing technologies, 321–322
    meat packaging technologies, 322
    superchilling, 317–318
    vacuum packaging, 322–324, 323–325*f*
    vacuum skin packaging, 326–327, 327*f*
  microbiology impact, 316–317
  packaging materials, 330–340
    antimicrobial packaging, 339–340, 340–347*t*
    barrier materials, 330–331
    gas barrier evaluation films, 332–335, 332–333*f*, 334*t*, 335*f*
    nanotechnologies, barrier packaging, 335–337, 336–337*f*, 338*t*
  smart intelligent packaging systems, 348–358, 349*f*, 350–352*t*, 353–355*f*, 356*t*, 357*f*
Strecker degradation, 253, 424–427
Sulfonamides, 594–595
Sustainable animal production systems, 729–739
  advantages and disadvantages, 737–739, 737*f*, 739*t*
  definition, 739–741, 740*t*
  intensive, 733–735, 734*f*
  maintenance, 742
  meat processing, benefits, 779–782
    economic, 780
    environmental, 781–782
    social, 780–781
    welfare, 782
  practices, 743–757
    agri-food, industrial by-products, 745–748, 746*t*
    fodder, tree legume, 751–755
    fresh, conserved grass pastures, 743–745
    genetic potential, 755–757
    low-nutritive high-fibrous roughages, 748–751, 750*f*
    perennialized grazing cereals, 757
  semiintensive, 735–736, 736*f*
  technologies/strategies, improvement, 757–769
    dietary-related subclinical diseases, 757–761
    methane gas emission, mitigation, 762–765, 763*f*
    secondary metabolites, bioactive compounds, 766–769
Sustainable meat production, holistic approach, 728, 728*f*

T

Tallow/sheep fat, 711
Taste, 196, 316, 421–422, 511–513, 514–520*t*
Tenderness, 393–413
  definition, 393–394
  measurement, 393–394, 413–414
  muscle type effect, 394–395, 394*t*
  postslaughter factors, 400–413
    aging, 406–407, 407*f*
    chemical intervention, 410–413, 412*t*
    muscle stretching, 403–406, 404*t*
    postmortem glycolysis, 400–403, 403*f*
    proteolytic mechanisms, 407–410
  preslaughter factors, 395–400
    age effects, 397–398, 398*t*
    gender effects, 398–399, 399*t*
    genotype effects, 395–397, 396*t*
    handling effects, 399–400
Testosterone, 34–35, 38, 93–95, 598
Tetracyclines, 595
Texturization/texturing, plant proteins, 804
Thaw rigor, 183–184, 230–231
Thermal process, 219–220
Thermal technologies, 220, 240
Thermoextrusion approach, 805
Thyreostats, 598
Tissue inhibitors of metalloproteinases (TIMPs), 81
Tolerable daily intake (TDI), 606
Total body electromagnetic conductance (TOBEC), 23–24
Toxic equivalent factor (TEF), 601
Toxins, 352, 554–569
Traceability, 628, 630*t*, 649–651
Trace element, 653–654
Traditional Speciality Guaranteed (TSG) products, 653–654
Transcription activator-like effector nuclease (TALEN), 30–31
Transmissible spongiform encephalopathies (TSE), 576
Trends, developments, 6–16
  animal welfare, 11–12, 12*t*
  cultured meat, 15–16

kosher, halal, 13–15
meat production, 7–9, 7–10*t*
plant-based meat alternatives, 16
sustainability, 12–13
Typhoid/enteric fever, 567–568

U

UK Food Standards Agency, 666
Ultra-performance liquid chromatography (UPLC), 617–618, 633
Ultrasound-assisted extraction (UAE), 607
Umami, 421–422, 711–712
United Nations Economic Commission for Europe (UNECE), 53
United States Department of Agriculture (USDA), 836
United States Department of Agriculture's Food Safety and Inspection Service, 563
UPLC. *See* Ultra-performance liquid chromatography (UPLC)

V

Vacuum packaging, 201–202, 322–324, 324*f*
Variant Creutzfeldt-Jacob disease (vCJD), 576–577
Verocytotoxigenic *E. coli* (VTEC), 559–562
Very low density lipoprotein (VLDL), 690–691
Veterinary drug residues, 592–599
Virulent coronavirus strain, 572–573
Vitamin D, 673–675
Volatile compounds, 422–427, 423*f*
carbohydrate degradation reactions, 427
ribonucleotides, 427
thiamine, 427
lipid degradation (oxidative reactions), 422–424, 425*f*
Maillard reactions, 424–427, 426*t*
methodology, 428–435
animal species, aroma, 433–435
extraction, 428–432, 429*f*, 430–431*t*
identification, 432–433
Strecker degradation, 424–427, 426*t*

W

Warmed-over flavor, 252–253, 440, 441*t*, 513–521
Warner-Bratzler, 224*f*, 394*t*, 404*t*
Water binding, 488–489
Water-holding capacity (WHC), 256, 457
WBC factors, 484–493
amino acids, other additives, 492–493
high-pressure processing, 493, 494*f*
ions, ionic strength, 488–489
marination, 490*t*, 491–492
phosphates, 489–491
salt, 484–487, 486*f*
salts and bicarbonates pre rigor, 487–488, 488*f*
WHC comminuted meats, 484, 485*t*
WHC, cooking raw meat, 474–481, 474*f*
cook loss, aging, 478–479, 478–479*f*
high-pressure processing, 480–481
sarcoplasmic proteins, 479–480
sous vide cooking, 481, 482*t*
temperature water loss, 474–477, 475*f*, 477*f*
water loss, protein denaturation, 477–478
WHC, juiciness, 457–461, 459–460*t*
measuring methods, 493–500
external force, 495–498, 497*f*
no external force, 493–495, 495*f*
indirect, 498–499
juiciness, 500
WHC correlation, 499–500
raw muscle, 463–474
changes, aging, 467, 468*t*, 469–471*f*
cutting, packaging, temperature, 467–472
electrical stimulation, 465–467
freezing, thawing, 472–474, 473*t*
pH fall postmortem, PSE, DFD, 463–464, 464*f*
rigor, cold, heat shortening, 464–465, 465–466*f*
thaw rigor, 472
structural influences, 461–462
Wiltshire method, 307
Whole-transcriptome shotgun sequencing (WTSS), 52
World Health Organization (WHO), 605

Y

Yeasts, 36, 205–206, 290, 292*t*, 341–347*t*, 448–449

Z

Zinc, 677
Zinc finger nuclease (ZFN), 30–31
Z-line, 62–64, 120–121, 182–183, 401